DYNAMICS OF FOULING OF MICROPOROUS MEMBRANES BY PROTEINS

by

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ABSTRACT

Cross-flow filtration (CFF) through microporous membranes is an important downstream processing operation for the separation of proteins from cells or cell debris. A major drawback is membrane fouling which results in an irreversible decline in permeate flux and in the partial rejection of solutes that would otherwise pass through the membrane unhindered. This research project was initiated with the anticipation that an understanding of the fouling mechanism would lead to improvements in equipment design, membrane materials and geometry, or in operating conditions.

Preliminary CFF experiments using 0.2 \( \mu \text{m} \) track-etch membranes and 0.25 kg/m\(^3\) solutions of bovine serum albumin (BSA) in the absence of cells or cell debris resulted in an order of magnitude decrease in flux. The largest dimension of BSA (14 nm) is more than 10-fold smaller than the pore diameter; hence size exclusion of the protein was not expected, nor was it observed. However, the passage of protein through the pores makes microfilters especially susceptible to intra-pore blockage. Most previous studies on fouling focus on the reduction in pore diameter caused by protein adsorption but our calculations showed that a monolayer of BSA was insufficient to account for the observed flux decrease. This was confirmed by the negligible changes (< 5%) in permeability upon soaking the membranes in BSA solution for several hours. The faster flux decrease observed when using smaller feed volumes and the occurrence of an inflection in the flux-time profile with complete recycle could not be explained by conventional fouling theories that assume a constant foulant concentration in the feed.

We then investigated the possible introduction or generation of foulant during CFF. Quasi-elastic light scattering (QELS) analysis of the recirculated solution revealed a steady increase in the concentration of particles larger than 30 nm. Careful experimentation established a direct correlation between the rate of particle formation and the number of times the solution was passed through the peristaltic pump used for feed recirculation. Electrophoretic analysis under native and denaturing conditions, of the particles collected on the membrane surface identified them as aggregates of BSA.

This led us to hypothesize that the observed fouling was due to the convective deposition of aggregates generated during CFF by the action of the peristaltic pump. In addition to the QELS analysis, the effect of the peristaltic pump was confirmed by the 10- to 100-fold decrease in the fouling rate during dead-end filtration (DEF) or CFF with a diaphragm pump. The flux profiles were divided into two phases, the first characterized by pore occlusion and a decrease in the number of open pores and the second characterized by flow through a polarized layer of aggregates. The flux equations for the first phase developed under the assumption that most of the flow occurred through the open pores successfully predicted the rates of fouling. The equations for the second phase were not predictive because several important parameters were not known. These include the concentration of aggregates at the membrane surface, the specific resistance of these aggregates and their size distribution.
The aggregates constituted less than 0.5% (wt./wt.) of total BSA even after 320 passes through the peristaltic pump; hence, the observed transmission essentially refers to transmission of non-aggregated BSA. During the first phase of fouling, the transmission was 100% as expected when most of the flow occurs through open pores which are much larger than the protein. This implies that there should be no concentration polarization of BSA, a conclusion supported by the absence of an effect of cross-flow velocity on transmission. However, during the second phase, partial rejection of BSA by the deposited aggregates caused a decrease in observed transmission. This was successfully modelled by the conventional concentration polarization equation. The effect of cross-flow velocity on observed transmission was well described by incorporating the Levêque equation which predicts the mass-transfer coefficient to vary with the cube root of velocity.

This research project raised several questions about protein denaturation and aggregation by shear. A few early studies suggested that enzymes were inactivated by mechanical shear alone but more recent research indicates that shear probably acts in concert with interfacial effects. The narrow gap widths formed when the tubing in the peristaltic pump is compressed leaves open the possibility that a combination of shear and interfacial denaturation is responsible for aggregation. It is also possible that the peristaltic action aids the aggregation of denatured protein already existing in the original solution or generated elsewhere in the cross-flow system. However, further research is needed before definite conclusions about the aggregation mechanism can be made. Investigations of fouling with other membranes and proteins yielded results qualitatively similar to those obtained using BSA and track-etch membranes. Thermal denaturation/aggregation of BSA caused membrane fouling similar to that observed with aggregates generated by pumping. We speculate that any operation that causes protein denaturation/aggregation (e.g., agitation with aeration, presence of thermal and/or pH gradients, etc.) can have an impact on the performance of membrane separations. This reinforces the belief that biochemical process design must recognize the potential impact upstream processes have on downstream processes.

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Chapter 1  Introduction

1.1 Membrane separations

Microfiltration, ultrafiltration, and reverse osmosis are pressure driven membrane separation processes differentiated according to the size of the smallest feed component rejected by the membrane. This division is largely arbitrary since there is no precisely defined size cutoff for classifying a membrane. Secondly, size exclusion is not the only mechanism of rejection which is also influenced by interactions of the solute or particle with the membrane. The size ranges conventionally used in the literature (Porter, M.C., 1986) for classifying membrane separations and the types of solutes rejected and transmitted by each class of membrane are shown in Figure 1.1. Solute transport through reverse osmosis membranes occurs by a solution-diffusion mechanism as opposed to a convection-diffusion mechanism for ultrafiltration and microfiltration. As the ratio of solute size to pore diameter decreases the mathematical models describing the solvent flow emphasize the hydrodynamics instead of

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<th>REVERSE OSMOSIS</th>
<th>ULTRA-FILTRATION</th>
<th>MICRO-FILTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Water, salts, alcohols, amino acids, organic acids</td>
<td>Water, low mol. wt. solutes, proteins</td>
</tr>
<tr>
<td>REJECTED SPECIES</td>
<td>Salts, alcohols, amino acids, organic acids</td>
<td>Proteins, polysaccharides, cell debris, whole cells</td>
<td>cell debris, whole cells</td>
</tr>
<tr>
<td></td>
<td>10 Å (0.001 μm)</td>
<td>1000 Å (0.1 μm)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.1 Classification of membrane separation processes
thermodynamics of the system. Ultrafiltration is described using hydrodynamic gel-resistance models or a reduction in the effective trans-membrane pressure because of osmotic pressure gradients. Our research focuses on microfiltration where osmotic pressure effects can be neglected and the solvent flow modelled in terms of hydrodynamic resistances in series.

1.2 Cross-flow Microfiltration: Definitions and Description

Microfiltration is a pressure driven, membrane based separation of micron sized particles from suspension, a typical example of which is the separation of extracellular proteins from whole cells. Suspended particles and solutes that are rejected by the membrane form a porous layer on the surface resulting in a progressively greater hydrodynamic resistance to the convective flow of feed through the membrane. Under constant pressure operation, this results in a decrease in the flow through the membrane whereas under constant flux conditions, the trans-membrane pressure needs to be increased to compensate for the increased resistance. When Brownian diffusion is the only mechanism of removal of the rejected species from the membrane surface, the mode of operation is called Dead-End Filtration (DEF) which never reaches a steady state. Continuous operation necessitates the physical removal of the porous layer as is done during rotary vacuum filtration. An elegant technique of minimizing the accumulation of rejected species is to flow the feed parallel to the surface at a high shear rate thereby increasing the mass-transfer coefficient. This mode of operation, called Cross-Flow Filtration (CFF) or Tangential-Flow Filtration (TFF), facilitates the continuous, steady-state operation of the equipment. The precise mechanism of removal of colloidal or micron-sized particles by the cross-flow of feed is debatable and some of the prevalent theories such as enhanced diffusion, lateral migration, and axial 'cake' flow will be discussed in Chapter 3.

The stream that passes through the membrane is called the permeate and the stream that is enriched in the rejected species is called the retentate (see Figure 1.2). Under typical operating conditions, the permeate flow is only about 10% of the flow of suspension on the feed side of
the membrane and a single pass through the filter is insufficient to achieve a substantial concentration of solids in the retentate or a high recovery of soluble product in the permeate. Therefore, a part of the retentate is recycled, the recycle ratio being defined as the recycle flow divided by the feed flow. The concentration factor, defined as the ratio of the retentate concentration to the feed concentration, increases with the recycle ratio. Temperature increases caused by continuous recycling are controlled by a cooling unit (heat exchanger) especially when handling heat labile species. If the solutes are not heat labile, a situation rarely encountered in bio-processing, it is advantageous to operate at high temperatures because of the lower viscosity. The valve on the exit stream is used for regulating the trans-membrane pressure which is not uniform over the membrane surface because of an axial pressure drop. Only during vortex flow filtration (VFF) can the axial pressure drop and trans-membrane pressure be independently controlled because the shear is generated by rotating the membrane rather than by maintaining a high flow rate of feed.

Figure 1.2 Flow sheet of a typical CFF system
Two measures of filtration performance are the flux and transmission and they are defined as:

\[
\text{FLUX} = \frac{\text{Permeate flow (m}^3/\text{s)} \quad \text{Solute concentration in Permeate}}{\text{Projected membrane area (m}^2) \quad \text{Solute concentration in Retentate}}
\]

TRANSMISSION = \frac{\text{Solute concentration in Permeate}}{\text{Solute concentration in Retentate}}

During DEF, the transmission is based on the feed concentration since there is no retentate stream. The concentration of solids is based on the total volume of suspension whereas the dissolved solute concentration is expressed on a solid-free basis. This allows the transmission coefficient to be bounded between zero and one. For dilute suspensions of solids whose densities are greater than that of the suspending medium, the difference in concentration based on total volume or solid-free volume is small and this is the case in all our experiments (except perhaps near the membrane surface). The rejection coefficient is defined as '1 - Transmission'.

Cross-flow microfilters are available in a variety of configurations such as plate and frame, spiral wound, pleated sheet, hollow fiber, tubular and rotating cylinder. The choice depends on the scale of operation, the pressure rating, solids concentration, susceptibility to plugging of the feed channel, ease of cleaning, and their proven ability in similar processes. Microporous membranes are made from several polymeric materials such as polysulfone, polypropylene, polyvinylidene difluoride, nylon, etc., and even from ceramics and sintered metals. The choice of membrane depends on the available pore sizes, structural integrity under repeated filtration, sterilization and cleaning cycles, compatibility to feed components, and susceptibility to pore occlusion and solute (especially protein) adsorption. Details of the various membrane configurations and materials can be found in the review articles and books listed at the end of the 'References' section.
1.3 Applications of microfiltration

Cross-flow microfiltration is ideally suited for the separation/concentration of whole cells especially when the fermentation medium contains proteins and other macromolecules that cannot easily pass through ultrafiltration membranes. When whole cells are the product of interest, CFF is convenient for in situ washing away of extracellular contaminants by continuously adding fresh water (or buffered salt solution) to the feed at the same flow rate as the permeate. After the desired reduction in contaminant levels, the cell suspension can be concentrated to a fairly viscous slurry (15-20% dry wt.). Diafiltration can also be performed when complete recovery of expensive, extracellular product is crucial for the economic viability of the overall process. Of course, the greater the volume of fresh buffer added, the greater is the extent of dilution of the product in the permeate stream and costs associated with incomplete recovery must be balanced against costs of subsequent concentration and purification. Microfiltration can be used for concentrating whole cells before lysis for the recovery of intracellular products and as a means of retaining cells in continuous recycle fermenters (Cheryan, M. and Mehaia, M.A., 1986). There are a number of published reports of laboratory or pilot scale operation of cross-flow cell separation processes (Patel, P.N., et al., 1987; Hanisch, W., 1986; Zahka, J. and Leahy, T.J., 1985; O'Sullivan, T.J., et al., 1984; Henry, J.D. and Allred, R.C., 1972) but very few descriptions of actual industrial processes.

Other solid-liquid separation techniques include rotary vacuum filtration and centrifugation. Since the former operates in a dead-end configuration, filter aid (such as diatomaceous earth) must be added to improve the flux. This is obviously not practical when cells are the desired product making it necessary for subsequent removal of the filter aid. It also creates a waste disposal problem and increases the ash generation in an incinerator (Gravatt D.P. and Molnar, T.E., 1986; Bemberism, I., 1983). Centrifugation is economical when the density difference between the cells and the suspending medium is significant and when repeated washing/resuspension of the cells is not required. However, the purity of the streams is
usually much less than achieved by microfiltration. Zahka and Leahy (1985) obtained a 70-90% recovery of the influenza virus by filtration compared to only 40-50% by centrifugation. Kroner, K.H., et al. (1984) compared different solid-liquid separation methods for harvesting *E.coli* and found the energy consumption for CFF to be about three times that of a disk stack separator. However, the purity of the filtrate stream was very high compared to the other techniques. The scale-up options available with a centrifuge are limited to the few discrete sizes that are manufactured whereas with CFF, it is possible to get almost any desired scale, for example by the addition or deletion of plates in a plate and frame system. Unlike some centrifuges, the filtration system does not produce aerosols, a factor that can be important in processing recombinant products.

The bacterium *E. coli* is commonly used as a host for expressing recombinant proteins which often need to be released into the fermentation medium by cell lysis. Microfiltration can be used for the protein-cell debris separation instead of the high speed centrifuges otherwise required (Forman, S.M., et al., 1990; Gabler, R. and Ryan, M., 1985; Le, M.S., 1984a; Le, M.S., 1984b). However, the small size of cell debris can lead to pore occlusion and a deterioration in filtration performance. Nonspecific adsorption of protein to cell debris necessitates repeated washing in order to drive the protein into the liquid phase. Centrifugation is at a disadvantage because resuspension of cell debris is very cumbersome and secondly, a high speed, high capacity centrifuge would be required to handle the large liquid volume caused by the addition of wash buffer. In CFF one can perform a constant volume wash without process interruption. Le (1984b) found the membrane process to be 30-50% less expensive than centrifugation for harvesting *E. carotovora*. Labor costs were the most important factor in centrifugation (30-50%), while membrane replacement costs were significant for microfiltration (up to 50%). Kroner et al. (1984) found that the energy demand for the recovery of formate dehydrogenase from disrupted *Candida boidinii* cells was 2 to 5 times lower for CFF than for centrifugation.
CFF is also used for the sterilization of beverages, pharmaceuticals, and heat-labile fermentation media, preparation of ultrapure water for the semiconductor and pharmaceutical industry, and in plasmapheresis (with recycle of the blood cells to the donor). CFF can be combined with affinity separations for recovering high value proteins (Luong, J.H.T, et al., 1987).

1.4 Membrane fouling

The major drawback of membrane separation processes is the decrease in flux to 2-10% of the initial water flux upon filtration of protein solutions or cell suspensions. Some of this decrease can be attributed to the formation of a polarized zone by the reversible accumulation of feed components that are rejected by the membrane. The effect of the polarized zone may be to reduce the pressure driving force by an increase in osmotic pressure or to increase the hydrodynamic resistance by the formation of a 'gel layer' or 'porous cake'. This phenomenon, called concentration polarization or particle polarization, is essentially a transport problem and can be reversed by increasing the mass transfer coefficient at the membrane surface. For example, the use of high cross-flow velocities or the use of high speed rotating membrane devices minimize the accumulation of rejected species on the surface. Potential limiting factors are the high axial pressure drops that accompany high velocities, pump capacity, the temperature rise due to viscous dissipation, and the integrity of the membrane and mechanical seals at high shear rates and pressure drops. These limits are known in advance and one can usually design the filtration system to account for concentration polarization by using a larger membrane area. However, the loss of performance by fouling and the loss of product by shear damage cannot be compensated for in this manner.

Our focus is, therefore, on fouling which refers to the largely irreversible decrease in flux through the membrane. 'Irreversible' usually implies that changes in mass transfer coefficient have little effect on flux in the absence of chemical treatment, back flushing, or any other
membrane cleaning techniques. The difficulty in defining the term 'irreversible' may be
illustrated by the example of ultrafiltration of bovine serum albumin (BSA) discussed by
Reihanian (1983). The permeate flux during unstirred ultrafiltration followed the classical one
dimensional convection-diffusion model but there was little flux recovery when the pressure
was released and the flow interrupted for several hours; a theoretical calculation showed that
the polarized layer should have relaxed in a matter of minutes. Stirring the solution above the
membrane resulted in an immediate increase in flux. Thus, while Brownian diffusion alone
could not reverse the polarization, an increase in the mass transfer coefficient did. We use the
term 'fouling' to describe the flux decrease that cannot be attributed solely to concentration
polarization and that cannot be reversed by merely stopping the convective transport of solute
to the membrane surface or by changing the mass-transfer coefficient.

Fouling is usually a consequence of membrane-solute or solute-solute interactions such as
adsorption to the membrane and aggregation (or polymerization) leading to pore blockage. It is
also responsible for changes in the transmission characteristics of the membrane leading to the
partial rejection of solutes that otherwise pass through the membrane unhindered. This can
significantly affect the economics of the process especially when the rejected solute is the
desired high-value product. Restoration of membrane performance necessitates the frequent
cleaning and replacement of the membranes thereby increasing the operating costs.

Another mechanism of flux decrease, that is more common to the very hydrophilic
ultrafiltration and reverse osmosis membranes, is the change in pore structure upon swelling
due to water absorption. However, most microfiltration membranes are not very susceptible to
water absorption. Changes in pore geometry due to the structural failure of the membrane
under extremes of operating pressure, pH, temperature, etc., will not be considered although it
is an important factor in the choice of a filtration system especially when the membrane needs
to be repeatedly autoclaved, back-flushed, or chemically cleaned using acid or base.
1.5 Thesis outline

The focus of this work is on the fouling of polymeric, microporous membranes by proteins and we begin by a statement of objectives and a brief description of the problem in Chapter 2. Fouling of membranes has been extensively investigated especially in the context of protein adsorption to ultrafiltration membranes and some of the published work is summarized in Chapter 3. This chapter also contains a review of the literature on shear-denaturation of proteins because of its relevance to an understanding of protein aggregation which was determined to be the main cause of fouling in our experiments. Chapter 5 presents a detailed account of the experiments done to determine the mechanism of fouling using the model system and experimental protocol described in Chapter 4. The protocol underwent several modifications over the course of this project reflecting the evolution in our understanding of the mechanism. A discussion of the experimental results is postponed to Chapter 7 because many of the results are best explained using the flux and transmission equations developed in Chapter 6. Some of the assumptions made in deriving the equations are based on the experimental observations and hence the model is not completely general. The important conclusions are summarized in Chapter 8 and some recommendations for future work are made in Chapter 9.
Chapter 2  Problem Statement and Objectives

Cross-flow microfiltration is an important operation in the downstream processing of biological products. Its utility is greatest when the membrane can selectively allow the passage of macromolecules such as proteins or enzymes while retaining micron-sized particles such as whole cells. However, fouling of membranes by feed components can cause a substantial decrease in permeate flux and more importantly, change the transmission characteristics of the membrane. This can result in the partial rejection of the macromolecular product. It also decreases the throughput and separation efficiency and increases the costs associated with frequent cleaning and replacement of the fouled membranes.

Fouling could be caused by pore occlusion by cells, cell debris or protein aggregates, adsorption of macromolecules (proteins, nucleic acids, anti-foams, polysaccharides) to the membrane, or by polymerization of the rejected macromolecules in the polarized layer that may form on the membrane surface. The focus of this project is on the fouling of microporous membranes by proteins. Preliminary cross-flow experiments using solutions of bovine serum albumin (BSA) in the absence of cells or cell debris revealed an order of magnitude decrease in flux even though the protein transmission was 100% during the period of rapid flux decrease. BSA is an order of magnitude smaller than the pore diameter of 0.2 µm; hence size exclusion was not expected, nor was it observed. Previous studies on fouling focused on the possible adsorption of proteins to ultrafiltration membranes where because of the comparatively smaller pore size, even a monolayer of adsorbed protein can significantly decrease the pore diameter. Since we require the protein to pass through the microporous membrane, there was a distinct possibility of intra-pore blockage caused by protein adsorption. However, we estimated that a monolayer of BSA adsorbed to 0.2 µm membranes could cause the flux to decrease to 85% of its initial value, instead of the observed 10-fold decrease. This presented us with the problem of understanding the mechanism of fouling when size exclusion and monolayer adsorption of
native protein were not important. Previous research on microfiltration did not investigate the possible effect that upstream processes and peripheral equipment such as the feed and recycle pumps might have on fouling. We speculated that protein denaturation and aggregation caused by repeated recycling of the feed solution or by exposure to gas-liquid interfaces could lead to increased adsorption or pore occlusion. Thus, the primary motivation for this research project was the anticipation that a clear understanding of the fouling mechanism would lead to improvements in process and equipment design, operating procedures and in membrane material properties which would minimize the detrimental effect of fouling on flux and solute transmission.

To summarize, the specific objectives of this research project are:

1) Understand the mechanism of flux decrease during the cross-flow microfiltration of protein solutions when the hydrodynamic diameter of the protein is much smaller than the pore diameter (so that size exclusion is not important).

2) Investigate the effect of various operating conditions on the rate and extent of flux decrease. Examples of these variables are the trans-membrane pressure, cross-flow velocity, pore size and geometry, solute concentration, and pH.

3) Develop mathematical models to describe the dynamics of flux decrease and protein transmission and validate the model using the experimental data.

4) Use the knowledge of the fouling mechanism to suggest improvements in the operation and/or design of cross-flow microfiltration and in membrane properties.
Chapter 3  Literature Review

3.1 Membrane fouling by proteins

Most of the published work on membrane fouling deals with ultrafiltration but the fundamental concepts of membrane-solute and solute-solute interactions are applicable to microfiltration as well. The review by Matthiasson and Sivik (1980) cites numerous examples of fouling of reverse osmosis and ultrafiltration membranes by proteins as well as non-protein solutes. They make an important observation that "while the analysis of concentration polarization requires essentially mathematics, fouling requires knowledge about physical chemistry as well". A more recent review by Fane and Fell (1987), although not as exhaustive, summarizes the important causes of fouling such as protein adsorption to the membrane, precipitation in the polarized zone and pore occlusion by protein aggregates. Even when the main resistance to permeate flow is offered by a polarized cell layer (e.g., in cell-protein separations), it has been suggested that the adhesion of the cells to the membrane is mediated by a proteinaceous film on the surface (Hanisch, W., 1986). A sense of the complexity of fouling and the reasons for the lack of good, predictive models can be understood by examining a few published investigations of fouling. The techniques used for investigating fouling may be classified into two broad groups, namely fouling from stagnant or gently stirred solutions and fouling under actual filtration conditions where convective flow occurs through the membrane.

3.1.1 Fouling in the absence of convective flow.

These investigations involve exposing the membrane to a protein solution in the absence of a trans-membrane pressure gradient. Consequently, there is no convective transport of solution through the pores and any accumulation of protein on the membrane occurs by diffusion followed by adsorption. The extent of adsorption may be directly measured using radio-labeled proteins as tracers or by extracting the adsorbed protein into solution using
chemical reagents like sodium dodecylsulfate (SDS). Attempts to estimate protein adsorption by measuring the decrease in protein concentration in the contacting solution are usually unsuccessful because of the small amount of protein adsorbed. For example, the incubation of microporous membranes for 18 h in 1 kg/m³ solutions of BSA or IgG at pH 7.2 using the respective I¹²⁵-labeled proteins as tracers yielded the following results (Table 3.1).

<table>
<thead>
<tr>
<th>Membrane</th>
<th>mg/m² (projected area)</th>
<th>mg/m² (BET area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA</td>
<td>IgG</td>
</tr>
<tr>
<td>PVDF</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>Polysulfone</td>
<td>34</td>
<td>134</td>
</tr>
<tr>
<td>Nylon</td>
<td>1964</td>
<td>1906</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>2736</td>
<td>2382</td>
</tr>
</tbody>
</table>

The hydrophilic polyvinylidene difluoride (PVDF) membrane exhibited almost two orders of magnitude less binding than the hydrophobic nylon or nitrocellulose membranes. When the amount adsorbed is normalized to the total surface area measured by BET, the author finds the surface coverage to be less than 1% for the PVDF and polysulfone membranes but almost 100% for the nylon membranes. The binding to the nitrocellulose membranes could be explained only in terms of multilayer adsorption. Pitt also found that the binding increased about 20-fold over the protein concentration range of 0.1 - 10 kg/m³.

The effect of protein adsorption on fouling is usually measured by observing the change in the hydraulic permeability of the membrane before and after exposure to protein. Reihanian (1983) determined the change in hydraulic permeability of several UF membranes after a 30 min equilibration with BSA solutions at concentrations ranging from 0.1 to 10 kg/m³. At pH 7.4,
the hydraulic permeabilities of the hydrophobic Dynel XM-300, XM-50, and polysulfone PM-30 membranes decreased by 50-60% whereas, there was no change for the hydrophilic cellulose YM-30 and polyion complex UM-10 membranes. Equilibration of the PM-30 membranes in BSA solutions at pH 4.7 (isoelectric pH) resulted in a 70-75% decrease in permeability, about 1.5 times that observed at pH 7.4. In all cases a greater decrease in permeability was observed at higher BSA concentrations with the profiles showing a saturation behavior. Likewise, Matthiasson (1983) found a maximum 5% decrease in the hydraulic permeability of cellulose acetate membranes versus a maximum 35% decrease for polysulfone and polyamide membranes upon contacting them with 10 kg/m³ BSA solutions for 3 h.

A potential conflict in interpreting hydraulic permeability data is the assumption of uniform adsorption on the pore walls versus pore occlusion. When the solute is larger than the pores, adsorption can occur only on the external surface but for solutes smaller than the pores, as are typically encountered in microfiltration, adsorption can occur to the inner pore walls either uniformly over the pore length or predominantly at the pore entrance. The reduction in hydraulic permeability by uniform adsorption is attributed to a decrease in the pore diameter and is estimated by applying a well-established pressure-flow equation such as the Hagen-Poiseuille relation for laminar flow through tubes. This approach is well documented for polymer adsorption (Cohen, Y., 1982; Idol, W.K., 1986) and for protein adsorption (Yavorsky, D.P., 1981). Yavorsky explains the reduced diffusional transport of electrolytes after the exposure of microporous mica membranes to BSA solutions by postulating an adsorbed layer thickness of 4.3 nm. The fact that the decrease in the diffusional permeability was the same as the decrease in the hydraulic permeability is interpreted as being indicative of a dense, impermeable adsorbed layer composed of compact molecules. With elongated polymer chains, the change in hydraulic permeability is usually greater than that in the diffusional permeability because the density of the flexible polymer segments varies with distance from the pore walls (Idol, W.K., 1986). Yavorsky, however, was not able to offer a convincing
explanation for the changes in hydraulic permeability of the protein-coated membranes with pressure. For example, the normalized permeability decreased irreversibly from 90-95% at 10 kPa to 60-75% at 50 kPa although the thickness of the adsorbed layer (from conductimetric measurements) showed no change before and after the pressure flow. He attempts to explain these results in terms of the instantaneous changes in protein structure under the influence of fluid shear. Occlusion occurs when the initial adsorption of protein at the pore entrance constricts the pore diameter and prevents additional protein from reaching the interior. Changes in hydraulic permeability are usually analyzed in terms of the Kozeny-Carman equations for flow through a porous layer. An easy way to distinguish between occlusion and uniform adsorption is by reversing the flow through the membrane and measuring the change in hydraulic permeability. Uniformly adsorbed protein would offer equal resistance to flow in either direction. Protein blocking the entrance to the pore could be forced out when the flow is reversed resulting in an immediate increase in permeability. The authors cited in this section have not attempted to distinguish between these two mechanisms in this way. Yavorsky's observations of a decrease in permeability upon increasing pressure could be interpreted on the basis of pore occlusion as an increase in the specific resistance due to the compression of the porous protein layer. It is not clear how this mechanism would affect the interpretation of the electrolyte conduction measurements.

3.1.2 Fouling in the presence of convective flow.

The examples mentioned above involved protein adsorption from a stagnant or gently stirred solution and the transport of solute to the membrane surface was by diffusion alone. The following examples describe membrane fouling when the protein solution flows through the pores under the influence of a pressure driving force. Bauer (1982) observed an order of magnitude decrease in flux through 0.4 μm track-etch, Nuclepore membranes during the filtration of blood serum even though the pore diameter is much larger than the hydrodynamic diameter of the serum proteins. Similar 10-fold decreases in flux were observed during the
filtration of 0.1% BSA, pH 7.4, through partially permeable Amicon XM-100 membranes at 100 kPa (Fane, A.G., 1983). As a consequence of fouling, the rejection of BSA by these membranes increased from 3% to 50% in 7 min and approached 80% after 50 min. Even after cleaning the membrane with 0.1N NaOH solution, the water flux was about 5.6×10^{-5} m/s compared to the value of 3.3×10^{-4} m/s for a new membrane. Suki (1984) measured the amount of BSA bound to the retentive Amicon PM-30 and YM-30 membranes after 3 h of ultrafiltration of 0.1% BSA solution in a stirred cell (400 rpm) at 100 kPa. The protein was extracted from the rinsed membranes using SDS and quantified by the standard Lowry assay. The bound protein was about 50-150 mg/m² at pH 2 and pH 10. Since the protein is expected to bind only to the external surface of these retentive membranes, this amount is almost 10-50 times more than a monolayer. Devereux (1986) and Bentham (1988) investigated the flux and transmission of mixtures of soya protein precipitate and soluble soya protein and they attribute the fouling to both protein adsorption and pore occlusion by the precipitates. The observation that soluble protein transmission during unstirred batch filtration was less than 100% suggests that concentration polarization was a factor influencing flux in addition to any hydrodynamic resistance offered by the deposited precipitates.

Analysis of protein binding under actual filtration conditions is complicated by the inability to distinguish between true adsorption and convective deposition. For example, Suki (1984) found a strong correlation between BSA accumulation on the membrane and the product of the flux and feed concentration of BSA (i.e., convective deposition rate). This casts serious doubts on their interpretation of the fouling results in terms of protein adsorption. Unfortunately, the other authors who investigated fouling under actual filtration conditions do not explicitly state whether such a correlation was observed in their experiments. Another complication during filtration with retentive or partially permeable membranes is the increased concentration of rejected solute at the membrane surface due to concentration polarization. Even if true adsorption (and not convective deposition) were the only mechanism of protein
accumulation on the membrane, the adsorbed protein would be in equilibrium with the concentration in the polarized zone and not with the bulk concentration. This can lead to an incorrect interpretation of the data using adsorption isotherms based on feed concentration. At very high solute concentrations, the possibility of flocculation and precipitation of the protein exists. Suki (1986) has developed a model based on protein aggregation in the polarized zone adjacent to the membrane leading to the formation of a layer of reduced voidage and increased resistance. They provide photographic evidence in support of the hypothesis that the densest layers are at the membrane surface. Howell and Velicangil (1982) propose a fouling mechanism based on adsorption followed by polymerization in the gel layer. A deviation from true adsorption can also be caused by the physical entanglement of macrosolutes with the solute that is already adsorbed. This hypothesis is proposed by Barham (1986) to explain the thick, multiple polyethylene layers observed on the inner walls of a Couette viscometer. Such entanglement is more likely with fibrous proteins and elongated, flexible polymers rather than with compact, globular proteins unless the solution also contains denatured proteins. In this respect, the data from experiments done under non-flow conditions are much more representative of true adsorption, although they would not account for the possible effect of shear on protein conformation and solute orientation and thus, on the extent of adsorption.

3.1.3 Influence of pore geometry

A second problem in interpreting protein binding results is the uncertainty in pore geometry. When the pores are much smaller than the solute, it is safe to assume that adsorption occurs only on the external surface and one can get a reasonable estimate of the surface coverage by using the projected membrane area (especially when the surface is not very rough). However, for partially and completely permeable membranes, the solutes can also bind to the inner pore walls. Measurement of internal surface area is very difficult and estimates such as obtained by BET analysis do not depict the area that is accessible to macrosolutes such as proteins. Experiments with track-etch membranes yield more reliable
data on adsorption because of the well defined pore geometry. Membranes which are susceptible to intra-pore binding of solutes often exhibit much greater declines in flux and changes in rejection characteristics. For example, a comparison of the flux of lysozyme (permeating species) and BSA (non-permeating species) solutions through Amicon PM-30 membranes indicates that although the initial flux was higher during lysozyme filtration (5.6×10^{-5} vs. 3.9×10^{-5} m/s), the flux after the passage of 40 ml of permeate, was less than 1.9×10^{-5} m/s with lysozyme in contrast to 3.5×10^{-5} m/s for BSA (Fane, A.G., 1983). After the collection of 150 ml of permeate in a different experiment, the flux through an XM-100 membrane was 4.2×10^{-5} m/s, which is less than the 5.6×10^{-5} m/s obtained with the more retentive PM-30 membrane under identical operating conditions. The influence of pore geometry and structure when using different membranes made of the same polymer is illustrated by the data of Suki (1984). They found that the tendency to accumulate protein correlated well with surface heterogeneity for the three polysulfone membranes, Amicon PM-30, DDS GR61P, and Millipore PTGC, with the less heterogeneous membranes accumulating less protein. Reihanian (1983) could not correlate their results with membrane hydrophobicity and they speculated that surface porosity and microstructure may influence the hydraulic permeability of the polarized protein layer. These results are not amenable to quantitative comparison because of the difficulty in defining and measuring 'surface heterogeneity' and 'hydrophobicity'.

Reihanian (1983) explains the observed decrease in BSA rejection from 0.32 to 0.09 when the pressure across the Amicon XM-300 membrane was increased from 34 to 138 kPa in terms of the larger force exerted on the solute blocking the pores resulting in its being forced through the membrane at higher pressures. This phenomenon is more likely with membranes having straight through pores than with those having a tortuous pore structure. When membranes have a heterogeneous population of pores, the flux is sensitive to the loss of the larger pores by plugging. Occlusion of the larger pores is also encouraged by the considerably larger flow that
occurs through them resulting is a higher rate of solute deposition. For low porosity membranes, the solvent streamlines curve near the impermeable inter-pore region so as to pass through the pores. This can cause the deposition of solute at the entrance of the pores by impaction and short range solute-membrane interactions (Kao, J.N., 1988).

3.1.4 Effect of pH and ionic strength

Several authors have reported an increase in fouling at the isoelectric pH. During the filtration of 0.1% BSA (isoelectric pH = 4.8) through partially permeable Amicon XM-100 membranes at 100 kPa, Fane (1983) observed permeate fluxes of \(6.5 \times 10^{-5}\), \(2.9 \times 10^{-5}\) and \(5.8 \times 10^{-5}\) m/s at pH 3, 5, and 9 respectively for a 'non-ionic' solution. The fluxes are pseudo-steady state values after the passage of 200 ml of permeate. For the 'ionic' case, i.e., in 0.1M citric/phosphate buffer, the flux was \(2.9 \times 10^{-5}\) m/s (±5%) at all three pH values. The rejection of BSA in these experiments was 85-95% for the non-ionic case and 75-82% for the ionic case with a shallow minimum at the isoelectric pH. Interpretation of these results is complicated by the fact that pH and ionic strength affect the solute-membrane and solute-solute interactions, and also influence protein conformation and diffusivity. Yavorsky (1981) estimated the thickness of adsorbed layers of BSA on track-etch mica membranes using conductimetric (\(\delta_E\)), tracer diffusion (\(\delta_D\)), and hydraulic permeability (\(\delta_H\)) measurements. The results in Table 3.2 show an increase in the adsorbed layer thickness at lower ionic strengths.

<table>
<thead>
<tr>
<th>Measurement technique</th>
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<th>0.3M KCl</th>
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<td>(\delta_E)</td>
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<td>4.4</td>
<td>4.0</td>
</tr>
<tr>
<td>(\delta_D)</td>
<td>6.0</td>
<td>4.7</td>
<td>-</td>
</tr>
<tr>
<td>(\delta_H)</td>
<td>6.3</td>
<td>6.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Table 3.2 Thickness of adsorbed BSA layers, nm. (Yavorsky, D.P. 1981)
This effect is attributed to the expansion of the adsorbed layers at lower ionic strengths because of greater inter-molecular electrostatic repulsion caused by reduced charge screening. Such an effect has also been reported by Idol (1986) who observed a 10 to 20% increase in the hydrodynamic resistance through track-etch mica membranes over the range 0.1 to 0.01M KCl using polyacrylic acid and polystyrene sulfonate as the solutes. These results of Yavorsky and Idol are consistent with the 'uniform adsorption' hypothesis where an expansion of protein or polymer reduces the effective pore diameter and thus increases the resistance to flow. The results of Fane, on the other hand, suggest pore occlusion or gel layer formation where the permeate flow occurs through the protein layer. In this case expansion of the protein layer at low ionic strengths increases the voidage and thus decreases the resistance to flow.

3.2 Protein Adsorption

Protein adsorption has been widely studied because of its significance in chromatography, bio-compatibility, and immune response. Some evidence in support of the effect of adsorption on membrane fouling was presented in the previous section, but this work represents only a tiny fraction of the research on adsorption. Instead of citing the results of numerous investigations with a variety of protein-substrate combinations, this section attempts to summarize the principles that govern protein adsorption at solid-liquid interfaces.

3.2.1 Hydrophobic effect.

The spontaneous adsorption of proteins occurs because of a decrease in the free energy of adsorption, $\Delta G_{ad}$, which may be represented as:

$$\Delta G_{ad} = \Delta H_{ad} - T \Delta S_{ad}$$  \hspace{1cm} (3.1)

Both positive as well as negative values of the enthalpy, $\Delta H_{ad}$, have been reported even for a given protein-substrate pair depending on pH, ionic strength and other solution conditions (Macritchie, F., 1978; Norde, W. and Lyklema, J., 1979). In general, the temperature
dependence of the adsorption isotherms suggests that the phenomenon is driven by a large increase in the entropy, $\Delta S_{ad}$. The major contributions to the entropy term arise from the changes in hydration of the protein and substrate and from changes in protein conformation. Smaller contributions arise from the adsorption/desorption of ions and co-solutes. The contribution of hydration is thought to be greater for hydrophobic surfaces than for hydrophilic ones making them better adsorbents. After adsorption, the protein can change its conformation to maximize favorable interactions with the substrate (Soderquist and Walton, 1980). The structure of many globular proteins is such that the inner core contains a higher proportion of hydrophobic amino acid residues while the surface contains more of the charged and polar residues. The change in protein conformation upon adsorption often exposes the inner hydrophobic residues which have a lower free energy when contacting a non-polar substrate than when exposed to the polar aqueous medium. This can increase the stability of the adsorbed protein.

An obvious way of counteracting the hydrophobic effect is by using membranes made of hydrophilic materials, but sometimes other desired properties such as structural integrity or ease of manufacture favor the choice of hydrophobic polymers. In this case, surface modification techniques such as coating with a hydrophilic material or introducing polar groups on the surface by chemical treatment are used. Fane (1987) reports a 40% improvement in flux over repeated filtration cycles upon contacting the polysulfone, Amicon PM-30 membrane with a 0.1 kg/m$^3$ solution of nonionic nonylphenol polyethoxylate for 3 h and a 100% improvement when contacted with a soluble methyl cellulose solution. Bauser (1982) observed a slower rate of flux decrease when blood serum was filtered through 0.4 $\mu$m Nuclepore membranes that were coated with hydrophilic polyacrylonitrile or carbon. However the steady state flux was similar with the treated and untreated membranes.
3.2.2 **Irreversibility of adsorption.**

An important feature of protein adsorption is its apparent irreversibility especially with hydrophobic substrates (Cheng, Y.L., et al., 1985). This is typical of large macromolecules which adsorb via multiple points of attachment to the surface. Adsorption requires only a few of the weak bonds to be established but desorption requires the simultaneous disruption of all the points of attachment and consequently, has a higher activation energy. Arguments based on activation energy imply that the irreversibility is not a thermodynamic phenomenon but represents a kinetic limitation so that very little desorption is observed over reasonable time scales (Cheng, Y.L., et al., 1987). Changes in protein conformation upon adsorption can also cause irreversible binding (Norde, W., et al., 1986) by means of a kinetic limitation or by a thermodynamic barrier. The latter depends on whether the denatured protein is at the global minimum in free energy for the given system. Despite the apparent irreversibility, some researchers have observed Langmuir type adsorption isotherms whose basic premise is a dynamic equilibrium between adsorbed and bulk solute. It is not clear whether the Langmuir isotherms were obtained with both increasing and decreasing solution concentrations of protein. The amount adsorbed upon equilibrating a clean surface with solutions of different concentration can be quite different from that obtained by successively increasing the concentration of solution exposed to the same surface. A second factor that precludes Langmuirian adsorption is the variation in even the monolayer coverage with protein orientation. Golander and Kiss (1988) quote values of 2.5 and 6.6 mg/m² for the side-on and end-on adsorption of BSA which is a prolate ellipsoid of dimension 14 x 4 x 4 nm. The corresponding values for IgG (23.5 x 4.4 x 4.4 nm, prolate ellipsoid) are 2.7 and 13.7 mg/m² and for bovine fibrinogen (45 x 6 x 6 nm, rod) are 2.1 and 15.7 mg/m². Different plateau concentrations have been reported depending on the initial solution concentration, with the end-on configuration preferred at high concentrations. Some authors have suggested that only a fraction of the protein is irreversibly adsorbed. This is demonstrated by the partial exchange of protein between the adsorbed layer and bulk solution when a polyethylene surface, saturated
with $^{125}$I-albumin, was brought into contact with a solution of $^{131}$I-albumin (Macritchie, F., 1978). Similar behavior was observed for the adsorption of IgG on silica gel and polyethylene by Iordanski and co-workers (1983) using a spin-labelling technique. The results are explained either in terms of a heterogeneity of binding site energies or in terms of an irreversibly bound monolayer on which stand the reversibly adsorbed proteins.

3.2.3 Effect of solution and adsorption conditions.

Protein adsorption is influenced by several parameters such as pH, ionic strength, temperature, and exposure to shear. These parameters can influence the extent of adsorption by their effect on protein conformation and stability. In addition, pH affects the electrostatic interactions between the protein and the membrane and also between adjacent protein molecules in the adsorbed layer. For uncharged membranes, the protein-membrane electrostatic interaction is not as strong as the hydrophobic interaction. However, the interaction between adjacent adsorbed molecules can be strong enough to alter the monolayer coverage at different pH values. For example, the extent of adsorption often exhibits a maximum at the isoelectric pH where the lower electrostatic repulsion between the molecules permits them to assemble on the substrate in a more compact configuration (Lee and Ruckenstein, 1988; Shirahama and Suzawa, 1985). Multilayer adsorption, which is sometimes observed at the isoelectric pH is conceptually similar to aggregation and is also facilitated by the lower electrostatic repulsion. The effect of ionic strength is to modulate the electrostatic interactions by screening the charged residues on the protein molecule. Consequently, the adsorbed molecules can pack in a denser configuration at higher ionic strengths. Ionic strength and the nature of the ions also affect the hydrophobic interactions (Melander and Horvath, 1977). A recent review of the various interactions between polymer surfaces and other molecules is presented by Rätzsch, M., et al. (1990).

The effect of shear on adsorption is relevant to membrane fouling because of the existence of
flow fields in the pores and across the membrane surface. A detailed discussion of the shear effect is presented by Cheng (1985 and 1987) and Darst (1985) who measured adsorption using a technique called Total Internal Reflection Fluorescence (TIRF). In the absence of denaturation which is discussed in greater detail in Section 3.3, the primary effect of shear is to orient the molecules in solution and on the substrate in the direction of flow, thus affecting the surface coverage. Its effect on the rate of adsorption is apparent only when diffusion is rate limiting. Darst found that adsorption from 0.01 kg/m³ BSA solutions, pH 7.4, on surfaces of polydimethylsiloxane, polydiphenylsiloxane, and polycyanopropylmethysiloxane was diffusion limited up to the maximum shear rate investigated, namely 4000 s⁻¹, as evidenced from the linear relation between the initial rate of adsorption and (shear rate)¹/³. The adsorption on polystyrenesulfonate was diffusion limited only up to shear rates of 70 s⁻¹ and the adsorption on polymethylmethacrylate was limited by intrinsic adsorption kinetics.

Another parameter that effects adsorption but has not received much attention is the presence of solid-gas-liquid interfaces. Matthiasson (1983) measured BSA adsorption to several UF membranes by injecting a labeled protein solution into a stirred cell. When the cell was partially filled with saline solution before injecting the labeled solution, the resulting adsorption was 2 to 3 times less than when the cell was empty to begin with. A possible explanation is the denaturation of the protein at the gas-liquid interface present when the cell is initially empty.

3.3 Protein denaturation/aggregation

Unlike polymeric macromolecules such as polyethylene or polystyrene, the polypeptide chain in proteins and enzymes is folded into an elaborate three dimensional structure which is essential to the function or activity of the molecule. Denaturation refers to the loss in activity or functionality due to changes in this native structure but there is no unique denatured state. Denaturation of proteins is relevant to the study of membrane fouling for the following reasons: (a) Denatured proteins usually have a stronger affinity for the membrane surface because the
exposed, core hydrophobic residues have a lower free energy when contacting the membrane surface rather than the aqueous medium. (b) Denatured protein can aggregate through intermolecular hydrophobic interactions and disulfide exchange and these aggregates can foul the membrane by pore occlusion. (c) If the protein or enzyme is the desired, high-value product, denaturation obviously leads to product losses and in the case of therapeutics, can generate an undesirable immune response in the patient. Changes in protein conformation upon filtration, detected by circular dichroism and FTIR spectroscopy, have been reported by Truskey (1987) and Franken (1990). Details of protein structure and the factors governing denaturation are presented in several books such as the ones by Creighton (1984), Ghélis and Yon (1982), Schulz and Schirmer (1979), and Lapanje (1978).

3.3.1 Shear Effect.

During the cross-flow filtration of proteins, the molecules are constantly exposed to shear in the narrow feed channel, in the membrane pores, and in the associated pumps, tubing and pressure valves. Repeated recycling of the retentate stream increases the duration of this exposure. Any object placed in a shear field experiences a torque but it is difficult to calculate its magnitude for a free flowing protein molecule. Secondly, the magnitude of the mechanical force required to sufficiently deform a protein so as to change its activity is not known. Tirrel and Middleman (1978) estimate the force on urease to be $2.5 \times 10^{-15}$ N under their experimental conditions and although such a force has been shown to cause a 40% elongation in polyisobutylene, its effect on urease was not known.

Charm and Wong (1977) were among the earliest to investigate the effect of shear on enzymes and they proposed the following correlation based on the shear rate, $\gamma$, and exposure time, $\theta$:

$$\gamma \theta = a \left( \frac{\chi}{\chi_0} \right)^b$$

...(3.2)
\( \chi \) and \( \chi_0 \) are the final and initial enzyme activities. They claim that when \( \gamma \theta \leq a \), there is no shear inactivation. They validated this relation using a narrow gap coaxial viscometer with several enzymes such as catalase, rennet, carboxypeptidase, heparin, and urease and obtained values of 'a' between \( 2.2 \times 10^5 \) and \( 6.2 \times 10^5 \) and values of 'b' ranging from -2.1 to -7.7. When catalase and heparin solutions were pumped through sections of Teflon tubing (0.05 cm i.d. x 1.9 m for catalase, 0.11 cm i.d. x 2 m for heparin) at 4 °C using a finger pump, the loss in activity closely matched the predictions of Equation...(3.2). They specifically state that there was a small air-liquid interface in the viscometer but that the pump system was designed to avoid such interfaces. Secondly, they have subtracted any inactivation that occurs in the pump alone. So, assuming that the inactivation they observed in the Teflon tube is indeed caused by shear, Equation...(3.2) may be rewritten for the case of laminar flow as:

\[
\gamma \theta = 5.33 \frac{L}{D} N = a \left\{ \frac{\chi}{\chi_0} \right\}^b \quad ...(3.3)
\]

This equation implies that for a given pump and tubing, the extent of denaturation depends only on the number of passes through the pump. In fact, the authors claim that this relation also applies to intermittent pumping.

Tamada and Ikada (1985) circulated solutions of BSA, fibrinogen, IgG, and human fibronectin through a polyethylene tube using a peristaltic pump and found an increase in turbidity (\( \lambda = 320 \) nm). Using BSA as a model system, they investigated the effect of protein concentration, tube length, flow rate, and tube diameter. The turbidity did increase at a faster rate when the concentration was increased from 0.03 to 3.0 kg/m\(^3\) but no correlation with the shear rate in the tubing was observed. Increasing the tube length from 1 to 3 m did not significantly change the rate of increase in turbidity. Unfortunately, they do not state whether their results could be correlated with the number of passes through the pump. An important observation was that replacing the peristaltic pump with a piston pump significantly decreased the aggregation,
suggesting that most of the denaturation/aggregation was occurring in the peristaltic pump and not in the tubing. They found no difference in the circular dichroism spectra or in the gel permeation chromatographic results before and after pumping with the peristaltic pump indicating that the fraction of total protein aggregating was below the sensitivity of these techniques.

Reese and Ryu (1980) observed first order inactivation of crude cellulase extract (1.25 kg/m³, 0.025 M citrate, pH 5, 50 oC) for the first 48 h of recirculation with a peristaltic pump through glass and silicone tubing of 0.2 cm i.d. and 5.5 m total length. The inactivation constant increased from 0.0067 h⁻¹ at a flow rate of 0.6•10⁻⁶ m³/s to 0.0227 h⁻¹ at a flow of 1.8•10⁻⁶ m³/s. Since the authors used the same volume of solution (2•10⁻⁴ m³) in all of their runs, it may be presumed that the first order inactivation kinetics correspond to a linear correlation with the number of passes through the pump. The authors did not verify this by changing the volume of solution.

A series of papers by Dunnill and co-workers question the hypothesis that proteins are denatured by shear alone. They take particular exception to the use of 'mass average shear', γθ, by Charm and Wong because it implies that even low shear rates would inactivate enzymes if the solution is sheared for a sufficiently long time. Thomas and Dunnill (1978) sheared solutions of yeast alcohol dehydrogenase (YADH), catalase and urease in a concentric cylinder viscometer custom designed to minimize air-liquid interfaces and temperature increases. Their observations are summarized in Table 3.3. Although the data are insufficient to draw any conclusions about the effect of various parameters such as temperature, shear rate, and concentration, a general observation is that the inactivation of the enzymes is much less than predicted by the equation of Charm and Wong for comparable values of γθ. They also found that the 'open' configuration of the viscometer, i.e., with an air-liquid interface, caused almost 40% loss in activity of the 0.006 kg/m³ solution of catalase after 6 h at 683 s⁻¹ compared with
Table 3.3  **Inactivation results with closed viscometer**  
*(Thomas and Dunnill, 1978)*

<table>
<thead>
<tr>
<th>Protein</th>
<th>Shear Rate (s⁻¹)</th>
<th>Duration (h)</th>
<th>Loss of specific activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YADH, 1 g/l, 5°C</td>
<td>≤ 3440</td>
<td>8</td>
<td>≤ 6</td>
</tr>
<tr>
<td>Catalase, 1 g/l, &lt;40°C</td>
<td>683</td>
<td>3</td>
<td>= 0</td>
</tr>
<tr>
<td>Catalase, 6 mg/l, 4°C</td>
<td>683</td>
<td>6</td>
<td>≤ 8</td>
</tr>
<tr>
<td>Catalase, 1 g/l, 4°C</td>
<td>3440</td>
<td>6</td>
<td>≤ 2</td>
</tr>
</tbody>
</table>

less than 8% with the 'closed' system. Virkar, P.D., et al., (1981) extended the investigation of the inactivation of YADH to high shear rates (26000 s⁻¹) in a concentric cylinder viscometer. Once again, care was taken to control temperature and eliminate air-liquid interfaces. Taylor vortices were avoided by rotating the outer cylinder. The loss in protein and total activity was less than 5% after 1 h of shearing when the solutions were prepared from the freeze dried powder and about 30% when the stock solution was a crystalline suspension in ammonium sulfate. The authors speculate that the latter result may be due to the action of shear on the partially inactive enzyme present in the ammonium sulfate suspension after long storage. In this context, it may be mentioned as an aside that Penarrubia and Moreno (1987) suspect that the enhanced rate of inactivation of ribulose-1,5-bisphosphate carboxylase upon shearing is caused by the action of shear on the fraction of enzyme that has spontaneously inactivated. When YADH solution was sheared in a rotating disk reactor for 5 h, there was no loss in activity (error ± 10%) with the completely filled reactor and about a 40% loss with the partially filled reactor (without foaming) irrespective of whether the head-space contained air or nitrogen. Likewise, there was no significant (≤ 10%) loss in specific activity when YADH solutions were pumped up to 1500 passes through centrifugal, rotary, gear, screw and peristaltic pumps but the authors do not report whether any loss in total activity occurred.
Narendranathan and Dunnill (1982) investigated the shear effect encountered by YADH solutions when pumped through porous and non-porous capillaries and ultrafiltration hollow fibers. An initial 20-30% loss in protein was attributed to adsorption but, subsequently, there was no further loss in protein or specific activity.

The results of Lee and Choo (1989) for the inactivation of lipase in a stirred tank reactor with a flat, six-bladed turbine impeller corroborate the effect of the gas-liquid interface observed by Virkar in the rotating disk reactor. When 0.1% solutions of lipase were sheared at 150 s\(^{-1}\) for 30 min, the rate of inactivation was 97% lower when the reactor was filled to the brim compared to the half-filled reactor. Addition of 0.05 ml of polypropylene glycol (anti-foam) to the half-filled reactor (1360 ml) decreased the rate of inactivation by 93%. Changing the viscosity of the solution by the addition of starch (shear stress: 0.72 - 109.2 kg/m\(^*\)s\(^2\)) did not affect the rate of inactivation suggesting that the effect of polypropylene glycol was probably caused by changes in surface tension rather than a change in shear stress.

Talboys and Dunnill subjected a membrane-associated enzyme complex, progesterone 11 alpha-hydroxylase, to shear in a concentric cylinder viscometer and measured its specific activity. Low shear rates of 720 to 1430 s\(^{-1}\) had little effect on the enzyme complex but at higher shear rates (2870-5020 s\(^{-1}\)) there was a 20 to 60% increase in activity for the first 4 min followed by a steady decrease of 15-60% over the next 40 min. The loss in activity at 40 min was greater at higher shear rates. The authors also varied the gap width of the sealed viscometer and found the specific activity to be 60-70% using a 0.15 mm gap and 110-120% using 0.25 and 0.475 mm gaps over a 10 to 30 min period of shearing.

Based on the above observations, mechanical shear per se seems to have little effect on the activity of enzymes. Since the sensitivity of most of the enzyme activity measurements is only ± 10%, one cannot entirely rule out an effect of shear on native enzymes but it is evident that it
is far less significant than Equation ...(3.2) would suggest. Dunnill and co-workers propose that shear merely serves to increase the transport of the enzymes to and from the gas-liquid or solid-liquid interfaces which are responsible for the actual denaturation (see Section 3.3.2). For example, in the stirred reactor, the increased mixing at higher stirring speeds would cause the entrainment of denatured protein from the air-liquid interface and expose fresh, native protein from the bulk solution to the interface. One of the difficulties in interpreting the data published by Dunnill and co-workers is that they use the relative specific activity as a measure of inactivation instead of relative total activity. So, if the denatured enzyme forms aggregates which are removed from the samples to prevent interference in the spectrophotometric assay, then the remaining native enzyme would yield a lower total activity but the same specific activity. Enzyme activity can also give misleading results when shear preferentially acts on partially denatured molecules to cause their aggregation. In this case, removal of the aggregates may actually give a higher specific activity than the original sample.

3.3.2 Interfacial effects:

The rates of inactivation of enzymes in 'closed' and 'open' viscometers and in full and half-full stirred tank reactors presented in the preceding section strongly implicate the gas-liquid interface in the mechanism of enzyme denaturation and aggregation. It was also mentioned in Section 3.2, that enzymes adsorbed on solid surfaces often undergo conformational changes in order to maximize the favorable interactions with the substrate. Although the jury is still out on the precise role of shear in the inactivation process, it is clear that shear can greatly enhance the rate of inactivation by increasing the interfacial mass transfer rate. It is also conceivable that shear can exert a stronger force on molecules that are anchored at solid-liquid interfaces than those which are free flowing or adsorbed at gas-liquid interfaces. In this section, we summarize the important observations about enzyme denaturation at gas-liquid interfaces although generalization is difficult because of the variety of protein and interface characteristics encountered in the literature.
The driving force for adsorption of proteins at interfaces is thought to be the large increase in entropy caused by the dehydration of the protein and changes in the three dimensional structure of the molecule (see Section 3.2.1). Stellwagen (1984) estimates that between 2.5 and 10.5 kJ/mole of stabilization energy could be obtained per apolar residue removed from contact with water. The conformational changes are thought to be confined to the tertiary structure of the protein with very little, if any, change in the secondary structure (Macritchie, F., 1978; Clark, D.C., 1988). Protein concentrations at gas-liquid interfaces are typically about 0.7 to 0.85 mg/m² which corresponds to about 0.12 to 0.15 nm² per amino acid. This supports the notion that proteins are unfolded at interfaces to give an amino acid monolayer (Donaldson, T.L., et al., 1980). Most of the experimental evidence for denaturation comes from the analysis of desorbed protein using circular dichroism, fluorescence quenching, and enzyme activity but these experiments are limited to the irreversible changes in protein conformation. If the diffusional barrier is overcome, adsorption at gas-liquid interfaces is thought to be governed by an energy barrier related to surface tension. Molecules approaching the interface have to compress those previously adsorbed against this interfacial pressure to make room for themselves. Calculations of this initial 'room' indicate that it is much smaller than inferred from the size of the protein suggesting that only a small fraction of the molecule needs to penetrate the interface for adsorption to proceed spontaneously. In order to unfold, the native, globular proteins have to overcome the energy barrier associated with expansion against the surface pressure. This barrier increases with interfacial concentration. When the bulk concentration of protein is high, the interface will become saturated with native molecules faster than the rate at which the adsorbed protein unfolds. Consequently, the extent of denaturation would be lower at higher bulk concentration. Such an effect was reported by Thomas and Dunnill (1978) in their work on the shear effect on catalase (see Table 3.3). Clark, et al., (1988) who investigated the denaturation of BSA upon foaming state that the change in fluorescence properties of the collapsed foam was lower at 5 kg/m³ than at 1 kg/m³ of BSA which they interpret as being indicative of less unfolding.
The importance of surface tension can also be gauged from the effect of surface active agents on the adsorption and denaturation of proteins. Donaldson (1980) observed no interfacial denaturation of acid phosphatase (wheat germ) when BSA was added to the enzyme solution. It was mentioned earlier that Lee and Choo (1989) observed a 93% reduction in the rate of inactivation when 0.05 ml polypropylene glycol was added to 1360 ml of lipase solution in a stirred tank reactor. A detailed, thermodynamic treatment of the contribution of surface tension to the hydrophobic interaction at surfaces is presented by van Oss (1987).

Surface tension is not the only determinant of the energy barrier. When the protein has a net charge, the adsorbed layer can set up a repulsive electric potential that resists the approach of additional bulk protein to the interface. This explains the greater adsorption (and denaturation) observed at the isoelectric pH where the protein has no net charge (Watanabe, N., et al., 1986). The denatured, adsorbed protein at a gas-liquid interface can coagulate under some conditions (e.g., when a critical interfacial pressure is exceeded) to form an aggregate or precipitate. The critical pressure correlates with the free energy of the interface which explains the greater propensity of proteins to coagulate at oil/water rather than at air/water interfaces. The insolubility of the coagulated protein results from the large energy barrier that needs to be overcome in order to bring the denatured molecules into the soluble, native form and in this respect, it is similar to thermal coagulation. Another issue is the possibility of disulfide interchange leading to incorrect covalent bridges within a molecule and also to covalent links between molecules (Burton, 1989; Ueki, 1985).

Most of the observations stated above also apply to adsorption and denaturation at solid-liquid interfaces. However, it is believed that the conformational changes at the solid surface are not as great because the protein cannot penetrate the interface. Secondly, the adsorption on solids is thought to be localized at 'active sites' which need not all have a homogeneous affinity for the protein.
3.3.3 Thermal effects:

The repeated recirculation of solution during cross-flow microfiltration can cause local increases in temperature by viscous dissipation. Most proteins and enzymes are susceptible to denaturation and inactivation at high temperatures and consequently, can foul the membranes by increased adsorption. Proteins also tend to aggregate and gel at high temperatures and these aggregates can cause pore occlusion. For example, Shirahama and Suzawa (1988) report that BSA undergoes irreversible denaturation above 50 °C, aggregation with disulfide interchange above 60 °C, and gel formation above 70 °C. They also found that heat denatured BSA adsorbed about twice as much to polystyrene latex beads as native BSA. Thermal inactivation of enzymes has been extensively investigated with the purpose of improving their stability so that they may be successfully employed as industrial catalysts (Zale and Klibanov, 1984). The aggregation and gelation of proteins has also been investigated by small angle X-ray scattering and transmission electron microscopy (Clark and Tuffnell, 1980; Clark, et al., 1981). With the exception of ribonuclease, the authors found that gelation of BSA, insulin, lysozyme, and α-chymotrypsin involved a decrease in helical content and an increase in sheet structure. However, there is no consensus on the precise mechanism of inactivation and on the number of intermediates involved although it is generally agreed that conformational changes, disulfide exchange, and covalent disruption play a role.

3.4 Modelling of flux based on particle polarization

In the absence of fouling, the flux during cross flow filtration is usually modelled using the concentration polarization equation which is derived from a balance between the convective transport of solute to the membrane and the diffusive removal of rejected solute from the surface (Blatt, et al, 1970). At steady state, this equation is represented as:

\[ J = k \ln \left( \frac{C_m - C_p}{C_r - C_p} \right) \]  

...(3.4)
where,  
\[ J = \text{flux through the membrane (m/s)} \]
\[ k = \text{mass transfer coefficient at the membrane surface (m/s)} \]
\[ C_m = \text{solute concentration at membrane surface (kg/m}^3) \]
\[ C_p = \text{solute concentration in permeate (kg/m}^3) \]
\[ C_f = \text{solute concentration in feed (kg/m}^3) \]

This equation is widely used in the modelling of ultrafiltration where, it predicts the pressure independence that is generally observed beyond a certain limit, namely when the solute concentration at the membrane reaches a 'gel' concentration. There is considerable difference of opinion concerning the significance of the 'gel' concentration, whether it has any relation to the solubility limit, whether it is a constant for a given protein, and whether it is influenced by membrane properties. A detailed discussion of concentration polarization can be found in several of the review articles listed at the end of the References section. The nature of the 'gel' when the feed solution contains a mixture of proteins has been studied by van der Berg and Smolders (1989a) who found the resistance to depend on both the charge of the mixed solutes and their relative sizes. The dynamics of gel formation during dead-end filtration has been treated by Trettin and Doshi (1980) using film theory as well as an integral boundary layer method. The second approach to modelling ultrafiltration has been the use of a thermodynamic barrier expressed in terms of an osmotic pressure difference acting in opposition to the applied trans-membrane pressure (Vilker, 1981a).

However, since microfiltration deals with the separation of micron sized particles, the formation of a gel layer has little meaning. The particles are generally modelled as hard spheres whose maximum wall concentration is limited only by their closest packed configuration. The actual packing density will be less when there are repulsive forces present, for example, when the particles are charged protein aggregates. The osmotic pressure of large particles is usually negligible compared to the applied trans-membrane pressures; consequently hydrodynamic resistance models are used to predict the flux. For example, Doshi and Trettin (1981) arrived
at the following equation for the cumulative permeate flow during un-stirred batch filtration, when the controlling resistance is the particle layer adjacent to the membrane surface:

\[
V = A \, d_a \sqrt{\frac{2 \, \Delta P \, \varepsilon^3}{150 \, \mu \, (1-\varepsilon) \, C_s \, \{ \rho_s - \frac{C_s}{1-\varepsilon} \}} \, t} \quad \text{...(3.5)}
\]

where,
- \( V \) = permeate volume obtained in time \( t \) (m³)
- \( d_a \) = particle diameter (m)
- \( \Delta P \) = trans-membrane pressure (Pa)
- \( \varepsilon \) = void volume in particle layer
- \( \mu \) = solvent viscosity (kg/m s)
- \( \rho_s \) = particle density (kg/m³)

During CFF, flux predictions based on Equation...(3.4), where the mass transfer coefficient is estimated from the Brownian diffusivity of particles, are generally much below experimental observations. The only way the steady state flux could be higher is if the removal rate of particles accumulated on the membrane surface is higher. Several modifications to Equation...(3.4) have been suggested all of which involve alternative mechanisms of removal of the rejected particles.

Zydney (1985) proposed the use of an enhanced diffusivity (first developed by Eckstein, 1977) whose magnitude increases with particle size and wall shear rate.

\[
D = 0.03 \, \frac{d_a^2}{4} \, \gamma \quad \text{(valid for vol. fraction > 0.2)} \quad \text{...(3.6)}
\]

where,
- \( D \) = enhanced particle diffusivity (m²/s)
- \( d_a \) = particle diameter (m)
- \( \gamma \) = shear rate (s⁻¹)

Substituting this equation for diffusivity in the equation for mass transfer coefficient for laminar flow, Zydney arrived at the following equation for flux.
\[ I = 0.078 \left\{ \frac{d_a^4}{16 \cdot L} \right\}^{1/3} \gamma_m \ln \left\{ \frac{C_m}{C_f} \right\} \]  

...(3.7)

This equation states that the flux is proportional to the wall shear rate, \( \gamma_m \). Belfort and Altena (1983) tested the 'flux' versus 'shear rate' relation for several suspensions and found the exponent to vary from 0.25 to 0.97. Secondly, Leighton and Acrivos (1987) found that the shear enhanced diffusion coefficient does not level off for solid fractions above 0.2 and in fact, at solid fractions of 0.4 the diffusivity could be five times greater than reported by Eckstein.

Romero and Davis (1990) have developed a steady state and a transient model for cross flow microfiltration based on a combination of shear enhanced diffusivity and an axially migrating cake. The transverse displacement of particles by particle-particle interactions in the shear field causes a swelling of the cake. The swelling results in a lower viscosity and facilitates the axial motion. They observe that as filtration proceeds, the moving bed of particles is backed by a stagnant layer that offers increased hydrodynamic resistance. Although the moving layer develops quite rapidly, the stagnant cake can take several minutes or even hours to form. Ogden and Davis (1990) provide experimental estimates of the hydrodynamic resistance of a packed bed of latex spheres and yeast cells. Zydney (1985) has provided similar estimates for red blood cells.

Altena and Belfort (1984) propose an alternative model for filtration where the convective deposition is balanced by a hydrodynamic lift force. The basis for this approach is the observation that when a dilute suspension of non-settling particles flows through a tube, the particles move radially under the influence of an inertial force to form an annular region of increased concentration between the centerline and the wall, a phenomenon referred to as the 'tubular pinch effect'. Being an inertial effect, the lift force is prevalent only for particle Reynolds' numbers greater than unity unlike the shear enhanced diffusivity which occurs at all
particle Reynolds' numbers. The lift velocity is sensitive to particle size, channel width, cross-flow velocity and distance from the wall and its maximum value as given by Belfort (1989) is,

\[ V_L = 0.1182 \frac{U_m^2 a^3}{\nu l^2} \]  

(3.8)

where,

- \( V_L \) = maximum hydrodynamic lift velocity (m/s)
- \( U_m \) = maximum undisturbed axial velocity (m/s)
- \( a \) = particle radius (m)
- \( \nu \) = kinematic viscosity (m²/s)
- \( l \) = gap width (m)

The true permeate flux is obtained by adding this lift velocity to the flux predicted by particle polarization (Equation...3.4).

Le and Howell (1984c) have developed a model based on the dynamic blocking and unblocking of membrane pores by the rejected solute. The fraction of time that a pore is blocked depends on a balance between the convective flow through the pore and the diffusive removal of the blocking particle. Their approach not only includes the dependence of flux on solute concentration and mass transfer coefficient (similar to the gel polarization model) but also accounts for the variations observed when membranes of different pore size or porosity are used. They explain the gradual approach to a limiting flux as pressure is increased in terms of the increased probability of blocking by hypothesizing that a greater fraction of the pores remain blocked for a longer time. However, this model has not been validated with several systems and it lacks predictive capabilities.

Yet another approach is based on the resistance of the polarized solute layer evaluated from sedimentation coefficient data (van der Berg and Smolders, 1989b). This is based on the correspondence between the permeability of the solvent through the concentrated, polarized
layer during filtration and the permeability of the solute through a stagnant solution during sedimentation. Based on their simulations and comparisons to experimental data (using BSA as the solute), the authors determined that the resistance of the polarized layer and the wall concentration were very sensitive to changes in applied pressure (over the range $1 \times 10^5$ to $5 \times 10^5$ Pa) but not very sensitive to bulk concentrations (over the range 1 to 6 kg/m$^3$).

The mass transfer coefficient that appears in Equation (3.4) is evaluated by analogy with the corresponding heat transfer phenomena during flow through closed conduits. These relations are generally correlations between the Sherwood number, $Sh$, with the Reynolds, $Re$, and Schmidt, $Sc$, numbers.

$$Sh = \frac{k}{D} = f(Re, Sc, d/l) \quad ...(3.9)$$

where, $d/l$ is the ratio of the characteristic transverse dimension to the characteristic axial dimension. For laminar flow, the Leveque or Gröber correlations are generally used.

$$Sh = 1.62 \left\{ \frac{Re \cdot Sc \cdot d}{l} \right\}^{1/3} \quad ...(Leveque)$$

$$Sh = 0.664 \left\{ \frac{Re \cdot d}{l} \right\}^{1/2} Sc^{1/3} \quad ...(Gröber)$$

For turbulent flow, the Dittus-Boelter equation is commonly used.

$$Sh = 0.023 \, Re^{0.8} \, Sc^{1/3} \quad ...(Dittus-Boelter)$$

A comprehensive review of the mass transfer coefficients for turbulent flow and a discussion of various factors such as membrane porosity and surface roughness that can affect the mass transfer coefficient is presented by Gekas and Hallström (1987). The major drawback to the use of the above correlations for mass transfer is that they were developed for flow through non-porous ducts. Consequently, the effect of the transverse velocity and the change in the
physical properties of the solution (diffusivity, viscosity) due to concentration polarization are not taken into account. Secondly, the behavior of suspensions can be different from that of solutions for which the equations were developed (Jeffrey and Acrivos, 1976; Ogden and Davis, 1990). Belfort (1989) has reviewed some of the approaches to increase the mass transfer coefficient using pulsatile flow and vortex flow.

3.5 Mathematical models of fouling

The polarization of rejected solutes and suspended particles described in the preceding section is a hydrodynamic phenomenon that can be reversed by altering the ratio of the flux to the cross-flow velocity. The effect of fouling on flux is usually superimposed on the polarization effects and its modelling involves a detailed knowledge of the physico-chemical interactions between the solutes themselves and between the solutes and the membrane. Matthiasson and Sivik (1980) have reviewed several models of fouling which are essentially empirical extensions of the concentration polarization model by incorporating a time-dependent term in the flux equation. For example, one approach is to assume a power law variation of the hydrodynamic resistance with time, that is,

\[ \frac{R_0}{R} \propto t^{-m_1} \quad \ldots(3.10) \]

Most of the models reviewed apply to reverse osmosis and a closer examination reveals that many of them are not fouling models but instead a different representation of the hydrodynamic effect. For example, the equation by Carter and Hoyland (referenced by Matthiasson and Sivik) is based on the assumption that the removal rate of 'fouling layers' that build up on the membrane is proportional to the wall shear stress and the thickness of the layer. The wall shear stress is calculated according to standard equations for flow through closed channels.

Some models of fouling are based on identifying the changes in the individual resistances to
permeate flow. These are not empirical equations but the numerous parameters involved effectively eliminates their predictive capability. For example, Suki, et al. (1986), have developed a model based on the slow aggregation of protein in the concentration polarization layer. They divide the polarized layer into 'k' sections each of thickness \( \delta_x \), with the total flux given by the following equation.

\[
J = \frac{\Delta P}{\mu \left( R_m + \sum_{x=1}^{k} \frac{\delta_x}{P_x} \right)} 
\]  

...(3.11)

The permeability of each section, \( P_x \), is calculated from the Kozeny-Carman equation for flow through a packed bed of hard spheres. The void fraction in each sub-section is evaluated from a knowledge of the number density and size of the aggregates which in turn, is obtained from flocculation dynamics. One of the key assumptions of this model is that permeate flow can occur through the individual aggregates and not just in the void space between the aggregates. The authors assume that the void fraction within an aggregate is about 0.3. To use this model one requires not only the initial size distribution of particles but also the spatial variation in the size distribution caused by polarization and the interaction parameters that govern flocculation.

Models based on the adsorption of protein to the pore walls causing a reduction in the effective pore diameter have been developed by Ethier (1986) and Nonaka (1988). Ethier took the more general approach by accounting for the permeability of the protein layer adsorbed to the pore walls using the Debye-Brinkman equation. He presents the theoretical results for the net permeability, \( K_{net} \), of a pore whose initial radius is reduced by the thickness of a protein layer of permeability \( K_{gel} \).
\[
\frac{K_{\text{net}}}{K_{\text{gel}}} = \left[1 + \frac{R^2}{8}\right]\left[\frac{R}{R_0}\right]^2 + 1 + 2B_1\left\{\frac{I_1(R_0)}{R_0} + \frac{I_0(R)}{2}\left[\frac{R}{R_0}\right]^2\right\}
\]

\[
+ 2B_2\left\{\frac{K_0(R)}{2}\left[\frac{R}{R_0}\right]^2 - \frac{K_1(R_0)}{R_0}\right\}
\]

\cdots(3.12)

where, \(B_1\) and \(B_2\) are constants evaluated from the boundary conditions, \(I_0\) and \(I_1\) are Bessel functions of the first kind, \(K_0\) and \(K_1\) are Bessel functions of the second kind, and \(R_0\) and \(R\) are the dimensionless initial and final pore radii normalized to the square root of the gel permeability. The adsorption kinetics are modelled using a Langmuirian adsorption isotherm valid for dilute solutions, i.e., where \(\Gamma \ll \Gamma_\infty\).

\[
\frac{d\Gamma}{dt} = k_1 c \Gamma_\infty - k_2 \Gamma
\]

\cdots(3.13)

where,

- \(\Gamma\) = mass of protein adsorbed per unit area
- \(\Gamma_\infty\) = maximum amount that can be adsorbed
- \(c\) = solution concentration of protein (assumed constant)
- \(k_1, k_2\) = adsorption and desorption constants

Combining Equation\cdots(3.12) and Equation\cdots(3.13) with the transport equations for flow through the pore, Ethier has developed a computer program to obtain the increase in membrane resistance with time. This model has 11 input parameters of which 6 are adjustable. Ethier (1986) has also developed the equations where the protein blocks the entrance to the pores rather than adsorbing to the inner pore walls. He considers two alternative geometries, namely, uniform deposition over the membrane surface and the formation of hemi-spherical gel caps over individual pores. The resistance of the gel in each instance is evaluated for both fibrous and spherical particles.

A detailed mathematical treatment of the capture of particles at the entrance to the cylindrical pores of Nuclepore membranes has been done by Kao, et al. (1988). Their model includes the
hydrodynamic as well as electrostatic and electrodynamic interactions between the particles and the pores unlike other models that include only the inertial impaction of particles on the membrane surface. It is useful in understanding how the process of pore bridging can be initiated even when the particles are much smaller than the pore radii. A key conclusion is that particle capture depends primarily on the range of molecular forces that come into play when the particle's inertia is not the dominant factor (i.e., Stokes numbers of $O(1)$). The electrostatic effects that govern particle deposition at solid-liquid interfaces (e.g., during adsorption) are reviewed by Jia and Williams (1990).

3.6 Modelling of solute transmission

Although there have been extensive experimental investigations of solute transmission through membranes (van den Berg and Hanemaaijer, 1987; Papamichael and Kula, 1987; Long, et al., 1981; Zeman, 1983) there are no good predictive models. Modelling of solute transmission under actual filtration conditions is complicated by the polarization of particles at the upstream face of the membrane. This polarized layer often behaves like a secondary membrane with a higher rejection coefficient than the polymeric membrane. Even in the absence of particle polarization, the solute transmission is affected by several parameters such as relative size of the solute and the pore, the tortuosity of the pore, solute-pore interactions, solute association and adsorption to the pore walls. For non-spherical solutes, the orientation of the molecules in the shear field at high pore velocities can make the transmission coefficient a function of the flux even in the absence of polarization. Much of the modelling on transmission deals with the restricted diffusion of solutes through idealized, cylindrical pores of large aspect ratio (Davidson and Deen, 1988; Mitchell, 1984). The average solute flux at steady state for the case of spherical particles is given as,

$$N_s = W(\lambda) V C_0 \left[ 1 - \left( \frac{C_L}{C_0} \right) e^{-PeL} \right] \left\{ \frac{1 - e^{-PeL}}{1 - e^{-PeL}} \right\}$$  \hspace{1cm} (3.14)
where, \[ P_{\infty} = \frac{W(\lambda) V L}{H(\lambda) D_{\infty}} \]

\[ W(\lambda) = 4 \int_0^{1-\lambda} G(\lambda, \beta) e^{-E(\beta)/kT} (1 - \beta^2) \beta d\beta \]

\[ H(\lambda) = 2 \int_0^{1-\lambda} K^{-1}(\lambda, \beta) e^{-E(\beta)/kT} \beta d\beta \]

In the above equations, \( H \) and \( W \) are the diffusive and convective hindrance factors, \( G \) is the 'lag coefficient', \( K \) is the 'enhanced drag', \( \lambda \) and \( \beta \) are the solute size and radial coordinate normalized to the pore radius, \( N_s \) is the solute flux, \( C_0 \) and \( C_L \) are the solute concentrations at the entrance and exit of the pore, \( V \) is the average velocity through the pore, \( E \) is the potential energy of interaction with the pore, \( k \) is the Boltzmann constant, and \( T \) is the temperature. In most microfiltration experiments, the axial Peclet number, \( P_{\infty} \), is much greater than unity; hence, the solute flux is then only a function of \( W(\lambda)V C_0 \). The difficulty in evaluating the radial dependence of the hindrance factors usually compels the use of centerline approximations, which in the special case of \( E(\beta)=0 \) become,

\[ H(\lambda) = K^{-1}(\lambda,0) (1 - \lambda)^2 \]

\[ W(\lambda) = G(\lambda,0) (1 - \lambda)^2 [2 - (1 - \lambda)^2] \]

Expressions for \( K(\lambda,0) \) and \( G(\lambda,0) \) are provided by Mitchell who also formulates the more general problem where \( E(\beta)\neq0 \). He also compares the prediction of this equation to ultrafiltration data using the globular protein, BSA, and Nuclepore track-etch membranes and finds that the theoretical prediction significantly underestimates the rejection coefficient for both dilute and concentrated solutions. Since most of our experiments involve polarization of the solute and solute-solute interactions, the applicability of the theoretical development is limited and hence, will not be considered in any more detail.
Chapter 4. Materials and Methods

4.1 Choice of protein

Bovine serum albumin (BSA) was selected as the model protein for the following reasons:

1. It has been extensively used by other researchers studying ultrafiltration (van den Berg and Smolders, 1989; Vilker, 1981a; Trettin and Doshi, 1980), plasmapheresis (Zydney, 1985), membrane fouling (Ethier, 1986; Suki, 1984; Fane, 1983; Matthiasson, 1983; Reihanian, 1983), adsorption (Lee and Ruckenstein, 1988; Cheng, 1987) and denaturation/aggregation (Franken, 1990; Damodaran, 1987; Ueki, 1985).

2. Its structure and physical/chemical properties are well documented (Peters and Sjöholm, 1978; Vilker, 1981b).

3. It is inexpensive and readily available in pure form.

4. Being a globular protein, its orientation in a shear field and possible effects of chain entanglement leading to multi-layer adsorption are unimportant compared to fibrous proteins.

The main disadvantages of BSA are the absence of a simple enzyme activity assay to gauge the extent of denaturation and its tendency to dimerize in solution up to about 10% (by weight).

The early experimental work, which was initiated when little was known about the fouling mechanism, was done with BSA purchased from Sigma Chemical Co. (St. Louis, MO, Catalog # A-7030). Subsequent experiments which required a more careful analysis of adsorption and aggregation effects were done with 99% pure BSA (Catalog # 81-028, Lot # P341, ICN Immunobiologicals, Lisle, IL). The chemical analysis for this lot of albumin, as provided by the manufacturer, is shown in Table 4.1. When a solution of this BSA (2.5 kg/m³ in 50 mM PO₄, 150 mM NaCl, pH 7.4) was analyzed using a Pharmacia preparatory scale chromatographic column (Superose 6, volume 100 ml), the monomer content was found to be only 90%. Within the limits of sensitivity of the assay, nothing larger than the dimer was
<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Retention Volume (mL)</th>
<th>% Area</th>
<th>Peak Height (% Full Scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67.88</td>
<td>4.61</td>
<td>16.5</td>
</tr>
<tr>
<td>2</td>
<td>68.32</td>
<td>0.04</td>
<td>16.5</td>
</tr>
<tr>
<td>3</td>
<td>68.33</td>
<td>4.30</td>
<td>16.5</td>
</tr>
<tr>
<td>4</td>
<td>70.17</td>
<td>0.41</td>
<td>12.5</td>
</tr>
<tr>
<td>5</td>
<td>73.05</td>
<td>82.33</td>
<td>124.0</td>
</tr>
</tbody>
</table>

Note: Peaks 1 to 3 were assumed to refer to the dimer and peaks 4 and 5 to the monomer. The multiple peaks are an artifact of the instrument. The 90% monomer content is calculated on a renormalized basis.

Elution Buffer: 50mM PO₄, 150mM NaCl, 0.05% NaAzide, pH 7.4.
Elution Rate: 0.2 ml/min
Column volume: 100 ml

Figure 4.1: Chromatographic analysis of BSA (ICN Immunobiologicals, Catalog # 81-028) using a Pharmacia Superose 6 column.
Table 4.1: Chemical analysis of BSA (specified by ICN Immunobiologicals, Cat. # 81-028, Lot # P341)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer content</td>
<td>96.6%</td>
</tr>
<tr>
<td>Purity (Albumin Fraction IV)</td>
<td>99%</td>
</tr>
<tr>
<td>By cellulose acetate electrophoresis</td>
<td></td>
</tr>
<tr>
<td>Protein (by nitrogen analyzer, Dry wt. basis)</td>
<td>99%</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>15.9%</td>
</tr>
<tr>
<td>Non-protein nitrogen</td>
<td>0.02%</td>
</tr>
<tr>
<td>Carbohydrates (calculated as hexose)</td>
<td>0.01%</td>
</tr>
<tr>
<td>Metabolites (by HPLC; sum of citrate, pyruvate and lactate)</td>
<td>0.004%</td>
</tr>
<tr>
<td>Lipids</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Table 4.2: Properties of the proteins used in this study

<table>
<thead>
<tr>
<th></th>
<th>BSA(^b)</th>
<th>Ribonuclease</th>
<th>Lysozyme(^c)</th>
<th>Catalase(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Wt.</td>
<td>66,700</td>
<td>13,700</td>
<td>14,300</td>
<td>247,000</td>
</tr>
<tr>
<td>(Daltons)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimensions (nm x nm x nm)</td>
<td>14 x 4 x 4</td>
<td>3.8 x 2.8 x 2.2</td>
<td>4.5 x 3 x 3</td>
<td>10.5 x 10.5 x 5</td>
</tr>
<tr>
<td>Diffusivity (x 10(^{11}) m(^2)/s)</td>
<td>6.7</td>
<td>10.7</td>
<td>11.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Frictional ratio (f/f(_0))</td>
<td>1.33</td>
<td>1.29</td>
<td>1.24</td>
<td>1.25</td>
</tr>
<tr>
<td>Isoelectric pH</td>
<td>4.8</td>
<td>9</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>S-S linkages</td>
<td>17</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>(35 Cys)</td>
<td></td>
<td>(8 Cys)</td>
<td>(8 Cys)</td>
<td>(16 Cys)</td>
</tr>
</tbody>
</table>

\(^a\) Creighton, T.E., 1984; Cantor, C.R. and Schimmel, P.R., 1980.
\(^c\) Imoto, T., et al., 1972.
\(^d\) Murthy, M.R.N., 1981.
detected in the pre-filtered samples (see Figure 4.1).

A few experiments were done with other globular proteins (enzymes) to gauge the generality of the results obtained with BSA and to use activity measurements as a measure of structural integrity. These proteins, a few of whose properties are listed in Table 4.2, are:

1) Lysozyme from chicken egg white (Catalog # L-6876, Sigma Chemical Co.)
2) Ribonuclease A (Catalog # 3433, Worthington Biochemical Corp., NJ)
3) Catalase from bovine liver (Catalog # C-40, Sigma Chemical Co., MO)

4.2 Choice of Membranes

Most of the CFF experiments were done with 7.2 cm\(^2\) (±5%) flat sheets of 0.2 μm polycarbonate, track-etch membranes (Catalog # 113506, Nuclepore Corp., Plesanton, CA). The DEF experiments were done with 2.5 cm diameter disks of the same membrane. The reasons for the choice of this membrane and configuration are:

1) The flat sheet configuration allows easy access to the membrane surface for the purposes of analyzing any protein deposited onto the membrane and for visualization of the surface by scanning electron microscopy.

2) The well defined pore geometry (cylindrical, straight through pores) makes the track-etch membranes an attractive choice for mathematical modelling of flux and transmission (Zydney, A., 1985; Ethier, R.C., 1986, Mitchell, B.D., 1984). For example, the defined surface area reduces the uncertainty in estimating the thickness of any uniformly adsorbed protein layers.

3) The polycarbonate membranes have a hydrophilic coating of polyvinyl pyrrolidone to minimize non-specific protein adsorption.

4) The lower costs of flat sheets compared to hollow-fiber cartridges allows them to be used once and discarded. This avoids the problems associated with imperfect cleaning of the fouled membranes which often results in wide variations in initial buffer fluxes.
The main disadvantage of the 0.2 μm track-etch membranes is the low pore density of $3 \times 10^{12}$ m$^{-2}$ (±10%) which corresponds to a porosity of about 10% on an area basis. The average pore density obtained from 8 scanning electron micrographs (SEMs) of new membranes was $3.2 \times 10^{12}$ m$^{-2}$ (±10%). The specified membrane thickness is 10 μm but the average pore length has been estimated to be about 6.8% larger due to the non-perpendicularity of the axis of some of the pores to the plane of the membrane (Mitchell, B.D., 1984). The maximum deviation from perpendicularity is specified to be 29°. Another feature of these membranes is the partial overlap of about 10-15% of the pores to form doublets and triplets (see Figure 5.6a). However, since the hydrodynamic resistance is inversely proportional to the fourth power of the pore diameter for laminar flow, the overlapping pores contribute significantly to the total flux through the membrane. For example, the effective pore diameter calculated from hydraulic permeability measurements is about 0.27 μm. The membranes have a negative zeta potential with external surface charge densities about $-4 \times 10^{-3}$ C/m$^2$ and about $-8 \times 10^{-3}$ C/m$^2$ inside the pores (Hernández, A. et al., 1987). A few experiments were also done with 0.4, 0.6 and 0.8 μm track-etch membranes.

The 0.2 μm hydrophilic, polyvinylidene difluoride (PVDF) Durapore membrane (Catalog # GVLP 0MS 10, Millipore Corp., Bedford, MA) and the 0.45 μm nylon-66 Ultipor (Type NXG, Pall Corp., Glen Cove, NY) membrane were used to explore the generality of the results obtained with the Nuclepore track-etch membranes. Unlike the straight-through, cylindrical pores of the latter, the Durapore and Ultipor membranes have an inter-connected network of tortuous pores. According to the manufacturers, the nominal pore size is an estimate of the largest pore size and the effective diameter of the smallest pores could be 10-15% lower. The thickness of the Durapore membranes is about 100-120 μm and that of the Ultipor membranes is about 130-150 μm but the expected greater hydrodynamic resistance compared to the 10 μm thick track-etch membranes is compensated by the greater porosity, typically about 85% on an area basis. The Ultipor membranes have about 375 m$^2$ surface
area/m² projected area and the Durapore membranes have about 150 m² surface area/m² projected area (obtained via personal communication from manufacturers).

Diaflo, 0.1 μm hollow fiber cartridges (Model H1MP01-43, Amicon Inc., Danvers, MA) were used in early experiments which first revealed the occurrence of a decrease in flux with protein solutions even in the absence of cells. These cartridges contain 55 polysulfone fibers, 20.3 cm long and with an internal diameter of 0.11 cm. The filtration area is about 300 cm². The cartridges have a pressure rating of 175 kPa and a cross-flow rating of 0.6 m/s (2 l/min).

4.3 CFF flat membrane apparatus

The custom designed flat membrane apparatus (Figure 4.2) is modelled after the one used by Zydney (1985) in his work on plasmapheresis. It consists of two 1.27 cm thick acrylic plates (12.7 cm x 7 cm) with semi-cylindrical channels (0.63 cm diameter) at both ends for the smooth entry and exit of solution. The membrane is supported by a 3.2 cm x 2.5 cm x 0.3 cm porous stainless steel slab (Grade D, 20 μm nominal rating, Pall Corp., Cortland, NY) which offers a negligible hydrodynamic resistance compared to the microporous membranes. The surface of the support is smooth enough not to visibly damage the membrane upon application of a trans-membrane pressure at least up to 35 kPa. A 0.06 cm thick silicone sheet (Silastic™ 500-5, Dow Corning Corp. Medical Products, Midland, MI) with a 2.6 cm wide channel cut into it, is placed between the acrylic plates to provide a water tight seal. Stainless steel shims, 0.06 cm thick, are placed in grooves cut into the silicone sheet to maintain the channel height when the acrylic plates are clamped together. This assembly results in a flow channel of cross-section 2.6 cm x 0.06 cm. Although the total channel length is 6.4 cm, the length above the porous support is only 2.5 cm resulting in an active filtration area of 6.5 cm². Repeated use of the silicone gasket causes the width of the flow channel to increase by about 10%; therefore, the area is measured every time the apparatus is assembled. The top acrylic plate has entry and exit ports for feed and retentate solutions respectively and two pressure
Figure 4.2  Schematic of cross-flow apparatus used with flat-sheet membranes.
connections on either side of the filtration area to measure the axial pressure drop. The permeate port is located below the stainless steel support and is open to atmospheric pressure.

4.4 Standard protocol for CFF with flat sheet apparatus

The experimental protocol evolved gradually over the course of this research project as our understanding of the fouling mechanism increased. For example, the early experiments with the hollow-fiber system were done without prefiltering the protein solutions but when we realized the importance of protein aggregation, we began pre-filtering the solutions through 0.2 μm track-etch membranes. The following represents the final set of procedures adopted for cross-flow filtration with the flat membrane apparatus.

4.4.1 Preparation of protein solution: Phosphate buffered saline solution was prepared using deionized water from a Milli-Q system (Millipore Corp., Bedford, MA) and the following concentrations of analytical grade salts: 0.2 kg/m³ NaH₂PO₄, 1.2 kg/m³ Na₂HPO₄, 8.76 kg/m³ NaCl, and 0.5 kg/m³ NaN₃. The pH of the buffered saline is about 7.3 and any adjustments, if required, were done using 4N HCl or NaOH before dissolving the protein, thus avoiding any denaturation by local extremes in pH that may exist before the added acid or base is well-mixed. The buffered saline solution was filtered through a 10,000 MWCO Romicon hollow-fiber cartridge. After adding protein to obtain the desired concentration, the solution was passed through a 4.7 cm diameter, 0.2 μm track-etch membrane (Catalog # 111106, Nuclepore Corp.) at 6.7 kPa in a dead-end configuration to remove any incompletely dissolved protein or aggregates that may be present in the original solution. All apparatus used for handling the protein solution was rinsed apriori with the filtered buffer solution.

4.4.2 Configuration of cross-flow system: Before assembling the flat membrane apparatus, air was expelled from the porous stainless steel support by pumping buffered saline solution through i.e permeate port. This served to prevent small bubbles from accumulating between
the support and the membrane. The membrane was then carefully centered over the support, the silicone sheet and metal shims positioned appropriately and the acrylic plates clamped together with six C-clamps placed over the shims. The pressure taps were connected to a mercury manometer which could measure up to 26.7 kPa (200 mmHg) with a least count of 0.13 kPa. Flexible silicone tubing (Catalog # N-06411-16, Cole Parmer Instrument Co., Chicago, IL) of 0.31 cm i.d. was used for all connections. A stainless steel needle valve, located at the retentate exit, was used for controlling the trans-membrane pressure. The feed solution, contained in a polypropylene reservoir, was circulated using a peristaltic or diaphragm pump. The peristaltic pump was connected directly to the feed entry ports of the cross-flow device, but because of excessive pulsation with the diaphragm pump, a partially filled stoppered flask serving as a fluctuation dampener, was placed between it and the cross-flow device (see Figure 4.3). This flask increased the system hold-up volume to about 200 ml from less than 20 ml in its absence.

4.4.3 Pumps : A Masterflex™ peristaltic pump (Catalog # N-07553-20, Cole Parmer Instrument Co., Chicago, IL) with a stainless steel rotor housed in a standard, polycarbonate pump head (Catalog # N-07016-21) was used for the majority of the experiments with the flat membrane apparatus. The rotor has three rollers which compress the tubing resulting in the liquid entrapped between two points of compression being pushed along the direction of rotation (see Appendix A). Either silicone (Catalog # N-06411-16) or Norprene (food grade, Catalog # N-06402-16) tubing of 0.31 cm internal diameter was used. The maximum flow rate was about 8 \cdot 10^{-6} \text{m}^3/\text{s} (480 \text{ml/min}) against pressures of 26.7 kPa at about 600 rpm.

A Teflon, diaphragm pump (Model 7090-42, Cole Parmer Instrument Co., Chicago, IL) was tested as an alternative to the peristaltic pump after the latter was found to be the source of protein aggregation leading to membrane fouling. This pump has a pressure rating of 345 kPa and a flow rate range of 1.3 \cdot 10^{-6} to 13.3 \cdot 10^{-6} \text{m}^3/\text{s} (80-800 \text{ml/min}). The drive mechanism is
Figure 4.3: Components of the cross-flow microfiltration system
composed of a double convoluted short-stroke Teflon diaphragm which flexes in response to a nutating bearing linked to a spring loaded spool. The pump-head has inlet and outlet ports for the check valves, each composed of a spring-loaded ball on a seat, all made of Teflon. The outlet port is positioned perpendicular to the diaphragm axis to avoid air entrapment. The dead volume in the pump head is about 16 ml.

4.4.4 Experimental protocol: Every experiment was begun with a new membrane and its permeability measured using buffered saline solution. This procedure also served to wet the membrane and expel air from the apparatus and associated tubing. Flow rates were measured by timed collection of the solution in a 10 ml graduated cylinder. After the membrane was fouled, the low permeate flow rates were measured using a glass pipette with a least count of 0.01 ml. Unless otherwise stated, all experiments were done at constant trans-membrane pressure with complete recycle of the retentate and permeate. The experiments were conducted at room temperature (22 ± 1°C) and despite repeated recirculation, the temperature increase during a typical experiment (2 - 4 h) was no more than 1 - 2 °C; hence a temperature control scheme was not employed. About 1.5 ml samples of permeate and retentate were collected periodically for analysis of protein concentration, enzyme activity, extent of aggregation, etc.

4.5 Dead-end filtration system and protocol

The apparatus consisted of a stoppered, thick-walled, glass flask partially filled with feed solution prepared according to the protocol described in Section 4.4.1. The feed was forced through the dead-end filter using nitrogen pressure measured by a mercury manometer. The 2.5 cm disks of polycarbonate, track-etch membranes were housed in a standard Swin-Lok™ holder (Catalog # 420210, Nuclepore Corp.). Unless otherwise specified, the permeate was not recycled. This caused the solution level in the feed reservoir to decrease necessitating a periodic adjustment of the height of the dead-end filter to coincide with the feed level in order to maintain the accuracy of the trans-membrane pressure (see Figure 4.4). Similar to the CFF
protocol, every experiment was begun with a new membrane whose permeability was
determined using buffered saline solution. Flow rate measurements and sample collection were
also similar to that described earlier.

4.6 Hollow-fiber system and protocol

Experiments with the Diaflo, 0.1 μm hollow-fiber cartridge described in Section 4.2
were done using a Cole-Parmer Masterflex™, digital drive, peristaltic pump (Model N-07523-
00) and silicone tubing of 0.79 cm i.d. (Catalog # N-06411-18). Recirculation rates were in
the range of 750-1500 ml/min (0.24-0.48 m/s). Pressure was measured using an oil-filled
gauge (max. pressure 30 psi or 207 kPa; least count 0.5 psi or 3.4 kPa) mounted on a fluctuation dampner. The protein solution was not pre-filtered before use and the same cartridge was reused after cleaning with 0.1N NaOH, 0.2-0.3% detergent, and buffered saline solution. The hydraulic permeability of the membrane was measured before protein filtration. The experiments were done at constant trans-membrane pressure with complete recycle of retentate and permeate.

4.7 Analytical techniques

4.7.1 Protein concentration:

With pure protein solutions, the concentration was determined from the UV absorbance at 280 nm where the aromatic residues tryptophan and tyrosine are close to their maximum absorbance. A Perkin-Elmer, Lambda 3A UV/VIS Spectrophotometer and high quality quartz cuvettes (1 cm path length, Catalog # WG-9B-Q-10, Wilmad Glass Co., Buena, NJ) were used for the absorbance measurements. A sample of buffered saline solution identical to the one in which the protein was dissolved, was used as a blank. The absorbance of BSA was linear with concentration at least up to 1 kg/m³ with a slope of 0.6 AU/(kg/m³) ± 2%.

In a few experiments, protein concentration was determined from the absorbance at 595 nm by the complex formed between the protein and Coomassie Brilliant Blue G-250 (Bio-Rad Chemical Division, Richmond, CA). Since the absorbance changes gradually with time, all samples were analyzed between 5 and 10 min after adding the dye. The preparation of the Coomassie solution and the ratio of dye to protein solution were according to standard procedures recommended by Bio-Rad. The blank reading was obtained by adding the dye in the same proportion to a sample of buffered solution not containing protein. Disposable, polystyrene cuvettes were used to minimize excessive coloration of the walls of the cuvette. The concentration-absorbance curve was linear at least up to 1 kg/m³ with a slope of 1.2 AU/(kg/m³) ± 5%.
4.7.2 **Enzyme assays**:

The activity of Ribonuclease-A was measured using its ability to catalyse the hydrolysis of cytidine 2':3'-phosphate to cytidine 3'-phosphate and measuring the rate of change in absorbance at 286 nm (Crook, E.M., et al., 1960). This wavelength represents the maximum in the difference spectra of the substrate and product. The substrate and enzyme solutions were prepared in phosphate buffered saline (10mM PO₄, 150mM NaCl, 0.05% NaN₃, pH 7.3) and pre-filtered through 0.2 µm Nuclepore, track-etch membranes. The substrate concentration was 0.1 kg/m³ and the enzyme concentrations in the standard solutions were 0.1 - 2.0 kg/m³. The spectrophotometer was zeroed using a blank consisting of 1.4 ml substrate solution and 0.1 ml of phosphate buffered saline in a quartz cuvette. 0.1 ml of enzyme solution was added to 1.4 ml of substrate solution, mixed rapidly by inverting the cuvette 3 times, and then

![Figure 4.5](image)

**Figure 4.5**: Standard curve of RNAse activity (initial slope of absorbance-time curve) versus concentration.
scanned as a function of time. For the purposes of this project, the initial slope of the absorbance-time curve was assumed to be proportional to enzyme activity. More accurate measurements of activity can be obtained by letting the reaction go to completion and then plotting 'ln(A - x)' versus 't', where A is the change in absorbance for the complete reaction and 'x' is the change at any time, t. The standard curve is shown in Figure 4.5.

The ability of Catalase to catalyse the reduction of hydrogen peroxide was used as a measure of its activity (Bergmeyer, H.U., 1965). Both the enzyme and substrate solutions were prepared in a buffered solution consisting of 3.52 kg/m³ KH₂PO₄ and 5.8 kg/m³ Na₂HPO₄ at pH 7.0. Sodium azide (NaN₃) was not added because it is an enzyme inhibitor. 0.2 ml of H₂O₂ (30% w/v) was added to 100 ml of buffer solution and the concentration adjusted to give an absorbance value between 0.52 and 0.55 at 240 nm. The enzyme solutions were all diluted to a final concentration of 0.01 kg/m³ which gave reasonable rates of reaction. After zeroing the spectrophotometer with buffer, 0.05 ml of enzyme solution was mixed with 1.45 ml of substrate solution by rapidly inverting the cuvette 3 times. The time, Δt, required for the absorbance to decrease from 0.450 to 0.400 was measured and the activity in the assay mixture was calculated as 17/Δt in accordance with the definition given by Bergmeyer.

4.7.3 Electrophoresis:
Qualitative analysis of protein samples to determine the existence of dimers and larger aggregates was done using polyacrylamide gel electrophoresis (PAGE) on PhastGel™ Gradient 8-25 media (Catalog # 17-0542-01) and native buffer strips (Catalog # 17-0517-01) on the PhastSystem™ (Pharmacia LKB, Uppsala, Sweden) using Coomassie as well as silver staining. The separation and development protocols and the concentrations of the solutions used in development were identical to those recommended by Pharmacia. In order to determine whether the particles deposited on the membrane surface were protein aggregates, they were analyzed by PAGE using sodium dodecyl sulfate (SDS) buffer strips (Catalog # 17-0516-01)
after treatment with denaturants such as SDS or urea and reducing agents such as dithiothreitol (DTT) or β-mercaptoethanol to break the disulfide bonds. The concentrations of the various reagents, duration of exposure to the denaturants, boiling times, etc. are presented in Section 5.13 where a better appreciation of the reasons for adopting certain experimental protocols can be obtained based on a discussion of the results.

4.7.4 Chromatography:

The chromatographic analysis of protein samples was done using cross-linked, agarose based columns (Superose-6 and -12) on an FPLC System (Pharmacia Fine Chemicals AB, Uppsala, Sweden) using a UV flow cell (280 nm) as the detector. Superose-6 was used for the verification of the manufacturer's specification for the monomer content of BSA as mentioned in Section 4.1. Later experiments to determine the effect of pumping on protein used a Superose-12 column, which has an optimal separation range of 1 to 300 kDaltons and permits faster analysis. The elution buffer (10mM PO₄, 150mM NaCl, 0.5 kg/m³ NaN₃, pH 7) was degassed after filtration through a 10000 MWCO hollow fiber and pumped through the column at 0.2 ml/min. After equilibrating the column with two column volumes of eluent, 200 µl of the BSA solution (1 kg/m³) was loaded for each run. The typical duration of a run was equivalent to the flow of 30-35 ml of eluent.

4.7.5 Quasi-Elastic Light Scattering (QELS):

The presence of aggregates in the protein solution was detected using a Coulter Model N4 Sub-Micron Particle Analyzer (Coulter Electronics, Inc., Hialeah, FL) which calculates the particle size distribution from the diffusion coefficient obtained by quasi-elastic light scattering. It uses a 4 mW helium-neon laser and a scattering angle of 90° to detect particles in the size range 3 - 3000 nm. The proteins investigated in this project are close to the lower limit of the size range (for example, BSA has a Stokes radius of 3.6 nm) and this possibly accounts for the 50-100% error in the absolute size reported by the instrument even for pre-filtered solutions
that were not subjected to pumping and hence, not expected to contain large aggregates. We found that the results could be used for a qualitative assessment of the changes in cumulative aggregate concentration if we defined aggregates as those whose particle diameter as reported by the instrument was at least 3-4 times larger than BSA, say about 30 nm.

The principle of operation is based on relating fluctuations in scattered light intensity to particle diffusivity and hence, to particle size. This involves generating an autocorrelation function, $G(\tau)$, defined below, by measuring fluctuations in the intensity of scattered laser light.

$$G(\tau) = I(t) \times I(t + \tau)$$

$I(t)$ is the time average scatter intensity at time 't' and $I(t + \tau)$ is the same at time 't + \tau$. For a monodisperse suspension, the autocorrelation function is a direct measure of the diffusivity, $D$.

$$G(\tau) = \exp \left( -D K^2 \tau \right)$$

$K$ is a constant for the instrument if all samples contain solvent of the same refractive index. The diffusivity can be related to particle size using the Stokes-Einstein relation:

$$D = \frac{k T}{3 \pi \eta d}$$

where, 'k' is the Boltzman constant, 'T' the temperature, 'η' the viscosity, and 'd' the particle diameter. When the sample is polydisperse, some uncertainty is introduced into the calculated size distribution because the instrument fits a smooth distribution curve to the data and constrains it to non-negative sizes using a Size Distribution Processor (SDP) and a program called CONTIN, further details of which are given in the product reference manual for the Coulter N4. The number of particles in each size range, $N_d$, is calculated from the scattered intensity using a relation that tends to overemphasize the smaller particles. For example, the program assumes that $N_d \propto d^{-6}$ for particles less than 200 nm (at a scattering angle of 90°), and $N_d \propto d^{-2}$ for larger particles. The 'dust' term reported by the instrument reflects the fact
that some aggregates were outside the size range specified and not necessarily that there was
dust actually present. We did not attempt to minimize the dust term by prefiltering the samples
before loading them into the instrument for fear of losing some of the protein aggregates.
Consequently, the size distribution results were not useful for quantitative purposes. Usually,
the best results are obtained when particles of only two or three sizes which are about a factor
of 3 apart from one another are present in the sample. Secondly, it helps to have an *apriori*
estimate of the sizes of the particles present.

4.7.6  **Protocol for radio-labelling of BSA**

$I^{125}$-BSA was used as a tracer to quantify the amount of protein adsorbed to or
deposited on the microporous membranes. This isotope has a half-life of 60 days and being a
strong $\gamma$-emitter, the activity can be measured easily without the need for scintillation liquids
or pretreatment. $I^{125}$ decays into Te$^{125}$ by electron capture followed by emission of a 35.5 keV
$\gamma$-ray and X-rays having energies of 27.2 to 31.8 keV. This radiation is not blocked by most
polymeric membranes and hence one can directly count the membrane-associated radioactivity
without fear of possible shielding effects. The disadvantage is that the high energy emissions
make it necessary to take great care in handling and storage of the material. Usually, a 0.5 mm
thick lead sheet is required to reduce radiation exposure from a 1 mCi source to safe levels.
The basic assumption in a tracer study is that the labelled and unlabeled protein behave
identically.

BSA (Catalog # 81-028, ICN Immunobiologicals) from the same lot as used in the other
filtration experiments was iodinated using the iodine monochloride method described below.

**Reagents**: Iodine monochloride (ICl). 50 $\mu$l of ICl (MW 162.36, $\rho^{29}_{4} = 3.1$) is added to
495.5 ml of freshly prepared 0.1N HCl. 1.0 ml of this solution is added to 4.5
ml of 0.1N HCl to give a final ICl concentration of 3.5 x $10^{-4}$ M.
Glycine buffer. 1.5 g glycine (MW 75.07) and 1 ml of 2N NaOH are diluted to 100 ml with distilled water giving a solution of pH 8.5. This is stored at 4 °C.

Saline-borate buffer (pH 8.0). 8.5 g NaCl, 0.96 g Na₂B₄O₇.10H₂O, and 0.34 ml HCl are diluted to 1 liter with distilled water. A stock solution 10 times this concentration can be prepared and diluted before use.

**Protocol:** A 10 mg/ml solution of BSA is filtered through a 0.22 μm Durapore membrane (Millipore Corp.) and 1 ml of it used for labelling.

1 mCi Na¹²⁵ solution (= 10 mCi/ml, Catalog # NEZ-033, New England Nuclear) is carefully added to 0.7 ml of the ICl solution in a stoppered test tube. (Caution: I¹²⁵ is volatile at this stage.) 0.1 ml of glycine buffer followed by 0.8 ml of 0.1N NaOH are mixed in. The pH of the labelling mixture (measured with a pH paper) should be about 8.5, else another drop of NaOH is added.

The labelling solution is added gradually, with intermittent mixing, to the protein solution. The reaction is complete within a few seconds.

The reaction mixture is dialyzed twice against 1 liter solutions of saline-borate buffer, the first dialysis solution also containing 0.02M KI since I₂ is soluble in I⁻. It is then dialyzed twice against phosphate buffered saline (10mM PO₄, 150mM NaCl, 0.05% NaN₃). The labelled BSA solution is stored at 4 °C where it is stable for several weeks.

Following such a procedure, a 4.5 kg/m³ I¹²⁵-BSA solution having a specific radioactivity of 0.025 μCi/μg was obtained. Trichloroacetic acid precipitation of the labelled BSA revealed that about 99.8% of the I¹²⁵ was covalently linked to BSA. The radioactivity was measured using an Auto-Gamma 500C Counter (Packard Instrument Co., Downers Grove, IL) which employs
a crystal of sodium iodide doped with thallium to generate scintillations of light upon interactions of the γ-rays with the electrons in the crystal. The photons are captured by a photomultiplier tube whose electrical output is sent to a processor for analysis and storage. The radioactivity is related to the counts per minute (CPM) as:

\[ 1 \text{ Curie (Ci)} = 2.22 \times 10^{12} \text{ CPM}. \]

4.7.7 Protocol for filtration experiments using radio-labelled BSA

About 30 to 50 μl of labelled BSA solution was added to 1-2 ml of 'cold' (i.e., unlabeled) BSA solution and this mixture was filtered through a 0.2 μm Durapore membrane into the remainder of the 'cold' solution, typically about 150 to 200 ml. The Durapore membrane was rinsed by filtering an additional 3 or 4 ml of 'cold' solution through it in order to remove any labelled solution contained in the hold-up volume of the filter cartridge. The radioactivity of the feed solution was measured using 200 μl samples of solution taken in stoppered, polypropylene, tubes before and after every filtration experiment. The 'cold' solution in all of these experiments was prepared using the standard protocol described in Section 4.4.1. Due to the evolutionary nature of the experimental protocol for determining the amount of BSA deposited on membranes during CFF, further details are presented in Section 5.15 where the reasons for adopting certain procedures such as glutaraldehyde fixing and forward flushing with saline can be better explained in terms of other experimental results.

4.7.8 Scanning Electron Microscopy :

Samples of new or fouled membranes were dried in a desiccator and cut into squares about 0.6 cm x 0.6 cm. They were directly placed on aluminum mounts without using double sided tape as is customary, and held in place by graphite paint applied to the edges of the sample. This procedure, recommended in a personal communication from Nuclepore Corp., minimized the charging of the non-conducting, polymeric sample even at high magnifications. After coating the samples with an 8 to 10 nm gold layer, they were viewed using a Cambridge
Stereoscan 240 Mk 3 Scanning Electron Microscope at a magnification of 15,000-20,000 and a beam voltage of up to 20 kV. These values were arrived at by trial and error. The images were recorded on Polaroid Type 55N B/W film.
Chapter 5 EXPERIMENTAL RESULTS

5.1 Flux & transmission during CFF with a peristaltic pump

A common feature of the cross-flow filtration (CFF) experiments in which protein solution was recirculated with a peristaltic pump, is the initial, rapid decrease in permeate flux even though the protein transmission is about 100%. This is followed by a comparatively slow decrease in flux accompanied by a decrease in transmission. The rate and extent of flux decrease depend on several parameters discussed later in this chapter; however, the following example serves to introduce the various features of the flux and transmission profiles. 500 ml of a 0.25 kg/m^3 BSA solution in 10 mM PO₄, 150 mM NaCl, 8 mM NaN₃, pH 7.3 was prepared according to the protocol described in Section 4.4. The CFF was done through a 0.2 μm, Nuclepore, track-etch membrane at a trans-membrane pressure of 6.7 kPa (50 mmHg). The feed recirculation rate was 310 ml/min which corresponds to a cross-flow velocity of 0.3 m/s. The flux of BSA solution initially was 2.8×10⁻⁴ m/s (1000 l/h-m²), equal to that obtained with buffered saline solution. However, it decreased to about 10% of its initial value in 47 min and continued to decrease slowly to about 6% of its initial value in 120 min without reaching a steady state (See Figure 5.1). This behavior was confirmed in other experiments done for longer periods of time.

As expected from the relative sizes of BSA (Stokes diameter 7.2 nm) and the membrane pore (200 nm), the initial transmission was 100%. The surprising observation was that it remained at about 100% during the first 47 min even though the normalized flux decreased to 10%. This meant that the flux decrease was probably not caused by concentration polarization of BSA via a size exclusion mechanism. Previous studies on membrane fouling (see Chapter 3) indicated that intra-pore protein adsorption could reduce the pore diameter and decrease the flux. We speculated that in our experiments the adsorbed amount was too small to cause a significant change in permeate concentration (measured using absorbance at 280 nm) and hence, was not
detectable as a decrease in BSA transmission. Thus, based on this experiment, we could not rule out the possibility of BSA adsorption. After 47 min, when the rate of flux decrease had slowed considerably (the 'pseudo steady state region'), the transmission decreased steadily to about 84% in 120 min.

Another interesting feature is an inflection in the flux-time profile during the initial period of rapid flux decrease. This inflection is clearly identifiable as a maximum in the rate of flux decrease about 22 min after the filtration was begun (Figure 5.2). Such a behavior has not been reported in the literature and could not be explained by any conventional filtration theory based on a constant concentration of solute in the feed solution (see Section 7.7).
Control experiments with prefILTERED buffer show a very low rate of flux decrease, reaching about 70-80% of the initial value in 120 min. This decrease can probably be attributed to the low porosity of the Nuclepore track-etch membranes which makes them susceptible to fouling by even small concentrations of contaminants. However, the order of magnitude difference in the rates of flux decrease obtained with protein and buffered saline suggests that trace contaminants present in the buffer could not be solely responsible for the rapid flux decrease observed with protein solutions. This decrease was not exclusive to the track-etch membranes and was also observed during CFF with membranes of higher porosity as discussed later in this chapter.
During the initial stages of this research project, we used a Diaflo, 0.1 μm polysulfone, hollow fiber cartridge for the CFF of protein solutions which were not pre-filtered before the experiment. Unlike the experiment described earlier, which was done with 99% pure BSA purchased from ICN Immunobiologicals, these experiments used BSA purchased from Sigma Chemical Co. (see Section 4.1). As an example, the flux and transmission profiles obtained during the CFF of 0.5 kg/m³ BSA solution recirculated at 1500 ml/min (cross-flow velocity: 0.48 m/s) are shown in Figure 5.3. The flux had decreased to 20% of the buffer flux (0.035 cm/s at 55 kPa) by the time of the first measurement at 1 min. It continued to decrease to about 7% of the buffer flux in 90 min and it is difficult to identify a sharp transition from a period of rapid flux decrease to a 'pseudo steady state region'. BSA transmission was constant at 96% for the first 50 min and then decreased steadily to about 84% in 90 min.

![Figure 5.3](image_url)

**Figure 5.3** Flux and transmission of 0.5 kg/m³ BSA during CFF through a Diaflo, 0.1 μm hollow fiber cartridge
5.2 Irreversibility of flux decrease

The flux of phosphate buffered saline through a 0.2 μm track-etch membrane that had been used for protein filtration provided important information about the reversibility of the flux decrease. Two feed reservoirs, one containing a 0.25 kg/m³ BSA solution (2.5 mM PO₄, 7.5 mM NaCl, pH 7) prepared without prefiltration and the other containing the phosphate buffered saline solution, were connected in parallel to the same peristaltic pump via a T-joint. After a certain period of CFF with the protein solution, the feed was switched to the saline solution without introducing any discontinuity in recirculation or trans-membrane pressure. This means that there were no sudden changes in flow rate or pressure during the switch. The flux of saline through the membrane was then measured for about 30-40 min without returning the retentate or permeate to the feed to ensure that any protein (or other component) removed from the membrane surface was not reintroduced into the system.

The flux profiles from three such experiments where the switch from BSA to saline was done after 5, 10 and 30 min are shown in Figure 5.4. The flux decreased rapidly during the BSA filtration reaching about 80%, 60% and 10% of the initial value in 5, 10 and 30 min respectively. However, after switching to saline solution, there was no increase in flux as would be expected if concentration polarization were the cause of flux decrease. In fact, the basis of concentration polarization is a dynamic equilibrium between deposition of solute on the membrane and diffusive removal. Hence, if protein deposition was causing the decrease in flux, switching to saline solution would have stopped further deposition. Diffusive removal then would be expected to cause the dissipation of the polarized layer and an increase in flux. The absence of a flux increase suggested that the feed component responsible for the flux decrease was not removed easily by diffusion and was probably associated with the membrane. This result coupled with the 100% protein transmission observed during the period of rapid flux decrease (see Section 5.1) indicated that some other mechanism such as irreversible adsorption or pore occlusion was probably responsible for the flux decrease.
Figure 5.4 Flux of saline solution through 0.2 µm track-etch membrane after CFF with 0.25 kg/m³ BSA solution.

According to our definition in Section 1.4, this lack of a recovery in flux even upon preventing the deposition of additional solute is attributed to fouling which usually occurs by solute adsorption or pore occlusion. The species responsible for fouling is called the foulant.

An early clue that the foulant was composed of protein was obtained from experiments similar to the ones described above but involving the use of protease solution after the switch to saline solution. This time, three feed reservoirs containing 0.25 kg/m³ BSA, buffered saline and 1 kg/m³ protease (Sigma P-5147) respectively were connected in parallel to the peristaltic pump. As was observed previously, the flux decreased to about 10% of its initial value in 30 min during the BSA filtration and there was no subsequent increase in flux when the feed was
switched to saline. However, when the feed was subsequently switched to the protease solution, the normalized flux increased within 2-3 min from about 10% to 65% and this increase was sustained upon further switching to the saline solution (see Figure 5.5). We speculate that the flux increase was caused by at least a partial proteolysis of the foulant, thus facilitating its removal from the membrane. This suggests that the foulant is composed of protein. The slight decrease in flux observed during the protease filtration is probably because of fouling by the protease itself. Similar experiments where a 10% ethanol solution was used instead of the protease solution did not result in any increase in flux. In fact, the ethanol solution has a higher viscosity than the saline solution and its flux was proportionately lower (about 73% of the saline flux).

![Figure 5.5 Effect of protease solution on flux recovery](image-url)
5.3 Scanning electron microscopy of the fouled membranes

An advantage of the flat membrane configuration is the easy accessibility of the surface for visualization. The filtration protocol was similar to the one used in Section 5.2. 150 ml of 0.25 kg/m³ BSA (ICN Immunobiochemicals) solution were subjected to CFF through 0.2 μm track-etch membranes at 6.7 kPa and at recirculation rates of 200 ml/min (0.2 m/s). After a predetermined duration of filtration, the feed was switched to saline solution to purge the system of bulk protein. As expected from the results in Figure 5.4 there was no increase in flux; therefore, we assumed that the foulant deposited on the membrane during protein filtration was not removed by rinsing. In order to prevent removal of foulant (thought to be proteinaceous) during subsequent handling of the membrane, we switched the feed from saline to a 2.5% glutaraldehyde solution to fix the protein. After 15-20 min, the system was again rinsed with saline and the membrane processed according to the protocol described in Section 4.7.8. Figures 5.6a-e provide a visual record of the fouling process, each SEM obtained from a separate experiment begun with a new membrane. The normalized fluxes reported are the final values before glutaraldehyde fixing. A major uncertainty in any quantitative comparison is the definition of a blocked pore and its contribution to the total flux. For example, some of the lightly shaded pores that appear filled but not covered by large particles may have a resistance quite different from the pores blocked by larger particles. In addition, the SEMs do not provide any clue regarding the thickness of the deposited layers either on the surface or within the pores. Initially, the foulant may form a very porous deposit with the underlying pores relatively open. Further deposition may fill up the void spaces in the initial porous structure resulting in completely blocking the underlying pores. The smooth nature of the surface in Figure 5.6e after the membrane is completely fouled may in fact be a manifestation of this 'void filling' phenomenon. The permeate side of the fouled membranes appears no different from the surface of a new membrane. This is qualitative support for a fouling hypothesis based on pore entrance blockage as opposed to intra-pore fouling throughout the pore length.
Membrane material : polycarbonate (with PVP coating)
Pore diameter : 0.2 μm (nominal)
Pore density : $3.2\times10^{12}$ pores/m$^2$ (measured)
Membrane thickness : 10 μm (nominal)

*Figure 5.6a*: Unused 0.2 μm track-etch membrane
Figure 5.6b,c: Membrane surface when normalized BSA flux is 80% and 40%
Figure 5.6d,e: Membrane surface when normalized BSA flux is 7% and 5%
5.4 Preliminary evidence on effect of recirculation of feed

An early clue that repeated feed recirculation affected the rate of flux decrease was obtained when different volumes of 0.25 kg/m³ BSA solutions were filtered through 0.2 μm track-etch membranes at 6.7 kPa (50 mmHg). The feed was recirculated at 38 ml/min (0.04 m/s) using a peristaltic pump. Smaller initial feed volumes gave a faster rate of flux decrease. For example, it took about 50, 75 and 100 min for the normalized flux to decrease to about 10% when using 100, 500 and 2000 ml of BSA solution (see Figure 5.7). The inflection in the flux profiles also occurred at earlier times with smaller feed volumes, namely at about 25 min for 100 ml, 32 min for 500 ml and 40 min for 2000 ml. This is evident from the maximum in the rate of flux decrease as shown in Figure 5.8. These rate data were calculated by assuming a linear gradient between adjacent flux measurements from Figure 5.7 and hence,

![Graph showing effect of feed volume on flux during CFF using peristaltic pump](image)

**Figure 5.7 Effect of feed volume on flux during CFF using peristaltic pump**
Figure 5.8 Slopes of the flux profiles obtained during CFF with different volumes of BSA solution

have a considerable amount of scatter. During the course of the experiment, the feed volume decreased by 20-25 ml because of retentate and permeate samples taken for measuring BSA concentrations.

Also shown in Figure 5.7 is the flux profile obtained during CFF of 0.25 kg/m$^3$ BSA solution without recycling the retentate or permeate. The cross-\(\phi\) rate (44 ml/min) and transmembrane pressure (6.7 kPa) were similar to those used in the experiments with 100-2000 ml of feed volume. Since the feed makes just one pass through the cross-flow system, this experiment is equivalent to using an infinite feed volume. The rate of flux decrease was much slower than observed when using a fixed feed volume, the normalized flux decreasing to only about 20\% in 150 min and about 11\% in 220 min at which point the experiment had to be
stopped because all the feed solution was exhausted. Furthermore, no inflection was observed in the flux profile.

The transmission profiles obtained at different feed volumes are qualitatively similar to the typical profile shown in Figure 5.1. The transmission was 100% during the initial period of rapid flux decrease. However, when the flux decrease had slowed considerably which in these experiments corresponded to the normalized flux decreasing below 10%, the transmission too began to decrease (see Figure 5.9). As shown in Figure 5.7, it took a shorter time for the normalized flux to decrease to 10% when using smaller feed volumes. Correspondingly, the transmission began to decrease at earlier times when using smaller feed volumes. These results show that there is a strong connection between the transition from a rapid to a slow flux

![Graph](image_url)

**Figure 5.9** Transmission profiles during CFF with different feed volumes
decrease and the mechanism of BSA rejection. In the filtration experiment done without recycle, the transmission was 100% during the entire 220 min which is consistent with the observation that the normalized flux had not yet decreased below 10%.

When cross-flow experiments using 0.25 kg/m^3 BSA and 0.2 µm track-etch membranes were done keeping the feed volume constant at 500 ml and changing the recirculation rate from 38 to 316 ml/min (0.04 - 0.3 m/s), faster rates of flux decrease were observed at higher cross-flow rates (see Figure 5.10). For example, the normalized flux decreased to about 10% in 50, 65 and 75 min at recirculation rates of 316, 106 and 38 ml/min respectively. However, the normalized pseudo steady state flux was about the same (5-6%) at all three cross-flow rates.

![Graph showing the effect of recirculation rate on flux during CFF of BSA through 0.2 µm track-etch membranes using peristaltic pump.]

**Figure 5.10** Effect of recirculation rate on flux during CFF of BSA through 0.2 µm track-etch membranes using peristaltic pump.
Figure 5.11  BSA transmission during CFF at different recirculation rates.

Of course, using a fixed feed volume implies that the retentate and permeate streams were completely recycled. Similar to the results obtained with different feed volumes and constant cross-flow rates, we find a correspondence between the time at which the normalized flux decreases below 10% and the time at which the BSA transmission begins to decrease from its initial value of 100% (see Figure 5.11).

5.5 Dead-end Filtration

A common theme that emerges from the cross-flow experiments is that repeated recirculation of feed solution affects the rate of flux decrease. The flux decreased faster when using either higher recirculation rates or smaller feed volumes. One could make an argument to
explain the former observation in terms of the differences in hydrodynamic conditions in the cross-flow channel at different velocities that perhaps affect the rate of deposition of foulant on the membrane. However, there was no obvious explanation for the effect of feed volume (see Section 7.7). In order to determine whether the observed fouling was unique to the cross-flow system, we carried out the dead-end filtration (DEF) of 0.25 kg/m³ BSA solutions through 0.2 μm track-etch membranes at the same trans-membrane pressure as before (6.7 kPa). The flux profiles from three successive experiments indicate a very slow flux decrease to about 70 ± 5% of the initial buffer flux in 120 min (Figure 5.12). These profiles are not very different from those obtained during the filtration of phosphate buffered saline. In fact, the rate of flux decrease is much slower than observed during the 'infinite' volume experiment in

![Graph](image)

**Figure 5.12** Flux profiles during the DEF of 0.25 kg/m³ BSA through 0.2 μm track etch membranes
which the normalized flux had decreased to about 30% in 120 min. The transmission of BSA was 100% throughout the experiment.

5.6 Effect of hydrodynamics above membrane surface

We speculated that the differences in the rates of flux decrease during CFF and DEF were due to the different hydrodynamic conditions above the membrane surface. Based on several reports in the literature on protein denaturation by shear and/or interfacial tension (see Section 3.3), we also suspected that repeated recirculation of the feed during CFF could lead to changes in protein conformation. This denatured protein could, by exposure of the core hydrophobic residues, adsorb to the membrane to a greater extent than the native protein. In addition, the denatured protein could form aggregates that block the membrane pores. In order to distinguish between the effect of hydrodynamics above the membrane and the effect of repeated recirculation, the following filtration configuration was set-up.

Two parallel feed streams were drawn from the same well-mixed feed reservoir with one stream entering the DEF apparatus and the other entering the CFF apparatus (see Figure 5.13). The retentate from CFF and both permeate streams were recycled. The feed reservoir was pressurized with nitrogen to provide the necessary driving force for DEF. In order to allow better control of the trans-membrane pressure in the cross-flow device accounting for pressure drops in the device, needle valve and connecting tubing, we introduced a break in the flow circuit by using an intermediate vessel to collect the retentate and permeate streams. A second peristaltic pump (P2) was needed to pump the recycle stream from the intermediate vessel back to the pressurized feed reservoir. In this configuration, the feed streams entering the DEF and CFF apparatus were identical; any denatured protein or other foulant introduced into the feed during CFF would now be expected to affect the performance of DEF as well. Thus, differences in flux or transmission could be attributed to the hydrodynamic conditions above the membrane.
Figure 5.13 Parallel DEF and CFF scheme to isolate the effect of surface hydrodynamics on rate of fouling.
Figure 5.14  Flux and transmission profiles using the parallel DEF and CFF configuration
The initial flux of the 0.25 kg/m$^3$ BSA solution was 0.028 cm/s at 6.7 kPa in both DEF and CFF. The flux during CFF decreased rapidly to less than 10% of its initial value in about 75 min (see Figure 5.14). The important observation is that the dead-end flux also decreased to 10% of its initial value in about 85 min unlike what was observed earlier in Figure 5.12. The slightly faster flux decrease with CFF could not be explained with the information available at the time of the experiment. Later, when we identified the peristaltic pump as the source of foulant generation, we realized that the faster decrease was because of the effect of the feed pump, P1, on the stream entering the cross-flow device and not because of any differences in hydrodynamics on the membrane surface. Both the CFF and DEF flux profiles exhibited an inflection at about 40-45 min as inferred from the maximum in the rate of flux decrease (see Figure 5.15). As will be shown later, such an inflection is not observed when DEF is done in

![Graph showing flux profiles](image)

*Figure 5.15  Inflection in DEF and CFF flux profiles when the feed is continuously recirculated*
isolation, that is when the feed is not being continuously recirculated. The similarity in the flux profiles suggests that under our experimental conditions, surface hydrodynamics had little effect on the rate of flux decrease and that the main influence was of repeated feed recirculation.

In both cases, BSA transmission was 100% during the initial phase of rapid flux decrease. Similar to earlier observations, the transmission during CFF started to decrease only when the normalized flux fell below 10%, which in this experiment took about 75 min. However, the transmission during DEF remained at 100% even after the normalized flux had fallen below 10%. This suggests that surface hydrodynamics affected BSA transmission through the 0.2 μm track-etch membranes only after the initial period of rapid flux decrease.

5.7 Identification of the possible sources of foulant.

The experimental results presented in the previous sections demonstrated the strong effect that repeated recirculation of protein solution had on the rate of flux decrease. We had mentioned that one possible effect of this recirculation is the denaturation/aggregation of protein leading to increased fouling by adsorption or pore occlusion. If denatured protein is indeed the foulant, we wished to identify the equipment along the recirculation pathway responsible for protein denaturation. During recirculation, the protein encounters the cross-flow apparatus, the needle valve, the peristaltic pump, and the connecting tubing.

Our initial hypothesis was that exposure to shear in the narrow channel of the cross-flow apparatus was responsible for protein denaturation. So, the cross-flow apparatus was removed from the parallel DEF-CFF filtration scheme shown in Figure 5.13 and the feed that formerly entered the cross-flow apparatus, was merely recycled through the two peristaltic pumps, P1 and P2 and the needle valve. 0.25 kg/m³ BSA solution contained in the pressurized (6.7 kPa) feed reservoir was used for DEF as before. The normalized dead-end flux profile was similar to the profile in Figure 5.14, decreasing rapidly to 10% in about 70-75 min and then decreasing
slowly to about 6% in 120 min. The protein transmission was 100% throughout the filtration. Thus, removing the cross-flow apparatus made little difference in the rate of fouling indicating that it is probably not the source of the foulant.

At this stage we decided to pump a 0.25 kg/m³ BSA solution through the peristaltic pump and subsequently use it for DEF. We hypothesized that foulant generated or introduced into the solution during pumping would cause a flux decrease during DEF. The pump operated against a pressure of 6.7 kPa regulated by a needle valve to simulate the pumping conditions in the earlier CFF experiments. The solution was passed 10 times through the pump and then used for DEF through 0.2 μm track-etch membranes at a pressure of 6.7 kPa. The normalized flux decreased rapidly to less than 10% in 45-50 min (see Figure 5.16) in contrast to the results in Figure 5.12 thus supporting our hypothesis that the foulant was generated or introduced into the solution during the pre-pumping. Furthermore, the flux profile did not exhibit an inflection unlike the results obtained during CFF (Figure 5.2) or DEF with simultaneous pumping of the feed solution (Figure 5.15). This suggests that repeated recirculation of the feed solution with the peristaltic pump while the filtration is in progress causes the inflection. In order to determine whether the needle valve had an effect, we repeated the pumping without the needle valve using a fresh solution of BSA. We, however, did maintain a back pressure of 6.7 kPa by pumping the solution 10 times into a stoppered flask pressurized with nitrogen. The dead-end flux profile using this solution is almost identical to the earlier one (Figure 5.16) indicating that the needle valve was not responsible for the observed fouling.

5.8 CFF using a Teflon diaphragm pump

Having eliminated the cross-flow apparatus and the needle valve as possible sources of the foulant, we turned our attention to the peristaltic pump. The model CFF system depicted in Figure 4.3 was used for the filtration of identical batches of 0.25 kg/m³ BSA solution, one recirculated with a peristaltic pump and the second with a Teflon diaphragm pump. The two
Figure 5.16  DEF using BSA solution pumped apriori with a peristaltic pump (in the presence and absence of a needle valve).
configurations are identical except for a stoppered flask between the diaphragm pump and the CFF apparatus to dampen out fluctuations in flow rate. Both experiments were done with complete recycle of retentate and permeate at a cross-flow rate of 200 ml/min (0.2 m/s) and at a trans-membrane pressure of 6.7 kPa.

With the peristaltic pump for feed recirculation, the normalized flux decreased rapidly to less than 10% of its initial value in 55 min and then continued to decrease at a slow rate (see Figure 5.17). In contrast, when the feed was recirculated with the diaphragm pump, the flux decreased slowly to about 70% of its initial value in 120 min. For comparison, the dead-end flux profile from Figure 5.12 also is shown and there seems to be little difference between the

![Graph](image)

**Figure 5.17** Comparison of CFF using a peristaltic or diaphragm pump for feed recirculation.
results of DEF and CFF using a diaphragm pump. In fact, both these flux profiles are similar to those observed with buffered saline solution and the slow decrease is probably because of trace impurities. However, the dramatic differences between the CFF flux profiles obtained with the peristaltic and diaphragm pump provide strong evidence that the peristaltic pump is the source of the foulant. The protein transmission was 100% during the 120 min of CFF with the diaphragm pump, perhaps reflecting the fact that the normalized flux was well above 10%, the point at which the transmission had been observed to decrease in other cross-flow experiments. With the peristaltic pump, the transmission was 100% initially and decreased only after the normalized flux fell below 10%.

An earlier experiment had revealed that DEF of pre-pumped BSA solution resulted in a rapid flux decrease (Figure 5.16) in contrast to the DEF of BSA solution that had not been pumped (Figure 5.12). This was attributed to the possible generation or introduction of foulant during pumping. We now decided to perform a similar experiment where BSA solution is initially passed through a peristaltic pump and subsequently used for CFF with a diaphragm pump. The flux profile thus obtained can be compared to the profile obtained by CFF of a fresh solution using a diaphragm pump, which is essentially the profile presented in Figure 5.17. 1000 ml of 0.25 kg/m³ BSA solution was passed 20 times through a peristaltic pump. The subsequent CFF of this solution using the diaphragm pump was done at a cross-flow rate of 200 ml/min and a pressure of 6.7 kPa. As anticipated, the normalized flux of the pre-pumped solution rapidly decreased to less than 10% in about 30 min in contrast to the slow decrease to about 70% in 120 min observed with fresh solution (see Figure 5.18). Furthermore, the flux profile did not have an inflection point unlike the profile obtained when CFF was done with the peristaltic pump. This supports our hypothesis stated earlier that the inflection is caused by the repeated recirculation of protein solution with the peristaltic pump during filtration. A detailed explanation of this effect will be deferred until Chapter 7 where we use the fouling model based on convective deposition to discuss many of our experimental results.
Figure 5.18 CFF with diaphragm pump using fresh BSA solution and solution pre-pumped with a peristaltic pump.
With pre-pumped solution, BSA transmission exhibited the typical profile seen in earlier CFF experiments; it was 100% for the first 25-30 min and then decreased to about 73% in 70 min after which it appeared to be close to a steady state value (see Figure 5.18). The time at which the transmission started to decrease corresponds approximately with the normalized flux switching from a rapid to a slow rate of decrease. These transmission results are in contrast to those observed during DEF of pre-pumped BSA solution, where the transmission was 100% even after the normalized flux had switched to a slow rate of decrease. These observations support the hypothesis that surface hydrodynamics affect BSA transmission in the pseudo steady state region but not during the initial period of rapid flux decrease.

5.9 Effect of number of passes through peristaltic pump on subsequent DEF.

Repeated recirculation of BSA solution using the peristaltic pump has already been identified as the main factor affecting the decrease in flux through 0.2 μm track-etch membranes. It remains to be seen whether a direct correlation exists between the rate of flux decrease and the number of times an element of solution encounters the peristaltic pump. In the previous experiments, continuous recycling of solution meant that not all fluid elements encountered the pump exactly the same number of times; there existed a residence time distribution characteristic of a CSTR. To add precision, we now pumped the feed from one flask to a second one so that all of the solution encountered the peristaltic pump only once (plug flow mode of operation). This was repeated till the desired number of passes through the pump was achieved. Between pumping, some solution was used for DEF through the 0.2 μm track-etch membranes at 6.7 kPa.

The initial flux at 6.7 kPa was about 0.028 cm/s (±5%) in all these experiments. The normalized flux profiles in Figure 5.19 clearly show a faster flux decrease with solutions that had been pre-pumped more number of times. The time taken for the normalized flux to
Figure 5.19  Effect of number of passes through a peristaltic pump on subsequent DEF of BSA.

decrease to about 10% is given Table 5.1.

<p>| Table 5.1  Effect of number of passes through peristaltic pump on time taken for normalized flux to reach 10% |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>No. of passes through pump</th>
<th>Time to reach 10% of initial flux (min)</th>
<th>No. of passes through pump</th>
<th>Time to reach 10% of initial flux (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>5</td>
<td>95</td>
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<tr>
<td>2</td>
<td>165</td>
<td>10</td>
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<td>3</td>
<td>145</td>
<td>15</td>
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<tr>
<td>4</td>
<td>105</td>
<td>20</td>
<td>30</td>
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At sufficiently long times, the extent of flux decrease was about the same in all cases (≈5% of initial flux), but a true steady-state was not reached. None of the flux profiles exhibited an inflection as expected from earlier results which linked the inflection to repeated recirculation of feed with the peristaltic pump during the filtration. In this set of experiments, the solution was pumped between successive filtrations and not while the filtration was in progress; hence, an inflection was not expected.

BSA transmission was 100% throughout the DEF, even after the normalized flux had fallen below 10% and switched from a fast to slow rate of decrease. As mentioned in Section 5.8, this behavior is different from that seen in the cross-flow experiments and confirms the effect of surface hydrodynamics on transmission during the period of slow flux decrease after the membrane is fouled.

5.10 Operating conditions that may effect the protein during pumping with the peristaltic pump

Having determined that the peristaltic pump had an effect on fouling, we then investigated specific pumping conditions such as axial flow rate, back pressure, external gas-liquid interfaces and tubing material that could influence the rate of fouling. Our hypothesis at this stage, was that protein was being denatured during the pumping and that adsorption of this denatured protein to the membrane caused the fouling.

The effect of axial flow rate was investigated by passing two identical batches of solution (2000 ml, 0.25 kg/m³ BSA, pH 7.3) 20 times through the peristaltic pump at 50 and 400 ml/min respectively. These flow rates correspond to axial velocities of 0.11 and 0.88 m/s (Re = 340 and 2700) through the Norprene tubing of 0.31 cm internal diameter. A back pressure of 6.7 kPa was maintained using a needle valve. Subsequently, the pumped solutions were used for DEF through 0.2 μm track-etch membranes at 6.7 kPa (50 mmHg). The flux profiles
Figure 5.20  **Effect of flow rate during pre-pumping with peristaltic pump on subsequent dead-end flux.**

with the two solutions are similar (see Figure 5.20) suggesting that differences in axial flow rates during pre-pumping do not significantly affect the flux decrease (at least over the range of flow rates investigated). If our hypothesis of protein denaturation is correct, this result implies that axial flow *per se* cannot be the major cause of denaturation. Likewise, pumping in the presence and absence of a back pressure of 6.7 kPa made little difference in the rate of fouling. We did not investigate higher back pressures.

The effect of the tubing material could not be established with certainty because of the difficulty in obtaining reproducible results whenever the tubing was changed. It did not seem to matter
whether the replaced tubing was made of the same material as existed earlier or was of a different material. The differences in the rate of flux decrease were sometimes as large as 50-100%. However, for a given set of experiments done successively without replacing the tubing, the results were reproducible to within 10-20%. Therefore, when two or more flux profiles are compared for example, to determine the effect of number of passes through the peristaltic pump on the fouling rate, the data were obtained without replacing the tubing or dismantling the peristaltic pump-head between experiments. We speculate that if protein denaturation is an interfacial phenomenon associated with the narrow gap width formed when the tubing is compressed in the pump, then it could be affected by differences in the internal tubing surface and in the way the tubing is loaded in the pump-head.

Another factor that could cause protein denaturation is the presence of an air-liquid interface in the feed reservoir. As mentioned in Section 3.3.2, proteins are known to denature at gas-liquid interfaces. We hypothesized that continuous mixing in the feed reservoir would cause denatured protein from the interface to be entrained into the bulk solution, thereby exposing more native protein to the interface. Preliminary evidence against this hypothesis was provided by the results of CFF of fresh BSA solution using a diaphragm pump for recirculation (see Figure 5.17). Unlike the flux profile obtained when using a peristaltic pump, a very slow flux decrease was observed. Since continuous replenishment of the interface in the feed reservoir occurs irrespective of whether a diaphragm or peristaltic pump is used, the differences in fouling suggest that external gas-liquid interfaces could not be the major factor. Independent confirmation was obtained by DEF of protein solution which was pre-pumped in the absence of an external gas-liquid interface. This was done by filling the feed reservoir (a stoppered flask) to the brim with solution and visually checking to make sure that no air bubbles were present. After 20 passes through the peristaltic pump using this closed system, the dead-end flux was measured at 6.7 kPa (50 mmHg). There seems to be no significant difference in the flux profiles whether the interface is present or not during pre-pumping (see Figure 5.21).
Figure 5.21  Effect on DEF of eliminating external air-liquid interfaces during pre-pumping.

In both cases the normalized flux decreased to 10% in about 25 min (±10%). This result along with the result of CFF with a diaphragm pump suggests that external gas-liquid interfaces, such as present in the feed reservoir or in the stoppered flask used as a fluctuation dampner, cannot be a major factor in the observed fouling.

5.11 Effect of using two peristaltic pump-heads in series.

The experimental results presented so far point to one parameter as having had the greatest effect on the rate of flux decrease, namely the number of times the solution was passed through the peristaltic pump (see Figure 5.19). This pump operates by compressing the tubing
against the pump-head by three stainless steel rollers. The solution contained between two points of compression is pushed along the tubing as the rollers rotate. The number of times that a given tubing is compressed and released during each pass of a fixed volume of solution through the pump is independent of axial flow rate, back pressure, tubing material, etc. We speculated that this peristaltic action may be involved in generating the foulant responsible for flux decrease. In order to change the frequency of compression keeping all other factors constant, one would ideally have to add or remove rollers from the pump-head. We took a simpler (but approximate) approach by adding a second pump-head in series on the same pump drive, thereby doubling the frequency of compression/release.

Two identical batches of solution (0.25 kg/m³ BSA, pH 7.3) were passed through the peristaltic pump mounted with one or two pump-heads in series. All other operating conditions (axial flow rate, tubing length, feed volume, back pressure, external air-liquid interfaces, etc.) were identical during the pumping of both batches. The pumped solutions were subsequently used for DEF through 0.2 μm track-etch membranes at 6.7 kPa (50 mmHg). A comparison of the flux profiles obtained using the two batches of solution indicates a doubling of the fouling rate with solution pre-pumped through two pump-heads in series (see Figure 5.22). In other words, the solution that was passed twice through the peristaltic pump mounted with one pump-head fouled the membrane at the same rate as the solution passed once through the pump mounted with two pump-heads. Since all other operating conditions were held constant, these results provide strong evidence that the foulant is generated by some phenomena directly associated with the peristaltic action.
Figure 5.22  Effect on DEF of using two pump-heads in series on the peristaltic pump during pre-pumping.
5.12 Changes in protein solution caused by pumping

So far, the effect of the peristaltic pump was inferred indirectly from the observation of rapid fouling only when the protein solution was pumped. There was a strong correlation between the extent of pumping and the rate of flux decrease but we had no direct evidence that pumping was either introducing a foulant into the feed solution or causing the denaturation/aggregation of protein. We, therefore, began a systematic analysis of the pumped protein solution first to detect any changes in composition and then to identify the foulant.

5.12.1 UV absorbance of pumped solution: 200 ml of 0.25 kg/m³ BSA solution (pH 7.3) was recirculated at 200 ml/min with a peristaltic pump operating against a back-pressure of 6.7 kPa maintained by a needle valve. About 1.5 ml samples of solution were withdrawn

![Graph](image)

*Figure 5.23* UV absorbance of BSA solution pumped with a peristaltic pump
periodically and scanned from 200 to 800 nm in a Hewlett-Packard diode array spectrophotometer. The absorbance at 240 nm (peptide bond) and 278 nm (aromatic residues) increased steadily with duration of pumping (see Figure 5.23). The base line absorbance at 656 nm, after subtracting the absorbance of the buffer solution, was about two orders of magnitude lower and showed no trend with the duration of pumping.

5.12.2 Chromatographic analysis: 150 ml of BSA solution (1 kg/m³) was recirculated at 200 ml/min with a peristaltic pump operating against a back-pressure of 6.7 kPa maintained by a needle valve. The pumping was done for 4 h, paused for about 10 h, and then continued for another 11.5 h. 200 μl samples, withdrawn periodically, were injected through a 0.45 μm syringe filter into a Superose 12 column and analyzed as described in Section 4.7.4. All of the chromatograms revealed the presence of only two components, one corresponding to monomer BSA (retention volume 11.47 ± 0.05 ml) and the second corresponding to the dimer (retention volume 12.75 ± 0.04 ml). Monomer BSA was found to account for 90±2 % of the area under these two peaks irrespective of the duration of pumping (see Figure 5.24). Also shown in the same figure are the ratios of the heights (measured as the percent full scale reading) of the monomer and dimer peaks. This ratio shows a slight decrease from about 8.5 : 1 at the beginning to about 7.8 : 1 after 15.5 hours of pumping. However, we speculate that this difference is probably because of a drift in the UV monitor which was used for detecting the protein. The sensitivity of the monitor was set at a high level to detect trace components that could be the foulant whose presence we were seeking to establish; hence, the monomer peaks in fact have a height which represents greater than 100% of full scale. We, therefore, do not place much emphasis on the ratio of the peak heights and conclude from the area under the peaks that there was no detectable change in the solution composition.

5.12.3 Electrophoretic analysis of pumped solution: 100 ml of BSA solution (0.25 kg/m³, pH 7.3) was recirculated at 200 ml/min with a peristaltic pump operating against a back
Figure 5.24 Chromatographic analysis of pumped BSA solution on a Superose 12 (Pharmacia) column

pressure of 6.7 kPa maintained by a needle valve. Solution samples (1 µl) were loaded onto the PhastGel 8-25 Gradient electrophoresis media and analyzed using native buffer strips (Catalog # 17-0517-01, Pharmacia). Lanes 1 to 4 contained fresh BSA solution, BSA solution pumped for 30 min, BSA solution pumped for 60 min and the high molecular weight (HMW) markers respectively. After separation, the protein bands were visualized by Coomassie staining (see Figure 5.25a). There was no qualitative difference in the bands for the fresh and pumped BSA solutions (Note: A faint band corresponding to dimer BSA was visible in lanes 1 to 3 on the original gels). Similar results were obtained when the gels were visualized by silver staining, except that the band corresponding to dimer BSA was more prominent.
NATIVE-PAGE OF PUMPED BSA SOLUTION

(with Coomassie staining)

5.0 G/L BSA

0.25 G/L BSA

THYROGLOBULIN (690000)
FERRITIN (440000)
CATALASE (232000)
LDH (140000)
BSA (67000)

Figure 5.25

(a) 4 3 2 1

(b) 7 6 5 4 3 2 1
The previous experiment was repeated with a 5 kg/m³ BSA solution with the anticipation that a higher protein concentration may lead to greater aggregation during pumping and also reveal the presence of trace amounts of larger aggregates upon electrophoresis. The solution samples were loaded onto the polyacrylamide gels without dilution; only Coomassie staining was done because of the high loading. The results are shown in Figure 5.25b where Lanes 1 to 7 correspond to fresh BSA solution, the HMW marker, and BSA solution pumped for 30, 60, 90, 120, and 180 min respectively. Since 100 ml of BSA solution was pumped at 200 ml/min, on average the solution made 2 passes through the pump every minute. Here, we can clearly see the BSA monomer and dimer bands and also a faint band corresponding to the trimer. However, there was no qualitative difference between the fresh and pumped solutions.

5.12.4 Quasi-Elastic Light scattering analysis of pumped solution: 400 ml of BSA solution (0.25 kg/m³, pH 7.3) was pumped at 200 ml/min with a peristaltic pump against a pressure of 6.7 kPa maintained by a needle valve. Samples of pumped solution were withdrawn periodically and analyzed using the Coulter Model N4 particle size analyzer (see Section 4.7.5). In this analysis, the size range was set at 3 to 300 nm, the sample time was 10 μs and the run time was 60 s. The results are presented as the cumulative weight percent of particles larger than 30 nm versus the extent of pumping (see Figure 5.26). Two important observations can be made: (a) The particle concentration increased with the number of passes of solution through the peristaltic pump. (b) Even after 320 passes through the pump (much greater than encountered in most of our CFF experiments), these particles constitute less than 0.6% (wt.) of the total protein. We have presented the results as the cumulative percentage of particles larger than 30 nm for the following reasons: (a) We wanted to clearly distinguish between the particles and monomer BSA which has a Stokes diameter of 7.2 nm. We knew that monomer BSA alone could not cause the observed flux decrease because very little fouling was observed when the solution was not pumped. (b) There was considerable scatter and irreproducibility in the size distribution as reported by the QELS instrument. (c) Assuming that
Figure 5.26 Quasi-elastic light scattering analysis of pumped BSA solution

the particles are the foulant, we do not know which size fraction is actually responsible for fouling. Even if we assume that size exclusion of the particles is the sole cause of fouling, it is obvious that as the membrane pores get progressively blocked the smaller particles too will be rejected and begin to participate in the fouling process.

5.13 Electrophoretic analysis of material taken from membrane surface

The quasi-elastic light scattering analysis indicated that particles larger than 30 nm were generated by recirculating the BSA solution with a peristaltic pump; it did not provide any information about their composition. Since there is very slow flux decrease during control CFF experiments with buffered saline solution, it seems unlikely that the particles originate in
the buffered saline solution or in the tubing and other equipment with which the solution comes into contact. We hypothesized that the particles were aggregates of BSA formed during pumping either because of protein denaturation or by aggregation of denatured protein existing in the original solution. We felt that chromatographic and electrophoretic analyses of the pumped BSA solution did not reveal any change in composition probably because the aggregates constituted too small a fraction of total protein to be detected by these techniques. Anticipating the deposition of the particles on the membrane surface to be the likely cause of fouling, we decided to analyze the material concentrated at the surface of a fouled membrane.

400 ml of BSA solution (1 kg/m³) was recirculated with the peristaltic pump at 200 ml/min for 8 h. This is equivalent to 240 passes through the pump. Subsequent DEF of the pumped solution at 6.7 kPa resulted in a rapid flux decrease. A protocol similar to the one described in Section 5.2 was used to rinse the fouled membrane. The feed was switched from protein to buffered saline solution contained in a second pressurized flask, by simultaneously clamping the protein feed tube and unclamping the buffer feed tube. This ensured that there was no discontinuity in feed flow or in trans-membrane pressure. The saline flux stayed constant during the 15-30 min of rinsing which served to purge any bulk protein solution from the membrane surface. The absence of a flux increase during rinsing confirmed that the foulant was not washed away. The track-etch membrane was then removed from the Swin-Lok holder and placed on a clean polyethylene sheet with the 'feed side' facing up. About 50 μl of buffered saline was placed on the membrane and gently agitated with a pipette tip to dislodge any deposited particles. This solution was then assayed using native or SDS-PAGE, after appropriate treatment with denaturants where necessary.

Shown in Figure 5.27 are the results obtained using native-PAGE and Coomassie staining using a sample volume of 1 μl. Lanes 1 to 4 correspond to bulk BSA solution before pumping, bulk BSA solution after pumping, solution taken from the fouled membrane surface,
NATIVE-PAGE OF PROTEIN TAKEN FROM MEMBRANE SURFACE
(with Coomassie staining)

THYROGLOBULIN
(669000)
FERRITIN (440000)
CATALASE (232000)
LDH (140000)
ALBUMIN (67000)

LARGE AGGREGATES

BSA DIMER
MONOMER

Lane 1 : BSA solution before pumping
Lane 2 : BSA solution after pumping
Lane 3 : Material taken from membrane surface
Lane 4 : High molecular weight marker

Figure 5.27
and the high molecular weight (HMW) marker. As reported in Section 5.12.3, there was no qualitative difference between the fresh and pumped BSA solutions (Lanes 1 and 2), both of which showed bands corresponding to monomer and dimer BSA but nothing larger. However, the solution taken from the membrane surface (Lane 3) revealed a distinct band corresponding to a molecular weight higher than even the 669,000 Daltons of thyroglobulin present in the HMW marker. In fact, this species was too large to enter the separation zone of the polyacrylamide gel (Phastgel 8 - 25 Gradient media, Pharmacia). Furthermore, Lane 3 did not have any bands corresponding to BSA monomer and dimer which suggests that these two species were washed from the membrane surface during the buffer rinse, leaving behind only the large particles. Since there was no flux increase during rinsing, the results of Lane 3 also imply that BSA monomer and dimer (which are no longer present on the membrane after rinsing) were not the foulant responsible for flux decrease. From the earlier experiments with protease (see Section 5.2) we knew that the foulant was composed of protein; but it remains to be seen whether the high molecular weight species in Lane 3 is an aggregate of BSA.

Additional evidence that the species in Lane 3 of Figure 5.27 was protein came from the results of treating it with trypsin which is a specific protease cleaving peptide bonds on the carboxyl-terminal side of lysine and arginine residues. Samples of fresh BSA solution and solution taken from the fouled membrane surface were treated with a stock trypsin solution (2.5 kg/m³ in phosphate buffer, pH 7.3) to a final trypsin concentration of 0.5 kg/m³. These mixtures were placed in a water bath at 37°C for 2 h and then analyzed using native-PAGE and Coomassie staining. Lanes 1 and 2 in Figure 5.28 correspond to fresh BSA solution and solution taken from the fouled membrane surface treated with buffer (as a control), while Lanes 3 and 4 correspond to the same solutions treated with trypsin. The large component in Lane 2 almost completely disappears upon trypsin treatment (see Lane 4). Comparing Lanes 1 and 3, it appears that trypsin hydrolyzes the dimer at a faster rate than the monomer, but this may merely reflect the smaller amount of dimer initially present.
TRYPTIC DIGEST OF PROTEIN TAKEN FROM MEMBRANE SURFACE

(with Coomassie staining)

Lane 1 : BSA solution
Lane 2 : Material from membrane surface
Lane 3 : BSA solution (after trypsin treatment)
Lane 4 : Material from membrane surface (after trypsin treatment)

Figure 5.28
Several attempts were now made to positively identify the large component in the material taken from the fouled membrane surface. Since we thought that this component consisted of BSA aggregates, our aim was to break it up using denaturants and reducing agents (to cleave disulfide bridges) and verify whether the resulting peptides corresponded to monomer BSA. The first attempt focused on the standard protocol for SDS-PAGE given by Pharmacia. Equal volumes of stock denaturant solution (5% SDS, 10% β-mercaptoethanol, in phosphate buffered saline at pH 7.3) and sample solution containing the large component were mixed and heated to 100 °C for 5 min. The standard protocol recommends that any insoluble material present must be removed by centrifugation to prevent streaking patterns in the gel. This step was not followed so as to check whether the aggregates were completely disrupted. The results (not shown) of SDS-PAGE with Coomassie and silver staining revealed that the aggregates were only partially disrupted. Whereas native-PAGE had yielded a single band corresponding to the large component, SDS-PAGE showed additional bands corresponding to BSA monomer and dimer.

Attempts were also made to completely disrupt the large component using a 4 : 1 volume ratio of a stock solution of urea-DTT (10 M urea and 50 mM DTT in 0.2 M Tris-HCl buffer, pH 8) and sample solution. The mixture was not boiled in this case but was allowed to stand for several hours. Once again, only partial disruption of the large component to BSA monomer and dimer was observed.

Finally, the solution collected from the fouled membrane surface was mixed in a 1:1 ratio with a stock solution of 10% SDS and 10% β-mercaptoethanol in phosphate buffer and boiled for 40 min. After cooling to room temperature (22°C) the mixture was analyzed by SDS-PAGE with Coomassie staining (Lane 4, Figure 5.29). The high molecular weight marker, fresh BSA solution and pumped BSA solution were also treated in an identical manner (Lanes 1-3, Figure 5.29). A single band corresponding to denatured monomer BSA was observed in
SDS-PAGE OF PROTEIN TAKEN FROM MEMBRANE SURFACE
(with Coomassie staining)

Lane 1: High molecular weight markers
Lane 2: BSA solution before pumping
Lane 3: BSA solution after pumping
Lane 4: Material taken from membrane surface

Figure 5.29
Lanes 2, 3 and 4 indicating that the large component and BSA dimers were completely disrupted. This result is strong evidence that the large component present on the surface of the fouled membrane consists of BSA aggregates. Of course, we cannot say with absolute certainty that they are 100% BSA and there is a possibility that they are associated with or generated by the presence of a trace impurity.

As an aside, it must be pointed out that the inability of the previous protocols to completely disrupt the aggregates deposited on the membrane surface from a pumped solution was also true for thermally generated BSA aggregates deposited on the membrane. In the latter case, the BSA solution was never exposed to the peristaltic pump.

5.14 Evidence linking deposited protein aggregates to flux decrease

The extent to which BSA solutions were pumped with a peristaltic pump was strongly correlated with the concentration of large particles (> 30 nm) in solution and also with the rate of flux decrease. So, it seemed a reasonable hypothesis that these particles were responsible for the flux decrease. The subsequent detection by native-PAGE of a large component in the material taken from the surface of a fouled membrane and the determination by SDS-PAGE that it was composed of BSA suggested that the particles fouling the membrane were aggregates of BSA.

A simple way to verify the hypothesis that the particles were responsible for fouling was to remove them from solution and check whether the fouling is prevented. 2000 ml of 0.25 kg/m³ BSA solution were pumped at 200 ml/min using a peristaltic pump for 200 min (which is equivalent to an average of 20 passes through the pump). A portion of this solution was used for DEF through 0.2 μm track-etch membranes at 6.7 kPa. The remaining solution was processed in a Sorvall centrifuge at 10,000 rpm for 60 min and the supernatant used for DEF under conditions identical to those during DEF of uncentrifuged solution. A comparison of the
flux profiles shows that the normalized flux decreased to 10% in about 22 min with the original pumped solution in contrast to 60 min with the supernatant after one centrifugation and 80 min with the supernatant after a second centrifugation (see Figure 5.30). The fact that the flux decreased even after centrifugation could be because of incomplete particle removal. This is corroborated by the observation that centrifuging the solution twice resulted in a slower rate of flux decrease than with the solutions centrifuged only once or not at all. The extent of flux decrease was similar in both cases (=5% of initial flux) and BSA transmission was 100% for the entire duration of DEF.

DEF was used as an alternative to centrifugation for removing particles from the pumped protein solution. 2000 ml of 0.25 kg/m³ BSA solution were pumped at 400 ml/min with a

![Graph showing flux profiles and centrifugation effects.](image)

*Figure 5.30  Effect of removing particles by centrifugation on DEF*
peristaltic pump for 100 min, which is equivalent to an average of 20 passes through the pump. A part of the pumped solution was then used for DEF through 0.2 μm track-etch membranes at a trans-membrane pressure of 6.7 kPa. The remainder of the pumped solution was passed through 47 mm diameter disks of 0.2 μm track-etch membranes in an attempt to remove the particles from solution. The membrane disks were replaced as soon as the normalized flux decreased to 15-20% in order to minimize any rejection of BSA by the fouled membrane. A part of this 'once filtered' solution was used for DEF at 6.7 kPa. The remaining part of the 'once filtered' solution was again passed through the 47 mm diameter membrane disks to further remove particles from solution. The dead-end flux of this 'twice filtered' solution was also measured. A comparison of the flux profiles indicates that particle removal by the first filtration step did slow (but not prevent) the fouling, the time taken for the normalized flux to

![Graph illustrating flux profiles](image)

**Figure 5.31 Effect of removing particles by successive filtration on DEF**
reach 20% increasing from 25 min to about 175 min (see Figure 5.31). In fact, the second filtration step did not cause a further slowing of the fouling process to any significant extent, the time to reach 20% of the initial flux increasing to only about 185 min. We have chosen 20% as the benchmark for comparison because the DEF with the 'once filtered' and 'twice filtered' solutions were stopped at this level. These results indicate that size exclusion of particles is not the sole mechanism of fouling since solution that passed through a 0.2 μm track-etch membrane was capable of fouling an identical membrane in a subsequent filtration. One possibility is that smaller particles may occlude the pores by bridging across their entrance via particle-membrane and particle-particle interactions. Such interactions were hinted at in Section 5.2 as a possible reason for the lack of flux recovery even after preventing additional deposition of particles by switching the feed from the protein solution to a saline solution.

5.15 Cross-flow filtration experiments with I\textsuperscript{125}-labeled BSA

Attempts were made to quantify the protein accumulating on the membrane surface during CFF using I\textsuperscript{125}-BSA as a tracer. Initial experiments used labeled BSA purchased from New England Nuclear but when the importance of the effect of the peristaltic pump on the protein was realized, the results from these experiments had to be discarded because of the lack of proper controls on the pumping conditions. As mentioned in Section 4.7.6, subsequent experiments used radio-labeled BSA prepared from the same batch of protein (Catalog # 81-028, Lot P341, ICN Immunobiologicals, IL) used in other microfiltration experiments. Our intention was to minimize any differences in the adsorption or aggregation behavior between the labeled and unlabeled protein.

The feed for all of the experiments presented in this section was prepared by adding 50 μl of stock I\textsuperscript{125}-BSA into 150 ml of 0.25 kg/m\textsuperscript{3} unlabeled BSA solution using the procedure described in Section 4.7.6. This yielded a solution having a radioactivity of 0.012 μCi/ml (5400 cpm per 200 μl sample) on average. The CFF through 0.2 μm track-etch membranes
was done at 6.7 kPa using a peristaltic pump to recirculate the feed at 200 ml/min. The experimental set-up was shielded by a transparent glass barrier doped with lead to absorb the emitted γ-radiation and no flux measurements were taken during the filtration of labeled BSA because of safety considerations. The flux of buffered saline was measured before and after filtering the labeled BSA solution for a predetermined time period. It has been shown in Section 5.2 that the saline flux is a good measure of the final protein flux as long as the switch from protein to saline is done without sharp changes in flow or pressure. This step also served to purge the labeled solution from the system leaving behind only the foulant deposited on the membrane surface.

Initially, the CFF was stopped after flushing the system with saline, the cross-flow apparatus dismantled and the membrane removed for measuring the radioactivity. The results obtained were not very reproducible. Further testing revealed that some of the foulant deposited on the membrane during filtration was lost when the flow of saline was stopped. In order to minimize this loss, we decided to cross-link any deposited protein with glutaraldehyde, CHO.(CH₂)₃.CHO, a commonly used fixative that rapidly reacts with active hydrogen amino and imino groups in the protein. This crosslinking step was included in the CFF protocol by adopting the procedure used in the protease experiments (see Section 5.2) except for replacing the protease solution with a 2.5% glutaraldehyde solution. After purging the labeled BSA solution from the system with saline, the feed was switched to the glutaraldehyde solution without any discontinuity in flow or trans-membrane pressure. After 15 minutes, the feed was switched back to the saline to purge the fixative from the system. It was now observed that repeated stopping and restarting the flow did not change the saline flux suggesting that there was no loss of foulant (protein) from the membrane surface even when the flow was stopped. Finally, a known area of the membrane was counted in the Auto-Gamma 500C.

In order to get a complete time profile of protein accumulation for just one set of operating
conditions (flow rate, pressure, concentration, pH, ionic strength, etc.), the experiment had to be repeated several times, each one with a different duration of filtration of the labeled protein solution. Shown in Figure 5.32 are the normalized values of the final saline flux plotted against the duration of protein filtration; the initial flux was 0.028 cm/s (±8%) in all cases. Each data point in this figure comes from a different experiment begun with a new membrane and a freshly prepared BSA solution; yet, the flux profile is remarkably smooth and shows a rapid decrease in flux for 10 min followed by a slow rate of decrease. In the lower half of Figure 5.32 are plotted the corresponding amounts of protein deposited on the membrane (calculated from the membrane radioactivity), expressed in terms of the projected membrane area. The rapid increase in protein deposition during the first 10 min correlates well with the rapidly decreasing flux. Thereafter, the BSA deposited on the membrane surface increases almost linearly reflecting the very small decrease in flux.

Permeate and retentate samples were collected during the CFF experiment done for 120 min. The transmission of labeled BSA through the 0.2 µm track-etch membrane was calculated by using the ratio of the radioactivities of the permeate to the retentate samples (see Figure 5.33). There is an abrupt change in the transmission profile around 10 min, from an initial, fairly constant value of 95% to one that decreases gradually to about 70% in 120 min. This transition coincides with the sharp change in the rate of flux decrease and in the rate of protein deposition around 10 min. Similar sharp changes in BSA transmission corresponding to a change in the rate of flux decrease were observed in all of the previous CFF experiments suggesting that a fundamental change in the mechanism of fouling is responsible for it.

5.16 BSA adsorption to 0.2 µm track-etch membranes

The CFF experiments with I\(^{125}\)-BSA as a tracer are not useful in distinguishing between protein adsorbed to the membrane and that which is merely deposited on the surface by the convective flow of permeate. The observation that there was no recovery in flux when
Feed: 0.25 kg/m$^3$ 'cold' BSA, pH 7.3
Radioactivity: 0.012 μCi/ml
Feed volume: 150 ml
Cross-flow rate: 200 ml/min
Pressure: 6.7 kPa

Figure 5.32 Correlation between flux and BSA deposition during CFF
the feed was switched from protein to saline solution could be explained by the irreversible adsorption of BSA to the membrane. We decided to investigate the role of protein adsorption in fouling in the absence of convective deposition of solute. A broader discussion of the adsorption versus deposition hypotheses is presented in Chapter 7.

$I^{125}$-BSA was used as a tracer to determine the adsorption of BSA to 0.2 μm track-etch membranes from a stirred solution. We anticipated that transport of BSA to the membrane surface and within the pores would occur only by diffusion since there was no convective flow through the membrane. 100 μl of stock $I^{125}$-BSA was added to 200 ml of 0.25 kg/m$^3$ unlabeled BSA solution (pH 7.3) using the procedure described in Section 4.7.7. Several 25 mm diameter membrane disks were pre-soaked in buffered saline solution to remove air.
initially present in the pores. This was done to avoid possible interferences from gas-liquid interfaces that have been reported to affect the extent of protein adsorption (see Section 3.2). The disks were transferred to the radioactive BSA solution contained in a polypropylene beaker. The solution was stirred gently using a Teflon coated, magnetic bar taking care to prevent foaming or excessive vortex formation. Three disks were withdrawn periodically, blotted on a paper towel to remove excess solution, and the bound radioactivity measured in the Auto-Gamma 500C. A second, identical, 200 ml batch of labeled BSA solution was prepared as mentioned above and recirculated at 200 ml/min with a peristaltic pump against a back pressure of 6.7 kPa maintained by a needle valve. The pumping was done for 60 min which corresponds to an average of 60 passes of solution through the pump. This pumped BSA solution was used in a second adsorption experiment to determine whether pumping caused any change in the adsorption profile. The results of the adsorption experiments expressed on the basis of the projected membrane area are shown in Figure 5.34. The total surface area may be calculated from the geometry of the 0.2 μm track-etch membranes to be 20 m²/m² projected area. The rate and extent of BSA adsorption from the pumped solution was similar to that from solution that was not pumped. Based on these results, we will demonstrate later (Chapter 7) that less than a monolayer of BSA is adsorbed during the course of a typical CFF experiment and that a monolayer is insufficient to account for the 10-fold decrease in flux.

Further information about the affect of adsorption on fouling was obtained by measuring the permeability of 0.2μm track-etch membranes before and after BSA adsorption. The flux of saline solution is a sensitive gauge of the extent of fouling. The membrane was soaked in a 0.25 kg/m³ BSA solution (pH 7.3) for a specified time and then its permeability to saline solution measured under cross-flow conditions (200 ml/min, 6.7 kPa) without recycling the retentate and permeate. There was no change in saline flux even after 20 h of exposure to BSA solution (see Figure 5.35) suggesting that under our experimental conditions adsorption was too slow to account for the rapid decrease in flux during CFF of protein solutions. There is, of
Figure 5.34  BSA adsorption to 0.2 µm track-etch membranes in the absence of convective deposition

course, the possibility that the protein was desorbed while measuring the saline flux. In that case, desorption should also occur in the experiments described in Section 5.2 where we measured the saline flux through a fouled membrane. Since no increase in flux was observed in those experiments, we may infer that either BSA was not being desorbed or that adsorbed BSA was not the main cause of flux decrease. The latter conclusion seems to be the likely explanation based on the adsorption experiments described earlier and on the strong correlation observed between the generation of large (> 30 nm) particles by pumping and the rate of fouling. We cannot rule out the possibility that adsorbed BSA somehow mediates or aids pore occlusion by the larger particles.
Figure 5.35  Effect of BSA adsorption on saline flux through 0.2 \( \mu \text{m} \) track-etch membranes

5.17 Experimental investigation of convective deposition hypothesis

We now decided to verify the alternative fouling hypothesis that convective deposition of particles generated by pumping was the cause of the observed flux decrease. According to this hypothesis, the rate of fouling is limited by the transport of particles which are carried to the membrane surface by the convective flow of permeate through the pores. Hence, we anticipate the rate of deposition to be proportional to the flux and to the particle concentration in the feed. It also implies that, in the absence of convective deposition, there should be no decrease in flux. We assume that the removal of deposited particles from the membrane surface is slow (or absent) as inferred from the absence of a flux increase when saline solution
is passed through a fouled membrane (Section 5.2). In the remainder of this section, we present the experimental evidence without discussion since the results are best explained using the flux equations developed in Chapter 6. A detailed discussion follows in Chapter 7.

5.17.1 Pressure Effect: One way of changing the convective deposition rate is by changing the permeate flux, for example by using different trans-membrane pressures. Prefiltered BSA solution (0.25 kg/m³, pH 7.3) was passed 10 times through a peristaltic pump at 200 ml/min. Then it was used for DEF through 0.2 µm track-etch membranes at transmembrane pressures of 6.7, 13.3 and 20 kPa. The flux profiles for the first 10 min, during which most of the rapid decrease occurred, are shown in Figure 5.36. As expected, the initial

![Diagram showing permeate flux vs time for different pressures](image)

**Figure 5.36** Faster flux decrease at higher pressures during DEF of pre-pumped BSA solutions
flux was proportional to pressure (0.025 cm/s at 6.7 kPa, 0.049 cm/s at 13.3 kPa and 0.076 cm/s at 20 kPa) and the profiles do indicate a faster flux decrease at higher trans-membrane pressures. It appears that the flux after 10 min was the same at all three pressures, but this is an artifact of the scale of the ordinate. The filtration was actually carried out for 60 min and the effect of pressure on flux after 10 min is discussed in Section 5.18.

5.17.2 **Deposition by diffusion alone**: As mentioned earlier, a second test of the convective deposition hypothesis is to check for a change in membrane permeability caused by particle deposition in the absence of permeate flow. In this case, we anticipate that deposition of particles would occur by diffusion or sedimentation. In the case of DEF, this is equivalent to the premise of the adsorption experiment done by soaking the membrane in pumped protein solution, since the particles can reach the membrane surface only by diffusion (Section 5.16). The saline flux (membrane permeability) did not decrease with longer exposure of the membrane to the pumped BSA solution, consistent with the convective deposition hypothesis.

The hydrodynamic conditions above the membrane surface are quite different during CFF and DEF. We felt that cross-flow might increase the diffusive deposition of particles on the membrane by increasing the mass transfer coefficient. Since it is not possible to have a zero trans-membrane pressure gradient while recirculating BSA solution through the cross-flow apparatus, we decided to prevent convective flow of permeate by clamping the permeate exit. After a predetermined period of recirculation, the cross-flow system was flushed with saline solution (to purge bulk BSA solution) and then the permeate exit unclamped. The flux of saline was used as a measure of the change in membrane permeability caused by diffusive deposition in the presence of cross-flow. The feed consisted of 0.25 kg/m³ BSA (pH 7.3) pre-pumped 10 times with a peristaltic pump to generate particles. It was recirculated through the cross-flow apparatus (with the permeate port shut) at 200 ml/min using a diaphragm pump. For convenience, the feed side pressure was maintained at 6.7 kPa throughout the experiment,
even when recirculating the BSA solution with the permeate port clamped. The switch from BSA to saline solution was accomplished smoothly without sudden changes in flow or pressure. During saline filtration, the retentate and permeate streams were not recycled. The saline flux decreased by less than 10% even after 4 h of recirculating the BSA solution (see Figure 5.37). This rate of decrease is one or two orders of magnitude slower than in the presence of convective permeate flow (at 6.7-20 kPa) and thus, supports the convective deposition hypothesis. In fact, some of the slow decrease in Figure 5.37 may be attributed to small amounts of BSA solution remaining in the system during the permeability measurements due to imperfect flushing with saline.

**Figure 5.37** Effect of diffusive deposition of particles during CFF on membrane permeability (saline flux).
5.18 Effect of trans-membrane pressure on flux and transmission

5.18.1 Dead end filtration: The effect of pressure on flux during the period of rapid flux decrease was described in Section 5.17.1. We had mentioned that the initial flux was proportional to pressure, the ratio at 6.7, 13.3 and 20 kPa being 1 : 1.94 : 2.99. We also observed a faster rate of fouling at higher pressures (that is, at higher absolute value of flux); this was interpreted as supporting the convective deposition hypothesis. We now present data on the effect of pressure on flux in the 'pseudo steady state region', that is after the flux decrease has slowed. These data are from the same experiments as before but for filtration beyond 10 min and are presented on an expanded ordinate scale (see Figure 5.38). The absolute value of flux was higher at higher pressure, but the ratio at 6.7, 13.3 and 20 kPa measured at 60 min was only 1 : 1.15 : 1.29. Thus, there was a sharp decrease in the sensitivity of the flux to pressure compared to the period of rapid flux decrease. Also shown in Figure 5.38 (lower half) are the normalized flux profiles, the normalization being with respect to the initial flux measured at the respective pressures. The normalized flux is lower at higher pressures.

The transmission of BSA through the 0.2 μm track-etch membranes was 100±1% throughout the filtration at all three pressures (see Figure 5.39). This result is no different from those obtained in all of our DEF experiments using BSA and 0.2 μm (or larger) track-etch membranes.

5.18.2 Cross-flow filtration using the hollow fiber system: The effect of trans-membrane pressure also was investigated using the Diaflo 0.1 μm, polysulfone hollow fibers. These experiments, representing the first stage of an evolving protocol, used 0.5 kg/m³ BSA (Sigma A-7030) in phosphate buffer (3.55 kg/m³ KH₂PO₄, 7.1 kg/m³ K₂HPO₄) without pre-filtration. The combination of maximum pressure and minimum cross-flow rate to be used in
**Figure 5.38  Pressure effect on flux during second phase of DEF**
Figure 5.39  BSA transmission during DEF through 0.2 \( \mu \)m track-etch membranes at different pressures

the experiments were selected to ensure a non-zero retentate flow when measuring the buffer flux. The maximum cross-flow rate was limited by the capacity of the pump. These criteria set the bounds on the inlet pressure from 27-69 kPa (4 to 10 psi) at each of the cross-flow rates investigated, namely, 750, 1100 and 1500 ml/min (0.24, 0.35 and 0.48 m/s).

The flux profiles at each cross-flow rate indicate that the flux decreased at a faster rate at higher trans-membrane pressures (see Figures 5.40a, b, c) as was the case during DEF through track-etch membranes. Similarly, although the absolute value of flux was greater at higher pressures throughout the filtration, the sensitivity to changes in pressure decreased considerably with
time. This is reflected in the flux profiles which seem to approach one another as the hollow fibers are progressively fouled. Similar to the results of DEF, the normalized flux during the second phase was lower at higher pressures, the normalization being with respect to the initial buffer fluxes at the respective pressures and flow rates. In all of the hollow-fiber experiments, the flux decreased to less than 30% of the buffer flux within 2 min, the time at which the first flux measurement was made. This is probably because of a combination of aggregates present in the BSA solution (which was not pre-filtered) even before pumping and the lower pore size of 0.1 μm compared to the 0.2 μm of the track-etch membranes.

The effects of trans-membrane pressure on the 'pseudo steady-state' flux, i.e., the flux measured after 90 min of filtration, are summarized in Figure 5.41. This captures the trend of higher absolute values of flux at higher pressures and contrasts it with the trend of lower normalized fluxes at higher pressures. The broad band in which the data points appear reflects the difficulty in obtaining reproducible results, probably a consequence of the variations in the membrane permeability caused by reusing the hollow fiber cartridge after cleaning.

The transmission of BSA through the 0.1μm hollow fibers started at about 95% but decreased steadily once the initial, rapid decrease in flux had stopped. Although this general trend was observed at all the pressures and cross-flow rates investigated, there were considerable variations in the extent of decrease in transmission and in the time at which it began to decrease. This is reflected in the large scatter in the 'pseudo steady-state' transmission data (measured at 90 min) which makes it impossible to detect any trend with respect to the effect of pressure or cross-flow rate (see Figure 5.42). This scatter is probably because of differences in membrane properties caused by repeated use of the same hollow fiber cartridge. Despite extensive cleaning with 0.1N NaOH, detergent or 0.1N HCl, it was difficult to reproduce the initial buffer fluxes. The scatter in the transmission data is greater than that in the flux data probably because the significant hold-up volume on the permeate side of the hollow-fiber
Figure 5.40a  Flux profiles at 750 ml/min through 0.1 μm hollow fibers
Figure 5.40b  Flux profiles at 1100 ml/min through 0.1 μm hollow fibers
Figure 5.40c  Flux profiles at 1500 ml/min through 0.1 μm hollow fibers
Figure 5.41 'Steady-state' fluxes during CFF through the 0.1 μm hollow-fiber cartridge at different pressures and cross-flow rates
cartridge made it impossible to detect instantaneous changes in the transmission. In fact, these were the main reasons for the switch from the hollow fiber cartridge to the flat sheet apparatus.

5.18.3 CFF through 0.2 μm track-etch membranes: These experiments with the flat membrane apparatus were done by recirculating the 0.25 kg/m³ BSA solution at 200 ml/min (0.2 m/s) using the diaphragm pump in order to minimize changes in the concentration of particles during filtration. The particles were generated by passing the BSA solution 20 times through a peristaltic pump prior to CFF. The flux profiles obtained at 6.7 and 13.3 kPa are...
shown in Figure 5.43; the two plots using different ordinate scales for the initial and later stages of filtration facilitate the comparison of fluxes. The initial fluxes at 13.3 and 6.7 kPa were 0.060 and 0.032 cm/s respectively which correspond to a ratio of 1.87 : 1. However, the flux decreased rapidly in the first 30 min to less than 10% of the initial values and its sensitivity to pressure also decreased. For example, after 90 min the flux at 13.3 kPa was 1.5\times10^{-3} \text{ cm/s} which is only 1.1 times the corresponding flux at 6.7 kPa. The profiles also reveal a faster rate of flux decrease at higher pressure, a result that was observed earlier during DEF and CFF with the hollow fiber system.

The transmission of BSA through the 0.2 \text{ \mu m} track-etch membranes was 100% for the first 30-35 min. When the flux switched from a rapid to a slow rate of decrease, the transmission began to fall. After 65 min, it appeared to reach a steady state value of 71% at 6.7 kPa and 64% at 13.3 kPa (see Figure 5.44). The lower transmission at higher pressures could be attributed to the greater amount of particles deposited on the membrane (because of the larger cumulative flow) and also to the greater compression of the particle layer formed on the membrane surface.

5.19 Effect of pressure cycling on 'steady state' flux and transmission

The following experiments were designed to investigate the response of flux and BSA transmission to changes in pressure during the 'steady-state' phase. A constant cross-flow rate of 200 ml/min was maintained with a diaphragm pump. The protein solution (0.25 kg/m^3 BSA, pH 7.3) was pre-pumped 20 times with a peristaltic pump before CFF. Two experiments were performed, one where the initial decrease in flux occurred at 6.7 kPa and the second where it occurred at 20 kPa. After the flux reached a 'pseudo steady-state', the pressure was cycled between 6.7 and 20 kPa, each pressure maintained long enough for the flux to reach a new 'steady state'. The response of flux and transmission were different in the two experiments (see Figure 5.45 and Figure 5.46). When the initial filtration was done at 6.7
Feed: 0.25 kg/m³ BSA, pH 7.3 pre-pumped 20 times with a peristaltic pump
Cross-flow rate: 200 ml/min (using diaphragm pump)

Figure 5.43 Pressure effect on flux during CFF with diaphragm pump
Figure 5.44  Pressure effect on BSA transmission during CFF with diaphragm pump

kPa, a hysteresis was observed in both flux and transmission, i.e., when the pressure was successively increased to 13.3 and 20 kPa and subsequently decreased, the flux and transmission did not return to the previous values. Such a hysteresis was not observed when the initial filtration was done at 20 kPa and the pressure successively lowered to 13.3 and 6.7 kPa and then brought back to 20 kPa. These observations can be explained as follows.

When the initial filtration is done at 20 kPa, more particles may be deposited on the membrane because of the higher cumulative permeate flow and the layer formed may be compressed to a greater extent compared to when the initial filtration is done at 6.7 kPa. If we assume that the
Figure 5.45 Response of 'steady state' flux to pressure changes
Figure 5.46  Response of 'steady state' transmission to pressure changes
deposition of the aggregates is irreversible (or that their removal from the membrane is very slow on the time scale of the experiment), then there would be little change in the resistance offered by the particle layer upon lowering the pressure. Hence, the permeate flux would decrease proportionately to the decrease in pressure. However, when the initial filtration is done at 6.7 kPa, which is the lowest pressure during the experiment, further deposition of particles and/or compression of the layer can occur upon subsequently increasing the pressure to 13.3 or 20 kPa. This additional deposition is again assumed to be irreversible so that the particle layer now offers a higher resistance to permeate flow even upon lowering the pressure back to its initial value. This results in a lower flux at 6.7 kPa than observed initially, i.e., before additional particles were deposited at the higher pressures. The argument made to explain the hysteresis in flux also applies to BSA transmission because an increase in resistance to the passage of solvent would also affect the passage of solute through the membrane.

In order to distinguish between deposition and compression of particles, the flux of buffered saline through the particle layer was measured at different pressures. It has been shown earlier that switching the feed to saline solution does not lead to an increase in flux and this was interpreted to mean that the deposited particles are not removed from the membrane surface. Any hysteresis observed upon cycling the pressure can be attributed to the compression or rearrangement of the pre-deposited layer and not to further deposition since there were no particles in the saline solution. The results of varying the pressure between 6.7 and 20 kPa during the initial phase of rapid flux decrease and during the 'steady state' phase are shown in Table 5.2. The decrease in the flux ratio at 'steady state' from 2.33 to 2.10 over three cycles of pressure is an indication of the compressibility/rearrangement of the particle layer. The constant flux ratio during the initial phase suggests that most of the permeate was not flowing through the particle layer but probably through the unfouled regions of the membrane. This supports the assumption made in modelling the flux that most of the flow during the initial phase occurs through 'open' pores (see Chapter 6). The larger flux ratio of 2.71 during the
| Table 5.2: Ratio of buffer fluxes through partially fouled membranes at 20 and 6.7 kPa |
|-----------------------------------------|-----------------|------------------|
|                                        | Initial Phase   | 'Steady state' Phase |
| First cycle                            | 2.71            | 2.33             |
| Second cycle                           | 2.71            | 2.14             |
| Third cycle                            | -               | 2.10             |

initial phase compared to 2.33-2.10 during the second phase corroborates the previously presented results (from DEF and CFF) on the decrease in the sensitivity of flux to pressure after the membrane is fouled. Of course, in the previous experiments the flux ratio during the second phase was much lower (about 1.3 : 1) because additional deposition was occurring continuously during the filtration. In the current experiment, there was no additional deposition because saline solution was used during the pressure variations.

### 5.20 Effect of cross-flow rate on flux and transmission

We wished to determine the response of flux and transmission to changes in cross-flow rate without the complicating factor of a continuously changing particle concentration during the pumping. Since very slow fouling was observed when using the diaphragm pump for CFF (see Section 5.8), we surmised that very few particles, if any, were generated with this pump and it was chosen to recirculate the feed in this experiment. The fccd was a 0.25 kg/m³ BSA solution that had been passed 20 times through the peristaltic pump in order to generate the foulant. The CFF was done at 13.3 kPa with 950 ml of feed at an initial cross-flow rate of 200 ml/min (0.2 m/s). After the rapid decrease in flux to a 'steady state', the cross-flow rate was cycled between 90 and 480 ml/min (0.1-0.5 m/s). There was no detectable change in flux over this 5-fold change in flow rate but the protein transmission varied from about 70% at 90 ml/min to about 52% at 480 ml/min (see Figure 5.47). There was no significant hysteresis in
Figure 5.47 'Steady state' flux and transmission versus cross-flow rate
the transmission when the flow was increased and then decreased.

The sensitivity of BSA transmission to changes in cross-flow rate and the lack of sensitivity of permeate flux to changes in flow rate is consistent with the various observations made earlier, namely, that concentration polarization of BSA does not govern the flux (although it affects transmission) and that deposition of the foulant (particles thought to be BSA aggregates) is a largely irreversible process as long as there is permeate flow through the membrane. Since the BSA aggregates constitute a very small fraction of total protein, their rejection by the membrane is not detectable as a change in protein concentration or transmission. A more detailed discussion of these phenomena follows the mathematical formulation of the problem.

5.21 Effect of pH on flux, transmission, and aggregation

So far, we have presented strong evidence to support the hypothesis that convective deposition of particles, thought to be BSA aggregates, on the membrane surface causes fouling. Since BSA, like most proteins, has several charged residues, it is reasonable to expect any aggregates of BSA to also be charged. Consequently, the packing density (or void volume) of aggregates deposited on the membrane surface would be influenced by electrostatic repulsion between aggregates. BSA has an isoelectric pH of about 4.7-5.1, the exact value depending on the number of fatty acid molecules associated with each protein molecule. This represents the pH at which the native protein has no net charge. Assuming that the aggregates reflect the charge of native BSA, the electrostatic repulsion between the aggregates would be minimum at about pH 4.8; correspondingly, the packing density would be at its maximum (if other factors such as pressure and ionic strength are constant) and the resistance offered by a packed layer of aggregates would be at its maximum. The feed in the experiments described so far had a pH of 7.2±0.1 where BSA has a net negative charge of about -20 esu (in 0.15 M NaCl). We now investigated the effect on flux and transmission of altering the pH so as to change the resistance offered by the aggregates fouling the membrane.
Two batches of 0.25 kg/m³ BSA solution, one at pH 4.8 and the other at pH 7.0 were passed 20 times through a peristaltic pump. These batches were used in identical cross-flow experiments using a diaphragm pump for recirculation of feed. The CFF was done through 0.2 μm track-etch membranes at cross-flow rates of 200 ml/min and trans-membrane pressures of 6.7 kPa. The initial fluxes were identical in both experiments, about 0.033 cm/s, hence the normalized flux is an appropriate description of the flux profiles. There were no significant differences in the flux profiles during the initial phase, but after about 35 min when the rate of decrease slowed abruptly, a higher flux was observed at pH 7.0 than at pH 4.8 (see Figure 5.48). Likewise, during the first 35 min, pH had no effect on BSA transmission which was constant at 100%. After 35 min, the transmission decreased steadily to about 62.5% at pH 4.8 and about 70.2% at pH 7.0 in 120 min.

The similarity in flux and transmission profiles at pH 4.8 and pH 7.0 for the first 35 min suggests that most of the permeate flow during the initial period does not occur through the deposited BSA aggregates but through unfouled regions of the membrane. The resistance of the latter is not expected to be sensitive to pH. This compares favorably to the results of the pressure cycling experiments using saline solution (see Section 5.19). During the 'steady state' phase, the lower flux and transmission at pH 4.8 compared to pH 7.0 is an indication of the higher packing density of aggregates resulting in a higher specific resistance to the flow of solvent and solute.

The effect of pH on the formation of particles by pumping was investigated using quasi-elastic light scattering (QELS). Two 400 ml batches of 0.25 kg/m³ BSA solution, one at pH 7.0 and the second at pH 4.8 were recycled at 200 ml/min with a peristaltic pump operating against a pressure of 6.7 kPa maintained with a needle valve. Samples of solution were withdrawn periodically and analyzed in the Coulter Model N4 Particle Size Analyzer. As mentioned in Section 5.12.4, the results are presented as the cumulative weight percent of particles larger
Figure 5.48  Flux and transmission profiles during CFF of BSA solution at pH 7.0 and pH 4.8
than 30 nm. The results in Figure 5.49 indicate that the rate of generation of particles was about 1.8 times greater at pH 4.8 compared to pH 7.0, and that the particles constituted less than 1% of total BSA even after 330 passes through the peristaltic pump. Similar to the effect of pH on flux and transmission, these light scattering results can also be explained in terms of the possible greater aggregation of BSA at its isoelectric pH because of lower electrostatic repulsion between the molecules. There may also be a greater interfacial denaturation of BSA at its isoelectric pH.

The QELS result indicates that pumping the BSA solution for a given number of passes should generate a higher concentration of particles at pH 4.8 than at pH 7.0. Hence, filtration of the

![Graph showing the effect of pH on the rate of generation of particles](image)

**Figure 5.49** Effect of pH on rate of generation of particles during recirculation of BSA solution with a peristaltic pump
two solutions under the same conditions of pressure and cross-flow rate should result in a greater rate of deposition of particles at pH 4.8 and consequently, in a greater rate of flux decrease. However, the flux profiles during the initial period of rapid fouling seem to be almost identical for both solutions (see Figure 5.48). A possible explanation is that when the solutions are pumped only 20 times, the difference in particle concentration at the two pH values may be insignificant as evident from the large scatter in the QELS data.

5.22 Effect of aggregate concentration on flux

According to the convective deposition hypothesis introduced earlier, the rate of decrease in flux is governed by the rate of deposition of foulant on the membrane surface. The latter depends on the permeate flux through the membrane and on the concentration of foulant in the feed solution. The dependence on permeate flux was demonstrated in Section 5.17 by using trans-membrane pressure to alter the initial flux. The concentration effect discussed in this section, serves to verify the convective deposition hypothesis especially in view of the identical flux profiles obtained at pH 4.8 and pH 7.0 despite the different rates of particle generation measured by QELS. However, the QELS data have a considerable amount of scatter and cannot be used to accurately measure particle concentration especially when a broad size distribution of particles exist in solution. This problem can be circumvented by precise dilutions of a stock solution containing particles followed by a comparison of the flux profiles obtained via filtration of the diluted solutions. Since the concentration of aggregates is very low even in the undiluted stock solution, it is assumed that further dilution of the stock solution with saline does not lead to the formation or break-up of existing aggregates.

The stock solution was prepared by passing the 0.25 kg/m³ BSA solution 10 times through a peristaltic pump. A part of the pumped solution was diluted 2-fold with saline solution and another part was diluted 4-fold. These three solutions were used in DEF through 0.2 μm track-etch membranes at 6.7 kPa. A comparison of the normalized flux profiles reveals a lower
rate of flux decrease with increasing dilutions of solution (see Figure 5.50). Since the foulant concentration is expected to be proportionately lower, these results are a justification of the convective deposition hypothesis. BSA transmission was 100% throughout the filtration as was the case in all of the DEF experiments described in earlier sections.

5.23 Filtration experiments with enzymes and proteins other than BSA

5.23.1 Lysozyme: An early indication that the rapid decrease in flux during CFF was not unique to BSA was obtained from hollow fiber experiments with lysozyme solutions. As mentioned in Section 5.18.2, the protein solutions were not pre-filtered to remove any particulates; at the time we were not aware of the effect of pumping on fouling or the presence
of protein aggregates. 2000 ml of 0.5 kg/m$^3$ lysozyme solution were recirculated at 750 ml/min (0.24 m/s) through the hollow fiber cartridge previously used in filtration experiments with BSA. The flux profiles show a rapid decrease from the initial buffer fluxes of 510, 700 and 930 l/h•m$^2$ (0.014, 0.020 and 0.026 cm/s) at 4, 6, and 8 psi (28, 41, and 55 kPa) respectively to about 107, 150, and 180 l/h-m$^2$ (3•10$^{-3}$, 4.2•10$^{-3}$ and 5•10$^{-3}$ cm/s) in the 'steady state' region (see Figure 5.51). These 'steady state' fluxes are much higher than observed with BSA (75 - 90 l/h-m$^2$) and show a greater sensitivity to trans-membrane pressure. The transmission of lysozyme exceeded 97% through most of the experiment. The slightly lower transmission observed during the first few minutes is probably because of dilution of the permeate by the buffer solution that is initially present in the membrane and in the permeate channels and also due to losses by adsorption to the polysulfone membrane.

Much later in our research, when we became aware of the effect of the peristaltic pump on fouling with BSA solutions, we decided to verify whether a similar effect was responsible for the flux decrease with lysozyme. Two identical batches of 0.105 kg/m$^3$ lysozyme solution (pH 7.0) were prepared using the standard protocol described in Section 4.4.1 (i.e., with prefiltration to remove particulates from the original solution). One of the batches was passed 20 times through a peristaltic pump. The two batches were then used for DEF through 0.2 µm track-etch membranes at a pressure of 6.7 kPa. With pre-pumped solution, the normalized flux decreased rapidly to less than 10% in about 90 min in contrast to the very slow decrease to 85% in 120 min observed with solution that was not subjected to pumping (see Figure 5.52). The transmission of lysozyme was 100% throughout the filtration in both cases. The results are qualitatively similar to those obtained from earlier experiments with BSA.
Figure 5.51 CFF of lysozyme solution through 0.1μm hollow fibers
5.23.2 Ribonuclease: Two identical batches of 0.2 kg/m³ ribonuclease (RNAse) solution, pH 7, were prepared using the standard protocol described in Section 4.4.1 (i.e., with prefiltration to remove particulates from the original solution). One of the batches was passed 20 times through a peristaltic pump. The two batches were then used for DEF through 0.2 μm track-etch membranes at a pressure of 6.7 kPa. With pre-pumped solution, the normalized flux decreased rapidly to less than 10% in about 70 min in contrast to the very slow decrease to 75% in 120 min observed with solution that was not subjected to pumping (see Figure 5.53). The transmission of RNAse was 100% throughout the filtration in both cases.

One of the advantages of using an enzyme such as ribonuclease is that its activity can be
monitored as a function of the duration of pumping in order to detect any denaturation. 94 ml of 1 kg/m\(^3\) RNAse solution (pH 7) were passed continuously through a peristaltic pump at 50 ml/min and samples of solution withdrawn periodically. The activity of RNAse in these samples was measured using the assay described in Section 4.7.2. As mentioned earlier, the initial rate of decrease in absorbance at 286 nm upon the conversion of cytidine 2′:3′-phosphate to cytidine 3′-phosphate was used as a measure of activity. There was no significant change in activity over almost 100 passes through the pump (see Figure 5.54).

In a separate experiment, 400 ml of RNAse solution (0.2 kg/m\(^3\), pH 7) were recirculated at 200 ml/min with the peristaltic pump. Solution samples, taken periodically, were analyzed for
the presence of particles using quasi-elastic light scattering (QELS). Ribonuclease (3.8 nm x 2.8 nm x 2.2 nm) is close to the lower detection limit of 3 nm for the Coulter Model N4 Particle Size Analyzer; this may account for the considerable scatter in the data even for solution that has not been pumped. For the reasons mentioned in Section 5.12.4 the results are presented as the cumulative weight percent of particles larger than 30 nm. There appears to be a trend of increasing particle concentration with increased duration of pumping as indicated by the best linear fit to the data (see Figure 5.55). However, we do not have much confidence in the absolute numbers because of the large scatter. Assuming that these particles are aggregates of RNase, their very low concentration (< 0.1% of total RNase after 300 passes through the pump) may be the reason for not detecting any significant change in enzyme activity.
Figure 5.55  Quasi-elastic light scattering analysis of ribonuclease solution pumped with a peristaltic pump

5.23.3 Catalase: Attempts at comparing the dead-end flux profiles using 0.5 kg/m³ catalase solutions, with and without pre-pumping, were not successful because the 0.2 µm track-etch membranes were fouled almost immediately even when the solution was not pumped. This made it impossible to pre-filter the solutions, a necessary step in ensuring that the flux decrease is due to particles generated by pumping and not due to those present in the original solution. For example, at 6.7 kPa, the dead-end flux decreased to less than 5% of the buffer flux in about 1.5 min. The results were similar even when track-etch membranes of a much larger pore diameter, namely 0.4 to 1.0 µm, were used. The flux at 6.7 kPa after 60 min was about \(2.2 \times 10^{-4} \text{ cm/s (±12%)}\) irrespective of the pore size. The transmission of catalase at 60 min,
calculated from concentrations determined by the UV absorbance at 280 nm, was about 70% through the 0.2 μm membrane and about 83% through the 1.0 μm membrane. This contrasts with the 100% transmission through 0.2 μm membranes observed during the DEF of BSA, RNase and lysozyme even after the membranes were fouled. Electrophoretic analysis of the fresh catalase solution on a native polyacrylamide gel (Pharmacia Phastgel 8-25) with Coomassie staining for visualization revealed only the band corresponding to the monomer. It is speculated that the observed fouling may be caused either by adsorption of catalase to the membrane or by some other non-protein component present in the original protein. Catalase is much larger than the other proteins investigated in this research project (MW ≈ 247,000 Daltons; 10.5 nm x 10.5 nm x 5 nm).

**Figure 5.56** Effect on catalase activity of pumping with a peristaltic pump
The effect on catalase activity of continuous recirculation of the solution using a peristaltic pump was, however, investigated. 100 ml of 0.5 kg/m$^3$ catalase solution (pH 7) were pumped continuously at 200 ml/min against a pressure of 6.7 kPa maintained by a needle valve. Solution samples were withdrawn periodically, diluted 50-fold with buffer solution, and assayed using aqueous hydrogen peroxide as the substrate. Details of the assay and buffer composition are given in Section 4.7.2. There appears to be no significant change in catalase activity over almost 200 passes through the peristaltic pump (see Figure 5.56), a result similar to that observed with ribonuclease.

### 5.24 Filtration of BSA solutions containing thermally generated aggregates

We wished to demonstrate the generality of the observation of flux decrease when solutions containing protein aggregates are filtered and show that the method by which the aggregates are generated is relatively unimportant. In this experiment, BSA aggregates were generated thermally and the protein solution was not exposed to the peristaltic pump at any time to eliminate the possibility that trace contaminants introduced during pumping were responsible for fouling. 800 ml of pre-filtered BSA solution (0.25 kg/m$^3$ BSA, pH 7.3) was heated in a 2 liter glass beaker placed in a constant temperature water bath maintained at 65 °C. The contents were well mixed using a Teflon coated magnetic stirrer. The temperature of the protein solution rose to 62.5 °C in 10 min. The solution was kept at 64.5 °C for an additional 90 min and then rapidly cooled to room temperature (22 °C) in an ice-water bath. Samples of solution taken during the 90 min of heating showed a steady increase in the UV absorbance (at 280 nm) from 0.151 to 0.159 AU, an increase similar to that noted earlier (in Figure 5.23) during the recirculation of BSA solutions with a peristaltic pump.

This pre-heated solution was used for DEF through 0.2 μm track-etch membranes at 6.7 kPa. The flux profiles obtained are compared to those obtained from the filtration of a solution that was not heated. With pre-heated solution, the flux decreased to about 8% of its initial value in
Figure 5.57  DEF of pre-heated BSA solution through 0.2 μm track-etch membranes

120 min whereas in the control experiment the flux decreased slowly to only about 70% of the initial value in 120 min (see Figure 5.57). BSA transmission was about 100% for the entire 120 min in both cases. Thus, the flux and transmission profiles are qualitatively similar to those obtained earlier using pre-pumped solution and a control with solution that was not pumped with the peristaltic pump.

Samples of the material deposited on the membrane surface after filtration of pre-heated BSA solution were analyzed by native-PAGE (Pharmacia Phastgel 10-15) with Coomassie staining. The protocol is identical to the one used earlier (see Section 5.13) except that the membrane
NATIVE-PAGE OF BSA AGGREGATES GENERATED BY HEATING

(with Coomassie staining)

THYROGLOBULIN (669000)
FERRITIN (440000)
CATALASE (232000)
LDH (140000)

ALBUMIN (67000)

LARGE AGGREGATES

BSA DIMER
MONOMER

Lane 1 : BSA solution not heated
Lane 2 : BSA solution pre-heated to 65 °C for 90 min
Lane 3 : Material from membrane surface (without rinsing)
Lane 4 : High molecular weight standard

Figure 5.58
was not rinsed with buffered saline after filtration. Lanes 1 to 4 in Figure 5.58 correspond to samples of fresh BSA solution (not heated), BSA solution pre-heated to 65 °C, material taken from the fouled membrane surface, and the high molecular weight marker respectively. Both the pre-heated solution and the material taken from the fouled membrane contain a component corresponding to a molecular weight of about 700,000 Daltons. In addition, the sample taken from the membrane surface contains a component too large to enter the separation zone of the gel, similar to what was observed when the membrane was fouled using a pre-pumped solution (see Figure 5.27). We believe that the large component, shown previously to be an aggregate of BSA, deposits on the membrane and causes the decrease in flux.

5.25 Use of different membranes

Most of the experimental results presented so far were obtained with 0.2 μm polycarbonate, track-etch membranes. A few experiments were done with a Diaflo 0.1 μm polysulfone, hollow fiber cartridge, but these involved repeated use of the same cartridge that led to differences in the initial flux depending on the efficacy of cleaning. Secondly, the experiments with the hollow fiber system did not use pre-filtered protein solution since the generation of particles by pumping was not known at the time. We now decided to verify the effect of pumping on the fouling of membranes of different pore sizes, pore geometry and materials of construction using the fully evolved experimental protocol described Section 4.4.

The first set of experiments involved the use of polycarbonate, track-etch membranes of different pore sizes (0.2 to 0.8 μm). The protein solution (0.25 kg/m³ BSA, pH 7.3) was passed 20 times through a peristaltic pump and then filtered in a dead-end configuration at 6.7 kPa. The initial fluxes were 0.027, 0.050, 0.087, and 0.118 cm/s for the 0.2, 0.4, 0.6, and 0.8 μm membranes respectively. The flux decreased in each of the experiments reaching about 1.1×10⁻³, 3.4×10⁻³, and 7.3×10⁻³ cm/s in 120 min with the 0.2, 0.4, and 0.6 μm membranes respectively (see Figure 5.59). The filtration through the 0.8 μm membrane had to be stopped
Feed: 0.25 kg/m$^3$ BSA, pH 7.3
pre-pumped 20 times with
a peristaltic pump
Pressure: 6.7 kPa

Figure 5.59 DEF of pre-pumped BSA solutions through 0.2 - 0.8 μm track-etch membranes
after 65 min because all the feed solution was used. The flux at this time was 0.042 cm/s. The observation that the flux decreased even with the larger pore sized membranes suggests that either some of the particles blocking the pores are larger than 0.8 μm or that smaller particles can block the pores by bridging across the entrance via particle-membrane and particle-particle interactions. The transmission of BSA was 100% for the entire duration of filtration with all the membranes.

The role of pumping was also verified using the 0.22 μm, polyvinylidene difluoride, Durapore membranes and the 0.45 μm, nylon-66, Ultipor membranes which are available only in the flat sheet configuration. CFF experiments were performed using fresh, pre-filtered 0.25 kg/m³ BSA solutions recirculated at 200 ml/min with either a peristaltic or a diaphragm pump. We have already shown that rapid fouling of the 0.2 μm track-etch membranes occurs only with the peristaltic pump (see Figure 5.17). A comparison of the normalized flux profiles measured at 6.7 kPa is shown in Figure 5.60 (Durapore membrane) and Figure 5.61 (Ultipor membrane). The flux is normalized with respect to the initial flux through the respective membranes, which was 0.018 cm/s through the Durapore membrane and 0.044 cm/s through the Ultipor membrane. The normalized flux through the Ultipor membrane decreased to 4% in 300 min when using the peristaltic pump in contrast to only 86% in 300 min with the diaphragm pump. The normalized flux through the Durapore membrane decreased to 20% in 360 min when using the peristaltic pump in contrast to only 93% in 360 min with the diaphragm pump. The characteristic time for flux decrease is about an order of magnitude greater than observed with the 0.2 μm track-etch membranes. A possible explanation may be the much greater amounts of foulant required to block the pores of the Durapore and Ultipor membranes because of the greater porosity (> 80% compared to 9.5% for the track-etch membranes). Secondly, these membranes do not have narrow, cylindrical straight-through pores like the track-etch membranes but instead have a tortuous network of interconnected pores that may facilitate intra-pore fouling as opposed to blocking at the pore entrance.
Figure 5.60  CFF of BSA through 0.22 μm Durapore membranes: comparison of peristaltic and diaphragm pumps

The transmission of BSA through both membranes was 100% for the entire duration of filtration when using the diaphragm pump. A similar result was obtained with the Durapore membrane when using the peristaltic pump. In these three filtrations, by the time the experiment was stopped, the flux had not yet switched from the period of rapid decrease to the 'steady state' phase. Previous CFF experiments with track-etch membranes had shown that the transmission starts to decrease only when the normalized flux has fallen below about 10% and this was not the case in the three experiments in question. However, when using the peristaltic pump for CFF through the Ultipor membrane the normalized flux falls below 10% in about 250 min (see Figure 5.62). Correspondingly, BSA transmission which was 100%
Figure 5.61 CFF of BSA through 0.45 μm Ultipor membranes: comparison of peristaltic and diaphragm pumps

initially, starts to decrease after 250 min to about 84% in 300 min. These observations demonstrate that although we chose the 0.2 μm track-etch membranes as our model system, the results are qualitatively similar with other membranes as well. The faster flux decrease observed with the Ultipor membranes compared to the Durapore membranes cannot be explained on the basis of pore size because the former, in fact, have the larger pores. The initial flux through the Ultipor membranes is almost 2.5 times that through the Durapore membrane; hence, the convective transport of foulant is correspondingly greater. However, in order to determine whether this difference in initial fluxes is sufficient to account for the differences in fouling rates, one needs a quantitative flux model which is developed in the next chapter.
Feed: 0.25 kg/m³ BSA, pH 7.3
Crossflow Rate: 200 ml/min
Pressure: 6.7 kPa
Recirculation: Peristaltic pump

Figure 5.62 BSA transmission through 0.22 µm Durapore and 0.45 µm Ultipor membranes during CFF with a peristaltic pump
Chapter 6 Mathematical Modelling

6.1 Initial membrane resistance

The hydrodynamic resistance of a new membrane is given by:

\[ R_o = \frac{\Delta P}{\mu J_o} \]  

...(6.1)

where, \( \Delta P \) = transmembrane pressure (Pa)  
\( \mu \) = solvent viscosity (kg/m.s)  
\( R_o \) = hydrodynamic resistance of new membrane (m\(^{-1}\))  
\( J_o \) = flux of pure solvent through new membrane (m/s)

The hydrodynamic resistance, \( R_o \), can be calculated by modelling the membrane as a system of parallel resistances. Such a description is best suited for the polycarbonate track-etch membranes which consist of distinct, cylindrical, straight-through pores, whose resistance can be described by the Hagen-Poiseuille equation for laminar flow through tubes.

\[ R_o = \frac{128 \ L_p}{\pi \ d_p^4 \ N_T} + \frac{24}{d_p^3 \ N_T} \]  

...(6.2)

where, \( L_p \) = pore length (m)  
\( d_p \) = pore diameter (m)  
\( N_T \) = total number of pores per unit membrane area (m\(^{-2}\))

The second term on the right represents the pore entrance and exit effects for \( L_p \approx d_p \). Of course, \( d_p \) and \( N_T \) cannot both be arbitrarily large because the total surface porosity must be less than unity (\( \pi \ N_T \ d_p^2 / 4 < 1 \)). If the membrane has a pore size distribution such that a fraction \( f_i \) of the pores have diameter \( d_i \), then the hydrodynamic resistance is given by the following expression.
\[ R_0 = \frac{128 L_p}{\pi N_T \sum_i f_i d_i^4} + \frac{24}{N_T \sum_i f_i d_i^3} \] ...\(6.3\)

The Diaflo hollow fibers and the Durapore and Ultipor flat membranes have a structure that may be described as a network of tortuous, interconnected cells making it difficult to define a pore of a specific radius and length. The nominal pore radius specified by the manufacturers of these membranes is an experimental value related to the size of particles rejected by the membrane under some standard operating conditions. For these membranes, the hydrodynamic resistance, \(R_0\), is best determined experimentally by measuring the flux of saline solution at specified pressures.

Changes in resistance due to structural failure (for example, at high pressures) or membrane swelling upon water absorption are not considered. The pressures used in our experiments (< 70 kPa) are well below the specified tolerance level for each membrane. Swelling by water absorption is more commonly associated with the very hydrophilic reverse osmosis and ultrafiltration membranes and not with the microporous membranes considered in this thesis.

6.2 Proposed mechanism of flux decrease

The flux decrease is attributed to the accumulation of protein aggregates (particles > 30 nm) on the membrane; the rate of decrease is assumed to be limited by the convective transport of these aggregates to the membrane surface. The experimental evidence supporting this hypothesis was presented in Chapter 5. However, we do not know the specific particle size fraction responsible for fouling mainly because of the uncertainty in the mechanism by which the particles transported to the membrane are retained. Size exclusion, though important, cannot be the only factor involved in particle rejection. This is supported by the results in Figure 5.31, which indicate that there was a flux decrease even when the permeate from a prior filtration through a 0.2 \(\mu\)m track-etch membrane was used in a subsequent dead-end filtration
through an identical membrane. According to the size exclusion mechanism, this permeate should not contain any particles larger than the pore size of 0.2 μm; consequently, there should be no particle rejection during the second filtration and hence, no flux decrease. Since the experimental evidence does not support such a conclusion, alternative rejection mechanisms such as pore occlusion by aggregate-aggregate and/or aggregate-membrane interactions also could be playing a role. In fact, the mechanism could be a function of membrane material and pore geometry. The large internal surface area and the tortuous pore structure of the Durapore and Ultipor membranes may facilitate particle accumulation within the pores in contrast to a pore entrance blockage suspected for the track-etch membranes.

The proposed flux model makes no overt assumptions about the mechanism of particle retention by the membranes. The experimental results in Section 5.2 revealed no flux increase when the feed to the CFF unit was switched from BSA to saline solution during the initial period of rapid flux decrease. This implies that over the time scale of our experiments, the initial particle deposition was largely irreversible. Hence, we treat these particles as an integral part of the membrane and model their effect on flux as an increase in membrane resistance. In order to calculate the membrane resistance as a function of particle deposition we need to know the precise changes in pore geometry caused by particle retention. For example, a common assumption in the ultrafiltration literature is that uniform solute adsorption over the entire pore length causes a decrease in pore diameter (e.g., Nonaka, 1988). This is not a good description of fouling of membranes with a tortuous network of pores. Ethier (1986) attempts to model protein accumulation at the pore entrance by assuming the formation of either a polarized layer of uniform thickness or hemi-spherical caps over each pore. Our approach sacrifices much of this detail which is difficult to verify experimentally to give a model that describes our results adequately with few adjustable parameters. Clearly, the model can be improved by incorporating many of these omissions.
Figure 6.1 Assumption about the change in the resistance of an individual pore with time due to particle deposition.

We assume that the time scale for the occlusion of any individual pore is small compared to the time scale of flux decrease. This means that at any given time we may consider the pore to be in one of two states, open or blocked. At time $t_i$ after the start of filtration, the resistance of the $i^{th}$ pore is assumed to change abruptly from the open pore resistance, $r_o$, to that of the blocked pore, $r_b$ (see Figure 6.1). Any further deposition of particles due to passage of solution through the blocked pore leads to a porous 'cake' on the membrane surface above the blocked pore. This causes a continuous change in resistance, the exact profile depending on assumptions made about the cake structure and on the net rate of particle deposition.

6.3 Dead-end flux with constant aggregate concentration in the feed

The proposed mechanism does not assume that all pores become blocked simultaneously but rather that there exists a distribution of times, $t_i$, at which a fraction of the
pores become blocked. Let \( \phi(t) \) be the fraction of total pores 'open' at time 't'. According to our convective deposition hypothesis, the rate of decrease in the number of open pores is proportional to the rate of particle deposition within or at the entrance to the open pores, which in turn depends on the flow rate through the open pores, the concentration of particles in the feed, and the transmission coefficient of particles relative to the open pores.

\[
\frac{d\phi}{dt} \propto J_0 \phi(t) C_{af} (1 - T_{oa}) \quad \ldots(6.4)
\]

where,
- \( C_{af} \) = concentration of particles in the feed (kg/m\(^3\))
- \( T_{oa} \) = Transmission coefficient of particles relative to open pores
- \( J_0 \phi \) = Flux through the open pores (m/s)

The transmission coefficient is a function not only of the particle and pore dimensions, but also of any interactions between the particles and the pore wall. Even in the absence of such interactions, the polydispersity in particle size and pore diameter makes it difficult to predict the true transmission coefficient. We therefore, treat \( T_{oa} \) as an average transmission coefficient for the particles included in the concentration term, \( C_{af} \). If the total quantity (kg) of particles required to block a pore is denoted by \( W_p \), then the proportionality in Equation...\( (6.4) \) may be replaced by the following equality.

\[
\frac{d\phi}{dt} = - \frac{J_0 C_{af} (1 - T_{oa})}{W_p N_T} \phi(t) \quad \ldots(6.5)
\]

\[
= - k_B \phi(t)
\]

where, \( k_B \) = rate constant of pore occlusion (s\(^{-1}\))

When the particle concentration, \( C_{af} \), does not change with time, the rate constant of pore occlusion, \( k_B \), is a constant and Equation...\( (6.5) \) may be easily integrated using the initial
condition, $\phi = 1$ at $t = 0$.

$$\phi(t) = e^{-k_B t} \quad \ldots(6.6)$$

The assumption of constant particle concentration in the feed is reasonable for most of our dead-end filtration experiments where because of the observation of irreversible deposition, the rejected particles remain on the membrane rather than enter the bulk solution. The total flux through the membrane may be represented by the sum of the fluxes through the open and occluded pores. This assumes that the pores are independent flow channels and that occlusion of one pore does not affect the resistance of adjacent pores. This description is especially valid for the track-etch membranes with isolated, straight-through pores. These membranes also have a very low porosity (~10%) which reduces the probability that particles deposited on the surface block more than one pore.

$$J(t) = J_B(t) + J_\phi \phi(t) \quad \ldots(6.7)$$

Total flux, Flux through blocked pores, Flux through open pores

The flux at time $t'$ through a pore that was instantaneously blocked at time $t_i'$, where $t_i < t$, depends on the blocked pore resistance, $r_B$, and on the extent of 'cake' formation above the blocked pore during the time $t - t_i'$. Here, we have implicitly assumed that cake formation follows pore occlusion but so far, we make no assumption about the structure of the cake. The combined 'cake' and pore resistance is:

$$R_i(t) = R_B + \int_{t_i}^{t} \rho_{aw} \, d\tau \quad \ldots(6.8)$$
where, \( R_i(t) = \) combined resistance of 'cake' and pores blocked at time \( t_i \) (m\(^{-1}\))
\( R_B = \) resistance of blocked pores (m\(^{-1}\)) (see Figure 6.1)
\( \rho_a = \) specific cake resistance (m/kg)
\( w'(\tau) = \) rate of accumulation of aggregates in the cake (kg/m\(^2\).s)

The fraction of the total flux at time 't', occurring through the pores blocked at time 't_i' is given by the following expression.

\[
dJ_{Bi} = \frac{\Delta P}{\mu R_i(t)} d\phi_i
\]

...(6.9)

The total flux through all the blocked pores is obtained by integrating Equation...(6.9) over all pores that are blocked at times \( t_i \leq t \). The variable of integration may be changed from the fraction of blocked pores \( (\phi_i) \) to time \( (t_i) \) by making use of Equation...(6.6).

\[
d\phi_i = - \, d\phi = k_B \, e^{-k_B t_i} \, dt_i
\]

...(6.10)

Substituting the results from Equation...(6.8) and Equation...(6.10) into Equation...(6.9) and integrating over the time period \( 0 \leq t_i \leq t \), we get,

\[
J_B(t) = \frac{\Delta P}{\mu} \int_{t_i=0}^{t_i=t} \left( \frac{k_B e^{-k_B t_i}}{R_B + \int_{t_i}^{t} \rho_a w' \, d\tau} \right) dt_i
\]

...(6.11)

The complete expression for the flux through the membrane is obtained by substituting Equation...(6.11) and Equation...(6.6) into Equation...(6.7).
\[
J(t) = \frac{\Delta P}{\mu} \int_{t_i=0}^{t_i=t} \frac{k_B e^{-\left(\frac{k_B t_i}{\mu}\right)}}{R_B + \int_{t_i}^{t} \rho_a w' \, d\tau} \, dt_i + \frac{\Delta P}{\mu R_o} e^{-\left(\frac{k_B t}{\mu}\right)} \quad \ldots(6.12)
\]

Equation...(6.11) and Equation...(6.12) are not explicit in flux because the rate of particle deposition, \(w'(\tau)\), is itself a function of flux. As flux decreases with time, so does the rate of deposition. This necessitates an iterative solution of Equation...(6.12) even when all the other parameters are known \textit{apriori}. However, two important parameters, namely, the blocked pore resistance, \(R_B\), and the specific cake resistance, \(\rho_a\), are not known \textit{apriori} and need to be estimated by fitting the equation to experimental data. In addition, estimation of \(\rho_a\) requires some assumption about the cake structure and geometry. The form of Equation...(6.12) does not lend itself to easy curve fitting; consequently, we abandoned the attempt to use a single equation to describe the entire flux-time profile. We proceeded to divide the flux profile into two phases, each of which could be described by equations derived from Equation...(6.12) with appropriate approximations.

6.4 Rationale for dividing the filtration profile into two phases

The inconvenience of using Equation...(6.12) to describe the entire flux profile provided an incentive to find alternative ways of modelling the flux. The important hints regarding the kinds of approximations to make were provided by the experimental data. Shown schematically in Figure 6.2, are typical flux and transmission profiles obtained from the filtration of solutions containing a constant concentration of foulant. In our experiments, this represents the case of using pre-pumped or pre-heated protein solutions in dead-end filtration
or cross-flow filtration with a diaphragm pump. The pre-pumping with a peristaltic pump or pre-heating generates particles (protein aggregates) whose concentration does not change appreciably during subsequent filtration. The experimental data suggested a division of the profiles into two phases. The first phase was characterized by a rapid, 10-fold decrease in flux while the transmission was constant at about 100%. After a certain time $t^*$, the rate of flux decrease slowed considerably and in the cross-flow experiments, the protein transmission started to decrease. The value of $t^*$ and the extent of decrease in flux and transmission depend on several operating conditions discussed later. The initial flux was proportional to pressure but its sensitivity decreased with time. In the dead-end filtration described in Section 5.18.1, the ratio of the initial fluxes at 6.7, 13.3 and 20 kPa was $1 : 1.94 : 2.99$ but after 60 min, the ratio decreased to $1 : 1.15 : 1.29$. (In these experiments, $t^*$ is about 10 - 15 min). During the

Figure 6.2 Schematic depicting the division of the flux and transmission profiles into two phases
first phase, neither flux nor transmission were sensitive to changes in pH but during the second phase, both were lower at the isoelectric pH, which in the case of BSA is about 4.8 (see Figure 5.48). As mentioned, protein transmission was constant at 100% during the first phase and was not sensitive to changes in cross-flow velocity. However, after the transition at $t^*$, we observed a lower transmission at higher cross-flow velocities (see Figures 5.11 and 5.47). In fact, the highest transmission of 100% was during dead-end filtration which may be considered to be the limit as cross-flow velocity tends to zero.

These experimental results indicate that there are real differences in the physics of the filtration phenomena during the two phases and that dividing the filtration profiles is not just a mathematical convenience. We hypothesize that during the first phase, most of the permeate flow occurs through open pores which have not yet been occluded and that the flux decrease occurs because of a decrease in the number of open pores in accordance with Equation ...(6.6). Assuming that the structural integrity of the membrane is not compromised, the open pore resistance is not expected to be a function of trans-membrane pressure, pH, ionic strength and other solution properties. This would explain the proportionality of the initial flux to applied pressure and the lack of sensitivity of both flux and transmission to pH. The constant 100% transmission observed merely reflects the true transmission of the proteins used in this study through the open pores. Note that the proteins were at least an order of magnitude smaller than the open pore diameter. Consequently, we expect very little concentration polarization of non-aggregated protein during the first phase and this would explain the lack of sensitivity of transmission to changes in cross-flow velocity.

We hypothesize that the transition time, $t^*$, corresponds to a breakdown of the assumption that most of flow occurs through open pores. For the case where the blocked pore resistance, $R_B$, is much greater than the open pore resistance, $R_o$, this breakdown would occur when most of the open pores have been blocked. Further deposition of particles results in a porous 'cake'
above the blocked pores. The flux is now governed by the combined resistances of the blocked pores and the porous cake and any operating condition that affects the specific resistance of deposited particles would be expected to have an effect on flux. For example, the packing density (void fraction) of the deposited aggregates and hence, their specific resistance, $\rho_a$, would be a function of pH with a maximum near the isoelectric point of the constituent protein. This can explain the observation of a lower flux and transmission during the second phase at the isoelectric pH. Partial rejection of protein by the deposited particles can lead to the formation of a concentration boundary layer. The lower observed transmission at higher cross-flow velocities may now be explained in terms of changes in the extent of concentration polarization. It must be emphasized that the observed transmission essentially refers to the ratio of the permeate and feed concentrations of non-aggregated protein because we have determined from quasi-elastic light scattering that the particles constitute a very small percentage (< 0.5% wt./wt.) of total protein. Concentration polarization of non-aggregated protein did not determine the flux as evidenced from the lack of any measurable change in flux upon changing the cross-flow velocity (see Figure 5.47). The flux was determined mainly by the resistance of the deposited particles (aggregates).

6.5 Flux equations for the initial phase

Given the assumptions in Section 6.4, the flux profile during the first phase may be described by neglecting the flow occurring through the blocked pores. In this case, the first term in Equation...(6.12) is omitted which results in the following equation.

$$J(t) = J_0 \phi(t) = \frac{\Delta P}{\mu R_0} e^{-\left\{\frac{J_0 C_{af} (1 - T_{na})}{W_p N_T}\right\} t} \quad \ldots(6.13)$$

This implies that a semi-log plot of the normalized flux as a function of time would be linear with a slope proportional to the rate constant of pore occlusion, $k_B$. 
\[
\ln \left( \frac{J(t)}{J_0} \right) = - \frac{J_0 C_{af} (1 - T_{oa})}{W_p N_T} t = - k_B t \quad \ldots \text{(6.14)}
\]

(Valid for \( t < t^* \), and \( R_B/R_O \) ≈ 1)

The rate constant, \( k_B \), is proportional to the convective deposition rate, \( J_0 C_{af} \), and inversely proportional to the weight of foulant required to block all the pores, \( W_p N_T \). The transmission term, \( (1 - T_{oa}) \), reflects the fact that not all of the foulant transported to the membrane need be rejected.

The transition time, \( t^* \), may be approximated as the time at which we can no longer neglect the flow through the blocked pores. This is illustrated below for the special case where cake formation during the first phase is negligible, i.e., \( R_i(t) \approx R_B \) for \( t < t^* \) in Equation (6.8). The flux through the blocked pores may be approximated as:

\[
J_B(t) = \frac{\Delta P}{\mu R_B} \left\{ 1 - e^{-k_B t} \right\} \quad \text{(t < t*, } R_i(t) \approx R_B)\)

Substituting this result in Equation (6.7), the total flux is given as:

\[
J(t) = \frac{\Delta P}{\mu R_B} \left\{ 1 - e^{-k_B t} \right\} + \frac{\Delta P}{\mu R_O} e^{-k_B t} \quad \text{(t < t*, and } R_i(t) = R_B)\)

Rearrangement of this equation using the definition of \( J_0 \) from Equation (6.1) gives,

\[
\frac{J(t)}{J_0} = \frac{R_O}{R_B} + \left\{ 1 - \frac{R_O}{R_B} \right\} e^{-k_B t} \quad \ldots \text{(6.15)}
\]

(Valid for \( t < t^* \), \( R_i(t) \approx R_B \))
The fraction, \( f \), of the total flux during the initial phase that occurs through the blocked pores is obtained from Equation (6.13) and Equation (6.15).

\[
f = \frac{\left[ \frac{J(t)}{J_0} \right]_T - \left[ \frac{J(t)}{J_0} \right]_{OP}}{\left[ \frac{J(t)}{J_0} \right]_T} = \frac{1 - e^{-k_B t}}{1 + \left( \frac{R_B}{R_0} - 1 \right) e^{-k_B t}} \quad \ldots (6.16)
\]

where, the subscripts 'T' and 'OP' refer to the total flux and the flux through the open pores respectively. For any given value of \( R_B/R_0 \) and \( k_B \), the fraction, \( f \), increases with time as shown in Figure 6.3. Let \( t^* \) be the time at which a 'significant' fraction of the total flux occurs through the blocked pores. Solving for the transition time \( t^* \), we get,

![Graph showing the fraction of total flux as a function of \( k_B t \) for different \( R_B/R_0 \) values.](image)

**Figure 6.3** Fraction, \( f \), of total flux during the first phase occurring through the blocked pores when \( R_f(t) = R_B \)
\[ t^* = \frac{1}{k_B} \ln \left[ 1 + \frac{f R_B}{R_0 (1-f)} \right] \]  

(...(6.17)

Clearly, \( t^* \) depends upon \( k_B \) and \( R_B/R_0 \), which are physical quantities. In addition, \( t^* \) depends on \( f \), that is upon the precise definition of what constitutes a 'significant' fraction of flux occurring through the blocked pores. If we define \( t^* \) as the time at which 50% of the total flux occurs through the blocked pores, then,

\[ t^*_{0.5} = \frac{1}{k_B} \ln \left[ 1 + \frac{R_B}{R_0} \right] \]  

(...(6.18)

Substituting for \( k_B \) from Equation...(6.5), we get,

\[ t^*_{0.5} = \frac{W_p N_T}{J_0 C_{af} (1-T_{oa})} \ln \left[ 1 + \frac{R_B}{R_0} \right] \]  

(...(6.19)

Physically, Equation...(6.19) is consistent with the expectation that the transition from the first to the second phase would take a longer time if more foulant is required to occlude all the pores, i.e., if \( W_p N_T \) is greater and take a shorter time if the rate of foulant deposition, \( J_0 C_{af} \), is greater. In addition, the transition time depends on the ratio of the resistances of the blocked and open pores (\( R_B/R_0 \)) as represented graphically in Figure 6.4. At larger values of \( R_B/R_0 \), the duration of the first phase is longer for the same value of \( k_B \).

In practice, we attempt to describe the experimental flux profile for the initial phase using an equation of the form,

\[ \frac{J(t)}{J_0} = e^{-k_{eff} t} \]  

\[ (t \ll t^*_{0.5}) \]
Figure 6.4  Duration of first phase as a function of the blocked and open pore resistances

where, \( k_{\text{eff}} \) is related to \( k_B \) for the special case of \( R_i(t) = R_B \) by the following equation.

\[
    k_{\text{eff}} t = - \ln \left( \frac{R_0}{k_B} + \left\{ 1 - \frac{R_0}{R_B} \right\} e^{-k_B t} \right) \quad ...(6.20)
\]

This equation shows that even when \( R_B/R_0 \) and \( k_B \) are constant, the linearization of a semi-logarithmic plot of flux versus time is an approximation because \( k_{\text{eff}} \) varies with time. However, our approximation becomes a fairly good description of the flux profile when \( R_B/R_0 \rightarrow \infty \) so that \( k_{\text{eff}} = k_B \) and Equation...(6.14) can be used for predicting the flux.
6.6 Flux equations for the second phase

In the region of transition from the first to the second phase, the contribution to the total flux of open and blocked pores would be equally important and a complete description of flux would result in Equation ...(6.12) which, as mentioned earlier, is difficult to evaluate. Hence, we only consider the later stages of the second phase \((t > t^*)\) where most of the flux is assumed to occur through the blocked pores, i.e., \(J_0\phi(t) \ll J(t)\). With this approximation, we can neglect the second term in Equation ...(6.12). The resulting equation describes the situation where all of the pores have been occluded, i.e., \(\phi(t > t^*) \approx 0\), and any further flux decrease is caused by an increase in the resistance of a porous 'cake' on the membrane. Consistent with our hypothesis that most of the flux during the first phase occurred through the open pores, we assume that most of the foulant accumulated on the membrane during the first phase participated in the occlusion of the open pores rather than in 'cake' formation. These assumptions enable us to simplify Equation ...(6.12) to:

\[
J(t) = \frac{\Delta P}{\mu \left\{ R_B + \frac{t}{t^*} \int \rho_a w' \, dt \right\}} \quad \ldots(6.21)
\]

(valid for \(t > t^*\))

In order to evaluate the integral in Equation ...(6.21), we need to make some assumptions about the 'cake' structure and geometry. A common assumption in the literature on filtration is that the 'cake' is a uniform layer of constant void fraction and that the effect of particle deposition is to increase the thickness of this layer (Doshi and Trettin, 1981, Ethier, 1986). Ethier (1986) also attempted to model it as the formation of hemi-spherical caps over individual pores, with the radius of the caps increasing with particle deposition. When there is a particle size distribution, an additional effect could be the filling-in of the void spaces in the 'cake' by some of the smaller particles. This effect implies that the 'cake' resistance would increase not only
because of changes in dimensions (e.g., increase in thickness) but also because of an increase in the specific resistance, $\rho_a$. The specific resistance is very sensitive to changes in void fraction $[\rho_a \propto (1-\varepsilon)^2/\varepsilon^3]$; consequently, even a small amount of deposition would cause a large change in flux. The cross-flow experiments using I$^{125}$-BSA as a tracer revealed that during the first phase, the accumulation of about 15 mg/m$^2$ of BSA caused a 10-fold flux decrease whereas during the second phase, the deposition of an additional 40 mg/m$^2$ of BSA caused only about a 10-15% flux decrease (see Figure 5.32). This suggests, but does not prove, that the filling-in mechanism may not be significant in our experiments. In any case, we do not have reliable particle size distribution data which may be used to validate a flux model based on this mechanism. Hence, we chose to model the 'cake' as a uniform layer of constant specific resistance.

The net rate of particle accumulation on the membrane surface is given by the difference between the rate of convective transport to the surface and rate of particle removal by back-diffusion.

$$w'(t) = \left\{ J(t) \ C_a \ (1 - T_{la}) + D_a \ \frac{\partial C_a}{\partial y} \right\}_{y=l_a(t)} \quad \ldots(6.22)$$

where,

- $C_a(t,y) = \text{concentration of particles in solution on the feed side (kg/m}^3\text{)}$
- $T_{la} = \text{transmission coefficient of particles through the fouled membrane}$
- $D_a = \text{average diffusivity of the particles (m}^2/\text{s}\text{)}$
- $l_a(t) = \text{'cake' thickness (m)}$

The concentration profile being modelled is depicted schematically in Figure 6.5. In order to determine the value of $w'(t)$, we need to solve the one dimensional solute conservation equation given below, with $\theta = t - t^*$. 
Figure 6.5 Schematic of the concentration profile of protein aggregates near the membrane surface

\[
\frac{\partial C_a}{\partial \theta} - J(\theta) \frac{\partial C_a}{\partial y} = D_a \frac{\partial^2 C_a}{\partial y^2}
\]  

...(6.23)

The boundary and initial conditions are:

\[
C_a = C_{al} \quad \text{for} \quad y = l_a(\theta), \quad \text{all} \ \theta \quad \ldots(6.24)
\]

\[
C_a = C_{af} \quad \text{for} \quad y = \infty, \quad \text{all} \ \theta \quad \ldots(6.25)
\]

\[
C_a = C_{af} \quad \text{for} \quad \text{all} \ y, \quad \theta = 0 \quad \ldots(6.26)
\]

\[
J(\theta) C_{al} (1 - T_{la}) + D_a \frac{\partial C_a}{\partial y} = C_{al} \frac{dl_a}{d\theta} \quad \text{for} \quad y = l_a(\theta), \quad \text{all} \ \theta \quad \ldots(6.27)
\]

where, \( C_{al} \) = concentration of aggregates in the 'cake' (kg/m³)
Equation (6.27) is identical to Equation (6.22) with \( w'(\theta) = C_{al} \frac{dl_a}{d\theta} \)

As mentioned earlier, we have neglected the 'filling-in' mechanism which results in a change in void fraction; therefore, \( C_{al} \) is constant. Since we do not have data on the diffusivity of the aggregates as a function of concentration, as a first approximation we treat it as a constant. In any case, this is an average diffusivity representing particles of different sizes. The above equations also assume that the feed and permeate have identical densities, which was true in our experiments with dilute protein solutions.

We attempt to find a solution to Equation (6.23) by using the following similarity transform.

\[
\eta = \frac{y - l_a(\theta)}{2 \sqrt{D_a \theta}} \quad \ldots(6.28)
\]

When Equation (6.23) is expressed in terms of the variable \( \eta \), we get,

\[
\frac{d^2C_a}{d\eta^2} + \frac{dC_a}{d\eta} \left\{ 2\eta + \sqrt{\frac{4 \theta}{D_a}} \frac{dl_a}{dt} + \sqrt{\frac{4 \theta}{D_a}} J \right\} = 0
\]

or,

\[
\frac{d^2C_a}{d\eta^2} + \frac{dC_a}{d\eta} \left\{ 2\eta + L_a + J_w \right\} = 0 \quad \ldots(6.29)
\]

where, \( L_a = \sqrt{\frac{4 \theta}{D_a}} \frac{dl_a}{d\theta} = \text{constant} \quad \ldots(6.30) \)

and, \( J_w = \sqrt{\frac{4 \theta}{D_a}} J = \text{constant} \quad \ldots(6.31) \)
The boundary and initial conditions given in Equations 6.24 to 6.27, when expressed in terms of $\eta$, become,

\[
\begin{align*}
C_a &= C_{al} \quad \text{for} \quad \eta = 0 \quad \ldots(6.32) \\
C_a &= C_{af} \quad \text{for} \quad \eta = \infty \quad \ldots(6.33) \\
\frac{dC_a}{d\eta} &= L_a C_{al} - J_w (1 - T_{la}) C_{al} \quad \text{for} \quad \eta = 0 \quad \ldots(6.34)
\end{align*}
\]

Integrating Equation...(6.29) once using the boundary condition given in Equation...(6.34), we get,

\[
\frac{dC_a}{d\eta} = C_{al} \left\{ L_a - J_w (1 - T_{la}) \right\} e^{-\left( \eta^2 + (L_a + J_w) \eta \right)}
\]

Integrating a second time over the range $0 \leq \eta \leq \infty$ and making use of the boundary conditions in Equation...(6.32) and Equation...(6.33), we get,

\[
\frac{C_{al} - C_{af}}{C_{al}} = \left\{ J_w (1 - T_{la}) - L_a \right\} \int_0^\infty e^{-\left( \eta^2 + (L_a + J_w) \eta \right)} d\eta
\]

The result can be expressed in terms of the complementary error function,

\[
erfc(x) = \frac{2}{\sqrt{\pi}} \int_x^\infty e^{-u^2} \, du
\]
\[
\frac{C_{al} - C_{af}}{C_{al}} = \sqrt{\frac{\pi}{2}} \left\{ J_w(1-T_{la}) - L_a \right\} e^{\left[ \frac{(L_a+J_w)^2}{4} \right]} \text{erfc} \left[ \frac{L_a+J_w}{2} \right] \quad \text{(6.35)}
\]

This represents the complete solution to the solute conservation equation and its associated boundary and initial conditions under the assumptions stated earlier. This equation does not give an explicit determination of flux, \( J(t) \), which appears as a part of the non-dimensional term, \( J_w \). In addition, \( J_w \) can be obtained only by numerical evaluation as a function of the term \( L_a \), which represents the rate of increase in 'cake' thickness. This makes validation of the flux equation by comparison to experimental data very difficult. A simplified form of Equation…(6.35) may be obtained by using the following approximate form of the complementary error function:

\[
\text{erfc}(x) = \frac{1}{\sqrt{\pi}} \frac{e^{-x^2}}{x} \left\{ 1 - \frac{1}{2x^2} + \frac{1+3}{(2x^2)^2} + \ldots \right\}
\]

So, Equation…(6.35) simplifies to:

\[
\frac{C_{al} - C_{af}}{C_{al}} = \frac{J_w(1-T_{la}) - L_a}{J_w+L_a} \left\{ 1 - \frac{1}{2} \left[ \frac{2}{J_w+L_a} \right]^2 + \ldots \right\} \quad \text{(6.36)}
\]

In order to neglect second and higher order terms in the expansion on the right side of Equation…(6.36), we must have \( J_w+L_a \approx 1 \). Since \( L_a \) is always positive, it is sufficient to show that \( J_w \approx 1 \), a criterion that is satisfied in all of our experiments for \( t \approx t^* \). For example, in our experiments with 0.25 kg/m³ BSA solutions filtered through 0.2 μm track-etch membranes, the flux during the second phase is between 3 and 10% of the initial value of 0.03 cm/s at 6.7 kPa. In order to play it safe, we choose a conservative estimate (i.e., high value)
for the aggregate diffusivity by setting it equal to that of native BSA. We then have,

\[ J_w = \sqrt{\frac{4 \theta}{D_a}} \quad J = \sqrt{\frac{4 \theta}{6.7 \times 10^{-7}} \text{ (cm}^2/\text{s})} \{ 10^{-3} \text{ (cm/s)} \} \]

For \( J_w \) to be greater than 10, \( \theta \) must be greater than about 1 s. Since we are attempting to model the flux behavior well into the second phase, this condition is satisfied and we are justified in neglecting the higher order terms. Equation...(6.36) then simplifies to:

\[ \frac{C_{af} - C_{sf}}{C_{af}} \approx \frac{J_w(1-T_{la}) - L_a}{J_w + L_a} \quad \text{...(6.37)} \]

or,

\[ L_a = \left\{ \frac{C_{af} - T_{la}C_{af}}{2C_{af} - C_{af}} \right\} J_w \]

Substituting for \( L_a \) from Equation...(6.30) we obtain an ordinary, first order, differential equation for the thickness of the aggregate layer.

\[ \frac{dl_a}{d\theta} = \frac{\sqrt{D_a}}{2} \left\{ \frac{C_{af} - T_{la}C_{al}}{2C_{al} - C_{af}} \right\} J_w \ \theta^{-1/2} \quad \text{...(6.38)} \]

Integrating this equation with the initial condition \( l_a = 0 \), at \( \theta = 0 \), gives,

\[ l_a(\theta) = J_w \sqrt{D_a} \left\{ \frac{C_{af} - T_{la}C_{al}}{2C_{al} - C_{af}} \right\} \sqrt{\theta} \quad \text{...(6.39)} \]

The flux equation for the second phase given in Equation...(6.21) may be expressed in terms of the thickness of the aggregate layer as shown below.

\[ J(\theta) = \frac{\Delta P}{\mu \left\{ R_B + \rho_a C_{al} l_a(\theta) \right\}} \quad \text{...(6.40)} \]
Substituting the result for \( I_a(\theta) \) from Equation \((6.39)\) into Equation \((6.40)\), we get,

\[
J(\theta) = \frac{\Delta P}{\mu \left( R_B + \rho_a C_{al} J_w \sqrt{D_a} \left( \frac{C_{af} - T_{la} C_{al}}{2 C_{al} - C_{af}} \right) \sqrt{\theta} \right)}
\]

...(6.41)

When the 'cake' resistance becomes much larger than the resistance \( R_B \) of the occluded pores, this equation reduces to the familiar inverse square root dependence of flux on time that is commonly reported in the literature. Equation \((6.41)\) may be rearranged and more conveniently written as :

\[
\frac{J_0}{J(\theta)} = \frac{R_B}{R_0} + K_1 \theta^{1/2}
\]

...(6.42)

where,

\[
K_1 = \frac{\rho_a C_{al} J_w \sqrt{D_a}}{R_0} \left( \frac{C_{af} - T_{la} C_{al}}{2 C_{al} - C_{af}} \right)
\]

Therefore, a plot of the reciprocal of the normalized flux during the second phase versus the square root of time should be linear if all our assumptions are satisfied. This equation lacks predictive capabilities because several of the parameters are not known. These include the specific 'cake' resistance, \( \rho_a \), the concentration of particles at the membrane surface, \( C_{al} \), the average particle diffusivity, \( D_a \), and the constant \( J_w \). The specific resistance for a packed bed of hard spheres is usually estimated from empirical correlations such as the Kozeny-Carman equation :

\[
\rho_a C_{al} = \frac{180 (1 - \varepsilon)^2}{\varepsilon^3 d_a^2}
\]

...(6.43)

where,

\[
\varepsilon = \text{void volume fraction}
\]

\[
d_a = \text{equivalent particle diameter (m)}
\]
This equation only accounts for flow occurring around the particles. For the case of porous protein aggregates, there is the possibility of flow through the aggregates themselves. Of course, not much is known about the void fraction within an aggregate although a value of 0.3 was assumed by Suki (1986) in a different context.

It must also be mentioned that $C_{\text{al}}$ and $\varepsilon$ are not independent and can be related in terms of the specific volume of the particles, $S_a$ (m$^3$/kg), which is the reciprocal of the density.

$$C_{\text{al}} S_a = 1 - \varepsilon$$  \hspace{1cm} \ldots(6.44)

6.7 Flux equations with linearly increasing particle concentration in feed

Quasi-elastic light scattering (QELS) analysis of protein solutions recirculated with a peristaltic pump revealed an increase in particle concentration with the duration of pumping (see Section 5.12.4). Subsequent electrophoretic analysis of the material deposited on the membrane surface during filtration identified them to be protein aggregates. In order to relate the particle concentration quantitatively to the extent of pumping, one needs an understanding of the aggregation mechanism; at the moment, this is still in the realm of speculation. The large scatter in the QELS data makes it difficult to derive any clues regarding the possible aggregation rate. Hence, as a first approximation, we assumed a linear increase in particle concentration with the extent of pumping as shown by the solid line in Figure 5.26. The QELS data did reveal that the total particle concentration even after 320 passes through the pump, was less than 0.5% (wt./wt.) of the total protein. Consequently, we may treat the protein concentration as a constant over the course of a typical filtration experiment. With these assumptions, we have,

$$\frac{dC_{\text{af}}}{dt} = k_a = \beta_a C_f \frac{F}{V_f} \hspace{1cm} \ldots(6.45)$$
where, \( k_a \) = zero\(^{th}\) order rate constant of particle generation (which for the special case of generation by pumping is given by the term on the right side) (kg/m\(^3\).s)  
\( \beta_a \) = fraction of protein aggregated per pass through the pump  
\( C_f \) = total protein concentration in the feed (kg/m\(^3\))  
\( F \) = feed recirculation rate (m\(^3\)/s)  
\( V_f \) = feed volume (m\(^3\)) (assumed to be constant for complete recycle of permeate and retentate)

The fraction, \( \beta_a \), depends on several parameters such as protein concentration, solution pH, temperature, presence of surface active agents, etc. However, for a given set of experimental conditions, we may consider \( \beta_a \) to be constant. For the case of recirculation of a fixed volume of solution at a constant flow rate, the rate constant \( k_a \) is a constant. In the case of cross-flow filtration, this represents the case of complete recycle of retentate and permeate.

Integrating Equation...\((6.45)\) with the initial condition, \( C_{af} = C_{ao} \), at \( t = 0 \), we get,

\[
C_{af}(t) = k_a t + C_{ao}
\]  
\[\text{(6.46)}\]

\( C_{ao} \) represents any particles present in the solution even before it is pumped with the peristaltic pump. Combining Equation...\((6.46)\) with Equation...\((6.5)\), we get the following relation that describes the rate of pore occlusion:

\[
\frac{d\phi}{dt} = - \frac{J_o (1 - T_{oa})}{W_p N_T} \left\{ k_a t + C_{ao} \right\} \phi(t)
\]  
\[\text{(6.47)}\]

Integrating this equation with the initial condition, \( \phi(t) = 1 \), at \( t = 0 \), we obtain the following equation for the fraction of pores open at time \( t \).

\[
\ln \left\{ \phi(t) \right\} = - \frac{J_o (1 - T_{oa})}{W_p N_T} \left\{ \frac{k_a}{2} t^2 + C_{ao} t \right\}
\]  
\[\text{(6.48)}\]
The above equation was developed for the case of dead-end filtration using a feed containing a linearly increasing particle concentration. This is the situation in the experiment described in Section 5.6, but in most other dead-end filtration experiments, the feed concentration of particles was constant. The flux equations based on Equation…(6.48) would be most useful if they could describe the performance of cross-flow filtration using a peristaltic pump for feed recirculation. Experimentally, we determined that cross-flow velocity per se had little effect on flux or on the rate of fouling. The faster flux decrease observed at higher recirculation rates (using a fixed volume of solution with complete recycle) is more a reflection of the greater rate of particle generation rather than the rate of convective deposition. We also show in Chapter 7 that under our experimental conditions, convective deposition far exceeds the diffusive removal of deposited particles. As a result, we find that flux equations derived from Equation…(6.48) describe the results of dead-end filtration and cross-flow filtration equally well.

6.7.1 Flux equation for the initial phase :

Using the expression for $\phi(t)$ obtained above, the flux equations for the initial phase of fouling corresponding to Equation…(6.14) and Equation…(6.15) are:

\[
\ln \left[ \frac{J(t)}{J_0} \right] = - \frac{J_0 \left(1-T_{0a}\right)}{W_p N_T} \left\{ \frac{k_a}{2} t^2 + C_{ao} t \right\} \quad \cdots(6.49)
\]

(valid for $t \ll t^*$, and $R_B/R_0 \rightarrow \infty$)

\[
\frac{J(t)}{J_0} = \frac{R_o}{R_B} + \left(1 - \frac{R_o}{R_B}\right) e^{- \frac{J_0(1-T_{0a})}{W_p N_T} \left\{ \frac{k_a}{2} t^2 + C_{ao} t \right\}} \quad \cdots(6.50)
\]

(valid for $t \ll t^*$, and $R_i(t) = R_B$)
Setting the second derivative to zero, we can calculate the time at which the flux profile exhibits an inflection (i.e., a maximum in the slope).

\[
t_{\text{inf}} = \sqrt{\frac{W_p N_T}{J_0 (1-T_{oa}) k_a}} - \frac{C_{ao}}{k_a} \quad \ldots (6.51)
\]

We next determine the time \( t^* \) at which the transition from the first to the second phase is assumed to take place. As before, it represents the time at which a fraction \( f \) of the total flux occurs through the blocked pores and it may be calculated as shown in Section 6.5 by assuming that \( R_i(t<t^*) = R_B \), so that,

\[
\phi(t^*) = \frac{(f - 1)}{(f - 1) - f} \frac{R_B}{R_0}
\]

For the special case where \( t^* \) is defined as the time at which 50% of the flux occurs through the blocked pores (i.e., \( f = 0.5 \)), the value of \( t^*0.5 \) can be obtained by substituting for \( \phi(t^*) \) from Equation\ldots(6.48) and solving the resulting quadratic equation.

\[
t^*0.5 = \sqrt{\frac{2 W_p N_T}{J_0 (1-T_{oa}) k_a} \ln \left[ 1 + \frac{R_B}{R_0} \right]} + \left[ \frac{C_{ao}}{k_a} \right]^2 - \frac{C_{ao}}{k_a} \quad \ldots (6.52)
\]

(valid when \( R_i(t<t^*) = R_B \))

6.7.2 Flux equation for the second phase:

Although the species conservation equation is the same as Equation\ldots(6.23), some of the boundary and initial conditions are different. Equation\ldots(6.24) and Equation\ldots(6.27) are still valid. However, Equation\ldots(6.25) and Equation\ldots(6.26) now become,
\[ C_a = k_a \theta + C_{af} \] for \( y = \infty, \) all \( \theta \) \hspace{2cm} \ldots(6.53)

\[ C_a = C_{af} \] for \( all \ y, \ \theta = 0 \) \hspace{2cm} \ldots(6.54)

where, \( C_{af} = k_a t^* + C_{ao} \), is the particle concentration in the feed at the end of the first phase. The condition at \( y = \infty \) is now different from the condition at \( \theta = 0 \); hence, the similarity transform used earlier is in general not applicable; a numerical solution is probably the only alternative. However, for the case where \( (k_a \theta + C_{af}) \ll C_{al} \), we may use the solution to the conservation equation obtained by the similarity transform technique to construct an approximate solution. The starting point of our derivation is Equation \ldots(6.37) which is modified to:

\[ \frac{C_{al} - C_{af}(\theta)}{C_{al}} = \frac{J_w - L_a}{J_w + L_a} \] \hspace{2cm} \ldots(6.55)

We have assumed that the transmission coefficient of the aggregates through the combined resistance of the membrane and the aggregate layer is zero (i.e., \( T_{la} = 0 \)). If the similarity transform is valid, then the right side of Equation \ldots(6.55) is a constant and hence, so must be the left side. With the assumption stated earlier, namely \( C_{af}(\theta) \ll C_{al} \), the left side is approximately unity and as a first approximation, we are justified in using Equation \ldots(6.55). Substituting for \( L_a \) from Equation \ldots(6.30) and expanding \( C_{af}(\theta) \) as \( k_a \theta + C_{af} \), we get,

\[ \frac{2}{\sqrt{D_a J_w}} \frac{d l_a}{d \theta} = \left\{ \frac{k_a \theta + C_{af}}{2C_{al} - (k_a \theta + C_{af})} \right\} \cdot \sqrt{\theta} \] \hspace{2cm} \ldots(6.56)

Let,

\[ b = \frac{C_{af}}{k_a} \] and \[ q = \frac{2C_{al} - C_{af}}{k_a}. \]

Equation \ldots(6.56) may now be written as,
\[ \frac{2}{\sqrt{D_a} J_w} \frac{dl_a}{d\theta} = \left\{ \frac{\theta + b}{q - \theta} \right\} \sqrt{\theta} \]

Integrating this equation using the initial condition that \( l_a = 0 \), at \( \theta = 0 \), we get,

\[ \frac{2}{\sqrt{D_a} J_w} l_a(\theta) = \left\{ \sqrt{q} + \frac{b}{\sqrt{q}} \right\} \ln \left| \frac{\sqrt{q} + \sqrt{\theta}}{\sqrt{q} - \sqrt{\theta}} \right| - 2 \sqrt{\theta} \quad \text{(6.57)} \]

The above equation for \( l_a(\theta) \) was derived under the assumption that \( k_a \theta + C_{af} \ll C_{al} \) which means that \( \theta \ll q \) and \( b \ll q \). Consequently, we can simplify Equation...(6.57) by expanding the logarithmic term in a Taylor series.

\[ \frac{l_a(\theta)}{\sqrt{D_a} J_w} = \sqrt{\theta} \left\{ 1 + \frac{b}{q} \right\} \left\{ 1 + \frac{1}{3} \frac{\theta}{q} + \frac{1}{5} \left( \frac{\theta}{q} \right)^2 + \ldots \right\} - \sqrt{\theta} \]

Neglecting second and higher order terms in the series and replacing 'b' and 'q' by their definitions given above, we get,

\[ l_a(\theta) = \frac{\sqrt{D_a} J_w}{2C_{al} - C_{af}} \left\{ C_{af} + \frac{k_a \theta}{3} \right\} \sqrt{\theta} \quad \text{(6.58)} \]

The first term on the right side grows as the square root of time and represents the contribution of the aggregates that are present in the feed at the beginning of the second phase. This is similar to the behavior predicted by Equation...(6.39) for constant particle concentration in the feed when \( T_{l_a} = 0 \). The second term represents the contribution from particles that are generated during the second phase. Combining Equation...(6.58) and Equation...(6.40) gives...
the following expression for flux during the second phase when the particle concentration increases linearly with time.

\[
J(\theta) = \frac{\Delta P}{\mu \left( R_B + \rho_a C_{al} \frac{\sqrt{D_a J_w}}{2C_{al} - C_{af}} \left( C_{af} + \frac{k_a \theta}{3} \right) \sqrt{\theta} \right)} \quad \ldots (6.59)
\]

(valid for \( t > t^* \), \( k_a \theta + C_{af} < C_{al} \))

6.8 Transmission behavior of non-aggregated protein

In the development of the flux models presented so far, the controlling hydrodynamic resistance was assumed to be either the membrane itself (i.e., open pores) or a combination of occluded pores and a porous 'cake'. The effect of non-aggregated protein was neglected because experimental evidence suggested that the extent of polarization was probably too low to significantly affect flux. For example, during the first phase the observed protein transmission was constant at 100% and was not affected by changes in cross-flow velocity. Furthermore, replacing the protein solution with a saline solution after the membrane was fouled caused no increase in flux (see Section 5.2) even though all the non-aggregated protein was washed from the filtration system as confirmed by native-PAGE of the material taken from the fouled membrane surface (see Section 5.13, Figure 5.27). This clearly indicated that the foulant was not the non-aggregated protein.

During the first phase, most of the flux was assumed to occur through the open pores. Hence, protein transmission is governed by the true transmission through the membrane, which in most of our experiments was 100%. This implies that there was no concentration polarization of protein during the first phase. Partial rejection of protein by the occluded pores and the
porous 'cake' causes the polarization of non-aggregated protein during the second phase. Although the extent of polarization is not significant enough to affect flux, it does have an effect on protein transmission. As mentioned earlier, the measured concentrations of protein used in calculating transmission essentially refer to the non-aggregated molecules because the QELS data had shown the fraction of particles to be less than 0.5% (wt./wt.) of the total protein. Polarization is governed by the rates of convective solute transport to the membrane and diffusion back into the feed. Because of the orders of magnitude difference in concentration, the convective transport of protein is proportionately greater than that of the particles. From the Stokes-Einstein equation, we estimate the diffusivities of particles that are 10- to 100-fold larger than BSA \((D_{BSA} = 6.7 \times 10^{-11} \text{ m}^2/\text{s})\) to be proportionately smaller. Consequently, the dynamics of polarization of non-aggregated protein are expected to be much faster than that of the particles. The significance of this conclusion is that changes in operating conditions (cross-flow velocity, trans-membrane pressure) during the second phase would cause much faster changes in the surface concentration of non-aggregated protein compared to the deposition or removal of the large particles. Hence we may treat the transmission as being in a pseudo steady state with respect to changes in particle polarization.

For convenience, we develop the equation describing the transmission of non-aggregated protein during the second phase for the case of cross-flow filtration and treat dead-end filtration as the limiting case when cross-flow velocity is zero. The standard concentration polarization equation yields,

\[
\frac{C_f - C_p}{C_f - C_p} = e^{\frac{J}{k}} \quad \ldots (6.60)
\]

where,

\(C_f\) = protein concentration at the feed-particle layer boundary (kg/m³)

\(C_p\) = protein concentration in the permeate (kg/m³)

\(C_f\) = protein concentration in the feed (kg/m³)
\[ J = \text{permeate flux (m/s)} \]
\[ k = \text{mass transfer coefficient based on protein diffusivity (m/s)} \]

Since we have assumed that flux is not governed by the polarization of non-aggregated protein, changes in mass transfer coefficient (of protein) do not affect the flux. However, when 'k' changes, the protein concentration, \(C_l\), at the feed-particle layer boundary changes. For example, \(C_l\) would decrease when the mass transfer coefficient is increased and vice versa. Of course, for \(C_l\) to increase when the mass transfer coefficient is decreased, the surface concentration would have to be below the gel concentration or solubility limit. We anticipate that this is the situation in our experiments because in the limit of zero cross-flow velocity (dead-end filtration), the observed transmission was 100%.

A schematic representation of the concentration profile is shown in Figure 6.6. The particle layer is modelled as a porous, secondary barrier formed above the membrane surface. The concentration profiles within the membrane and the particle layer are functions of the Peclet numbers, \(Pe_m\) and \(Pe_l\), in those regions (Bungay, P.M., 1984).

\[
\frac{C(\zeta) - C_p}{C_m - C_p} = \frac{1 - e^{-Pe_m (1 - \zeta)}}{1 - e^{-Pe_m}} \quad \text{where,} \quad Pe_m = (1 - \sigma_m) \frac{J_{lm}}{D_m}
\]

and,

\[
\frac{C(\chi) - C_m}{C_l - C_m} = \frac{1 - e^{-Pe_l (1 - \chi)}}{1 - e^{-Pe_l}} \quad \text{where,} \quad Pe_l = (1 - \sigma_l) \frac{J_{la}}{D_l}
\]

where, \(\zeta, \chi\) = length variables in the direction of flow within the membrane and aggregate layer respectively.

\(\sigma_m, \sigma_l\) = Staverman filtration reflection coefficients for the membrane and particle layer respectively

\(l_m, l_a\) = thickness of the membrane and particle layer respectively

\(D_m, D_l\) = protein diffusivities in the membrane and in the particle layer
When the Peclet numbers are zero, the concentration profiles would be linear in each region. At very large Peclet numbers, the concentration profiles will be flat over most of the thickness except for a thin zone on the permeate side of each region. These equations are of limited applicability when the pore is occluded because we do not know the exact geometry after blockage.

The true transmission of the occluded pores (which are treated as a part of the overall membrane resistance), $T_m$, and of the particle layer, $T_l$, are defined as:

$$T_m = \frac{C_p}{C_m} \quad \text{and} \quad T_l = \frac{C_m}{C_i} \quad \ldots(6.61)$$
These transmissions are related to the Staverman filtration reflection coefficients and the Peclet numbers by the following equations.

\[
T_m = \frac{1 - \sigma_m}{1 - \sigma_m e^{-Pe_m}} \quad \text{and} \quad T_1 = \frac{1 - \sigma_1}{1 - \sigma_1 e^{-Pe_1}} \quad \ldots (6.62)
\]

Once again, for the occluded pores, the above relation is only an approximation. The following generalizations can be made about the true transmission.

\[
\sigma = 0, \quad T = 1 \quad \text{and,} \quad \sigma = 1, \quad T = 0 \quad \text{(for all Pe)}
\]

\[
Pee = 0, \quad T = 1 \quad \text{and,} \quad Pee \gg 1, \quad T = 1 - \sigma \quad \text{(for } \sigma \neq 0)\]

From the definitions of \(T_m\) and \(T_1\) given in Equation\ldots(6.61), we have,

\[
\frac{C_p}{C_f} = T_m T_1 \quad \ldots (6.63)
\]

Using Equation\ldots(6.60) and Equation\ldots(6.63), we can solve for \(C_p\) in terms of \(C_f\). For \(T_m T_1 \neq 0\), the observed transmission, \(T_o\), can thus be expressed as:

\[
T_o = \frac{C_p}{C_f} = \frac{e^{\{J/k\}}}{1 + e^{\{J/k\}}} \quad \ldots (6.64)
\]

This equation is plotted in Figure 6.7. The following generalizations can be made about the observed transmission:

\[
T_m T_1 = 0, \quad T_0 = 0 \quad \quad\quad T_m T_1 = 1, \quad T_0 = 1 \quad \text{(for all } J/k)\]

\[
J/k \rightarrow 0, \quad T_o \rightarrow T_m T_1 \quad \quad\quad J/k \rightarrow \infty, \quad T_o \rightarrow 1
\]
Figure 6.7 Graphical representation of the observed transmission (from Equation 6.64)
The last relation only applies if the protein concentration at the surface has not reached its upper limit (i.e., either the gel concentration or the solubility limit) and provided that $T_m T_1 \neq 0$. Physically, it represents the case of dead-end filtration through a partially permeable membrane or particle layer. The ratio of flux to mass transfer coefficient can be changed by changing the cross-flow velocity or the trans-membrane pressure. However, in the latter case, interpretation of the results is usually difficult because of the dependence of $T_m T_1$ on pressure. These effects are discussed in greater detail in Chapter 7 taking specific examples from our experiments. A graphical comparison of experimental transmission data to Equation…(6.64) is facilitated by re-writing the equation as:

$$\ln \left[ \frac{1}{T_0} - 1 \right] = \ln \left[ \frac{1}{T_m T_1} - 1 \right] - \frac{J}{k} \quad \ldots(6.65)$$

The mass transfer coefficient, $k$, is related to the cross-flow velocity by using correlations applicable to flow through non-porous channels (see Section 3.4).

$$Sh = \frac{k h}{D} = a \ (Re)^b \ (Sc)^c \ \left\{ \frac{h}{L} \right\}^d \quad \ldots(6.66)$$

where,

- $h$ = hydraulic diameter of flow channel (m)
- $D$ = solute diffusivity (m$^2$/s)
- $Re$ = Reynolds number ($= h u / \nu$, $u =$ average flow velocity (m/s), $\nu =$ kinematic viscosity (m$^2$/s))
- $Sc$ = Schmidt number ($= \nu / D$)
- $L$ = length of flow channel (m)

The constants 'a', 'b', 'c', and 'd', depend on the flow regime and also on other parameters such as the surface roughness. Two of the commonly used sets of constants are:

- Laminar flow: $a = 1.62, \ b = c = d = 1/3$ (Graetz, Leveque)
- Turbulent flow: $a = 0.023, \ b = 0.8, \ c = 1/3, \ d = 0$ (Dittus, Boelter)
For laminar flow, Equation...(6.65) may be expressed in terms of the average cross-flow velocity as shown below.

\[
\ln \left[ \frac{1}{T_0} - 1 \right] = \ln \left[ \frac{1}{T_m T_l} - 1 \right] - 0.617 J \left\{ \frac{h L}{u D^2} \right\}^{1/3} \quad \text{(6.67)}
\]

Thus, if the assumptions that went into the derivation of Equation...(6.67) are correct, a semi-logarithmic plot of \([1/T_0 - 1]\) versus \(J\), or versus \(u^{-1/3}\) is expected to be linear.
Chapter 7  Discussion and Model Validation

7.1  Effect of protein adsorption on flux decrease

7.1.1  Reasons for investigating protein adsorption effects:

A rapid, 10-fold decrease in permeate flux was observed during the cross-flow filtration (CFF) of fresh, pre-filtered BSA solutions through 0.2 μm track-etch membranes using a peristaltic pump for feed recirculation. For example, when 5×10⁻⁴ m³ of 0.25 kg/m³ BSA solution (pH 7.3) was subjected to CFF at a pressure of 6.7 kPa (50 mmHg) and at a recirculation rate of 5.2×10⁻⁶ m³/s (0.3 m/s), the permeate flux decreased to about 12% of its initial value of 2.8×10⁻⁴ m/s in about 40 min (see Figure 5.1). During this period of rapid flux decrease, the observed BSA transmission was 100% as expected from the relative sizes of BSA (prolate ellipsoid, 14 nm x 4 nm x 4 nm) and the pore (nominal diameter 0.2 μm = 200 nm). Consequently, the initiation of flux decrease could not be attributed to the concentration polarization of BSA caused by size exclusion alone. The basis of concentration polarization theory is the reversible accumulation of solute on the membrane until, at equilibrium, there exists a balance between convective deposition and diffusive removal. We observed no increase in flux even when additional protein deposition was prevented by switching the feed from BSA to saline solution (see Figure 5.4). Consequently, we began to investigate alternative mechanisms of flux decrease.

There exists an enormous body of literature on membrane fouling, some of which was reviewed in Chapter 3. Most of the focus is on protein adsorption to ultrafiltration membranes where the protein and pore dimensions are comparable. The driving force for adsorption is thought to be the increase in entropy caused by changes in hydration of the protein and the adsorbing material, the so called 'hydrophobic effect'. Adsorption is also affected by electrostatic effects and hence, is a function of pH and ionic strength. Most synthetic membranes adsorb proteins to varying extents depending on their hydrophobicity and surface
charge. However, a quantitative criterion for selecting the membrane material is lacking because of the difficulty in measuring 'hydrophobicity'. For example, contact angle measurements of surface tension generally require a smooth, non-porous surface.

For the case of microporous and partially permeable ultrafiltration membranes, where the protein has access to the internal pore area, adsorption is thought to cause a decrease in pore diameter. Assuming that the adsorbed protein layer is impermeable, we can estimate the extent of flux decrease by using the Hagen-Poiseuille equation for laminar flow through cylindrical pores. Some of the parameters relevant to estimating the hydrodynamic conditions within the pore are summarized below:

- Pore diameter : 0.2 \( \mu \)m (2\( \times \)10\(^{-7} \) m)
- Pore length : 10 \( \mu \)m (10\(^{-5} \) m)
- Initial flux : 2.8\( \times \)10\(^{-4} \) m/s
- Pore number density : 3.2\( \times \)10\(^{12} \) pores/m\(^2\)
- Solution density : 1010 kg/m\(^3\)
- Solution viscosity (aqueous) : 10\(^{-3} \) kg/m s

From these data, we estimate the average initial velocity through a pore to be 2.8\( \times \)10\(^{-3} \) m/s which corresponds to a Reynolds number (based on pore diameter) of 5.6\( \times \)10\(^{-4} \). Clearly, the flow is in the laminar regime. Using Equation...(6.2) and neglecting entrance effects, we find that the flux is proportional to the fourth power of pore diameter; consequently, changes in diameter caused by protein adsorption can have a significant effect on flux.

7.1.2 Magnitude of the adsorption effect:

Yavorsky (1981) has reported the thickness of adsorbed BSA layers on track-etch mica membranes to be between 4 and 6 nm. This thickness would cause the normalized flux through the 0.2 \( \mu \)m membranes to decrease to about 85%. Even if we assume that BSA adsorbs in an end-on orientation so that the adsorbed layer thickness is 14 nm, the normalized
flux would decrease to 55% which is still considerably higher than the observed value of 6% after 120 min. Cross-flow experiments using \( \text{I}^{125}\)-BSA as a tracer indicate that the amount of BSA on the membrane is 60 - 80 mg/m\(^2\) of projected area when the flux has reduced to about 6% of the initial value (see Figure 5.32). If the protein is assumed to be uniformly distributed over the total surface area (=20m\(^2\) total/m\(^2\) projected), the coverage becomes 3 - 4 mg/m\(^2\) which is within the range of monolayer adsorption. As estimated earlier, the thickness of a BSA monolayer would vary between 4 and 14 nm depending on side-on or end-on adsorption and this was shown to be insufficient to cause the observed flux decrease. The thickness would have to be at least 50 nm to cause the normalized flux to decrease to 6%. This corresponds to 4 - 12 layers of BSA depending on orientation and assuming the molecules to be touching one another.

The rapid decrease in flux during the filtration of freshly prepared, pre-filtered BSA solutions was only observed when a peristaltic pump was used for recirculating the protein solution. The decrease in flux during dead-end filtration (DEF) or during CFF with a diaphragm pump was no greater than observed with buffered saline solutions (see Figure 5.17). We attempted to explain these observations in terms of the adsorption of denatured protein, the latter thought to be generated by recirculating the solution with the peristaltic pump. It is well known that denatured proteins adsorb to a greater extent than native proteins because of the exposure of core hydrophobic residues to the external environment. These exposed residues have a lower free energy at hydrophobic interfaces than when surrounded by water; this increases the affinity of the protein for the adsorbent. An investigation of BSA adsorption to 0.2 \( \mu \)m track-etch membranes from stirred solutions revealed no significant differences in the rate or extent of adsorption between fresh solution and solution that had been pumped with a peristaltic pump (see Figure 5.34). The characteristic time \((L^2/D)\) for diffusion of BSA \((D = 6.7\cdot10^{-11} \text{ m}^2/\text{s})\) along the pore length (10 \( \mu \)m) is of the order of seconds where as the observed time scale for adsorption was of the order of hours; so, the observed kinetics are probably limited by the
intrinsic adsorption rate. In this case, BSA concentration would be uniform in the pore volume and equal to the external concentration, \( C_\infty \), of 0.25 kg/m\(^3\). The rate of adsorption may be expressed as:

\[
\frac{dC_s}{dt} = k_1 \left[ C_{\text{max}} - C_s \right] C_\infty - k_2 C_s
\]

where,

- \( C_s \) = surface concentration of protein (kg/m\(^2\))
- \( C_{\text{max}} \) = maximum surface concentration (kg/m\(^2\))
- \( k_1 \) = adsorption rate constant (m\(^3\)/kg s)
- \( k_2 \) = desorption rate constant (s\(^{-1}\))

Integrating with the initial condition, \( C_s = 0 \) at \( t = 0 \), we get,

\[
C_s = \frac{k_1 C_\infty C_{\text{max}}}{k_1 C_\infty + k_2} \left\{ 1 - e^{-\left(k_1 C_\infty + k_2\right)t} \right\} \quad \ldots(7.1)
\]

This equation describes the results of Figure 5.34 with a correlation of 0.99 and the parameter values obtained from the fit are:

- \( k_1 C_{\text{max}} \) = \( 1.152 \times 10^{-8} \) m/s
- \( k_1 C_\infty + k_2 \) = \( 1.42 \times 10^{-5} \) s\(^{-1}\)

Assuming that these parameter values also apply to the case of adsorption during CFF, the amount of BSA adsorbed after 10 min (normalized flux < 10%) is estimated to be 1.7 mg/m\(^2\) compared to the measured amount of about 55 mg/m\(^2\), based on projected area. Even after 120 min, which was the duration of the experiment, the adsorbed BSA is estimated to be about 20 mg/m\(^2\) projected area. When converted to a total area basis, this amount is still less than the monolayer coverage of 3-5 mg/m\(^2\). Clearly, uniform adsorption cannot explain our results.

Further evidence against uniform adsorption was obtained by measuring the saline flux through membranes soaked in BSA solutions for up to 20 h. No significant decrease in membrane
permeability was observed. As mentioned earlier, no increase in flux through the fouled membranes was observed when the feed was switched from protein to saline solution without any discontinuity in flow or trans-membrane pressure (see Section 5.2). On the other hand, there was an immediate increase in normalized flux from 5% to about 75% when back-flushing was used to clean a fouled track-etch membrane. This difference in behavior suggests that the foulant was plugging the pore entrance and that desorption of uniformly adsorbed protein was not the mechanism of flux recovery during back-flushing. Note that protein desorption, if any, should occur irrespective of the direction of flow. We speculate that protein occluding the pore entrance withstands the forces exerted by the flow of saline from the feed-side to the permeate-side of the membrane because the protein was deposited under similar flow conditions. However, they cannot resist the forces exerted by flow in the opposite direction and this causes the 'unplugging' of the pores. (One may draw an analogy between these observations and attempts to open a stoppered bottle by trying to force the stopper into the bottle instead of pushing it out. The stopper can withstand the applied force in one direction but pops out when the force is applied in the opposite direction).

7.2 Summary of fouling hypothesis

Details of the several experiments done to identify the foulant and the source of foulant generation were presented in Chapter 5. Here, we only summarize a few key results. A steady increase in particle concentration (>30 nm) was observed using quasi-elastic light scattering analysis of BSA solutions recirculated with a peristaltic pump (Section 5.12.4, Figure 5.49). The particles constituted less than 1% (wt./wt.) of total BSA even after 320 passes through the pump; hence we were unsuccessful in detecting changes in native BSA concentration using standard analytical techniques such as electrophoresis and chromatography (see Section 5.12). Likewise, we found no significant change in the activity of ribonuclease or catalase upon pumping (Section 5.23). An early indication that the foulant was protein was provided by the observation of an immediate increase in permeate flux upon contacting the fouled membranes
with a protease solution which presumably hydrolyzed the foulant (Figure 5.5). Electrophoretic analysis (native-PAGE) of material collected from the surface of a fouled membrane revealed the presence of a component too large to enter the separation zone of the polacrylamide gel (molecular wt. cut off. \( \approx 700 \) kDaltons). Under denaturing and reducing conditions, the same sample yielded a single band corresponding to BSA monomer (see Section 5.13). This led us to conclude that the particles generated by pumping a BSA solution with a peristaltic pump and collecting on the membrane surface during filtration were BSA aggregates.

The material from the fouled membrane surface was collected for native-PAGE analysis after rinsing the membrane with saline solution according to the protocol described in Section 5.2. As mentioned earlier, there was no increase in saline flux during the rinsing (by forward-flow), implying that the foulant was not washed away. Since native-PAGE yielded only a single band corresponding to a large component and no bands corresponding to BSA monomer or dimer, we felt justified in hypothesizing that the large component, which was shown to an aggregate of BSA, was the foulant in question. This hypothesis was also supported by the observation that lowering the particle concentration in the feed by filtration or centrifugation resulted in a slower rate of flux decrease (Section 5.14).

The observation of a decrease in flux during the filtration of solution that had been previously filtered through an identical membrane, suggested that particles smaller than the pore size could cause fouling. This implied that in addition to size exclusion other fouling mechanisms such as pore occlusion by particle-particle and/or particle-membrane interactions may be playing a role. As discussed in Section 6.2, we propose a model that does not depend on the precise mechanism of particle rejection by assuming the time scale for occlusion of any individual pore to be small compared to the time scale of the observed flux decrease. This assumption simplified the description of the partially fouled membrane to one consisting of a bimodal pore
distribution, namely, 'blocked' or 'open' pores. Therefore, instead of using a continuous function to model the increase in pore resistance, we used a step change from the open pore resistance, $r_0$, to the blocked pore resistance, $r_B$ (see Figure 6.1). We did not assume that the blocked pores were impermeable, but only that their resistance was much greater than that of the open pores. The observed irreversibility of flux decrease under forward flow conditions and the lack of sensitivity of flux to cross-flow velocity, enabled us to treat the particles participating in pore occlusion as an integral part of the membrane resistance. Further rejection of particles by the passage of solution through the occluded pores was assumed to cause a porous 'cake' above the blocked pores (see Section 6.2). Therefore, the overall decrease in flux resulted from a combination of pore occlusion and 'cake' formation. For convenience, the flux profile was divided into two phases, the first phase being dominated by the pore occlusion mechanism and the second being dominated by 'cake' formation. The division is not clear cut and around the transition region both mechanisms could be important. As discussed in Section 6.4, the response of flux and transmission to changes in operating conditions (pressure and cross-flow velocity) and feed properties (such as pH) was different in the two phases. This provided a physical justification for dividing the flux profile into two phases.

The equations describing the flux during the two phases were developed on the assumption that the rate limiting step was the convective transport of particles to the membrane surface. This means that the rate of fouling is proportional to the product of the convective flow of permeate and the concentration of particles in the feed. Experimental evidence in support of such a hypothesis was presented in Sections 5.17 and 5.22 but a quantitative analysis was deferred until the development of the flux equations. Having completed the modelling in Chapter 6, the remainder of this chapter is devoted to validating the model (and the underlying assumptions) using the experimental data presented in Chapter 5.
7.3 Validation of the flux equations for the initial phase

We hypothesized that the flux decrease during the first phase occurs because of a decrease in the number of open pores. When the resistance of the blocked pores is at least 10 times greater than the resistance of the open pores, Equation (6.14) may be used to describe the flux. This equation, reproduced below as Equation (7.2), predicts that the flux will decrease exponentially with time at a rate that is proportional to the rate of convective deposition of foulant and inversely proportional to the total amount of foulant required to block all pores.

\[
\ln \left[ \frac{J(t)}{J_0} \right] = - \frac{J_0 \, C_{af} \, (1 - T_{oa})}{W_p \, N_T} \, t 
\]  

...(7.2)

Figure 7.1 Semi-logarithmic flux profile for DEF of BSA solutions prepumped with a peristaltic pump. (Data from Figure 5.19).
where, \( J(t) \) = flux at any time 't' (m/s)
\( J_0 \) = flux at time \( t = 0 \) (equal to the initial saline flux) (m/s)
\( C_{af} \) = concentration of aggregates in the feed (kg/m\(^3\))
\( T_{oa} \) = transmission coefficient of aggregates through the open pores.
\( W_p \) = mass of protein required to block (bridge) one pore (kg/pore)
\( N_T \) = surface pore density (pores/m\(^2\))

We use the dead-end flux data from Figure 5.19 to test the validity of this equation by re-plotting them on a semi-logarithmic plot of normalized flux versus time (see Figure 7.1). The profiles appear to be linear only until the normalized flux decreases to about 10-12%; then the rate of flux decrease slows considerably. Since the flux equations for the second phase do not predict these profiles to be linear, we have qualitative support that there is indeed a change in

![Figure 7.2 Linearity of the flux profiles during the first phase of dead-end filtration (Solid lines are the best-fit through the data)](image)
fouling mechanism and that the division of the flux profiles into two phases is not arbitrary. We now analyze the data for the first phase of flux decrease in greater detail. For clarity, these data have been re-plotted in Figure 7.2 along with the solid lines representing the best fit of Equation...(7.2). The correlation coefficient is better than 0.99 in all cases. The slopes, $k_B$, of the best fit lines are a measure of the rates of convective deposition.

$$k_B = \frac{J_0 C_{af} (1 - T_{oa})}{W_p N_T} \quad \ldots(7.3)$$

The only experimental condition that was varied to yield the different profiles was the number of times, $N_{pump}$, the feed solution was passed through a peristaltic pump before DEF. As shown in Figure 5.26, there is a steady increase in the concentration of particles with increasing number of passes of solution through the pump. This suggests that the different slopes, $k_B$, observed in Figure 7.2 are a consequence of the different concentrations, $C_{af}$, of particles present in the feed. In order to quantitatively relate $C_{af}$ to $N_{pump}$, we use the approximations discussed in Section 6.7 for the case of continuous recirculation of feed, namely, that the rate of particle formation is linear with extent of pumping and that the total protein concentration, $C_I$, is a constant. The latter merely reflects the observation that the particles (aggregates) constitute less than 0.5% (wt./wt.) of the protein and hence, loss of protein through aggregation results in a negligible change in its concentration. We now have:

$$C_{af} = \beta_a C_I N_{pump} \quad \ldots(7.4)$$

where, $\beta_a = \text{fraction of protein aggregated per pass through the pump}$

From a linear fit to the data in Figure 5.26, we estimate $\beta_a$ to be $1.35 \times 10^{-5}$ (± 15%). Combining Equation...(7.4) with Equation...(7.3) we get the following relation between the slopes of the flux profiles and the number of passes of feed through the peristaltic pump:
**Figure 7.3** Correlation between the slope of the flux profiles and the extent of pumping of the feed solution before filtration.

\[ k_B = \frac{J_0 (1 - T_{oa})}{W_p N_T} \beta_0 C_f (N_{pump}) \]  

...(7.5)

According to Equation...(7.5), \( k_B \) is expected to vary linearly with \( N_{pump} \) and this is confirmed in Figure 7.3 where the data points correspond to the values of \( k_B \) from Figure 7.2 and the solid line is a best fit through the points. The equation for this best fit line is given below.

\[ k_B (\text{min}^{-1}) = 7.5 \times 10^{-3} + 3.7 \times 10^{-3} (N_{pump}) \]  

...(7.6)

The intercept on the ordinate axis is probably caused by the presence of trace amount of
foulant in the feed even before it was pre-pumped. From the magnitude of the intercept, we

can estimate this initial concentration of particles to be about 6.5×10^{-6} \text{ kg/m}^3.

The dead-end flux profiles shown in Figure 7.1 were all obtained at a trans-membrane pressure

of 6.7 \text{ kPa} at which the average value of the initial flux, \( J_0 \), was 2.8×10^{-4} \text{ m/s} (± 7\%). The total BSA concentration in the feed, \( C_f \), was 0.25 \text{ kg/m}^3. The pore density, \( N_T \), estimated

from the scanning electron micrographs of unused track-etch membranes is 3.2×10^{12} \text{ pores/m}^2.

In order to use Equation...(7.5) to predict the values of \( k_B \), one needs the values of two more

parameters, namely, the transmission of the particles through the open pores, \( T_{oa} \) and the

amount of particles required to block a pore, \( W_p \). As a first approximation, we assume that \( T_{oa} \)

\ll 1. The value of \( W_p \) can be obtained from the protein deposition measurements under actual

CFF conditions using \(^{125}\text{I}-\text{BSA} \) as a tracer (see Section 5.15). Since the flux model for the

first phase assumes that most of the flow occurs through the open pores, we may also assume

that most of the aggregates deposited on the membrane during the first phase participate in the

occlusion of the open pores rather than in 'cake' formation. This means that the rate of

accumulation of aggregates on the membrane surface is proportional to the rate of pore

occlusion and hence, to the rate of flux decrease. As a result, we obtain the following relation

between the amount, \( W \), of aggregates occluding the pores and the normalized flux \( J/J_0 \):

\[
W(t) = W_p N_T \left( 1 - \frac{J(t)}{J_0} \right) \quad \ldots (7.7)
\]

According to this equation, when the normalized flux is 100\%, there are no aggregates

occluding the pores. The \(^{125}\text{I}-\text{BSA} \) tracer technique measures all of the protein associated with

the membrane and not just that which occludes the pores. This is reflected in the the data of

Figure 5.32 where extrapolation to zero time gives a value of about 38 \text{ mg/m}^2 for the

membrane bound BSA even though the normalized flux is 100\%. Hence, in order to use
Figure 7.4 Correlation between normalized flux and aggregates occluding the pores during the first phase. (Data from Figure 5.32)

Equation...(7.7), we can either subtract this initial binding from the data or we can ignore the absolute values and just consider the slope which should be equal to \( W_p N_T \). We have chosen to adopt the former procedure for the graphical representation of Equation...(7.7) so that the ordinate will be a measure of the protein occluding the pores (see Figure 7.4). From the slope of the best fit line we estimate \( W_p N_T \) to be 14.7 mg/m².

Equation...(7.5) for \( k_B \) can now be related to \( N_{pump} \) without using any adjustable parameters.

\[
k_B \ (s^{-1}) = 6.4 \times 10^{-5} \ N_{pump}
\]

or,

\[
k_B \ (\text{min}^{-1}) = 3.8 \times 10^{-3} \ N_{pump} \quad \text{...(7.8)}
\]
The proportionality coefficient, $3.8 \times 10^{-3} \text{ min}^{-1}$ in Equation... (7.8) compares favorably with the value of $3.7 \times 10^{-3} \text{ min}^{-1}$ obtained from the linear fit to the experimental data in Figure 7.3. However, the model prediction does not account for aggregates that may be present in solution before pumping. Hence, unlike the best fit line, the model prediction (dotted line) does not have an intercept on the ordinate and, as a result, it underpredicts the value of $k_B$. The particle concentration, $C_{ao}$, before pumping varies from one batch of solution to another and cannot be predicted \textit{apriori}.

From the value of $W_p N_T$ estimated above, we can calculate the amount of aggregates required to occlude a pore.

$$W_p = \frac{W_p N_T}{N_T} = 5 \times 10^{-18} \text{ kg/pore}$$

For comparison, if the method of flux decrease is by uniform adsorption along the pore wall, then the amount of protein required to cause the normalized flux to decrease to 5% would be about $(1-2) \times 10^{-16} \text{ kg}$ which is about 20-40 times greater than $W_p$. This supports our hypothesis that the pore is not filled along the entire length and that the initial blocking (occlusion) occurs near the pore entrance.

### 7.4 Effect of operating conditions on dead-end flux (first phase)

#### 7.4.1 Aggregate concentration:

In the previous section, we had implicitly discussed the effect of aggregate concentration, $C_{af}$, on flux because this was the only solution property that changed with the extent of pumping. The linear relation observed between $k_B$ and $N_pump$ is a manifestation of the proportionality of $k_B$ to $C_{af}$. Since the initial flux was the same in all the filtrations, this result is a validation of the convective deposition hypothesis.
Figure 7.5 Effect of changing aggregate concentration (by dilution) on flux.
We now consider the case where the concentration of aggregates in the feed was changed by diluting the feed solution to various extents (see Section 5.22). The experimental flux profiles from Figure 5.50 for the first phase of fouling are shown in Figure 7.5 on a semi-logarithmic plot of the normalized flux versus time. Equation...(7.2) describes these profiles with a correlation coefficient better than 0.99. Also shown in Figure 7.5 are the slopes of the semi-log flux profiles (kB) as a function of aggregate concentration normalized to the concentration in the undiluted solution. In accordance with Equation...(7.3), we find that kB varies linearly with the extent of dilution (i.e., normalized aggregate concentration); this result again supports the convective deposition hypothesis.

7.4.2 Effect of trans-membrane pressure:

An alternative test of the convective deposition hypothesis is to verify whether kB varies linearly with the initial flux, Jo, while keeping all other parameters including the aggregate concentration, Ca, constant. Experimentally, this is done by preparing identical batches of feed solution and performing the dead-end filtration at different trans-membrane pressures. The data from such an experiment (see Figure 5.36) are reproduced in Figure 7.6 as a semi-logarithmic plot of the normalized flux versus time. Once again, such a representation yields a linear set of flux profiles. The slopes, kB, of these semi-log flux profiles and the initial flux, Jo, are plotted against the trans-membrane pressure in Figure 7.7. The data point at zero pressure represents the slope, kB, of the flux profile obtained by diffusive deposition of aggregates (data from Figure 5.37). We find that kB does not increase linearly with the trans-membrane pressure unlike the initial flux, Jo. In other words, the change in kB is less than predicted by Equation...(7.3) for the observed change in Jo. This suggests that trans-membrane pressure has an effect on some of the other parameters in Equation...(7.3) in addition to its effect on Jo. We speculate that the transmission coefficient, Toa, of the aggregates through the open pores is a function of pressure. There is, of course, some precedent for such a hypothesis since other researchers investigating the passage of proteins
Figure 7.6 Dead-end flux profiles (first phase) at different transmembrane pressures (Data from Figure 5.36).

through non-retentive membranes have reported a pressure effect on transmission. For example, Reihanian (1983) observed an increase in the transmission of BSA through the Amicon XM-300 membranes from 68% to 91% when the pressure was raised from 34 to 138 kPa. They explain this increase in terms of the greater force exerted on the solute blocking the pores resulting in its being forced through the membrane at higher pressures.

Since we do not have a measure of the absolute value of $T_{oa}$, we use the experimentally determined values of $k_B$ to estimate the transmission at 13.3 and 20 kPa relative to its value at 6.7 kPa. The results, summarized in Table 7.1, suggest that the rejection coefficient $(1-T_{oa})$ decreases with pressure, i.e., $T_{oa}$ increases with pressure.
Figure 7.7 Effect of trans-membrane pressure on initial flux and on the slope of the flux profiles.

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>$k_B$ (min$^{-1}$)</th>
<th>$J_0$ ($x 10^4$ m/s)</th>
<th>$(1 - T_{oa})$ relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7</td>
<td>0.17</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>13.3</td>
<td>0.25</td>
<td>4.9</td>
<td>0.8</td>
</tr>
<tr>
<td>20</td>
<td>0.31</td>
<td>7.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>
We speculate that the reason for the estimated increase in $T_{oa}$ is similar to the one given by Reihanian. Pore occlusion involves the formation of stable bridges across the pore entrance and the net rate of occlusion is affected by the rate at which the aggregates approach the pore (i.e., by $J_0$) and the rate at which the occluded aggregates are forced through the pore (i.e., by $J_0T_{oa}$). When pressure is increased, both of these rates increase but perhaps, not by the same ratio; hence, the slope $k_B$ does not increase linearly with pressure.

A second mechanism by which pressure affects transmission is when concentration polarization of the solute is important. The higher flux at higher pressures increases the convective transport of solute and thus increases the equilibrium concentration of solute at the membrane surface. If the true transmission does not change, then the higher surface concentration causes an increase in the observed transmission. However, our hypothesis is that most of the transmission during the first phase of fouling occurs through the open pores and that 'cake' formation (i.e., polarization) is negligible during this phase. Hence, the effect of pressure on transmission via a change in polarization is also assumed to be insignificant during this phase.

7.4.3 Effect of membrane pore size:

The experimental data from Figure 5.59 for the first phase of fouling during the DEF of BSA through 0.2 - 0.8 μm track-etch membranes is reproduced in Figure 7.8 on a semi-logarithmic plot of flux versus time. The linearity of the plots indicates that our fouling hypothesis applies to the larger pore size membranes as well. Analysis of the flux profiles using Equation...(7.3) is complicated by the fact that all the parameters that determine $k_B$ change simultaneously when the pore size is changed. We do not have experimental values of $W_pN_T$ for the 0.4 - 0.8 μm membranes; however, we can get an estimate of this quantity relative to its value of 14.7 mg/m$^2$ for the 0.2 μm membrane by assuming that the amount of protein occluding the pores scales as the pore area. We do have experimental values for the
Figure 7.8 Effect of membrane pore size on flux during the first phase

initial flux, $J_0$, and nominal values for the pore number density, $N_T$ for these membranes. Furthermore, since the same batch of solution was used with all four membranes, the aggregate concentration, $C_{af}$, is identical. We can thus represent Equation...(7.3) in a form that is useful for estimating the rejection coefficients through the 0.4 - 0.8 μm membranes relative to its value for the 0.2 μm membrane:

$$\frac{1 - T_{oa}}{1 - T_{oa}}_{0.2\mu m} = \frac{k_B d_p^2 N_T / J_0}{k_B d_p^2 N_T / J_0}_{0.2\mu m} \quad \ldots(7.9)$$

The values of the parameters in Equation...(7.9) and the calculated relative rejection coefficients, $(1-T_{oa})$, are shown in Table 7.2. As expected, the relative transmission of
Table 7.2 Effect of pore size on transmission of aggregates

<table>
<thead>
<tr>
<th>Pore diameter $d_p$ (µm)</th>
<th>Pore density $N_T$ (x10^{-12} m^{-2})</th>
<th>Initial flux $J_0$ (x10^4 m/s)</th>
<th>Slope $k_B$ (x10^4 s^{-1})</th>
<th>$(1 - T_{oa})$ relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>3</td>
<td>2.7</td>
<td>10.4</td>
<td>1.0</td>
</tr>
<tr>
<td>0.4</td>
<td>1</td>
<td>5.1</td>
<td>3.9</td>
<td>0.3</td>
</tr>
<tr>
<td>0.6</td>
<td>0.3</td>
<td>8.7</td>
<td>3.5</td>
<td>0.1</td>
</tr>
<tr>
<td>0.8</td>
<td>0.3</td>
<td>12.2</td>
<td>2.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

aggregates is greater for membranes with the larger pores but the reason for observing no difference between the 0.6 and 0.8 µm membranes is not clear. We speculate that the similar value of transmission through the 0.6 and 0.8 µm membranes may be due to a fortuitous combination of the relative particle and pore size distributions. For sufficiently large pore sizes (not investigated), the observed transmission is expected to be unity.

7.5 Hydrodynamic regime for cross-flow filtration

Using the parallel DEF-CFF scheme shown in Figure 5.13, we demonstrated that the hydrodynamic conditions above the membrane surface had little effect on the rate of flux decrease (see Figures 5.14 and 5.15). The faster flux decrease observed at higher recirculation rates during CFF with a peristaltic pump (Figure 5.10) is caused by the higher rate of particle formation due to pumping rather than a higher deposition rate due to differences in cross-flow velocity. In this section we compare the permeate flux to the mass transfer coefficient in the cross-flow apparatus and show that the rate of fouling is dominated by the convective deposition rate. Consequently, we find that the flux equations developed for the case of DEF also describe the rate of flux decrease during CFF.
We begin by establishing the hydrodynamic regime in the cross-flow apparatus during the CFF experiments.

Channel cross-section : 2.8 cm (±5%) x 0.06 cm (± 20%)
Cross-sectional area : 1.7\cdot10^{-5} \text{ m}^2
Hydraulic diameter : 1.2\cdot10^{-3} \text{ m}
Channel length : 6.4 \text{ cm (total)}; 2.6 \text{ cm (above membrane)}
Recirculation rate (typical) : 3.3\cdot10^{-6} \text{ m}^3/\text{s (200 ml/min)}
Cross-flow velocity : 0.2 \text{ m/s}
Reynolds number : 230

The axial pressure drop under these conditions was less than 0.7 kPa compared to the trans-membrane pressures of 6.7 - 20 kPa and hence, was neglected. The initial flux through the 0.2 \mu m track-etch membranes at a trans-membrane pressure of 6.7 kPa is 2.8\cdot10^{-4} \text{ m/s} and it decreases to about 0.15\cdot10^{-4} \text{ m/s} after the membrane is fouled.

Since the flow is laminar, we use Equation...(6.66) to calculate the mass transfer coefficient using the exponents given by Leveque.

\[ \text{Sh} = \frac{k \cdot h}{D} = 1.62 \left( \text{Re} \cdot \text{Sc} \cdot \frac{h}{L} \right)^{1/3} \quad \ldots(7.10) \]

where,
- \( h \) = hydraulic diameter of flow channel
- \( D \) = solute diffusivity (= 6.7\cdot10^{-11} \text{ m}^2/\text{s} for native BSA at pH 7)
- \( \text{Re} \) = Reynolds number
- \( \text{Sc} \) = Schmidt number (= \( \nu /D = 1.5\cdot10^4 \) for dilute BSA solutions)
- \( L \) = length of flow channel

Substituting the values of the various parameters, the mass transfer coefficient based on the diffusivity of native BSA is estimated to be 4\cdot10^{-6} \text{ m/s}. We have already determined that the flux is governed by the deposition of particles (aggregates > 30 nm) and not native BSA.
Hence, we need to estimate the mass transfer coefficient based on particle diffusivity which is assumed to vary inversely with particle size according to the Stokes-Einstein equation. We do not have reliable data on the particle size distribution; nor do we know which size fractions are actually involved in the fouling. For an order of magnitude estimate, we assume the particles to be, on average, 10 times larger than BSA and the diffusivity to be 10 times lower. With this assumption, we estimate the mass transfer coefficient (for particles) to be about $1 \cdot 10^{-6}$ m/s.

In order to compare the rates of convective deposition and diffusive removal, we use the solute conservation equation and estimate the predicted steady state flux, $J_{ss}$, for a reasonable value of the particle concentration at the membrane surface, $C_{al}$. If the predicted flux is much lower than the measured flux, we infer that convective deposition dominates.

$$J_{ss} = k \ln \left( \frac{C_{al}}{C_{af}} \right)$$

We use the gel concentration of BSA (350 kg/m$^3$) as the upper limit on $C_{al}$. The value of $C_{af}$ depends on the extent of pumping but it is of the order of $10^{-5}$ to $10^{-4}$ kg/m$^3$ for 10 to 100 passes through the pump. With these values, we estimate the steady state flux based on the polarization of BSA aggregates to be of the order of $10^{-5}$ m/s. Clearly, this value is at least an order of magnitude lower than the flux during the first phase of fouling. This implies that the rate of fouling is dominated by the convective deposition of aggregates and it explains why we do not observe an effect of cross-flow velocity on the fouling rate.

The preceding analysis assumes that particle deposition is reversible. This is not actually the case as seen from the absence of a flux increase during about 30 min of passage of saline through the fouled membrane (Section 5.2). In this case, we are all the more justified in assuming that the fouling is dominated by convective deposition. Consequently, we can use Equation...(7.2) which was developed for DEF to describe the rate of fouling during CFF.
7.6 Flux profiles during the first phase of CFF using a diaphragm pump

The experimental results demonstrating the absence of a measurable effect of surface hydrodynamics on fouling rate (Section 5.6) and the quantitative analysis based on mass transfer coefficients (Section 7.5) enable us to analyze the flux profiles during the first phase of CFF using equations developed for DEF. We first consider the case of CFF using a diaphragm pump where the particle concentration $C_{af}$ does not vary during the filtration. The particles are generated before CFF by passing the protein solution through a peristaltic pump. The analysis of the flux profiles is similar to that done earlier for DEF using Equation...(7.2) and Equation...(7.3). The data from Figure 5.18 are reproduced below on a semi-logarithmic plot of normalized flux versus time.

![Graph](image)

**Figure 7.9** Semi-logarithmic plot of normalized flux for CFF using diaphragm pump (Data from Figure 5.18)
The linearity of the semi-log plot (see Figure 7.9) not only supports the convective deposition hypothesis but also our use of Equation ...(7.2) which was originally developed for DEF. The slope, $k_B$, of 0.084 min$^{-1}$ compares favorably with the value of 0.078 min$^{-1}$ obtained from DEF of BSA solution pre-pumped 20 times (see Figure 7.3). As mentioned in Section 5.10, even when using similar protein solutions, we can assume that the same extent of pumping gives the same value of $C_{af}$ only when the experiments are done without replacing the tubing in the peristaltic pump between experiments. This is because of the unpredictable variations in $\beta_a$ when the tubing is replaced, irrespective of whether the replaced tubing comes from the same stock as the new one or from a totally different material (e.g., silicone versus Norprene).

### 7.7 Flux profiles during the first phase of CFF using a peristaltic pump

When CFF was done with complete recycle of the retentate and permeate using a peristaltic pump for feed recirculation, the rate of flux decrease was affected by the feed volume and the flux profile exhibited an inflection point (see Sections 5.1 and 5.4). At the time of the experiments, we could not explain these results by any conventional theory of membrane fouling. For example, fouling by solute adsorption or pore occlusion is expected to depend on solution properties such as concentration, pH, ionic strength, temperature, etc., solute properties such as molecular weight, shape, surface charge and hydrophobicity, solute-membrane interaction parameters such as adsorption constants and on the hydrodynamic conditions above the membrane surface. When the feed volume is changed, none of these parameters is expected to be different and the rate of fouling is expected to be unchanged. As it turns out, the main reason for our inability to explain the experimental results at that time was our belief that the foulant was present in the original feed solution. Indeed, many of our early experiments were designed under the impression that BSA was the foulant. Now that we know the cause of fouling to be the convective deposition of aggregates generated in the feed during the recirculation, we can explain both the effect of feed volume as well as the occurrence of an inflection in the flux profile.
With complete recycle of the retentate and permeate, the concentration of aggregates in the feed solution increases continuously at a rate that depends on the average number of passes of the feed through the peristaltic pump in a given time. When the feed volume is changed keeping the recirculation rate the same, we are changing the frequency of passes of solution through the pump. Consequently, the rate of aggregate formation is different and this affects the fouling rate. Likewise, when the recirculation rate is changed keeping the feed volume the same, we once again alter the frequency of passes of solution through the peristaltic pump.

The inflection can be explained only if there are at least two competing factors that affect the rate of fouling. According to the convective deposition hypothesis, the rate of fouling is proportional to the product of the flux and the foulant concentration in the feed. If the foulant concentration is constant, the continuous decrease in flux would result in a monotonically decreasing rate of fouling and no inflection would be observed. This is the case during DEF or CFF with a diaphragm pump. However, when using a peristaltic pump for feed recirculation, the foulant (aggregate) concentration increases continuously. We now have two competing factors: (a) the decrease in flux tending to decrease the rate of deposition, and (b) the increase in feed concentration of foulant which tends to increase the rate of deposition. Depending on the relative magnitudes of the two effects, we observe an inflection in the flux profile during the first phase.

As justified earlier, we analyze the fouling rate during CFF with a peristaltic pump using flux equations developed for DEF but with a continuously increasing foulant concentration (see Section 6.7). When the rate of foulant generation is linearly related to the extent of pumping, we can use Equation...(6.49) which is reproduced below as Equation...(7.11) by expanding the rate constant for foulant generation in terms of the recirculation rate, \( F \), feed volume, \( V_f \), and the fraction, \( \beta_a \), of protein aggregated per pass through the pump. This yields an equation that clearly shows the influence of feed volume and recirculation rate on the flux profile.
\[
\ln \left[ \frac{J(t)}{J_0} \right] = -\frac{J_0(1-T_{pa})}{W_p N_T} \left( \beta_a C_f \frac{F}{2V_f} t^2 + C_{ao} t \right)
\]

\[\text{...}(7.11)\]

\(C_f\) is the total protein concentration in the feed and the other parameters are defined as before (see Equation...7.2). The equation assumes that \(V_f, F, \) and \(\beta_a\) are constant with time during the course of any particular filtration. The concentration of aggregates present in the feed at the start of the filtration \((C_{ao})\), determines the initial rate of fouling and hence, it cannot be ignored even when using a fresh solution that has not been exposed to the the peristaltic pump before the experiment. However, as the feed is recirculated, additional aggregates are generated and after sufficient time these aggregates far outnumber those initially present. The scatter in the quasi-elastic light scattering data made it difficult to get a reliable value of \(C_{ao}\); hence, we use it

\[\text{Figure 7.10 Flux profiles at different feed volumes during first phase of CFF with peristaltic pump (Data from Figure 5.7).}\]
Figure 7.11  Flux profiles at different recirculation rates during first phase of CFF with peristaltic pump (Data from Figure 5.10)

as the only adjustable parameter in describing the flux profiles.

Equation...(7.11) is validated using the experimental data from Figures 5.7 and 5.10 which are reproduced in Figures 7.10 and 7.11 respectively. Figure 7.10 represents the results of CFF obtained at a recirculation rate of $0.63 \times 10^{-6}$ m$^3$/s (38 ml/min) and at feed volumes ranging from $0.1 \times 10^{-3}$ to $2.0 \times 10^{-3}$ m$^3$ (100 to 2000 ml). The data in Figure 7.11 were obtained at a constant feed volume of $0.5 \times 10^{-3}$ m$^3$ (500 ml) and at recirculation rates varied between $0.63 \times 10^{-6}$ and $5.27 \times 10^{-6}$ m$^3$/s (38 to 316 ml/min). The dotted lines in Figures 7.10 and 7.11 represent the fit of Equation...(7.11) to the experimental data using $C_{ao}$ as the only adjustable parameter,
constrained to be non-negative. The values of the other parameters used for curve fitting are listed below.

\[
\begin{align*}
J_0 & = 2.8 \cdot 10^{-4} \text{ m/s (measured)} \\
W_p N_T & = 14.7 \cdot 10^{-6} \text{ kg/m}^2 \text{ (from } \Gamma^{125}\text{-BSA tracer studies)} \\
\beta_a & = 1.35 \cdot 10^{-5} \text{ (from QELS analysis)} \\
C_f & = 0.25 \text{ kg/m}^3 \text{ (measured)}
\end{align*}
\]

The average value of \( C_{ao} \) obtained from this analysis is \( 9 \cdot 10^{-6} \text{ kg/m}^3 \) (\( \pm 5.6 \cdot 10^{-6} \text{ kg/m}^3 \)) which compares favorably with the value of \( 6.5 \cdot 10^{-6} \text{ kg/m}^3 \) obtained earlier from the intercept in Figure 7.3. The fit is good to within \( \pm 15\% \) except for the case where the feed volume was \( 2 \cdot 10^{-3} \text{ m}^3 \). In the latter case, the average time (\( V_p/F \)) taken for the feed solution to make a single pass through the pump is 52 min; consequently, in the 90 min taken for the normalized flux to decrease to about 10\%, the feed solution has undergone less than 2 passes. It is unlikely that the value of \( \beta_a \) obtained by a linear fit to the QELS data collected over 320 passes, is an accurate description for the first 2 passes since the linearization of the rate of particle generation is only an approximation.

The solid lines represent the fit of Equation...(7.11) to the data when both \( C_{ao} \) and \( \beta_a \) are treated as adjustable parameters. The flux equation now describes all of the experimental profiles with less than 5\% error. The values of \( \beta_a \) and \( C_{ao} \) obtained by this analysis are tabulated in Table 7.3. The value of \( C_{ao} \) is between \( 3.4 \cdot 10^{-6} \text{ and } 9.5 \cdot 10^{-6} \text{ kg/m}^3 \) which is, once again, comparable to the value of \( 6.5 \cdot 10^{-6} \text{ kg/m}^3 \) estimated from the DEF experiments. The value of \( \beta_a \) is between \( 0.9 \cdot 10^{-5} \text{ and } 2.7 \cdot 10^{-5} \text{ which is comparable to the value of } 1.35 \cdot 10^{-5} \) obtained from the QELS analysis of the pumped solutions. The sole exception is the value of \( \beta_a \) (\( 9.9 \cdot 10^{-5} \)) obtained when the feed volume was \( 2 \cdot 10^{-3} \text{ m}^3 \) and the possible reason for this difference is the long turnover time of 52 min mentioned earlier.
Table 7.3  Estimation of the rate of aggregation and the initial aggregate concentration during CFF with a peristaltic pump

<table>
<thead>
<tr>
<th>F (x 10^6 m^3/s)</th>
<th>Vf (x 10^4 m^3)</th>
<th>Ca0 (x 10^6 kg/m^3)</th>
<th>b_a (x 10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.63</td>
<td>1</td>
<td>7.0</td>
<td>1.6</td>
</tr>
<tr>
<td>0.63</td>
<td>5</td>
<td>9.5</td>
<td>2.7</td>
</tr>
<tr>
<td>0.63</td>
<td>20</td>
<td>3.4</td>
<td>9.9</td>
</tr>
<tr>
<td>1.77</td>
<td>5</td>
<td>7.1</td>
<td>1.5</td>
</tr>
<tr>
<td>5.27</td>
<td>5</td>
<td>6.2</td>
<td>0.9</td>
</tr>
</tbody>
</table>

7.8  Flux profiles during the second phase of filtration

The flux profile during the second phase is given by Equation...(6.42) which is rewritten as Equation...(7.12) in terms of the normalized flux and the mass of aggregates deposited on the membrane surface.

\[
\frac{J_0}{J(\theta)} = \frac{R_B}{R_0} + \frac{\rho_a}{R_0} W_a(\theta) \quad \ldots(7.12)
\]

where,
\(\frac{J(\theta)}{J_0}\) = normalized flux at time \(\theta\) (where, \(\theta = t - t^*\))
\(\frac{R_B}{R_0}\) = ratio of resistances of occluded and open pores
\(\rho_a\) = specific resistance of aggregate layer (m/kg)
\(W_a\) = weight of aggregates deposited during second phase (= \(C_{al} l_a\)) (kg/m^2)

This equation may be used to analyze the experimental data in Figure 5.32 for the amount of
aggregates deposited on the membrane surface during the second phase. Figure 7.12 shows the reciprocal of the normalized flux as a function of the extent of deposition that was measured using $^{125}$-BSA as a tracer. The slope of a linear fit to the data is a measure of $\frac{\rho_a}{R_o}$.

$$\frac{\rho_a}{R_o} = 2 \times 10^3 \text{ kg/m}^2$$

The resistance of the open pores, $R_o$, is calculated from Equation...(6.1) to be $2.4 \times 10^{10} \text{ m}^{-1}$ using the measured values of the initial flux ($2.8 \times 10^{-4} \text{ m/s}$) and trans-membrane pressure (6.7 kPa) and assuming that the viscosity of the dilute protein solution is the same as that of water ($10^{-3} \text{ kg/m} \cdot \text{s}$). We thus get an order of magnitude estimate of the specific 'cake' resistance to be $10^{13} \text{ m/kg}$. An independent estimate of $\rho_a$ may be obtained by assuming that the aggregates

![Diagram](image)

**Figure 7.12** Correlation between aggregate deposition and normalized flux during the second phase of filtration (Data from Figure 5.30).
are hard spheres and that the flow through the 'cake' can be described by the Kozeny-Carman equation given in Equation... (6.43). In order to relate the void volume fraction, $\varepsilon$, to the particle concentration, $C_{al}$, in the 'cake' using Equation... (6.44), we need an estimate of the specific volume (or density) of the aggregates. If we assume that this density is bounded by that of water (1000 kg/m$^3$) and that of BSA (1340 kg/m$^3$), we can relate $\varepsilon$ to $C_{al}$, as shown below.

\[
1000 (1 - \varepsilon) < C_{al} < 1340 (1 - \varepsilon)
\] ... (7.13)

Since the exact value is not known, we choose the mid-point of this range to be representative of $C_{al}$. The specific resistance, $\rho_a$, is thus a function of only the diameter of the aggregates and

---

**Figure 7.13** Specific resistance of a packed bed of aggregates predicted by the Kozeny-Carman equation
the void volume fraction. This relationship is shown graphically in Figure 7.13. It is clear that for the range of particle sizes and void volumes shown, the specific resistance is of the order \((10^{13} \text{ m/kg})\) estimated earlier. The absence of a precise measurement of \(C_{al}\) and particle diameter and the considerable scatter in the data representing the weight of aggregates deposited on the membrane makes it impossible to get better than an order of magnitude precision in \(\rho_a\).

To get a rough estimate of the 'cake' thickness, let us assume that the void fraction is 0.8, so that \(C_{al}\) is about 230 kg/m\(^3\). Note that this value is less than the gel concentration of BSA, 350-500 kg/m\(^3\), which we assume to be the upper bound for \(C_{al}\). This upper bound on \(C_{al}\) gives us the corresponding lower bound on \(\varepsilon\) as 0.7-0.6. When the weight of aggregates contained in the 'cake' is 40 mg/m\(^2\) (see Figure 5.32), the 'cake' thickness is about 0.2 \(\mu\text{m}\).

The lack of precision in the estimates for the various parameters makes it difficult to predict the flux profiles during the second phase. We can, however, verify whether these profiles are of the form suggested by the flux equations developed in Chapter 6. For constant aggregate concentration (dead-end filtration), the reciprocal of the normalized flux is expected to be linearly related to the square root of the elapsed time according to Equation...(6.42), that is,

\[
\frac{J_0}{J(t)} \propto \sqrt{t - t^*}
\]

...(7.14)

The experimental data from Figure 5.19 for the case where the feed solution was pre-pumped 10 and 20 times with a peristaltic pump are used for validating Equation...(7.14). We have considered only these two cases because the experiments were carried out long enough to obtain sufficient flux data for \(t \gg t^*\). A linear fit through each set of data shows that our hypothesis of a 'cake' model to describe the flux decrease during the second phase \((t \gg t^*)\) is reasonable. The intercepts of the flux profiles shown in Figure 7.14 provide an estimate for the ratio, \(R_B/R_0\), of the resistances of the occluded and open pores. In these two experiments,
Figure 7.14  
Validation of the flux equation for the second phase of DEF 
(Data from Figure 5.19)

this ratio is about 10. Using Equation...(6.18) and substituting for $k_B$ from Equation...(7.6), we can now evaluate the time, $t^{*0.5}$, at which about $50\%$ of the total flux occurred through the blocked pores.

\[
t^{*0.5} \text{ (min)} = \frac{2.4}{0.00745 + 0.00373 \left( N_{\text{pump}} \right)} \quad \cdots(7.15)
\]

The results for $1 \leq N_{\text{pump}} \leq 20$ corresponding to the experimental filtration profiles shown in Figure 5.19 are tabulated below.
Table 7.4  Duration of first phase of filtration ($t^{0.5}$)

<table>
<thead>
<tr>
<th>$N_{pump}$</th>
<th>$t^{0.5}$ (min)</th>
<th>$N_{pump}$</th>
<th>$t^{0.5}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>214</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>161</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>129</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>107</td>
<td>20</td>
<td>29</td>
</tr>
</tbody>
</table>

The effect of operating conditions such as trans-membrane pressure and pH on the flux during the second phase is difficult to predict because their effect on parameters such as the specific resistance is not known. For example, if the aggregate layer is compressible, then the specific resistance, $\rho_a$, would be greater at higher pressures. If we assume that $\rho_a \propto (\Delta P)^n$, the flux during the second phase, given by Equation... (6.40), can be expressed as:

$$J(t) \approx \frac{\Delta P}{\mu \left\{ R_B + K_2 (\Delta P)^n W_a(t) \right\}} \quad \cdots (7.16)$$

This equation shows that for $n > 0$, the sensitivity of flux to changes in pressure decreases with time (see Section 5.18.3 and 5.19). As long as $0 < n < 1$, flux will increase when the pressure is increased, but it is conceivable that for highly compressible layers ($n > 1$), an increase in pressure may actually decrease the flux. A similar analysis applies to the effect of pH on flux. In this case, we would expect the specific resistance to show a maximum at the isoelectric pH where because of the minimum electrostatic repulsion, the void volume fraction, $\varepsilon$, would be at its minimum (for a given particle size distribution). Unfortunately, we do not have data on the dependence of $\varepsilon$ on pH. If as a first approximation, we assume that the flux during the second phase is inversely proportional to $\rho_a$ and that changing the pH only affects $\rho_a$, then from the data in Figure 5.48 we have:
\[
\frac{J_{4.8}}{J_{7.0}} \propto \frac{\{\rho_a\}_{7.0}}{\{\rho_a\}_{4.8}} \propto \left(\frac{\varepsilon_{4.8}}{\varepsilon_{7.0}}\right)^3 = 0.64
\]

which implies that the void fraction at pH 4.8 is about 86% of the void fraction at pH 7.

7.9 Analysis of protein transmission (pseudo steady state)

7.9.1 First phase:

Even complete removal of aggregates, which constitute less than 0.5% (wt./wt.) of total protein, does not cause an appreciable change in measured protein concentration. Consequently, the observed transmission essentially refers to that of non-aggregated BSA. Our analysis has shown that most of the flux during the first phase occurs through open pores through which the true transmission of non-aggregated BSA is 100%. Hence, during the first phase, the observed transmission also is 100% and there is no concentration polarization of non-aggregated BSA as confirmed by the absence of an effect of cross-flow velocity on transmission.

7.9.2 Second phase:

Like our analysis of flux, we do not consider the intermediate region where open and occluded pores both contribute significantly to the flow of permeate. During CFF, the decrease in transmission beginning about \( t = t^* \), can be evaluated if we know the fraction of flow occurring through the blocked pores and the true transmission coefficient for BSA passing through the blocked pores. Since, neither of these quantities is known, we only consider the transmission behavior well into the second phase. At sufficiently large times, \( t > t^* \), we assume that all pores are occluded and the permeate flow occurs through the occluded pores and the porous 'cake' that forms above them. Partial rejection of non-aggregated BSA by the fouled membrane results in concentration polarization as evident from the effect of cross-flow
velocity on observed transmission (see Section 6.8 for further details). We now proceed to validate Equation...(6.67) which relates the observed transmission, $T_o$, to the flux, $J$, and cross-flow velocity, $u$.

\[
\ln \left[ \frac{1}{T_o} - 1 \right] = \ln \left[ \frac{1}{T_m T_l} - 1 \right] - K_3 \frac{J}{u^{1/3}} \quad \ldots(7.17)
\]

where,
- $T_o$ = observed transmission
- $T_m T_l$ = true transmission across fouled membrane
- $u$ = average cross-flow velocity (m/s)
- $J$ = permeate flux (m/s)

The constant $K_3$ depends on channel geometry, diffusivity of non-aggregated BSA, and the constants associated with the equation for mass transfer coefficient. Equation...(7.17) is not predictive because we do not have an apriori estimate of the true transmission, $T_m T_l$, through the fouled membrane.

The experimental 'pseudo steady state' transmission data from Figure 5.47 are reproduced in Figure 7.15 as a semi-logarithmic plot of $[1/T_o - 1]$ versus $u^{-1/3}$ and a linear fit describes the profile as suggested by Equation...(7.17). This validates our hypothesis that BSA transmission during the second phase is governed by concentration polarization. From the intercept at $u^{-1/3} = 0$, we estimate the true transmission through the fouled membrane to be about 30%. As mentioned earlier, the observation that changes in cross-flow velocity caused changes in $T_o$ without affecting flux is a reflection of the fact that the measured transmission relates to non-aggregated BSA whereas the flux is governed by the deposition (perhaps irreversible) of aggregated BSA. We find that in the limit as cross-flow velocity goes to zero (i.e., during dead-end filtration), the observed transmission tends to unity; this was indeed observed in all of the dead-end filtration experiments with BSA and track-etch membranes.
Figure 7.15 Effect of cross-flow velocity on observed BSA transmission (Data from Figure 5.47)

These observations imply that BSA concentration at the surface of the fouled membrane must be less than its maximum value (gel concentration or solubility limit). Only then can a decrease in cross-flow velocity cause an increase in observed transmission by increasing the surface BSA concentration. Since concentration polarization of non-aggregated BSA had little effect on flux, we expect its concentration at the fouled membrane surface to be low enough for osmotic effects to be insignificant (e.g., at 10 kg/m³, π ≈ 0.5 kPa ≪ 6.7 - 20 kPa ΔP).

An alternative approach to validating Equation...(7.11) is to keep the cross-flow velocity, u, the same and vary the flux, J, by changing the trans-membrane pressure. The effect on transmission of cycling the pressure between 6.7 and 20 kPa and the hysteresis observed when
the pressure was first increased and then decreased was discussed in Section 5.19. In order to validate Equation ...(7.11), we only use the data from the experiment where a hysteresis was not observed. When the transmission data from the lower part of Figure 5.46 are plotted as \( \ln(1/T_0 - 1) \) versus the flux, \( J \), we find that the profile deviates from linearity (see Figure 7.16) although the expected trend of a higher transmission at higher flux is confirmed. This deviation from linearity suggests that pressure has an effect on some other parameter in Equation ...(7.11) in addition to its effect on flux. We speculate that the true transmission, \( T_m T_h \), of non-aggregated BSA through the fouled membrane is affected by pressure due to a compression of the porous 'cake'.

![Diagram](image-url)

**Figure 7.16** Validation of the transmission equation by changing the flux at constant cross-flow velocity (Data from Figures 5.46)
The effect of pH on observed transmission during the second phase (see Figure 5.48) is similarly explained by changes in flux, J, and true transmission, $T_mT_1$. The pseudo steady state values of flux and transmission at pH 4.8 and pH 7 are summarized below:

<table>
<thead>
<tr>
<th>pH</th>
<th>J / $J_0$</th>
<th>$T_o$</th>
<th>$-\ln \left[ 1/T_0 - 1 \right]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>0.026</td>
<td>0.62</td>
<td>0.49</td>
</tr>
<tr>
<td>7.0</td>
<td>0.039</td>
<td>0.70</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Since we have only two sets of data, it is difficult to draw quantitative conclusions about changes in $T_mT_1$. However, we expect the void fraction of the deposited aggregates to be lower at the isoelectric pH of 4.8 because of the lower electrostatic repulsion. Consequently, compared to pH 7.0, the true transmission, $T_mT_1$, is expected to be lower at pH 4.8.

7.10 Estimation of fouling capacity of Ultipor and Durapore membranes

CFF experiments with 0.22 μm Durapore and 0.45 μm Ultipor membranes using a peristaltic pump for feed recirculation had yielded flux profiles qualitatively similar to those obtained with the track-etch membranes (see Figures 5.60 and 5.61). In order to predict the flux profiles using Equation...(7.11) we need an estimate of $W_pN_T$ for each membrane. Since we had not carried out the $^{125}$-BSA tracer experiments with these membranes, we did not have apriori estimates of this quantity. Therefore, we decided to fit Equation...(7.11) to the experimental flux profiles and estimate $W_pN_T$ from this fit. The initial flux, $J_0$, was $4.4 \times 10^{-4}$ m/s for the Ultipor membrane and $1.8 \times 10^{-4}$ m/s for the Durapore membrane. The other parameters, common to experiments with both membranes, are shown below:

- $\beta_a = 1.35 \times 10^{-5}$ (from QELS data)
- $C_f = 0.25$ kg/m$^3$ (measured)
- $F = 3.33 \times 10^{-6}$ m$^3$/s (measured)
- $V_f = 5 \times 10^{-4}$ m$^3$ (measured)
- $C_{ao} = 6.5 \times 10^{-6}$ kg/m$^3$ (assumed equal to value estimated from DEF)
Figure 7.17  Estimation of fouling capacity, \( W_p N_T \), for the Ultipor and Durapore membranes using the flux equation

Shown in Figure 7.17 are the experimental data from Section 5.25 along with the dotted lines which represent the best fit of Equation...(7.11) to these data using the parameter values stated above. This fit was obtained using only one adjustable parameter, namely, \( W_p N_T \), and as can be seen from the plot, the deviation of the data from the fit is no more than 5%. The values of \( W_p N_T \) estimated in this manner are shown below:

<table>
<thead>
<tr>
<th>Membrane</th>
<th>( W_p N_T ) (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45 ( \mu )m Ultipor</td>
<td>560</td>
</tr>
<tr>
<td>0.22 ( \mu )m Durapore</td>
<td>700</td>
</tr>
<tr>
<td>0.2 ( \mu )m Nuclepore</td>
<td>15  (from ( I^{125} )-BSA tracer expts.)</td>
</tr>
</tbody>
</table>
The values of $W_p N_T$ estimated above represent the upper limits since we have implicitly assumed that $T_{oa} \ll 1$, similar to the assumption made with the track-etch membranes. If, for example, $T_{oa}$ is 0.4, then the values of $W_p N_T$ for the Ultipor and Durapore membranes would be about 340 and 420 mg/m$^2$ respectively. The Ultipor and Durapore membranes require much more foulant to completely block the membrane compared to the 0.2 $\mu$m Nuclepore track-etch membrane. This is to be expected from the higher porosity of 80-85% compared to 10% for the track-etch membranes. Furthermore, we expect the foulant deposition to occur not only at the pore entrance but also within the membrane because of their inter-connected, tortuous pore structure in contrast to the cylindrical, straight through pores of the track-etch membranes.
Chapter 8 Conclusions and Significance

This research project was initiated with the intent of better understanding the factors that affect the performance of cross-flow microfiltration for the separation of proteins from cells or cell debris. Hence, the scope of the project was constrained to membranes which would readily permit the passage of proteins through their pores. Preliminary cross-flow experiments with protein solutions in the absence of any cells or cell debris revealed an order of magnitude decrease in flux within a few minutes. We, therefore, initiated a detailed investigation of this phenomenon and found that the precise rate and extent of flux decrease depended on several operating conditions such as the feed flow rate, feed volume, trans-membrane pressure, concentration, pH, and membrane geometry. Attempts to explain the flux decrease and changes in observed transmission in terms of size exclusion or uniform adsorption of the original protein were unsuccessful. A systematic investigation using bovine serum albumin (BSA) as the model protein and Nuclepore, track-etch, polycarbonate membranes as the model filters led us to an understanding of the cause of fouling under our experimental conditions. The results using the model system qualitatively agreed with the results with other proteins (ribonuclease, lysozyme) and membranes (PVDF Durapore, nylon Ultipor) that were investigated. The final conclusions of our investigation are summarized below:

Aggregates were generated by the repeated recirculation of protein solution with a peristaltic pump.

A steady increase in particle (>30 nm) concentration was detected by quasi-elastic light scattering (QELS) analysis of BSA solution recirculated with a peristaltic pump. However, these particles constituted less than 0.5% (wt./wt.) of the total BSA even after 300 passes through the pump and changes in solution composition were not detected by chromatographic and electrophoretic analyses.

The rate of particle generation was greater at pH 4.8 (isoelectric point of BSA) than at pH 7.3.
Native-PAGE analysis of the material deposited on the membrane surface revealed the presence of a protein component too large to enter the separation zone of the gel, i.e., greater than 700 kDaltons. This component could be visualized by Coomassie staining and hydrolyzed by trypsin. We anticipate that this component corresponds to the particles deposited on the membrane during filtration.

When pre-heated BSA solutions, that had never been subjected to pumping, were filtered and the deposited material analyzed by native-PAGE, a similar large component was observed. Therefore, the large component could not be a contaminant introduced during pumping.

SDS-PAGE analysis of the large component (after boiling in 5% SDS and 5% β-mercaptoethanol for 40 min) showed a single band corresponding to monomer BSA indicating that it was an aggregate of BSA.

*Deposition of the protein aggregates on the membrane during filtration caused the observed flux decrease.*

- Contacting the fouled membrane with a non-specific protease solution caused an immediate recovery of flux suggesting that the foulant was proteinaceous. Such a recovery was not observed upon treating the membrane with saline or 10% ethanol solution.

During the filtration of fresh protein solutions (i.e., not pre-heated or pre-pumped), rapid fouling was observed only when a peristaltic pump was used for feed recirculation. The rate of flux decrease during dead-end filtration from a pressurized feed reservoir or cross-flow filtration using a diaphragm pump was 10- to 100-fold slower.

Reducing the particle (aggregate) concentration in the feed by dilution, centrifugation or pre-filtration reduced the rate of flux decrease.

*The aggregates probably deposit in the entrance region of the pores.*

Scanning electron micrographs showed a progressive deposition of foulant on the feed side of the membrane but no visible difference in membrane geometry when viewed
from the permeate side.

Forward flushing with buffer did not cause any flux recovery. Backflushing caused an almost instantaneous recovery of flux from 5-10% to 70-80% of the initial value.

*The rate of flux decrease correlated well with the rate of convective deposition of aggregates.*

Convective deposition rate \( = (\text{Flux}) \cdot (\text{aggregate concentration in feed}) \)

No significant change in membrane permeability was observed when diffusion to the membrane surface was the only mechanism of aggregate accumulation. Diffusive deposition was simulated either by soaking the membranes in BSA solution or by recirculating the solution through the cross-flow device with permeate exit port closed to prevent convective flow through the membrane. Saline flux was used as a measure of permeability.

Faster fouling was observed at higher absolute values of flux obtained by changing the trans-membrane pressure and with a higher feed concentration of aggregates.

An inflection in the flux profile observed only during cross-flow filtration with a peristaltic pump can be explained by the competition between a decrease in flux trying to lower the deposition rate and an increase in aggregate concentration trying to increase the deposition rate. During dead-end filtration or cross-flow with a diaphragm pump, the aggregate concentration does not change with time and no inflection is observed.

*A transition in the flux and transmission profiles after the decrease in flux can be explained by a change in the relative importance of flow through open and occluded pores.*

At the beginning of filtration, flux was insensitive to pH and proportionally to pressure. Since the open pore resistance does not vary with pH or pressure, most of the permeate flow was assumed to occur through the open pores. Likewise, BSA (non-aggregated) transmission was 100% and not affected by cross-flow velocity. This indicates an absence of concentration polarization as expected from the relative sizes of BSA (14 nm x 4 nm; prolate ellipsoid) and the open pores (diameter: 200 nm).

After rapid fouling, the flux and transmission were lower at the isoelectric point.
Transmission was affected by cross-flow velocity and the sensitivity of flux to pressure decreased. These results indicate that most of the flow was occurring through occluded pores or through a porous 'cake' above the occluded pores. SEMs of the membrane surface revealed a completely covered surface.

The first phase of flux decrease was well described by an equation based on the convective deposition of aggregates leading to a decrease in the number of open pores.

When the aggregate concentration does not change during filtration, the equation predicts an exponential flux decrease and includes as independent parameters, the initial flux, the aggregate concentration in the feed, the transmission of aggregates through open pores and the amount of aggregates required to occlude all pores. For the model system, with the aggregate transmission assumed to be negligible and all other parameters measured independently, the equation predicted the flux decrease to within ±10%.

With a continuously increasing aggregate concentration, the equation predicts a sigmoidal flux profile and in addition to the parameters mentioned above, it requires the rate of aggregate generation. For the model system, with the aggregate transmission assumed to be negligible and using the initial concentration of aggregates as the only adjustable parameter, the equation describes the flux profiles to within ± 15% in most cases. The major source of error is in the rate of aggregate generation by the peristaltic pump. If this is used as a second adjustable parameter, the model describes all of the experimental results to within 3-5%.

In order to test the equations with other membranes and proteins, one needs values for the amount of aggregates required to occlude all the pores and the rate of aggregate formation upon pumping for each protein-membrane combination. These values were not determined independently; however, the equation qualitatively describes these flux profiles as well.

The second phase of flux decrease is described by an equation based on the formation of a porous 'cake' above the membrane surface.
This equation lacks predictive capability because the values of several parameters are not known. These include the concentration of aggregates at the membrane surface, the specific resistance of the porous 'cake', and the sizes of the aggregates causing the flux decrease. Secondly, the equation applies only well into the second phase where all of the open pores can be considered to be completely blocked. Limited tests of the equation for the case of constant aggregate concentration in the feed (dead-end filtration) showed that the flux decreases inversely with the square root of time.

*Transmission of non-aggregated BSA during the second phase is well described by the conventional concentration polarization equation.*

As mentioned earlier, transmission of non-aggregated protein during the first phase was 100% and there was no concentration polarization. This represented the trivial case of protein transport through open pores which are much larger than the protein.

During the second phase, partial rejection of BSA by the fouled membrane leads to concentration polarization. The observed transmission is now determined by the true transmission through the fouled membrane and by the hydrodynamics above the membrane surface which alters the surface concentration of non-aggregated BSA. The standard concentration polarization equation, rearranged to give an explicit expression for observed transmission, correctly described the effect of cross-flow velocity.

The equation also predicts that the observed transmission should increase with flux; this too was observed experimentally, although not to the extent predicted by the equation probably because of the effect of pressure on the true transmission through the fouled membrane. (Pressure was changed to alter the flux).

*During the second phase, the flux was determined by the deposited aggregates and not by the concentration polarization of non-aggregated BSA.*

When the protein solution was replaced with a saline solution and the flux through the fouled membrane measured, there was no increase in flux although all the non-aggregated protein was washed away.
The true transmission of BSA through the fouled membrane was estimated in one experiment to be about 30%. Since the observed transmission cannot exceed 100%, the concentration of non-aggregated BSA at the membrane surface will be at most 3.3 times the feed concentration of 0.25 kg/m³ BSA. Thus, the surface concentration is well below the gel concentration (350-500 kg/m³) and well below the level at which osmotic effects become important (e.g., at 10 kg/m³, $\pi = 0.5$ kPa $\ll \Delta P$ of 6.7 - 20 kPa).

**Significance**

The most significant result of this research project in terms of understanding the fouling of microporous membranes is that the source of the problem can be in any of the upstream unit operations that precede filtration or in the peripheral equipment associated with the membrane separation unit. Our work re-emphasizes the recent realization that the artificial division of a process into upstream and downstream processes can thwart the optimal design and choice of equipment especially when the performance of one unit is closely linked to the output of the other. For example, in our experiments we demonstrated that pore occlusion by protein aggregates generated during the repeated recirculation of feed solution with a peristaltic pump was the cause of the observed flux decrease. We also observed similar results when the aggregates were generated by pre-heating the protein solution. We may extrapolate these observations to include any potential cause of protein denaturation and aggregation such as aeration, agitation, centrifugation, heat sterilization, local extremes in pH due to poor mixing during acid or base addition, etc., as a potential source of fouling problems during membrane separations. The first three operations listed involve exposure of the protein solution to high shear fields in the presence of air-liquid interfaces and are therefore, prime candidates for causing protein denaturation and aggregation. The loss of native protein may be less than 1% as in our experiments and hence, not detectable by using enzyme assays, but it may be sufficient to block all the membrane pores.
Although all of our experiments were done with microporous membranes, the pore occlusion mechanism of fouling may also apply to ultrafiltration, where the membranes are not completely retentive. For example, when ultrafiltration is to be used for the fractionation of a mixture of proteins, some of the proteins are expected to pass through the pores. Proteins or aggregates that are lodged within the pore, albeit merely at the pore entrance, do not experience the full force of the shear fields generated by the cross-flow in the feed channel and hence, are difficult to remove by increasing the cross-flow velocity. One has to resort to backflushing or chemical cleaning techniques to recover the membrane performance. In this context, it may be advisable to use completely retentive membranes when concentration and not fractionation or purification is the goal. For example, when the objective is to concentrate cells and not separate them from any extracellular macromolecules, smaller pore sized membranes which prevent any macromolecular species from entering the pores may give a steadier flux performance over long times although the absolute value of flux may be lower.

Although we did not investigate the mechanism of pore occlusion, it was evident that aggregates that passed through the membrane were capable of occluding the pores of a similar membrane in a subsequent filtration. This suggests that size exclusion of the aggregates was not the sole cause of occlusion. Membranes which have a strong attractive interaction potential for proteins would have similar interactions with aggregates and this would facilitate both pore occlusion and internal adsorption. Hence, although our research indicated that adsorption of native protein to the pores was not the major cause of fouling at least in our system, it is preferable to minimize aggregate-membrane interactions by using hydrophilic, low protein binding membranes.
Chapter 9. Recommendations for future work

This research project has demonstrated the importance of protein aggregation in the fouling of microporous membranes. Future work in the area of membrane separations must account for interferences from aggregates or other particulate material even when their concentration in the feed is very low. Selective rejection of these components can, over time, lead to their accumulation within the pores or on the membrane surface and affect both flux and solute transmission through the membrane. Since size exclusion need not be the only mechanism of rejection, care must be taken to minimize possible interactions between the feed components and the membrane material. We speculate that the faster fouling of 0.45 μm Ultipor membranes compared to 0.22 μm Durapore membranes (see Figure 7.17) when both were challenged by the same feed solution, is because of the greater interactions of BSA and its aggregates with the more hydrophobic, nylon surface of the former in contrast to the low protein binding, polyvinylidene difluoride (PVDF) material of the latter. Note that these interactions are indirectly accounted for in the convective deposition flux model by the transmission term, $T_{OA}$, which is a measure of the ease of passage of the foulant through the membrane. If strong interactions lead to accumulation of foulant within or on the membrane, the value of $T_{OA}$ will be less. However, due to the complexity of the fouling process and our inability to characterize the surface interactions between aggregates and the membrane material, we did not evaluate $T_{OA}$ from first principles. Future work in the area of measuring or theoretically predicting the magnitude of foulant-membrane interactions would be very useful in providing a quantitative basis for the selection of membranes based on material properties and not just on pore size or a vaguely defined concept of 'hydrophobicity'.

Although this research project has successfully met the overall objective of understanding the reason for the flux decrease when pure protein solutions were subjected to cross-flow filtration through microporous membranes, it has created an awareness of the serious lack of
understanding in an apparently unrelated area, namely, the denaturation and aggregation of proteins during pumping. Our focus was on membrane fouling and we did not fully address the issue of protein aggregation during recirculation with a peristaltic pump. We suspect that the denaturation/aggregation of protein in the tubing of the peristaltic pump is a shear related interfacial effect caused by the narrow gap widths formed when the tubing is compressed by the rotating rollers in the pump-head. We discuss this issue in greater detail in Appendix A where, some experiments that provide preliminary clues to the possible mechanism of aggregation are presented with the intention of providing future researchers with a starting point for a more thorough investigation. Understanding the mechanism of aggregation caused by the peristaltic action along with a knowledge of protein-protein interactions would perhaps enable the apriori estimation of the fraction, $\beta_a$, of protein aggregated per pass through the pump. In our work, we obtained $\beta_a$ by a linear fit to the data obtained by quasi-elastic light scattering analysis of pumped protein solutions under a limited set of operating conditions. $\beta_a$ is expected to be different for different proteins, perhaps even for the same protein obtained from different sources, and also a function of solution properties such as concentration, pH, ionic strength, temperature, etc. Its experimental determination would involve an enormous amount of work and in some cases here we resorted to using $\beta_a$ as an adjustable parameter.

We have seen that the term, $W_pN_T$, that appears in our flux models is a measure of the amount of foulant required to completely occlude all the pores per unit area of the membrane. This may be viewed upon as a measure of the fouling potential of the membrane. Those membranes with a higher value of $W_pN_T$ with respect to a given foulant, will foul at a slower rate. We estimated this fouling potential for the Ultipor and Durapore membranes by fitting the flux model to the experimental flux profiles. It would be useful to validate these results by extending our experiments with $^{125}\text{I}$-BSA as a tracer to quantify the amount of protein bound to these membranes as a function of the normalized flux. We did such experiments only with the $0.2 \mu m$ track-etch membranes (see Section 5.15).
The flux model that we developed is a first attempt to explain the observed flux decrease; hence, several simplifying assumptions were made. We were, in fact, pleasantly surprised by how well the model predicted the flux decrease during filtrations with a constant aggregate concentration in the feed and how well it described the flux profiles when the aggregate concentration was increasing with time due to continuous recirculation of the feed by a peristaltic pump. However, there is ample scope for improving the flux model and some suggestions are given below:

1) **Incorporate partially blocked pores**: For simplicity, we assumed that the membrane pores were either 'open' or 'blocked' and that the transition from the former to the latter state occurred instantaneously for any given pore. One could extend the model to include the possibility of partially blocked pores whose resistance increases continuously with time rather than as a step change from $r_0$ to $r_B$ as shown in Figure 6.1. This would, however, necessitate the estimation of the geometry of partially blocked pores and relating the changes in geometry to the hydrodynamic resistance. In the literature on membrane fouling by protein adsorption, it is commonly assumed that the pore diameter decreases continuously as a consequence of uniform adsorption along the entire pore length. Such a description might be appropriate if the pores are distinct, straight through entities (e.g., for track-etch membranes) but it will be a gross approximation when the membranes have a network of tortuous, inter-connected pores. Indeed, with such membranes it is difficult to define what constitutes a single pore. One option to overcome this limitation would be to treat the membrane as a porous bed in which the void fraction continuously decreases because of solute adsorption.

2) **Incorporate particle size distribution**: Again for simplicity and because of the lack of reliable size distribution data, we treated the aggregates as one component with an average set of properties such as concentration, $C_{af}$, transmission coefficient, $T_{oa}$, diffusivity, $D_a$, etc. Since we were aware that aggregates were not being generated during dead-end filtration, we treated $C_{af}$ as a constant. However, even when the total aggregate concentration is constant, the effective foulant concentration can change with time. For example, suppose we assume
that size exclusion is the only mechanism of particle rejection. At the beginning of the filtration, only those particles which are larger than the open pores will be rejected and contribute to the flux decrease. Hence, the initial foulant concentration is the concentration of these large particles. However, as the pores become partially blocked, some of the smaller particles also will be rejected, thus increasing the effective foulant concentration. We expect to find that a semi-logarithmic plot of the normalized flux versus time will no longer be linear even for dead-end filtration. In fact, the slight deviation from linearity seen in many of our results may be accounted for by the existence of a particle size distribution. An alternative approach to incorporating the particle size distribution is to define $C_{af}$ as the total aggregate concentration (as was done in this project), but to assume that $T_{oa}$ effectively decreases with time as fewer particles are transmitted because of progressive fouling.

3) **Incorporate changes in specific resistance**: We assumed that the second phase of fouling was characterized by the build-up of a porous 'cake'. We modelled the decrease in flux during the second phase as an increase in the thickness of the cake caused by particle deposition without any change in the specific resistance of the cake. This may be a reasonable assumption if the particles were hard spheres all of the same diameter. When there exists a particle size distribution, additional deposition may not only cause an increase in thickness but also a change in specific resistance due to the 'filling-in' of the void spaces in the cake by the smaller particles.

Even with a homogeneous particle population and in the absence of particle-particle or particle-membrane interactions, insufficient information exists on the foulant concentration in the polarized layer and the hydrodynamic resistance offered by the polarized layer. The modelling of even the steady-state flux during cross-flow filtration is complicated by the ambiguity surrounding the mechanism of removal of particles from the membrane surface. As mentioned in Chapter 3, different authors have taken different approaches to estimating the removal rate such as the use of a shear enhanced diffusivity (Zydney, 1985), tubular pinch effect (Altena and Belfort, 1984), and tangentially moving polarized layer (Romero and Davis,
1990). There is a need to verify these models using a variety of non-fouling, non-interacting (via electrostatic forces) particles of different shapes, deformability, and density. Perhaps, a first step may be to use a monodisperse suspension of latex beads which have been surface modified to neutralize any charges. Although such theoretical work would be of limited use in a practical situation such as separating proteins from cell debris where complex interactions among multiple solutes can make flux predictions extremely difficult, it would prove useful in at least evaluating the validity of the numerous, detailed hydrodynamic models that exist in the literature.

Our attempts to obtain thin sections (parallel to the surface) of the track-etch membranes using glass knives on the microtome were unsuccessful because of the low thickness (10 μm) and brittle nature of the material. We adopted the standard protocol used in obtaining thin sections of tissues and it involved fixing the deposited proteins with glutaraldehyde, rinsing with appropriate buffers, drying the membranes in a desiccator after successive rinsing with ethanol, and embedding the membrane in resin. It would be useful to obtain such sections of the track-etch membranes in order to verify whether the protein is indeed located near the pore entrance. The protein may be quantified using a technique such as autoradiography.

On a different note, our identification of the deposition of protein aggregates as the cause of flux decrease may help understand some unexplained results of Ethier (1986) who investigated the cause of the increase in intraocular pressure (i.e., pressure within the eye). It is believed that this increase can damage the optic nerve and cause blindness in glaucoma patients and is usually attributed to the greater resistance to the outflow of aqueous humor which is the fluid that nourishes the lens and cornea. Aqueous outflow occurs through a porous tissue called the juxtacanalicular meshwork which Ethier sought to model as a microporous membrane. An order of magnitude increase in resistance was observed when aqueous humor was filtered at a constant flowrate through 0.2 μm Nuclepore, track-etch membranes. Since the known
molecular species in solution are much smaller than the pore size, size exclusion was not anticipated. So, Ethier attempted to explain the increased resistance in terms of solute adsorption to the membrane but was not very successful in describing the results quantitatively using models based on in-pore as well as surface blockage. It also was observed that freshly collected calf serum and plasma did not block the membranes. Based on our results, we hypothesize that small amounts of protein aggregates present in the aqueous humor but not in the fresh plasma or serum may be responsible for the observed fouling. It would be extremely useful to verify this hypothesis by electrophoretic analysis of material collected on the filter surface and quasi-elastic light scattering analysis of the solutions. Direct analysis of the aqueous humor using chromatographic or electrophoretic techniques may not indicate the presence of aggregates if their concentration is very low as was the case in our experiments. If such aggregates are detected, it would be interesting to determine their source; unfortunately, we do not know enough about the biology of the eye and the perfusion system to speculate about the possible causes of aggregation in vivo.
NOMENCLATURE

(Only important symbols used in modelling flux and transmission are listed)

\( \beta_a \) : foulant generation (as a fraction of total protein) per pass through the peristaltic pump

\( \Delta P \) : trans-membrane pressure (Pa)

\( \varepsilon \) : void volume fraction

\( \phi \) : fraction of pores which are open

\( \mu \) : solvent or solution viscosity (kg/m s)

\( \rho_a \) : specific 'cake' resistance (m/kg)

\( \theta \) : time variable for the second phase of fouling (= t - t*) (s)

\( C_f \) : total protein concentration in feed (kg/m\(^3\))

\( C_{af} \) : foulant (aggregate) concentration in feed (kg/m\(^3\))

\( C_{al} \) : foulant (aggregate) concentration in the cake at the membrane surface (kg/m\(^3\))

\( C_{ao} \) : foulant (aggregate) concentration in feed before pumping (kg/m\(^3\))

\( d_a \) : foulant (aggregate) diameter (m)

\( d_p \) : pore diameter (m)

\( D_a \) : foulant (aggregate) diffusivity (m\(^2\)/s)

\( f \) : fraction of total flow during first phase occurring through occluded pores

\( F \) : feed recirculation rate (m\(^3\)/s)

\( h \) : cross-flow channel height (m)

\( J \) : permeate flux at any time based on projected membrane area (m/s)

\( J_B \) : flux through occluded pores based on projected membrane area (m/s)

\( J_o \) : initial permeate flux based on projected membrane area (m/s)

\( J_w \) : constant term in second phase flux equation representing flux

\( k \) : mass transfer coefficient in the cross-flow channel (m/s)

\( k_a \) : zero\(^{th}\) order rate constant of foulant generation (kg/m\(^3\)/s)
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_B$</td>
<td>rate constant of pore occlusion ($s^{-1}$)</td>
</tr>
<tr>
<td>$l_a$</td>
<td>cake thickness (m)</td>
</tr>
<tr>
<td>$L$</td>
<td>length of flow channel in cross-flow device (m)</td>
</tr>
<tr>
<td>$L_a$</td>
<td>constant term in second phase flux equation representing rate of increase in cake thickness</td>
</tr>
<tr>
<td>$L_p$</td>
<td>pore length (m)</td>
</tr>
<tr>
<td>$N_{pump}$</td>
<td>number of passes through peristaltic pump</td>
</tr>
<tr>
<td>$N_T$</td>
<td>pore number density ($m^{-2}$)</td>
</tr>
<tr>
<td>$R_B$</td>
<td>resistance of occluded pores ($m^{-1}$)</td>
</tr>
<tr>
<td>$R_o$</td>
<td>resistance of open pores ($m^{-1}$)</td>
</tr>
<tr>
<td>$Re$</td>
<td>Reynolds number</td>
</tr>
<tr>
<td>$S_a$</td>
<td>specific volume of foulant (aggregates) ($m^3/kg$)</td>
</tr>
<tr>
<td>$Sc$</td>
<td>Schmidt number</td>
</tr>
<tr>
<td>$Sh$</td>
<td>Sherwood number</td>
</tr>
<tr>
<td>$t$</td>
<td>time of experiment (s)</td>
</tr>
<tr>
<td>$t^*$</td>
<td>time of transition from first to second phase of fouling (s)</td>
</tr>
<tr>
<td>$t^{*0.5}$</td>
<td>transition time where 50% of the flow occurs through blocked pores (s)</td>
</tr>
<tr>
<td>$T_l$</td>
<td>true transmission coefficient of non-aggregated protein through porous 'cake'.</td>
</tr>
<tr>
<td>$T_m$</td>
<td>true transmission coefficient of non-aggregated protein through occluded pores</td>
</tr>
<tr>
<td>$T_o$</td>
<td>observed transmission coefficient of non-aggregated protein</td>
</tr>
<tr>
<td>$T_{la}$</td>
<td>transmission coefficient of foulant (aggregates) through the fouled membrane</td>
</tr>
<tr>
<td>$T_{oa}$</td>
<td>transmission coefficient of foulant (aggregates) through open pores</td>
</tr>
<tr>
<td>$u$</td>
<td>cross-flow velocity (m/s)</td>
</tr>
<tr>
<td>$V_f$</td>
<td>feed volume ($m^3$)</td>
</tr>
<tr>
<td>$W$</td>
<td>weight of foulant per unit projected area of membrane ($kg/m^2$)</td>
</tr>
<tr>
<td>$W_p$</td>
<td>weight of foulant (aggregates) required to occlude one pore (kg)</td>
</tr>
<tr>
<td>$y$</td>
<td>distance measured from the membrane surface into the feed solution (m)</td>
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</tbody>
</table>
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**Review Articles on Membrane Separations**


**Books on Membrane Separation Processes**

Bungay, P.M., Lonsdale, H.K., de Pinho, M.N. (eds.), 1986; *Synthetic Membranes*


Appendix A. Clues to the mechanism of aggregation by pumping

A steady increase in the concentration of particles, later identified to be protein aggregates, was observed when protein solution was recirculated with a peristaltic pump. The particles constituted less than 0.5% (wt./wt.) of total BSA when the solution consisted of 0.25 kg/m³ BSA in phosphate buffered saline (10 mM PO₄, 150 mM NaCl, 8 mM NaN₃, pH 7). We did not determine whether the aggregates were formed directly from the native monomer or from denatured protein already existing in the solution. We do know that this phenomenon was common to at least the three proteins investigated, namely, BSA, ribonuclease and lysozyme; hence, it cannot be attributed to specific contaminants in any particular protein sample. However, we cannot entirely rule out the possibility that aggregation is aided by trace components introduced into the system during pumping. The rate of aggregation was related to the action of the rollers in the peristaltic pump head and was unaffected by the axial flow rate or the pressure against which the pump acted (see Sections 5.9 and 5.10). When two pump heads were used in series, thus doubling the frequency of compression of the tubing, the rate of aggregation was also doubled although the operating conditions, configuration of the rest of the apparatus and total length of tubing were identical in both cases. The extent of aggregation per pass of BSA solution through the pump was greater at the isoelectric pH of 4.8 ($\beta_a = 2.5 \times 10^{-5}$) than at pH 7 ($\beta_a = 1.35 \times 10^{-5}$) (see Section 5.21).

There is considerable debate in the literature whether mechanical shear per se, can cause protein denaturation. However, more recent evidence suggests that shear probably acts in concert with interfacial effects to denature proteins (see Section 3.3). In our case, external air-liquid interfaces such as occurring in the feed reservoir could not be responsible for the observed aggregation which was prevented by merely replacing the peristaltic pump with a diaphragm pump. When the external interfaces were eliminated by filling the stoppered flask to the brim and expelling any air bubbles present in the tubing, there was no change in the rate of
Figure A.1 Schematic of peristaltic pump head depicting the compressive action of the rollers on the tubing
aggregation during pumping with a peristaltic pump (Figure 5.21). This leads us to hypothesize that the aggregation results from protein denaturation occurring in the peristaltic pump head shown schematically in Figure A.1.

Shown in the bottom half of Figure A.1 is an expanded view of the region of compression of the tubing. As the roller rotate, the tubing ahead of the rollers gets compressed whereas the tubing immediately preceding the roller is released. We suspected that this continuous compression and release of the tubing contributes to protein aggregation. Estimating the shear as a consequence of compression is difficult because we do not know the precise gap width formed, although for all practical purposes the tubing is assumed to be completely pinched. If we draw an analogy between the roller-tubing geometry and the slipper-pad bearing problem commonly studied in lubrication theory, we know that the pressure forces can be very large:

\[ \Delta P \propto \frac{\mu \omega R_f L}{H_0^2} \]  

...(A.1)

where,  
\( \Delta P = \) pressure force generated in the compressed tubing (kPa)  
\( \mu = \) solution viscosity (kg/m s)  
\( \omega = \) frequency of rotation (s\(^{-1}\))  
\( R_f = \) radius of the rotating assembly (m)  
\( L = \) characteristic dimension representing length of compressed region (m)  
\( H_0 = \) characteristic gap width (m)

The above relation shows that the pressure forces, ahead of the rollers, become enormously large when the gap width becomes small and we anticipate that the shear forces also become very large. We speculate that, in the region just preceding the point of compression, we can have the opposite trend, i.e., negative pressures and this can cause cavitation by the spontaneous formation of vapor or gas bubbles.
For comparison, when the recirculation rate in our system is about 200 ml/min, the shear in the feed channel of the cross-flow apparatus is about 2000 s\(^{-1}\), and the shear in the non-compressed tubing (0.31 cm i.d.) is about 1140 s\(^{-1}\). At trans-membrane pressures of 6.7 kPa, the initial flux through 0.2 μm track-etch membranes is about 2.8\(\times\)10\(^{-4}\) m/s. This corresponds to a pore velocity of 2.8\(\times\)10\(^{-3}\) m/s, a pore Reynolds number of 5.6\(\times\)10\(^{-4}\) and a wall shear rate of 112,000 s\(^{-1}\). Clearly, much higher shear rates exist within the pore although the Reynolds numbers are very low compared to the feed channel or uncompressed tubing. If mechanical shear alone is responsible for protein denaturation/aggregation most of which occurs in the peristaltic pump, then the shear in the pump would have to, at the very least, exceed that in the pore. However, we suspect that interfacial effects caused by small gap widths or cavitation phenomena are also involved in the observed denaturation/aggregation.

The effect of protein concentration on the rate of aggregation provides some clue whether the limiting step in the process is the initial denaturation of protein or the association of denatured molecules to form aggregates. If the latter were true, one would expect the rate to be at least second order in protein concentration unless the aggregates are broken up by shear. For want of more information on the possible break-up of the aggregates, let us assume that the process is much slower than the aggregation and hence, can be ignored. Solutions of BSA in phosphate buffered saline (10mM PO\(_4\), 150mM NaCl, 0.05% NaN\(_3\), pH 7.3) were prepared at concentrations of 0.25, 1.0 and 2.5 kg/m\(^3\). After prefiltering the solutions through 0.2 μm track-etch membranes, the solutions were pumped 5 times with the peristaltic pump in order to generate aggregates. The flux of this prepumped solution was measured during dead-end filtration through 0.2 μm track-etch membranes at 6.7 kPa (50 mmHg). The results shown in Figure A.2 indicate that a slower rate of flux decrease was observed at the higher total protein concentration. Having demonstrated earlier that protein aggregates and not the total protein was responsible for flux decrease in our experiments, the flux profiles suggest that the extent of aggregation was lower at higher protein concentrations. Using Equation...7.3, we can
**Figure A.2** Effect of total BSA concentration on dead-end flux using solutions pre-pumped to the same extent with a peristaltic pump.

estimate the concentration of aggregates in the feed after 5 passes through the peristaltic pump assuming that the transmission of these aggregates was zero (see Table A.1). The percentage of BSA aggregated at each of the three concentrations was also estimated by quasi-elastic light scattering analysis of the pumped solutions. Once again we find that a smaller fraction was aggregated at higher concentrations of total protein (see Figure A.3). There is considerable scatter in the data and the best linear fits are only shown as an indicator of the relative trends. The slopes of these best fit lines are $14.3 \times 10^{-4}$, $3.8 \times 10^{-4}$ and $2.5 \times 10^{-4}$ at BSA concentrations of 0.25, 1.0 and 2.5 kg/m$^3$ respectively. This trend is in qualitative agreement with the results reported in the literature on the effect of solute concentration on protein denaturation by
Table A.1  Effect of total BSA concentration on rate of flux decrease and aggregate concentration

<table>
<thead>
<tr>
<th>BSA Concentration (kg/m³)</th>
<th>Initial flux, J₀ (x 10⁴ m/s)</th>
<th>- Slope, k_B (x 10⁴ s⁻¹)</th>
<th>Aggregate Conc. (x 10⁵ kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 (Run 1)</td>
<td>2.58</td>
<td>18.9</td>
<td>10.7</td>
</tr>
<tr>
<td>0.25 (Run 2)</td>
<td>3.08</td>
<td>18.3</td>
<td>8.7</td>
</tr>
<tr>
<td>1.0 (Run 1)</td>
<td>3.02</td>
<td>8.68</td>
<td>4.2</td>
</tr>
<tr>
<td>1.0 (Run 2)</td>
<td>2.87</td>
<td>9.07</td>
<td>4.6</td>
</tr>
<tr>
<td>2.5 (Run 1)</td>
<td>3.06</td>
<td>5.15</td>
<td>2.5</td>
</tr>
<tr>
<td>2.5 (Run 2)</td>
<td>3.06</td>
<td>4.28</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Figure A.3  QELS analysis of pumped BSA solutions at different concentrations.
Prior to our realization of the possibility of protein aggregation by pumping, we had investigated the effect of organic solvents on the rate of flux decrease during cross-flow filtration with a peristaltic pump with the idea of changing the hydrophobic environment in order to change the extent of protein adsorption. Consequently, these experiments did not have proper controls on extensive variables such as the feed volume and the results presented below are good only for qualitative purposes. Solutions of BSA (0.25 kg/m$^3$ based on total volume, Sigma A-7030) were prepared in phosphate buffered saline (0.05 g/l NaH$_2$PO$_4$, 0.3 g/l Na$_2$HPO$_4$, 0.44 g/l NaCl, pH 7) containing 0-10% (v/v) of ethanol or isopropanol. The cross-

![Graph showing effect of ethanol on rate of flux decrease during CFF with a peristaltic pump.](image)

*Figure A.4 Effect of ethanol on rate of flux decrease during CFF with a peristaltic pump*
Figure A.5 Effect of isopropanol on the rate of flux decrease during CFF with a peristaltic pump.

Flow filtration was done at 6.7 kPa (50 mmHg) using about 500 ml ($5 \times 10^{-4}$ m$^3$) of protein solution recirculated at 200 ml/min ($\pm 10\%$) with a peristaltic pump. The permeability of the 0.2 $\mu$m polycarbonate, track-etch membranes was measured using both pure buffered saline as well as saline/organic solvent mixtures in order to account for differences in viscosity. The flux profiles, normalized to the initial value, indicate a significant effect of the organic solvents on the rate of flux decrease (see Figure A.4 and Figure A.5). Based on our current awareness of the effect of pumping on aggregation, these data may be interpreted as being indicative of solvent effects on the interfacial denaturation of proteins prior to aggregation. However, further work needs to be done in order to draw any definitive conclusions. For example,
quasi-elastic light scattering analysis of the pumped solutions or dead-end filtration of prepumped solutions may yield useful information on the rates of aggregation in the presence of different organic solvents.

The flux profiles presented above may be analyzed using Equation... (7.11) which was developed for the case of cross-flow filtration with a peristaltic pump assuming that the aggregation proceeded linearly with the duration of pumping. Let us assume that the amount of protein required to block all the pores is 14.7 mg/m² which was the amount obtained using the I¹²⁵ BSA as a tracer (see Figure 7.4). The fraction of protein aggregated per pass through the pump, βₐ, and the initial aggregate concentration, Cₐ₀, are used as adjustable parameters. The results of fitting Equation... (7.11) to the experimental flux profiles are shown in Figures A.4 and A.5 as the dashed curves. The values of βₐ and Cₐ₀ obtained by this fit are shown in Table A.2.

<table>
<thead>
<tr>
<th>% Ethanol</th>
<th>Buffer Flux, Jₐ (x10⁴ m/s)</th>
<th>Initial flux, J₀ (x10⁴ m/s)</th>
<th>βₐ (x 10⁵)</th>
<th>Cₐ₀ (x 10⁶ kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.51</td>
<td>3.51</td>
<td>6.5</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>3.56</td>
<td>3.02</td>
<td>5.0</td>
<td>3.2</td>
</tr>
<tr>
<td>10</td>
<td>4.26</td>
<td>3.18</td>
<td>2.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Isopropanol</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>2.8</td>
<td>58.1</td>
<td>25.2</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>2.7</td>
<td>31.3</td>
<td>17.6</td>
</tr>
<tr>
<td>10</td>
<td>3.6</td>
<td>2.5</td>
<td>2.8</td>
<td>-</td>
</tr>
</tbody>
</table>
The results with ethanol yield consistent values of $C_{ao}$ and hence may be more reliable than those obtained with isopropanol. In both cases, $\beta_a$ decreases with increasing solvent concentration but $\beta_a$ with 1% and 5% isopropanol is higher than in the absence of any solvent. The ratio of $J_o$ to $J_w$ reflects the ratio of the viscosities of the two solutions, that is, in the presence and absence of solvent. One of the major sources of error in this analysis is that the feed volume reported (500 ml) is the volume of solution prepared before pre-filtration. Some solution was lost during this prefiltration step but it was not recorded because the importance of such extensive variables was not known at the time of the experiments. Secondly, the BSA used was purchased from Sigma and is different from the $^{125}$I-BSA which was prepared from protein purchased from ICN Immunobiologicals. The latter was used to obtain the value of $W_p N_T$ as 14.7 mg/m$^2$. It would be useful to repeat these experiments under more carefully controlled conditions.

The effect of total protein concentration and the effects of organic solvents probably reflect the denaturation of proteins at interfaces. These could either be the gas-liquid interfaces formed by cavitation during the decompression of the tubing in the peristaltic pump or the solid-liquid interface provided by the tubing itself. Interfacial effects could be coupled with the effect of shear in the narrow gaps in the compressed tubing to denature proteins. There is clearly a need to investigate the aggregation of proteins using a more well defined apparatus such as a concentric cylinder viscometer with narrow gap widths. Since the fraction of protein aggregating is very low, such experiments should use a direct measurement of aggregation (such as light scattering) and not rely on changes in the activity or concentration of native enzymes as was done by Dunnill and co-workers (see Section 3.3.1).