Mechanisms of Flux Decline in Skim Milk Ultrafiltration: A Review

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Abstract

Skim milk ultrafiltration (UF), used for milk protein concentration, is one of the most important unit operations in dairy processing. However UF performance is severely reduced by flux decline resulting from concentration polarisation (CP) and fouling, the mechanisms of which are still not fully understood for the complex colloidal fluid of skim milk. In this review, we analyse published observations of CP and fouling of relevance to skim milk UF to examine the underlying mechanisms. Current approaches for modelling the flux decline caused by CP and fouling are reviewed. Focussed discussion is given to the current state of understanding of CP and fouling in relation to the physicochemical properties of skim milk and the effectiveness of chemical cleaning. This review identifies the roles of various milk components in CP and fouling behaviour and offers insights into the mechanisms that govern flux decline.

1 **Keywords**

2 Ultrafiltration; Skim milk; Concentration polarisation; Fouling; Modelling.

3 **Abbreviations**

AAS	Atomic absorption spectroscopy
ATR-FTIR	Attenuated total reflection Fourier transform infrared spectroscopy
BSA	Bovine serum albumin
CCP	Colloidal calcium phosphate
CFD	Computational fluid dynamics
CFV	Cross-flow velocity
CIP	Cleaning-in-place
CM	Casein micelles
CN	Casein
CP	Concentration polarisation
DF	Diafiltration
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy dispersive X-ray
GISAXS	Grazing incidence small-angle X-ray scattering
IEP	Isoelectric point
MF	Microfiltration
MPC	Milk protein concentrate
MWCO	Molecular weight cutoff
NPC	Native phosphocaseinate
PAN	Polyacrylonitrile
PEG	Polyethylene glycol
PES	Polyethersulfone
PSf	Polysulfone
PVDF	Polyvinylidene difluoride
SAXS	Small-angle X-ray scattering
SEM	Scanning electron microscopy
SMUF	Simulated milk ultrafiltrate
TMP	Transmembrane pressure
TN	Total nitrogen
UHT	Ultra-high temperature
UF	Ultrafiltration
VRR	Volume reduction ratio (or volume concentration factor, VCF)
WP	Whey proteins
WPI	Whey protein isolate

1. Introduction

Skim milk ultrafiltration (UF) is a key unit operation in dairy processing in which milk proteins are concentrated through the removal of lactose, salts, peptides and other solutes, and water. UF exploits size differences in the milk components to preferentially concentrate the proteins, which cannot be achieved by evaporation alone, allowing more flexible control over product composition. In addition, filtration is considerably less energy-intensive [1,2], and avoids prolonged heat exposure, allowing the functional properties and sensory qualities of milk to be well preserved [3]. It is used extensively in the production of cheese [2,4–7] and milk protein concentrates (MPC) [5–9], and also for protein standardisation [5–8,10].

Skim milk UF is particularly susceptible to poor operational efficiency [8] due to flux decline resulting from concentration polarisation (CP) and fouling (Fig. 1). CP is the accumulation of retained particles at the membrane surface, while fouling occurs due to adsorption or deposition of colloidal particles on the membrane surface and in the membrane pores [2,11]. CP and fouling contribute resistance to permeation flow, and can be responsible for severe reductions in flux and changes in rejection properties, ultimately resulting in lower throughput and altered product quality. The CP layer is a direct result of flux and is usually reversible in the sense that it will quickly diffuse if flux across the membrane is halted. However, severe CP can also result in the formation of a gel layer formed through particle-particle interactions and such a layer dissipates slowly, if at all, when the flux is halted. From an operational perspective CP is unavoidable but it can be minimised by improving particle convection away from the membrane [2,12,13]. On the other hand, fouling is irreversible (upon cessation of flux), and its removal requires back washing or often even chemical cleaning. This interrupts operation, lowers productivity, consumes large amounts of water and chemicals, and reduces membrane life [2].

The optimisation of skim milk UF (both operation and cleaning) requires an understanding of the mechanisms of flux decline in relation to the physicochemical properties of skim milk, however this is still not fully understood despite the widespread use of skim milk UF for over 30 years. In recent years substantial progress has been made in understanding milk chemistry, and separately, the physics of CP and membrane fouling by proteins. In addition significant advances have been made in the development of mathematical models to describe filtration behaviour, in particular via computational fluid dynamics (CFD). However, this whole body of work has yet to be brought together in the context of skim milk UF and relatively few studies have been made specifically investigating CP and fouling mechanisms in skim milk UF. Past reviews on skim milk UF or dairy filtration have mostly focused on applications and avenues for product development, and very little has been dedicated to the discussion of CP and fouling in relation to the physicochemical properties of skim milk.

In this review we bring together results from recent studies of CP, membrane fouling, and filtration modelling with existing knowledge to extend our understanding of the fundamental

mechanisms of flux decline in relation to the physicochemical properties of skim milk. Aspects of membrane cleaning and processing factors affecting flux decline are also discussed. Through the analysis of the available literature, we hope to derive new insights that will allow further optimisation of skim milk UF.

2. The Skim Milk System

Some knowledge of milk chemistry is necessary for discussing how flux decline behaviour can be affected by changes in milk properties. This section provides an overview of the major milk proteins, mineral equilibria, and casein micelle structure and interactions with the surrounding milk serum.

Skim milk is a complex colloidal suspension of proteins in an aqueous solution of lactose and minerals, and some minor components including residual milk fat globules. Milk proteins make up 3.5 wt% of milk, of which 80% are casein (CN), defined as proteins insoluble at pH 4.6 and 20 °C, while the remaining 20% are whey proteins (WP) (Table 1). Caseins are comprised of α_{s1} -CN, α_{s2} -CN, β -CN and κ -CN, in approximate proportions of 38%, 10%, 35% and 12% respectively [14]. They generally have high phosphoserine content which can interact with the calcium phosphate in milk (except κ -CN), and proline content which limits their ability to fold into secondary (α -helices and β -sheets) and tertiary structures. Consequently, caseins generally have an open structure, and the majority of them are present in milk as casein micelles (CMs) (see Section 2.2).

Whey proteins are comprised of about 60% β -lactoglobulin (β -LG), 20% α -lactoglobulin (α -LA), 10% bovine serum albumin (BSA) and 10% immunoglobulins (Ig). Other proteins such as lactoferrin, peptides, hormones and enzymes are also present in skim milk in minor amounts. Unlike caseins, whey proteins generally have tertiary and quaternary structures, which as discussed later, can influence CP and fouling behaviour in skim milk. Under physiological conditions of milk, β -LG exists as dimers held together by hydrophobic interactions (Lewis-acid-base interactions), but also exhibits different aggregation states depending on the pH. It is also known to dissociate into monomers and undergo reversible conformational changes (Tanford transition) between 40-55 °C [14]. α -LA is a compact globular protein that has a very strong affinity for binding calcium (association constant, K_a at 25°C $\sim 3 \times 10^8 \text{ M}^{-1}$ [15]), which is important for its structure and stability [14]. BSA is a large elongated molecule with high disulphide content.

Table 1: Major proteins in skim milk [16].

Protein	Concentration in skim milk (g/L)	Molecular Weight (kDa)
Caseins		
α_{s1} -CN	12-15	23.6

α_{s2} -CN	3-4	25.2
β -CN	9-11	24.0
κ -CN	2-4	19.0
Whey Proteins		
β -LG	2-4	18.3
α -LA	0.6-1.7	14.2
BSA	0.4	66.4
Ig	0.4	150-1,000

2.1 Mineral Equilibria

Milk contains a large variety of salts, principally sodium, potassium, calcium, magnesium, phosphate, chloride, sulphate, carbonate and citrate [17]. These salts are not fully dissolved nor fully ionised in the milk serum [4] – dissolved salts are present in the aqueous serum phase as free ions or associated with proteins, lactose and other salts; undissolved salts are associated with CMs. In addition, α -LA and lactoferrin contain ion binding sites that specifically bind calcium and iron respectively.

Of major importance is calcium phosphate, which is sparingly soluble (solubility of CaHPO_4 in milk = ~ 1.8 mmol/L; $\text{Ca}_3(\text{PO}_4)_2$ = ~ 0.06 mmol/L) [4] and is present in milk at supersaturated concentrations. It is thermodynamically unstable but natural precipitation is prevented by complex associations of calcium with other salts and milk components. About two-thirds of the calcium is undissolved and incorporated into CMs along with half of the inorganic phosphate and other undissolved salts, collectively referred to as colloidal calcium phosphate (CCP). The remaining calcium is present in the serum as ionic calcium or complexed with free soluble caseins, other salts (e.g. citrate), proteins [18,19], and also lactose [20]. All these fractions exist in a dynamic and complex equilibrium influenced by temperature, concentration and pH [21–23]. Stabilisation of calcium phosphate is provided by the presence of CMs, however the partitioning of calcium and phosphate between serum and CMs is also responsible for many of the alterations to CM properties reported in literature (see below).

2.2 Casein Micelles

At native pH and room temperature, about 95% caseins are associated as colloidal assemblies of micelles. CMs are comprised of all the casein fractions in milk, and the caseins make up approximately 93% of the total dry matter of the CM. The remaining 7% is CCP, which is present in the form of nanoclusters. CMs are also highly voluminous, occupying about 4.4 mL/g casein at 25 °C, and are considerably hydrated (3.7g water/g casein) [24]. In addition, CMs are highly polydisperse, with sizes ranging from about 50 to 300 nm in diameter. On a volumetric basis, the average diameter of CM is about 150-200 nm [25].

The exact structure of the CM has not been unequivocally elucidated. A number of models have been developed over the decades that attempt to describe the CM structure and the interactions of its components with the serum. These models have been continuously refined, and has been discussed in a number of reviews [25–31]. For the purpose of this review, knowledge of the precise CM structure is not crucial. Rather, the key attributes of CMs that must be accounted for in any proposed mechanistic or mathematical model of skim milk UF are described below.

It is generally accepted that almost all κ -CN is present on the micelle exterior, inferred by the higher κ -CN content in smaller CMs compared to larger CMs [32–34], but the κ -CN is not evenly distributed on the CM surface [27]. Long hydrophilic sections of the κ -CN protein extend into the serum, giving rise to a so-called ‘hairy layer’. This ~7 nm thick layer provides steric stabilisation for CMs [4,14]. Meanwhile, the CM interior is considered to be a network of α - and β -CN linked to nanoclusters of calcium phosphate of about 2-3 nm in diameter [30,35,36]. Both CCP content and hydrophobic interactions are necessary for micellar integrity [31]. This is indicated by observations of micellar disintegration upon sufficient removal of calcium [37–39], or if surfactants or urea are added [14]. The voluminous and hydrated nature of CMs also suggests that the interior is fairly porous. This is supported by recent permeability measurements of CM dispersions by Bouchoux et al. [40] that were found to fit better to hard sphere models by considering the CMs to be 8.8 nm particles, whereas large deviations were seen when an average CM size of 100 nm was used. Importantly, CMs have been shown to be soft and deformable under compressive and flow stresses [41–45] – this is discussed in more detail in Section 4.

As the integrity of the CM structure depends on CCP and hydrophobic interactions, CM properties (e.g. composition, size, hydration) can be altered by shifts in calcium phosphate equilibria as well as changes in the strength of hydrophobic interactions [4,25]. For instance, decreasing the temperature and pH increases the solubility of calcium phosphate, which results in the dissolution of CCP and release of calcium from the micelle [46]. The irreversible removal of CCP from the micelle is also observed as a result of diafiltration [47]. Meanwhile, hydrophobic interactions are weakened when the temperature is decreased, causing the dissociation of β -CN from the CM [4].

3. Fouling

The term ‘membrane fouling’ is used here to describe changes brought about by interactions between colloidal particles and the membrane (e.g. protein adsorption and mineral precipitation). The resultant membrane-fouling conglomerate has different physicochemical properties compared to the membrane, for instance altered surface charge [48–50] and reduced pore sizes [51]. Consequently, its separation/fractionation properties are also changed. Fouling can manifest as complete or partial pore blockage/plugging and formation of a deposit on the membrane surface, depending on the relative sizes of particles and membrane pores [11]. It is important to note that this deposit formation is considered here as a direct interaction of the colloidal particles

with the membrane, and should not be confused with the layer that can result from particle-particle interactions due to concentrative effects of CP (i.e. gel/cake layer formation, see Section 4). Understanding the mechanisms of flux decline in skim milk requires knowledge of the roles of the various skim milk components (CMs, whey proteins and minerals) in relation to these different types of fouling.

3.1 Composition of the Fouling Layer

3.1.1 Fouling of organic membranes

Controlled studies identifying the components contributing to fouling in skim milk UF are surprisingly scarce. Fouling characterisations discussed here are mainly from independent studies using different milk sources, membrane materials and processing conditions (experimental details summarised in Table 2 below), and these parameters may affect the fouling behaviour (discussed in Section 6). Nevertheless, a few studies have shown that irreversible fouling of polymer (organic) membranes is caused almost exclusively by proteins [51–53]. Begoin et al. [51] analysed the surfaces of polyethersulfone (PES) membranes fouled by fresh/pasteurised skim milk at 50 °C. Scanning electron microscopy-energy dispersive X-ray detection (SEM-EDX) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) were used to detect minerals and quantify proteins respectively. The irreversible fouling layer was found to consist of 99.6% proteins and only 0.4% minerals, with no phosphorus detected in the mineral fraction. Rabiller-Baudry et al. [53] reported similar results from static fouling experiments (i.e. contact between membrane and fluid with no application of pressure) with skim milk at 20 °C. Koutake et al. [52] detected significant amounts of protein and little to no minerals in the Ultrasil 25 solution (alkaline cleaner) after chemical cleaning of polyacrylonitrile (PAN) and polysulfone (PSf) membranes fouled by fresh pasteurised and reconstituted skim milks at 50 °C.

Within the protein fraction, whey proteins appear to be the dominant foulant. Tong et al. [54] reported that whey proteins accounted for ~95% of the protein fouling on a PES membrane (pasteurised whole milk, 49 °C). This whey protein fraction comprised of roughly 80% α -lactalbumin (α -LA) and 20% β -lactoglobulin (β -LG). They highlighted contrasts with observations reported by Patel & Reuter [55], who detected significant amounts of casein on polysulfone (PSf) membranes fouled by whole milk at 30 °C. The discrepancies were attributed to differences in sampling methods. Whereas Tong et al. [54] extracted the foulants directly from the membrane, Patel & Reuter [55] analysed the proteins extracted from cleaning solutions. In the latter case, large errors can be introduced by small residual amounts of milk in the filtration equipment. Another reason could be because analysis by Patel & Reuter [55] was only carried out when flux ceased – at this point a significant casein micelle gel layer is likely to have formed (see Section 4), which may have been the subject of the analysis. The preferential fouling of α -LA over β -LG has also been observed in whey UF [56], though reasons for this are unclear.

Hanemaaijer et al. [57] has previously observed that more α -LA absorbed onto hydrophobic polysulfone globules over β -LG at pH 5.5, suggesting the preferential adsorption of α -LA over β -LG (at this pH, both β -LG and α -LA are negatively charged, and there are no known differences in protein structure compared to that at the native milk pH).

3.1.2 Fouling of inorganic membranes

The fouling behaviour of ceramic (inorganic) membranes differs slightly from organic membranes in that some mineral fouling is observed. Vetier et al. [58] found that the salt-to-total-nitrogen ratios in the irreversible fouling layer of alumina MF membranes fouled by raw whole milk were higher than that in milk, which suggested the presence of mineral fouling. However, they were not able to detect minerals from static fouling using milk ultrafiltrate, suggesting that mineral fouling only occurs in the presence of proteins. Separately, Daufin et al. [59] also reported mineral fouling on zirconia (ZrO_2) UF membranes by fresh/pasteurised skim milk at 50 °C. Furthermore, the protein foulant composition on ceramic membranes appears to be different to that of organic membranes (~95% whey protein). In contrast, on ceramic membranes, Vetier et al. [58] determined that casein comprised about 40-50% of the total protein (as total nitrogen) in the irreversible fouling layer removed by immersing in an ultrasonic bath. The authors considered the casein to be bound to the membrane via salt bridges. At the same time, the CN proportion in the fouling layer is lower than that in milk, once again indicating preferential fouling by whey proteins.

Table 2: Experimental details of studies investigating foulant composition in skim milk UF and MF.

Milk Source	Membrane	Processing Conditions	Analysis Methods	Observations	Ref.
Organic Membranes					
Skim milk (Pasteurised)	PES (5-10kDa)	50 °C, P_{in} =5bar, VRR=2, 2-8h	Minerals: SEM-EDX, Protein: ATR-FTIR	Mainly proteins, minor presence of minerals.	[51]
Skim milk (1/3 diluted)	PES (5-10kDa)	Static conditions, 20 °C	Minerals: SEM-EDX, Protein: ATR-FTIR	Mainly proteins, no minerals detected	[53]
Skim milk (Pasteurised / Reconstituted)	PSf (30-40kDa) PAN (10-20kDa)	50 °C, TMP=3bar, CFV=2.4m/s, up to VRR=6	Protein: Kjeldahl analysis & ashing of cleaning solution; Amino-acid analysis	Proteins, no minerals;	[52,60]
Whole milk (Pasteurised)	PSf (10kDa)	49 °C, TMP=4bar	Extraction with PAGE buffer (10mM Tris-HCl at pH 6.8, 1% SDS, 20% glycerol, 0.2%	Whey proteins 95% whey proteins; 80% of total protein foulant as α -LA	[54]

Skim milk (Pasteurised)	PSf (50kDa)	30 °C, TMP=1.2bar, flux→0	bromphenol blue) followed by SDS- PAGE Proteins: Kjeldahl method, SDS-PAGE of cleaning solution Minerals: autoanalyser	Caseins, calcium, phosphate	[55]
Inorganic (Ceramic) Membranes					
Whole milk (Raw)	Alumina (0.2µm)	20 & 50 °C, TMP=2bar, CFV=3.3m/s, 5min-2h	TN: Kjeldahl; Ca: AAS; P: Ammonium phosphomolybdate colorimetric method SDS-PAGE	CN = 40-50% TN Mineral content increased with temperature	[58]
Skim milk (Fresh / pasteurised)	Zirconia (10 & 150kDa)	50 °C, CFV=4m/s, up to VRR=1.6	IR Spectroscopy, XPS	Proteins, phosphate	[59]

1

2 3.2 Protein Adsorption

3 During filtration, proteins can adsorb directly onto the membrane surface (primary adsorption),
4 resulting in fouling [53,61–64]. Protein adsorption is a complex phenomenon, but is generally
5 considered to be an entropically driven process in which the free energy of the system is
6 minimised through: (1) charge redistribution on protein-surface contact, (2) hydrophobic
7 interactions resulting in the release of surface-adsorbed water and ions from parts of the protein
8 and membrane, (3) structural rearrangements of the protein molecule [65–67]. Adsorbed proteins
9 may undergo additional conformational changes to further reduce free energy, causing the
10 protein to be more tightly bound to the surface, gradually becoming more resistant to elution [68].
11 These conformational changes can also potentially reveal additional active sites (e.g. thiol groups,
12 disulphide bonds) that can serve as nucleation sites for aggregation or secondary adsorption
13 reactions, and this has been observed to significantly affect the fouling behaviour in the filtration
14 of model protein systems [69–71].

15

16 Proteins are amphoteric molecules – they carry both basic and acidic functional groups which
17 exhibit different extents of dissociation depending on the pH, and contribute to the protein's
18 overall charge. At the natural milk pH of 6.6–6.8, all major milk proteins and majority of the
19 membranes commonly used in skim milk UF carry net negative charges (Table 3). This implies
20 that lateral protein-protein and protein-membrane electrostatic interactions are mostly repulsive
21 by nature. However, adsorption is still observed to occur, especially on hydrophobic surfaces,

indicating the dominant influence of hydrophobic interactions over electrostatic interactions [72]. In this case, protein adsorption will be accompanied by the co-adsorption of protons and cations as a means of local charge regulation [65]. Protein adsorption in the presence of salts has been observed to be more resistant to desorption or elution [73], indicating the role of salts in further stabilisation of the adsorbed proteins.

Table 3: Isoelectric points (IEP) of major milk proteins and membrane materials commonly used in skim milk UF [4,11,14,74–77].

Proteins	IEP	Charge at native milk pH (≈ 6.7)
α_{s1} -casein (α_{s1} -CN)	4.96	–ve
α_{s2} -casein (α_{s2} -CN)	5.27	–ve
β -casein (β -CN)	5.2	–ve
κ -casein (κ -CN)	5.54	–ve
α -lactalbumin (α -LA)	4.6	–ve
β -lactoglobulin (β -LG)	5.35	–ve
Bovine serum albumin (BSA)	4.7	–ve
Immunoglobulin G (IgG)	6.1-8.5	+ve/–ve
Membrane materials		
Polysulfone (PSf)	3.6	–ve
Polyethersulfone (PES)	2.2-2.4	–ve
Zirconium (IV) Oxide (ZrO_2)	3.5-5	–ve
Alumina (γ - Al_2O_3)	9.5	+ve

The reasons for the preferential adsorption of whey proteins over caseins, and that of α -LA over β -LG have not been fully elucidated. However, this can perhaps be explained by differences in free energy reductions obtained by different proteins exhibiting conformational changes at a surface. As caseins have high proline content which limits their ability to fold [4,78], they do not have tertiary structures, and thus have much lower driving forces for free energy reduction and adsorption compared to whey proteins. Distinction among whey proteins is more difficult. At first glance, a comparison of thermal denaturation temperatures of whey proteins (Table 4) suggests that β -LG is more conformationally stable than α -LA and BSA, and hence less prone to conformational changes and adsorption [65,68]. However, β -LG, which normally exists as a dimer at milk pH, can dissociate into monomers and undergo reversible conformational changes (Tanford transition) between 40-55 °C [14,79]. These conformational changes increase its hydrophobicity and expose the thiol group normally embedded within the protein, making it more accessible for thiol-disulphide interactions which result in protein aggregation (discussed in more detail in the next section). Yet, considering that the foulant identification studies discussed earlier have mainly been conducted at 50 °C, which is the typical processing temperature used in industrial skim milk UF (the alternative being <10°C), prevalent α -LA fouling is observed

despite the increased β -LG hydrophobicity and reactivity. A possible reason for this, as will be discussed in Section 3.4 later, is the accessibility of α -LA to a larger portion of pores due to its small size compared to other proteins, which increases the surface area available for adsorptive fouling.

Table 4: Thermal denaturation temperatures (T_d) of major whey proteins in skim milk [14,17,80].

Whey protein	T_d ($^{\circ}\text{C}$)
α -LA	63
α -LA (Ca depleted)	41
β -LG	78
BSA	63

3.3 Protein Denaturation and Aggregation

Relating to protein conformational stability, protein denaturation and aggregation processes can be detrimental to filtration. Several authors have reported significant and progressive flux decline during BSA MF (0.22 μm PVDF) and UF (10, 40, 100 kDa PSf) in the presence of BSA aggregates in phosphate buffer solution (PBS) at room temperature [70,81,82]. Kelly & Zydney [70,81] found that these aggregates can form in bulk solution, or through attachment of native BSA to existing BSA adsorbed onto the membrane surface. They demonstrated that BSA aggregation is caused by intermolecular thiol-disulphide exchange reactions. Similar results were reported by Maruyama et al. [83] for BSA dissolved in deionised distilled water (6 kDa PSf), which was observed to undergo changes in secondary structure as a result of aggregation.

Thiol-disulphide exchange reactions, which are also responsible for protein aggregation commonly seen in high-temperature and high-pressure processing [17,84], involve the oxidation of a disulphide bond ($-\text{S}-\text{S}-$) by a protonated thiol group ($-\text{SH}$). Thiol groups are present as side chains on cysteine amino acid residues [4]. BSA contains 35 cysteine residues, of which 34 are linked pairwise as 17 intramolecular disulphide bonds, with one free thiol group near the NH_2 -terminal end of the molecule. As such, intermolecular thiol-disulphide exchanges between BSA monomers result in the formation of a BSA dimer with a free thiol group, which can participate in further thiol-disulphide exchanges and propagate aggregate growth. The presence of a free thiol group has been shown to be necessary to initiate thiol-disulphide exchanges. Severe flux decline has been observed with other proteins containing thiol groups (e.g. β -LG, ovalbumin), but not for proteins either without thiol groups (e.g. lysozyme, pepsin) [71] or with thiol groups capped, for instance by a cysteinyl group or a carboxymethyl group [69,70,81] (in all cases pre-existing aggregates were removed by pre-filtration). Consistent with this, decreasing the reactivity of the thiol group, for instance by increasing pH, or chelation of divalent copper salts that reportedly can catalyse the thiol-disulphide exchange during storage, reduced the severity of flux decline [69].

Among milk proteins, only BSA, β -LG and κ -CN contain free cysteine groups (Table 5), meaning that only these three proteins are capable of initiating thiol-disulphide exchanges. In milk, κ -CN exists as a series of intermolecular disulphide bonded aggregates on casein micelles, with 10% of the total cysteine residues free and therefore theoretically able to participate in thiol-disulphide reactions [78]. Despite this, κ -CN is not known to initiate thiol-disulphide reactions, perhaps due to steric hindrances in the hairy layer of the CM surface. However, denatured whey proteins can initiate thiol-disulphide bond formation with κ -CN on the CM surface, which increases the hydrodynamic diameter of the CMs [85] (discussed further in Section 6.1). In β -LG, the cysteine group is only accessible upon protein unfolding. Thermally this occurs between 40-55 °C, following dissociation of the β -LG dimer and reversible unfolding (Tanford transition) [86]. Interestingly, its flux decline behaviour at room temperature is reported to be similar to BSA [71], implying that both adsorbed and pre-aggregated β -LG may be sufficiently unfolded to reveal the free cysteine group for thiol-disulphide reactions.

Table 5: Cysteine content (linked and available) of major proteins in skim milk [4].

Protein	Cysteine Residues	Intramolecular Disulphide Bonds	Cysteine Units Available for Disulphide Bonding
α_{s1} -CN	0	0	0
α_{s2} -CN	2	1	0
β -CN	0	0	0
κ -CN	2	0	2
α -LA	4	2	0
β -LG	5	2	1
BSA	35	17	1

Here, several factors must be taken into account when interpreting observations from simple protein systems to more complex dairy systems such as skim milk. Firstly, the native protein structure of pure protein fractions may be compromised during manufacture. Secondly, protein stability is also influenced by the composition of the background buffer – lactose and minerals present in milk serum can help stabilise protein structure [87] reducing the likelihood of denaturation compared to using water or phosphate buffer solutions, as in the aforementioned studies. After all, milk proteins are stable under bovine physiological conditions (38.0-39.3 °C [88]), meaning that thermal unfolding and/or protein aggregation reactions in the bulk solution should not occur below 40 °C.

1 Although thiol-disulphide interactions in skim milk UF have not been directly investigated, a few
2 studies have indicated its presence in the filtration of other dairy fluids. Lee & Merson [89]
3 observed a significantly higher flux for UF of cottage cheese whey (10 kDa PSf) when 0.0015 M
4 of N-ethylmaleimide (NEM) was added, which inhibits thiol-disulphide reactions by irreversibly
5 reacting with thiol groups. These observations were supported by SEM micrographs of
6 chemically-treated whey samples mounted on 0.4 μm Nuclepore membranes showing
7 significantly reduced protein deposition [89]. In a more recent study, Steinhauer et al. [90]
8 showed that formation of heat-induced β -LG aggregates resulted in lower fluxes in whey MF
9 (0.1 μm PES) and UF (30 kDa PES). However, aggregation was not seen with native β -LG
10 capped with NEM, analogous to the BSA MF studies by Kelly & Zydney [69–71] discussed
11 earlier. In investigating the influence of temperature on β -LG MF, Steinhauer et al. [91] reported
12 a marked increase in fouling resistance from 30 to 35–40 $^{\circ}\text{C}$. This was attributed to increased
13 aggregate deposition from increased thiol reactivity resulting from dissociation of the β -LG
14 dimer. However, the increased fouling could have also been due to calcium phosphate
15 precipitation, as the UF permeate used for solubilising β -LG in this study was obtained at 10 $^{\circ}\text{C}$.
16 UF permeates obtained at lower temperatures have higher calcium content due to increased
17 solubility of calcium phosphate, and as such precipitation may occur at elevated temperatures.

18
19 Separately, and more related to CP (Section 4), Steinhauer et al. [92] observed a decrease in the
20 specific resistance of CM dispersions in the presence of β -LG. This was attributed to the
21 attachment of β -LG to κ -CN on the surface of CMs and/or an increase in osmotic pressure – the
22 former was supported by GISAXS observations. However, this change was not observed in the
23 presence of NEM, indicating that the attachment was due to thiol-disulphide reactions. Given
24 that the experiments were conducted at room temperature, thermal unfolding is an unlikely cause
25 for the unfolding and exposure of the thiol group in β -LG in this case. Instead, the unfolding was
26 likely caused by concentrative effects in the interstitial spaces between CMs in the CP layer.

27
28 The severity of fouling caused by protein aggregation can depend on the membrane pore size. A
29 comparison of MF and UF studies on whey proteins indicates that flux decline is greater during
30 MF (~70% decline) than UF (10–20% decline) [70,82,90]. In MF, which is only partially
31 retentive or non-retentive of native whey proteins, protein aggregation results in new particles
32 that may be larger than the membrane pores, potentially blocking pores and changing retention
33 properties. However in fully-retentive UF, the presence of aggregates will not change the
34 (complete) retention of the native particles, but the packing behaviour (CP) may be altered by the
35 new particle size distribution.

36
37 Lastly, are there any other sources of protein denaturation during filtration that may result in
38 aggregation and fouling? A number of studies suggest that protein unfolding may also be
39 induced by shear effects near or within the membrane pores. During cross-flow MF (50 nm,
40 zirconium oxide) of a 0.2% β -LG solution in constant flux operation, Marshall et al. [93]

observed a correlation between filtration resistance and protein transmission through the membrane. The data was consistent with model predictions based on preferential protein deposition near the membrane pores, and the behaviour was thus attributed to shear-induced unfolding of β -LG near the pore entrance. As the calculated shear rates within membrane pores were of the same order of magnitude as the wall shear stresses during cross-flow operation, they also concluded that the unfolding was likely to have been caused by a combination of shear stresses and interactions with the pore geometry. The shear-induced protein unfolding of β -LG and α -LA as a result of permeation through PES membranes has also been reported by Van Audenhage et al. [94].

The studies above show that milk proteins can denature and aggregate via thiol-disulphide interactions, and protein denaturation can occur either prior to (e.g. heat treatments) or during filtration (e.g. Tanford transition at elevated temperatures, exposure to hydrophobic surfaces, concentrative and shear effects). However, because protein aggregation introduces a new population of larger colloidal particles, its impact on filtration behaviour can vary depending on the membrane pore size used. Comparisons between MF and UF of whey or model protein solutions indicate that protein aggregation is significantly less detrimental to UF than it is to MF, though these results may not be directly translatable to skim milk since it has a different native population of particle sizes. A comparison of skim milks subjected to different levels of heat treatment may provide a better picture of the effects of thiol-disulphide interactions and protein aggregation on skim milk UF behaviour. This is discussed in Section 6.1.

3.4 Location and Impact of Fouling

The manifestation of fouling as an adsorbed surface deposit and/or pore blockage and its impact on filtration behaviour depends on the relative sizes of the colloidal particles and the membrane pores. Even though UF membranes are designed to be fully-retentive of proteins and should have smaller pores sizes than whey proteins, pore blockage or constriction has been observed. AFM measurements of 5-10 kDa PES membranes by Begoin et al. [51] showed a decrease in mean pore diameter from 5.1 nm to 3 nm after fouling by skim milk (50 °C, ~5 bar, 2-8 hr). As a comparison, the smallest whey protein, α -LA, has physical dimensions of 2.3 nm $\times$ 2.6 nm $\times$ 4.0 nm [15], which is slightly smaller than the pore sizes reported by Begoin et al. [51]. SEM observations of fouled 10-20 kDa PAN and 30-40 kDa PSf membranes by Koutake et al. [52] (pasteurised and reconstituted skim milk, 50 °C) also show considerable pore blockage, though this is to be expected given the slightly larger cut-offs. Pore plugging of a fully-retentive membrane may also be due to the imperfect pore size distribution of membranes or the non-spherical or non-circular shape of proteins and membrane pores respectively, which may allow them to enter the pores [11]. The ability of proteins to penetrate the pores is also substantiated by reports of α -LA being detected in the permeate of skim milk filtered with a 10 kDa UF membrane [95]. In another study, Rabiller-Baudry et al. [96] found that streaming potentials along the membrane surface (10 kDa PES) and through the pores had values close to the IEPs of

1 β -LG and α -LA respectively, which suggests surface fouling by β -LG and pore fouling by α -LA.
2 The indication of pore fouling by α -LA is also consistent with expectations based on protein
3 sizes. The accessibility of the smaller α -LA to a greater percentage of the membrane pores than
4 the other larger milk proteins may also be a contributing factor to the prevalence of α -LA in the
5 fouling layer [54] as discussed earlier (Section 3.1).

6
7 Pore blockage also appears to have a more significant impact on flux/resistance than the
8 adsorbed protein deposit on the membrane surface. Removal of this surface protein deposit by
9 gentle scraping was found to not improve water fluxes [52], indicating the dominance of pore
10 blocking. This is perhaps because pore blocking directly obstructs permeate flow, whereas
11 surface adsorption generally occurs on non-porous areas of the membrane. Additionally, pore
12 fouling would have implications for cleaning, since turbulence and shear-mixing effects typically
13 applied to improve mass transfer at the membrane surface is less likely to propagate into the
14 porous matrix. Pore blocking is an important phenomenon in skim milk UF in which the typical
15 cut-off is 5-10 kDa. Unfortunately, there is limited scope to address this problem by changing the
16 membrane pore size. Increasing the size of the pores reduces protein retention, while membranes
17 with smaller pores may be more susceptible to fouling by other low molecular weight particles
18 present in skim milk (e.g. peptides).

19 3.5 Mineral Fouling

20 In Section 3.1, it was pointed out that mineral fouling in skim milk UF is minor (especially for
21 organic membranes). However, mineral fouling is generally considered to be a dominant factor
22 in a number of other dairy systems including whey, UF permeate, or simulated milk ultrafiltrate
23 (SMUF) [2,8,57]. Mineral fouling in dairy membrane filtration was first reported for whey
24 streams, and is attributed to the precipitation of sparingly soluble salts, particularly calcium
25 phosphate [2,8,97]. Hanemaaijer et al. [57] demonstrated that flux decline during filtration of
26 SMUF could be eliminated upon removal of calcium. Calcium phosphate also has a reverse
27 solubility, meaning that its solubility decreases with increasing temperature. Accordingly,
28 mineral precipitation and fouling has been observed to be less severe following calcium
29 demineralisation [57,98], or operation at lower temperatures and pH [99,100], where calcium
30 phosphate is more soluble.

31
32 In skim milk, although calcium is present at supersaturated concentrations in milk, precipitation
33 does not occur naturally primarily due to associations with CMs, as well as with other salt
34 species, whey proteins and lactose, which provides a huge buffering capacity for the solubility of
35 calcium phosphate (see Section 2.1). Furthermore, Liu et al. [21] has demonstrated that mineral
36 re-equilibration in milk, including that of calcium, occurs very rapidly (within the response time
37 of a pH probe) following temperature changes. The high buffering capacity and responsiveness
38 of the calcium phosphate equilibria suggest that the calcium phosphate equilibrium between the
39 serum and micelles would be established at the operating temperature prior to membrane

1 separation. In this case the calcium concentrations in the serum phase would be at equilibrium.
2 Since UF pores are too large for any significant enrichment of mineral ions to occur (if at all),
3 membrane fouling in skim milk UF via mineral precipitation in the bulk fluid seems rather
4 unlikely. This differs for CM-deficient dairy streams such as whey, UF permeate or SMUF, for
5 which most mineral precipitation studies have been performed [57,97,99,100]. They may not
6 have sufficient buffering capacity to cope with drastic shifts in the calcium equilibrium, for
7 instance when processed at elevated temperatures.

8
9 Maubois [97] suggested that precipitation of calcium phosphate can still occur within the
10 membrane pores as the CMs and whey proteins are no longer in proximity to provide stability.
11 Minerals may also indirectly contribute to fouling. Kessler et al. [73] observed that adsorbed
12 proteins are more resistant to desorption at higher calcium levels, suggesting that calcium may
13 act as cement or salt bridges to protein deposits, though a more cohesive deposit may also have
14 been formed due to the increased ionic strength from calcium addition (see Section 6.1.3). In
15 addition, the differences in mineral fouling behaviour of organic and inorganic membranes
16 discussed earlier (Section 3.1) could imply the influence of membrane-specific interactions (e.g.
17 ionic interactions). Nevertheless, mineral fouling does not appear to be a major factor in skim
18 milk UF owing to the stabilising influence of CMs.

19 **4. Concentration Polarisation and Gel Layer Formation**

20 During filtration, there is an accumulation of retained colloidal particles at the membrane surface
21 giving rise to a concentration gradient of particles perpendicular to the membrane surface, known
22 as concentration polarisation (CP) [2,8,11,13]. This concentration gradient is the driving force
23 for diffusion of the particles back to the bulk, which at steady state, is in balance with the bulk
24 movement of particles to surface. This layer of concentrated particles generates a resistance to
25 fluid flow, as a physical barrier or increased osmotic pressure which reduces the effective
26 transmembrane pressure.

27
28 CP is a reversible phenomenon that is controlled by the balance between mass transfer towards
29 and away from the membrane. For instance, increasing turbulence (e.g. by increasing the cross-
30 flow velocity) reduces the thickness of the boundary layer and thus the effects of CP, while
31 increasing flux has the opposite effect [13]. This gives rise to the typical flux-pressure
32 correlation seen during cross-flow filtration (Fig. 1). At low transmembrane pressures (TMP),
33 flux and CP are both low, and flux is observed to increase linearly with pressure (pressure-
34 controlled region). Conversely, at high TMP, CP is severe, and flux starts to plateau with
35 increasing pressure (mass transfer-controlled region). Here, flux increases are only possible if
36 mass-transfer properties are improved, for instance by increasing the cross-flow velocity or
37 temperature (increases diffusivity).

Fig. 1: Typical flux-pressure correlation in cross-flow filtration showing pressure and mass transfer controlled regions (Modified from Cheryan [2])

In skim milk UF, CMs and whey proteins are retained. CMs are considered the major constituent of the CP layer in skim milk UF [101]. Compared to whey proteins, CMs occupy approximately 17 times the particulate volume fraction (10% vs 0.6%) and have lower diffusivity due to their larger size [4].

CP is also often associated with the irreversible formation of a gel/cake layer if high enough concentrations are achieved [2,11,40]. Gel formation in skim milk UF has been reported based on observations of gel layers that remain on the membrane after filtration, but which can generally be removed by water rinsing [102–106]. According to Steinhauer et al. [104], 60-80% of CM deposits formed during dead-end filtration could also be removed by self-diffusion into a drawn solution of UF permeate, corresponding to a weakly bound CP layer, with the remainder of the CP layer present as a more cohesive gel layer only removable by cross-flow rinsing.

Even though gel formation is reported to occur during filtration, little has been described in regards to the physical appearance (e.g. texture, consistency, adhesiveness) of the gel/cake formed which would allow one to identify or distinguish it from other types of deposits that may form during filtration, for instance the aggregation of thermally denatured proteins. Furthermore, the preconditions for its formation are still not fully elucidated. Of particular importance is the concentration of CMs necessary for gels to form, and how this relates to the actual CM concentrations attained at the membrane surface. Due to the reversible nature of CP upon relaxation of TMP, accurate assessment of this layer requires in-situ observations. This has only recently been made possible through the application of small angle X-ray scattering (SAXS). Early applications have allowed the monitoring of the evolution of concentration profiles near the membrane surface [107–109], however its resolution (especially at the membrane surface)

has been limited by interferences from the membrane. SAXS has also been used to probe the behaviour of CMs near the membrane surface during filtration, which is discussed in the following section.

CP has been investigated using mathematical models of filtration flux performance [2,11,110] (discussed in Section 5). However, the accuracy of these models depends on incorporating an understanding of the close-packing and compressional behaviour of CMs, which are not hard spheres. Instead, CMs are deformable, porous, compressible, and have a ‘hairy’ surface layer of κ -CN (as described in Section 2). Therefore the packing behaviour of CMs is expected to be vastly different from that of model hard spheres. There are a number of recent works that have investigated the packing behaviour of CMs, which are discussed below.

4.1 Compressive Behaviour of Casein Micelles

Characterisation of the compressive behaviour of CMs has been performed by Bouchoux and co-workers [40–42,111]. In these studies, CM dispersions of different concentrations were obtained via osmotic stressing, providing conditions similar to what would be expected near the membrane surface during skim milk UF. CMs dispersed in UF permeate were dialysed at 10 kDa against a known concentration of polyethylene glycol (PEG) in UF permeate until no further concentration could be achieved. Of particular importance, a solid-liquid phase transition of CM dispersions was observed in oscillatory shear experiments at $\sim 178 \text{ g}_{\text{casein}}/\text{L}$ ($\Pi = 10 \text{ kPa}$), which was thereby considered to be the gel point (Π_{gel}) [111]. This concentration (solids volume fraction, $\phi = 0.78$) also corresponds to the random-closed packing concentration of CM dispersions assuming a CM voluminosity of $4.4 \text{ g}_{\text{water}}/\text{g}_{\text{casein}}$ and a polydispersity of 30-40% [111–113], and is well within the random closed packing fraction of monodisperse ($\phi = 0.64$) and fully polydisperse ($\phi = 1$) hard-sphere particles. Below the gel point, CMs generally behave as hard spheres, indicated by the good agreement of shear-viscosity measurements with hard sphere models. Slight deviations were observed at higher shear rates, which were attributed to either polydispersity effects or shear deformation of the CMs [111]. Also, at concentrations below the gel point, SAXS measurements indicated no changes in CM internal structure [42]. Above the gel point, CMs are highly compressible [106]. Further concentration beyond the gel point leads to overlapping contact of CMs, resulting in the collapse of the exterior κ -CN hairy layer and subsequently fusion of CMs and the visible loss of turbidity [41,114]. These cohesive interactions appear to result from ionic and hydrophobic forces at the CM surface, as CM gels were not able to fully redisperse even at long time scales (>15 hours) [41,114], whereas complete redispersion is observed with gels formed by sodium caseinate, which exist as polymer chains [41]. SAXS measurements indicated that compression also led to the removal of micellar water and shrinkage of CMs [42]. In addition, compression was shown to be non-affine, meaning that some parts of the micelle were more resistant to deformation than others.

The occurrence of an apparent gel point at $\Pi = 10$ kPa implies that irreversible flux decline due to gel formation should occur if the osmotic pressure at the membrane surface exceeds 10 kPa. However, discrepancies have been reported in dead-end filtration experiments by Qu et al. [106]. While irreversible flux decline was observed at a critical osmotic pressure at the membrane surface, it was higher than expected (31-35 kPa). The difference was attributed to the gel formation kinetics which may be slow at low osmotic pressures due to reduced interactions between micelles. For reference, the osmotic stressing experiments were conducted over the course of weeks, whereas the dead-end filtration experiments only lasted for several hours. The authors also suggested that gel formation may be disrupted by hydrodynamic forces present during filtration meaning a higher osmotic pressure may be necessary for gel formation. Relating to this, the gel point measured is an order of magnitude lower than the TMP typically used in industrial UF and MF (MF: 100-200 kPa, UF: 300-500 kPa), implying that gel formation can easily occur, though it could be hindered by the cross-flow shear forces present in the membrane module.

4.2 *Spatial Arrangement of Casein Micelles at the Membrane Surface*

The spatial arrangement of CMs at the membrane surface is likely to affect flux and is also influenced by filtration forces. AFM micrographs and GISAXS measurements by Gebhardt et al. [44,45] showed that CMs deposited on non-retentive micro-sieves (0.88 μm) appeared elongated perpendicularly to the membrane, and was arranged in a hexagonal lattice. Similar observations were reported for CMs deposited as thin films on silicon wafers [115]. In addition, GISAXS and light scattering measurements suggested that the deposit was formed initially by larger CMs, and the gaps between large CMs were filled by smaller CMs, increasing the packing density of the deposit layer [44]. The elongation was attributed to the shear forces induced by permeation drag, as well as the lateral compression due to high packing densities [43,45]. This elongation was also observed to be more pronounced at porous areas of the micro-sieve than at non-porous areas, where localised permeation flow rates (and hence shear forces) are expected to be greater [43]. In addition, CMs exhibited more of an oblate (flattened) shape when the compressive pressure was increased from 40 to 80 kPa [43], indicating that the shape and orientation depends on the relative contributions of permeate drag and compressive pressure on the deposit. Shear deformation of CMs has also been cited by Bouchoux et al. [111] as a reason for minor deviations in the viscoelastic behaviour of CMs from hard sphere models at high shear rates, and could explain the discrepancies between gel points estimated from the osmotic stressing [111] and dead-end filtration experiments [106] mentioned earlier.

Gebhardt et al. [43] also observed that deformations did not result in any reduction in CM size despite operation at TMPs above the gel point, and highlighted disagreements with the sponge model proposed by Bouchoux et al. [42]. Reasons for the disagreement were not elucidated. It is possible that the shrinkage of CMs could not be observed by Gebhardt et al. [43] because the model used for data fitting assumed an ellipsoid form factor with a fixed particle volume (equal

to that of spherical CMs with a diameter of 150 nm). The discrepancy may also be due to differences between the two compressive systems. During osmotic stressing experiments, the serum phase is only removed from the (concentrated) CM matrix, and there is little freedom for packing or deformation due to squeezing of the dialysis bag from all directions. In addition, measurements or analyses are conducted at the experimental end-point (steady-state). In contrast, during dead-end filtration, serum permeates through the CM matrix, and there is more freedom for particle movement. This constant replenishment of the serum in the CM interior and packing flexibility may explain why the sponge-like shrinking of CMs was not observed.

4.3 *Influence of Whey Proteins on Deposit Properties*

To this point we have only considered the intrinsic filtration properties of CMs. However, WPs are also retained during skim milk UF and thus it is important to understand their influence on the packing behaviour of CMs. Since WPs are significantly smaller than CMs, they can fill up the interstitial spaces within the CM matrix, thus a more densely packed deposit is expected to be formed. Interestingly, experimental observations of Steinhauer et al. [91,92,104] show that the specific resistance of CM deposits is observed to decrease in the presence of β -LG [92,104] or whey protein isolate (WPI) [104]. The decrease in specific resistance with β -LG was not observed in the presence of NEM, suggesting that β -LG attached to the CMs via disulphide linkages. β -LG denaturation could not be due to thermal effects as the experiment was conducted at room temperature, and could be due instead to concentrative effects. Supporting GISAXS measurements also indicates the formation of a fractal network, and this was attributed to the agglomeration of CMs resulting from surface attachment by β -LG [92]. Meanwhile, the CM deposit was observed to be slightly more easily diffused in the presence of WPI, meaning that CMs were less bound as a networked gel or less compressible. The reduced compressibility of the CM deposit in the presence of WPs may have been due to osmotic pressure contributions of WPs, which are significantly higher than that of CMs due to their smaller size.

The distinct size difference between WPs and CMs also implies different magnitudes of forces (e.g. hydrodynamic, electrostatic, Brownian diffusion) experienced by these particles, which may give rise to variations in the local compositions of CMs and WPs within the CP layer. Given the influence of WPs on the compressible behaviour of CMs, the distribution of WPs within the CM matrix may become important, especially from a modelling perspective to accurately describe the behaviour of the CP layer. To our knowledge, this has not been explored.

The studies currently available have provided significant insight into CP formation during skim milk UF, the key characteristics of which are illustrated in Fig. 2. However many uncertainties remain. Dewatering properties of CMs, the main component of the CP layer, have been quantified via osmotic stressing, however there are discrepancies between expectations from osmotic stressing experiments and actual filtration observations which needs to be addressed. Dead-end filtration experiments and direct observations of CMs via SAXS suggest that filtration

forces influence gel formation and the spatial arrangement of CMs, however the precise interactions are not fully understood. To this end, it is important to note that the studies so far pertain to dead-end filtration. Hydrodynamics in cross-flow filtration are more complex (especially with the inclusion of spacers in spiral wound modules), and the kinetics of gel formation and stability of the gel formed is expected to be affected by cross-flow shear forces. For the latter case, the mechanical strength of these gels could be important.

Whey proteins have been shown to influence the compressible behaviour of CMs, which is an important step from model CM dispersions to real skim milk feeds. The interactions between WPs and the packing behaviour of CMs are also relevant to skim milk MF, as WP rejection can vary with processing conditions [116,117], which has a direct influence on the packing density of the polarised CM layer, potentially altering the composition of the CP layer. For this, a more comprehensive characterisation of the impact of WPs on CM dewaterability is necessary. Lastly, CM properties can be influenced by processing conditions and natural variations, however its impact on dewatering properties of CMs have not been studied.

Fig. 2: Schematic representation of concentration polarisation and fouling phenomena during skim milk UF showing the formation of a gel-like layer and deformation of casein micelles (CMs) that occur beyond the gel point (Π_{gel}), as well as the attachment of whey proteins (WPs) to the CM surface during concentration. Π_{bulk}

and Π_{membrane} correspond to the osmotic pressures of CMs (+WPs) in the bulk fluid and at membrane surface respectively. Particles are not drawn to scale.

5. Mathematical Modelling of Skim Milk UF

Mathematical models that can describe filtration behaviour from knowledge of fluid and particle properties are required for predicting and optimising filtration performance. Central to describing this performance, the flux has to be related to the hydraulic resistance resulting from fouling and CP. A large body of work has been devoted to developing mathematical models of cross flow filtration. In this section, basic filtration models are first briefly described and discussed. These models are then discussed in the context of skim milk properties and skim milk UF. Progress towards comprehensive modelling of skim milk UF is considered, with a focus on accounting for the key complicating factors such as the deformable and polydisperse nature of milk colloids.

5.1 Basic Filtration Models

5.1.1 Film Model

The film model was one of the earliest models proposed to quantify the effect of CP. According to the film model, the boundary layer is in a dynamic equilibrium, with the convective flux of the solids normal to the membrane ($J_f c$) balanced by the diffusive flux back into the bulk [13,118]. Solids diffusion is attributed to its Brownian motion. It is assumed that the wall concentration is constant along the channel length and the boundary layer is very thin compared to the height of the channel [119]. At steady-state, the mass balance over the boundary layer is given as [13,118,120]:

$$J_s = J_f c - D \frac{dc}{dy} = 0 \quad (1)$$

where J_f , J_s , c and D are solvent flux, solids flux, concentration and diffusivity respectively. Integration of Eq. (1) over the polarised boundary layer, assuming a uniform concentration boundary layer thickness δ , gives

$$J_f = k \ln \left(\frac{c_w}{c_b} \right) \quad (2)$$

where c_w and c_b are solid concentrations at the membrane and in the bulk, respectively, and k is an effective mass transfer coefficient defined by

$$k = \frac{D}{\delta} \quad (3)$$

Mass transfer coefficients used in Eq. (2) is usually determined using theoretical or empirical correlations [13], expressions for which are available in the literature for different geometries and operating conditions [121]. Amongst them, Leveque's expression for a fully developed flow in tubes and Grober's expression for a developing flow on a flat plate are close approximations

to membrane filtration applications [119]. However, these expressions are valid only across a thin boundary layer with constant wall concentration under a laminar regime.

5.1.2 Gel-polarisation Model

The gel-polarisation model is based on the film model and takes into account the formation of a gel when the gel concentration is reached [118]. The model can be divided into two regimes: a pre-gel polarised regime and a gel polarised regime. In the pre-gel polarised regime, the wall concentration is much lower than the gel concentration and hence, the permeate flux is limited by the hydraulic resistance offered by the polarised layer which is a function of c_w . In the gel-polarised regime, the wall concentration is equal to the gel concentration ($c_w = c_g$ in Eq. (2), that is, the highest concentration possible) and any further solid build-up occurs by thickening of the gel layer.

In attempts to use the gel-polarisation model to model the UF of skim milk (and several other colloidal systems), wall concentrations relative to the bulk (c_w/c_b) were found to be unrealistically large ($\sim 10^5 \times$) [118], suggesting deficiencies in the model. This was attributed to other transport mechanisms (e.g. shear-induced diffusion or inertial lift) that augment back-transport of the species and are not included in the model. Alternatively, the permeate flux may have been limited by additional mechanisms (e.g. osmotic pressure) which are unaccounted for by the gel, boundary layer and membrane resistance terms. Apart from mechanistic factors, other assumptions required to make the problem mathematically tractable may have also contributed to this discrepancy. The uniform solute transport equation (Eq. (1)) is strictly only valid for dilute solutions in which diffusivity is independent of concentration. In the boundary layer, the concentration can be many folds larger than bulk values and hence this assumption may be questionable.

5.1.3 Shear-induced Diffusion Model

Building upon the models discussed above, the shear-induced diffusion model considers the particles deposited at the membrane surface to not flow readily until sufficient shear stress is imposed on the deposited layer. Upon reaching this sufficient shear stress, the particles tumble over each other, resulting in back transport. Zydney [122] proposed that this was the dominant mechanism for the back-transport of solids from the polarised region into the bulk. In this model, the diffusivity term in Eq. (1) is replaced with a shear-induced diffusion coefficient proportional to the applied shear rate to account for the back-transport [123]:

$$D_{SI} = 0.33\phi^2 d_p^2 \gamma (1 + 0.5e^{8.8\phi}) \quad (4)$$

Here ϕ is the solids volume fraction, d_p is the particle diameter and γ is the shear rate on the deposited solids. This model provides more insight into the influence of particle-particle interactions in the polarised region.

5.1.4 Osmotic Pressure Model

Osmotic pressure is defined as the stress within the matrix of solid particles caused by solid-solid interaction [40]. It is a function of solute concentration and can be represented by a power series for colloids as:

$$\pi = A_1c + A_2c^2 + A_3c^3 \dots \quad (5)$$

where c is the solids concentration (g/L) and A_1 , A_2 , A_3 are virial coefficients that may be determined experimentally. At any point within the boundary layer, momentum conservation shows that the sum of fluid pressure and osmotic pressure is approximately constant [40]. Hence, as c increases near the membrane, osmotic pressure increases (due to Eq. (5)), resulting in a decrease in the fluid (solvent) pressure. This decrease in pressure at the membrane results in a decline of permeate flux, modeled as [124,125]

$$J_f = \frac{\Delta P - \Delta \pi}{\mu R} \quad (6)$$

where $\Delta \pi$ is the osmotic pressure difference between the bulk and the membrane surface and R in denotes the membrane resistance only (i.e. $R = R_m$). The concentration distribution within the boundary layer is determined simultaneously from the solute transport equation (Eq. (1)).

Osmotic pressures of larger colloids (with high molecular weight) such as proteins are quite small (magnitude of 10^1 Pa) at low concentrations, however they can become significant (similar magnitudes to the TMP applied) at high solid concentrations that are observed at the membrane [126], thereby significantly reducing the net driving force for filtration. This non-linear increase in osmotic pressure at high concentrations is not accounted for in earlier models such as the gel-polarisation model [118]. It has also been shown that the flux decline caused by an increase in osmotic pressure was equivalent to an increase in polarisation or gel resistance [127,128].

5.2 Modelling Skim Milk UF

Although considerable work has been undertaken in modelling the UF of colloids, only a few attempts have been made to develop models specifically for skim milk UF. Studies have suggested that the generalised mass transfer coefficients (discussed previously) underpredict the permeate flux for milk [129,130]. Hence, attempts have been made to obtain mass transfer coefficients suitable for milk UF. Chiang [130] measured experimental flux data to obtain an empirical mass transfer coefficient, derived using the film model. In their study, an empirical correlation between diffusion coefficient and concentration was incorporated to capture the effect of solids concentration on mass transfer. In a similar study, Clarke [129] suggested that permeate flux exhibits two distinct flow behaviours: a pressure-dependent regime in which flux decline is ascribed to the decrease in driving force as given in Eq. (6), and a pressure-

independent regime, where permeate flux is controlled by mass transfer and defined by Eq. (2). Pressure and osmotic pressure terms (the latter assumed to be linear with concentration) were introduced in the Sherwood equation (mass transfer coefficient) to compensate for the pressure effects in the transition region between pressure-dependent and pressure-independent regions. Separately, Samuelsson [131] investigated the importance of various back-transport mechanisms in skim milk MF assuming CMs as the only retained species, and found that a sum of Brownian and shear-induced diffusion coefficients predicted the limiting flux most accurately. Brownian diffusion is found to be most effective for particles smaller than 0.5 μm , while shear-induced diffusion is dominant for slightly larger particles ranging between 0.5 and 20 μm [125,132]. In the case of skim milk MF, the average size of CMs is around 0.2 μm [4], thereby making both Brownian and shear-induced diffusion mechanisms significant.

In the discussions above, even though these semi-empirical filtration models may fit reasonably well with the experimental data, these models have not fully taken into account the physical properties of the colloidal particles. CMs are considered the major constituent of the CP layer in skim milk UF [101], and are polydisperse, porous and deformable [4,41,42,106,111] (see Section 2 & 4). Additionally, WPs which are also retained during UF add to the polydispersity of the system. These properties influence the hydrodynamics and performance of the system. Hence, the development of predictive models of skim milk UF should ideally account for these distinct properties.

5.2.1 Deformable Particles

During the filtration of deformable colloids, particles generally behave as hard spheres until the maximum packing fraction is exceeded, and then undergo deformation [111,134]. In the case of native skim milk ($\sim 35 \text{ g}_{\text{casein}}/\text{L}$), CMs occupy a volume fraction of about 0.1 and hydrodynamically largely behave like hard spheres. As discussed in Section 4.1, maximal closed packing of CMs occurs at $\sim 178 \text{ g}_{\text{casein}}/\text{L}$ (corresponding to the gel point) [111], above which further concentration or compression leads to the deformation of CMs.

The rate and extent of solids compression is dependent on a balance between the viscous drag force associated with the permeate flow through the deposited layer and the particle stress developed within the deposit (i.e. compressibility) [135]. The viscous drag force may be defined in terms of a deposit resistance [40,136], which is also a measure of deposit permeability. The average deposit resistance is typically assumed to be a product of the mass of solids deposited and the specific deposit resistance [137], where the specific deposit resistance for compressible solids is determined experimentally and depends on the applied pressure or deposit porosity [40,136–138]. Meanwhile, the non-hydrodynamic particle stress is equivalent to the osmotic pressure for colloidal suspensions [135,139], which in turn can be determined either experimentally [41,140] or as the sum of attractive and repulsive interactions based on the DLVO theory [141,142]:

$$\pi(\phi) = \pi_{\text{ent}}(\phi) + \pi_{\text{vdw}}(\phi) + \pi_{\text{ele}}(\phi) \quad (7)$$

Here the terms on the right hand side of the equation denote contributions from entropic, van der Waals and electrostatic interactions respectively [142]. For a given particle size, entropic and electrostatic (repulsive) contributions are higher at low volume fractions, however, beyond a critical volume fraction (i.e. gel concentration), van der Waals' (attractive) interactions dominate resulting in gel formation. On gel formation, the particle stress is defined by the compressive pressure of the gel/cake, which increases steeply with volume fraction [41,139].

This approach has been used by Bacchin et al. [140] to model cross-flow UF of deformable latex particle suspensions (120 nm diameter – similar in size to CMs) using computational fluid dynamics (CFD). In their model, an effective diffusion coefficient was defined using the particles' osmotic pressure and drag force, via the Stokes-Einstein law:

$$D = \frac{V_p}{m(\phi)} \frac{d\pi}{d\phi} \quad (8)$$

where V_p is the particle volume and $m(\phi)$ is the mobility of a particle (which is the reciprocal of the friction factor) [142]. A hindered settling function given by the Happel's function was introduced to account for the effect of particle-particle interactions in the concentrated regime [142,143]. The values of critical flux (defined as the flux above which an irreversible deposit begins to form) predicted by this model were approximately four times higher than the experimental results. This discrepancy was claimed to be due to the heterogeneity of the membrane that influenced the local permeate flux. However, the influence of particle deformability/compressibility on its mobility, which is not captured by the hard-sphere Happel's function [144], may also have contributed to the error.

A similar approach was followed by Bouchoux [40] to model the dead-end filtration of native phosphocaseinate (NPC) dispersions. The viscous drag on the fluid phase was expressed in terms of the permeability of the CM deposit layer. An empirical expression for permeability as a function of casein concentration was determined based on data from osmotic stressing and dead-end experiments. This permeability expression accounts for particle deformation and compression, and hence should overcome some deficiencies in previous model by Bacchin et al. [140]. Flux and concentration profiles predicted by this model were in the same range as the corresponding experimental results, with the difference between the two mainly attributed to the use of different conditions for the measurement of osmotic pressure and actual filtration experiments: osmotic pressures were determined for CMs dispersed in UF permeate via osmotic stressing and dead-end filtration using a 10 kDa MWCO, while the filtration experiments were conducted using CMs dispersed in water and 100 kDa MWCO membranes.

5.2.2 Polydispersity

Proteins in skim milk are polydisperse – CMs alone range from 60-300 nm and whey proteins are around 3-8 nm in size [4]. Polydispersity of a particle dispersion influences the rate of settling, shear-induced diffusion and most importantly the packing void fraction (in turn the permeability of the CP and cake/gel layers). Also, since the specific hydrodynamic drag on smaller particles is higher, and the countering specific shear-induced diffusion of larger particles is lower, the rate and extent of deposition strongly depends on particle size.

Typically a characteristic length based on a mean particle diameter is used to define various parameters such as cake permeability, drag force and shear-induced diffusion in modelling studies [145–147]. However, this approach yields average properties of the cake and thus is not capable of modelling deposition dynamics of each particle size group. To overcome this deficiency, an area-averaged iterative approach known as the predictive aggregate transport model proposed by Baruah [148] could be considered. This model is capable of predicting the permeate flux, while accounting for the transmission of smaller particles through the cake matrix comprising larger particles. In this approach, permeate flux is calculated based on different back-transport mechanisms (Brownian and shear-induced diffusion) for particles of different sizes. The minimum of these calculated fluxes is used to imply a wall concentration. After this wall concentration exceeds the maximum packing limit, the resulting pore diameter is calculated and the transmission of particles smaller than the pore diameter is evaluated using a sieving coefficient. Model predictions of permeate flux matched reasonably well with the experimental data from MF of goat milk [149], a polydisperse system comprising three retained species: fat globules, CMs and immunoglobulin. Although this model provides information on the transmission of smaller species through the cake, it does not account for a simultaneous deposition of different particle species, which could alter the behaviour of flux decline.

In this regard, a multifluid framework which has been used a few independent studies [150,151] to model the flow of polydisperse suspensions in fluidised beds might be applicable. In this approach, each set of particles (phase) is considered as an interpenetrating continuum. The continuity and momentum equations are solved for each phase to obtain individual flow fields simultaneously, and the interaction between different phases is modelled by inter-phase momentum terms. This approach is capable of modelling the rate of settling and diffusion of individual phases by defining drag models and solid stress for each phase. Extensive studies have been undertaken to establish closure relations necessary to model fluidised beds [152]. As yet, only a few studies have attempted to apply this framework to filtration, namely to monodisperse systems [153,154] by employing well-established drag models (valid for rigid spheres). While the solids stress is not considered in these models, the permeate flux showed reasonable agreement with experimental data [153]. Although this approach has not been applied to milk filtration yet, results from the polydisperse fluidisation and monodisperse filtration studies suggest that it may be able to account for the polydispersity in filtration applications. However,

development of closure relations for drag and solid stress terms specific to skim milk are required for this modelling approach.

5.2.3 Modelling Flow Non-uniformities

Due to the pressure drop along the membrane length resulting from the cross-flow velocity, the permeate flux varies along the length, resulting in a non-uniform solids concentration at the membrane surface. The UF models discussed in the previous sub-sections employ an area-averaged flux to determine the concentration at the membrane. These models do not account for local variations in the flow and its effects along the membrane.

Two-dimensional models were developed to determine the flow and corresponding concentration along the membrane length. In this approach, the Navier-Stokes equation along with a convection-diffusion equation for solids concentration are solved [155]:

$$\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot \rho \mathbf{u} \mathbf{u} = -\nabla p + \nabla \cdot \mu [\nabla \mathbf{u} + (\nabla \mathbf{u})^T] \quad (9)$$

$$\frac{\partial c}{\partial t} + \nabla \cdot c \mathbf{u} = \nabla \cdot [D \nabla c] \quad (10)$$

Different methodologies have been followed to solve this set of equations. Initially, the Navier-Stokes equation (Eq. (9)) was solved for an approximate velocity distribution along the length of the membrane, which was then applied to the solids transport equation (Eq. (10)) for the concentration profile [156]. Computational Fluid Dynamics (CFD) methods based on the finite difference method and the finite volume method have also been employed to obtain simultaneous solution of flow and concentration equations [157,158] throughout the membrane channel. The above governing equations (Eq. (9) and (10)) are solved using suitable boundary conditions. In filtration applications, the fluid velocity at the membrane is calculated either by Eq. (4) or Eq. (8) with no-slip walls [140,159,160]. The inlet flow is either set to be a fully developed flow or a plug flow [160] with a fixed velocity.

One of the main advantages of CFD is that it takes into account the effects of geometric complexities on the flow. As such, CFD models are capable of modeling additional mechanisms such as fouling and turbulence in a reasonably detailed manner [160,161]. Irreversible fouling has been found to be low at regions with higher shear rate [102], such as around structural non-uniformities. Utilising this property, mesh spacers have gained importance and become a key feature of spiral-wound modules that are commonly used for skim milk UF. Spacers are filamentous structures that keep the membrane leaves apart and destabilize the boundary layer, thereby enhancing mass transfer. However, the improvement in mass transfer comes at the expense of increased pressure drop along the channel. Spacers may be of different shapes and configurations to promote mixing, sometimes even inducing turbulence. CFD studies have been

conducted to optimize the design of spacers and associated operating conditions. A detailed review of CFD models for various spacer configurations is given in [161].

5.2.4 Fouling Resistance

In addition to CP, the evolution of fouling resistances over time should be accounted for in a predictive filtration model. As discussed in Section 3, fouling can manifest as a surface deposit or pore blocking, and simple fouling models such as complete/partial/intermediate pore blocking models and cake filtration models are available to describe either of the two types of fouling mechanisms [11,162]. In these models, the time-dependent fouling resistances are related to the particle deposition rate. For protein filtration systems (such as skim milk UF) where membrane fouling is also contributed by primary adsorption of proteins, the rate of protein deposition may also be modelled via irreversible adsorption kinetics [160,163].

However, these simple models are insufficient for describing fouling in skim milk UF, since both surface deposition and pore blocking are observed (see Section 3.4). More sophisticated combined models which take into account both pore blocking and cake formation may be more appropriate, such as the transient area-averaged protein filtration model developed by Ho & Zydney [164] or the concurrent combined model developed by Iritani et al. [165]. The former considers fouling to be a two-stage process with localised pore blocking prior to build-up of a surface deposit, whereas the latter considers both pore blocking and cake formation to occur concurrently. To our knowledge, these models have yet to be applied to skim milk UF, but have demonstrated excellent agreement with experimental data obtained for whey UF [166,167] and latex dispersions [165] respectively. In both cases, it was observed that resistance due to pore blocking dominated initially, while cake resistance becomes more significant in the later stages of filtration.

6. Processing Factors Affecting Flux Decline

The purpose of this section is to discuss how processing can affect the properties of skim milk, and how in turn filtration behaviour is affected by these changes.

6.1 Influence of Feed Pre-treatment and Feed Modifications

6.1.1 Heat Treatment

Skim milk is often subject to heat pre-treatment prior to filtration, in the form of pasteurisation, thermisation or heat-and-hold treatment. These heat treatments are not done specifically for the purpose of improving UF performance, and consequently filtration studies involving this spectrum of mild heat treatment are limited. Nonetheless, it is known that heat treatment affects the denaturation/aggregation state of whey proteins as well as the mineral balance between the serum and casein micelles. Following heat treatment, denatured whey proteins can aggregate with other whey proteins, or attach to the surface of CMs [85] via disulphide linkages (see

Section 3.3). The degree of denaturation depends on the intensity and duration of heat treatment [85,168], but even mild pasteurisation (72 °C/20s or 73 °C/15s) can cause denaturation of ~7% milk proteins [169]. Naturally, these effects are generally more pronounced with skim milks from heat-treated sources (e.g. reconstituted, UHT) as they undergo more severe heat treatments during manufacture. All these types of milks are commonly used in filtration experiments, including those investigating the effects of thermal treatment on filtration behaviour.

Heat treatment is generally seen to improve filtration performance. Renner & El-Salam [110] stated that a long heat-and-hold period of milk prior to UF increased the operation time between cleaning cycles in UF-cheese factories. El-Salam & Shahein [170] compared fluxes of skim milk reconstituted from milk powders of different heat treatments with fresh pasteurised skim milk, and found that the fluxes increased with powders produced using more intense heat treatments. In contrast, even though UHT processing causes significant denaturation of whey proteins (50-90%) [84], no differences in fluxes were observed between UHT and pasteurised skim milk [171]. This may be due to the different collective heat exposures or thermal histories experienced by the milks in their respective manufacturing processes, resulting in different milk properties.

Different gelation behaviour is also observed with heat-treated skim milks. Koutake et al. [52] observed a more adhesive protein gel deposit which could not be removed by water rinsing with reconstituted skim milk – this was not observed with pasteurised skim milk. Alternatively, the observed differences in gelation may result from differences in the casein micelles from fresh and reconstituted skim milk [172]. Paugam et al. [171] observed about 75% less proteins adsorbed on PES membranes fouled with UHT milk compared to pasteurised milk despite having similar fouling resistances, suggesting that different deposit structures were formed. In particular, UHT skim milk formed a less open or less hydrophilic fouling layer.

There is insufficient information in these studies to draw definitive conclusions about the detailed mechanisms of the influence of heat treatment on CP and fouling. Nonetheless, this can be discussed based on known physicochemical alterations that occur during heat treatment. For instance, the attachment of aggregated WPs to CMs increases the hydrodynamic size of CMs [85] changing the particle polydispersity in skim milk, which may impact the packing behaviour of the CP layer. The ‘stabilisation’ of WPs by attachment onto CMs also potentially means that there are less native WPs that can adsorb onto and foul the membrane. On the other hand, it was mentioned earlier that WPs can attach to the CM surface during filtration via disulphide interactions, resulting in the formation of a fractal network [45] which appears to be less compressible [104]. Since the fraction of WPs that are pre-attached onto the CMs prior to filtration are different in native and heat-treated skim milks, it is unclear if this pre-attachment would give rise to any differences in the dewatering properties of the CMs, for instance the networked structure formed beyond the gel point.

6.1.2 Acidification

When UF is used prior to cheese making, skim milk is sometimes pre-acidified to pH 5.9-6.1 before filtration to increase solubilisation and removal of calcium [110]. Acidification results in the dissolution of CCP and a decrease in zeta potential, however there are no marked changes in CM size between pH 4.7-6.7 [173,174]. Lower fluxes and increased protein fouling are observed as pH is lowered [110,173–176], which can be explained by weaker protein-protein and protein-membrane electrostatic repulsions which result in denser packings in both the CP and fouling layers. However this trend cannot solely be attributed to the decrease in zeta potential of the CMs. According to Kühnl et al. [177], electrostatic repulsions can be neglected under high ionic strength conditions in milk. Based on their calculations, there appears to be no significant differences in electrostatic interactions beyond a particle separation of 1 nm assuming a smooth planar surface. While not an accurate representation of the CM surface, on which a hairy κ -CN layer of about 7 nm is present, the actual electrostatic interactions are expected to be even less significant. Instead, they attributed the reduced fluxes to decreased hydrophilic repulsion due to a decrease in surface hydrophilicity as a result of reduced CM charge. That said, the influence of electrostatic interactions also depends on the degree of packing achieved in the CP layer. MF and UF operating pressures typically fall within the 100-500 kPa – at these pressures a portion of CMs in the CP layer would exceed the random closed packing fraction [111] and would be in close enough proximity such that electrostatic interactions are relevant. Meanwhile, it is unknown if the dissolution of CCP causes any changes in the CM internal structure that would influence its compressible behaviour.

While irreversible fouling was observed to increase with decreasing pH [174,175], no significant differences in fouling persistence could be inferred from cleaning comparisons [178]. This would suggest that the binding of foulants to the membrane is not strongly influenced by pH, at least not within this pH range.

6.1.3 Diafiltration

Diafiltration involves dilution of skim milk retentate with water followed by refiltration for the increased removal of lactose and salts, resulting in the reduction in ionic strength. CM size is not observed to change as a result of DF [179], however the dissolution and release of CCP from the CM has been observed through SAXS [47]. The latter result conflicts with observations by Ferrer et al. [179], who indirectly determined the CM calcium concentration via the differences between total calcium and soluble calcium in the serum of the ultracentrifuged sample. However this method is likely to be less accurate than direct SAXS observations, as it considers all non-soluble calcium, including that bound to whey proteins, as insoluble calcium within the CM.

Related filtration studies mainly investigated the effect of increasing ionic strength. Jimenez-Lopez et al. [117] observed that CMs (pasteurised skim milk) were more prone to deposition at higher ionic strengths (by NaCl addition), and also formed a more cohesive CP deposit. The

observations were attributed to weaker electrostatic interactions, as they measured no change in CM size and a decrease in zeta potential from an ionic strength of 72 to 372 mM. This is consistent with lower limiting fluxes (higher CP resistance) observed by Rabiller-Baudry et al. [176] at higher ionic strengths, although in their case no significant change in zeta potential was observed above an ionic strength of 172 mM (by NaCl addition). A point to note is that mineral additions also have an influence on the calcium phosphate equilibria. In particular, the addition of NaCl induces the dissolution of CCP from the CMs, while more calcium is incorporated into the CMs when CaCl_2 is added [39,180]. In contrast, minerals in the aqueous phase are diluted as a whole during DF, and this appears to ultimately result in the removal of CCP from CMs. Since CCP is integral to the CM structure, changes in CCP composition within the CM might influence its compressible behaviour, however this has not been explored.

On the other hand, in a study by Rabiller-Baudry et al. [53], foulants present on a UF PES membrane fouled by 1/3 diluted skim milk under static conditions were identified to be proteins (see Section 3.1.1). This fouling composition is the same as that caused by skim milk, suggesting that the fouling behaviour is not affected by salts and lactose removal during DF. Comparisons of foulant protein composition and quantities were not available. To our knowledge, there are no other studies that have directly investigated the effects of very low ionic strength or the effect of lactose removal resulting from DF on CP and fouling. However, it can be inferred from the aforementioned studies that a reduction in ionic strength due to mineral removal would enhance (repulsive) interactions, resulting in a less compact CP layer.

6.2 *Influence of Operating Conditions*

6.2.1 *Temperature*

Skim milk UF is most commonly conducted at 50 °C, though in some parts of the world cold filtration (<10 °C) is preferred. The vast majority of the milk filtration studies relevant to fouling have generally been conducted at 50 °C. Processing temperature has an effect on CMs and the structural stability of whey proteins. In particular, calcium solubility decreases with temperature, which causes more calcium to be incorporated into the CMs as CCP and increases the average CM size (from 200 nm to 220 nm between 10-40 °C) [21]. This results in compositional differences in UF retentates produced at different temperatures [181]. However, it is not known whether this change in size is sufficient to induce noticeable differences in filtration behaviour given the high polydispersity of CMs. In addition, increased dissociation of β -CN from CMs is observed at low temperatures (<10 °C) [4], but its effect on filtration behaviour has not been studied. On the other hand, increased temperature increases the likelihood of protein denaturation and aggregation, for instance the increased thiol reactivity resulting from the dissociation of β -LG dimers into monomers which occurs above 40 °C (Section 3.3). Conversely, this suggests that fouling may be less pronounced in skim milk UF at low temperatures. However to our knowledge, no comparisons between the fouling behaviours at 10 and 50 °C have been made.

While higher fluxes are generally seen with higher temperatures, flux does not provide any indication of changes to CP and fouling due to significant viscosity changes which masks all other compounding effects. According to data reported by Chiang & Cheryan [130], a minor decrease in CP resistance was seen with increasing temperatures (40-60 °C), while no significant differences in fouling resistance were observed. Meanwhile, lower cleaning efficiencies were generally observed with fouling at higher temperatures [178], suggesting that the fouling layer formed at higher temperatures is more strongly bound to the membrane surface.

6.2.2 Hydrodynamics

System hydrodynamics are not known to cause any alterations in skim milk properties, but they do affect flux decline behaviour. Higher fluxes (reduced CP) are observed at lower TMP and higher cross-flow velocity (CFV) [130,182], similar to trends described in Fig. 2. Furthermore, the degree of CP and distribution of fouling along the membrane module is influenced by hydrodynamics. Piry et al. [183,184] investigated the variation of flux and resistances along the channel length of the membrane module during skim milk MF (0.1 µm cutoff, 1.2m long, divided in four sections), and observed generally higher reversible flux resistances (CP) at the module inlet, which gradually decreased along the channel length. This was attributed to higher local TMPs at the membrane inlet compared to the outlet. Comparatively, irreversible resistances (fouling) did not vary significantly with local TMP [130,183,184]. Overall this suggests that operating at high cross-flow velocity, which is generally favoured to minimise CP, potentially results in downstream sections of the module operating at sub-optimal TMPs. Unfortunately hydrodynamic limitations due to pressure drops along the membrane module are inherent to the module. As such they can only be addressed by improvements in membrane module design, for instance design improvements in spacer (turbulence promoter) geometry to maximise mass transfer while minimising pressure drops, or special modules that allow uniform pressure drops (e.g. ceramic membranes with longitudinal porosity gradients) [2,12].

In regards to fouling distribution, Delaunay et al. [102] investigated the distribution of charcoal particles during the UF of a charcoal suspension, and observed that regions predicted by CFD modelling to have higher shear rates had reduced particle deposition. They reported similar trends for the deposition of whey. In a similar study, Rabiller-Baudry et al. [185] investigated the distribution of deposited protein along the channel length of a Koch HFK-131 membrane (5-10 kDa cutoff, 1m long) fouled by UHT skim milk, and observed greater protein deposition towards the module inlet. No particular trend could be determined between the localised protein deposition and local TMP ($TMP_{in}=3.5$ bar, $TMP_{out}=2$ bar), which is consistent with observations of Piry et al. [183,184] discussed earlier. It should be noted that the differences in inlet and outlet TMPs may not be large enough to cause significant differences in irreversible fouling. Wemsy-Diagne & Rabiller-Baudry [186] observed that the amount of protein in the irreversible fouling layer produced during skim milk UF (UHT) increased with applied TMP (between 1.5 to 4 bar).

1 However, no differences in protein fouling were seen between 2 and 3.5 bar, which were the
2 outlet and inlet TMPs in the study by Rabiller-Baudry et al [185]. They also found that
3 irreversible fouling formed at higher TMPs was resistant to chemical cleaning [103,186],
4 presumably due to the formation of a more compact deposit and hence less penetrable by
5 cleaning solutions.

6
7 The relationship between modifications of the physicochemical properties of skim milk and flux
8 decline mechanisms has been studied to some extent. For instance, acidification and diafiltration
9 both affect the electrostatic interactions between milk particles, and their impact on CP and
10 fouling has been discussed alongside the general colloidal properties of CMs (size, zeta potential,
11 ionic strength). On the other hand, the influence of heat treatment on filtration behaviour has not
12 been studied in sufficient detail to facilitate a proper discussion on its impact on CP and fouling,
13 however, some speculations have been made based on known effects of heat treatment on milk
14 properties. Nonetheless, a recurring question with these studies on physicochemical
15 modifications is whether the resultant alterations in CM size, polydispersity, composition and/or
16 structure have an impact on its intrinsic dewatering properties (packing behaviour, permeability),
17 as analyses of flux or CP resistance alone do not provide sufficient insight due to various
18 compounding effects such as hydrodynamics or viscosity.

19
20 From an operational perspective, there are only a limited number of processing variables that can
21 be adjusted to optimise filtration operation, though their interactions with CP and fouling have
22 not been completely explored. In particular, despite common application of skim milk UF at 10
23 and 50 °C, majority of the fouling studies are based on skim milk UF at 50 °C. Even though
24 there are temperature-dependent milk properties that are relevant to CP and fouling (e.g. CM size,
25 protein denaturation), to our knowledge the flux decline behaviour at this temperature has not
26 been investigated in detail. Meanwhile, hydrodynamic improvement is largely subject to
27 hardware limitations, though a deeper understanding on the interactions between hydrodynamic
28 forces in cross-flow filtration and the dewatering properties of CMs (e.g. packing, gelation
29 behaviour) as well as fouling might allow more precise optimisation of hydrodynamic conditions.
30 Finally, given that thermal pre-treatments influences protein structural stability, and can have a
31 positive impact on flux and fouling, there could be potential applications of thermal (or similar)
32 pre-treatments tailored to optimise filtration performance while still maintaining the desired
33 product properties (e.g. pasteurisation, flavour) for downstream processing.

34 **7. Membrane Cleaning**

35 Membrane cleaning is an integral part of membrane processing operations. Fouling not only
36 reduces filtration productivity, it also promotes microbial growth posing a contamination risk for
37 the processed product [8]. As such, regular chemical cleaning is necessary during skim milk UF
38 operation. Fouled membranes are commonly rejuvenated via cleaning in-place (CIP) procedures.
39 A cleaning regime in the dairy industry typically involves an initial water rinse to remove

residual milk and loosely bounded material, followed by a series of chemical cleaning steps: alkaline cleaning → water rinse → acid cleaning → water rinse → disinfection/sanitisation under chlorinated alkaline conditions → final water rinse [8,187]. Cleaning requires the use of large amounts of water and chemicals are consumed, and incurs significant operational downtime (2-3 hours per 8 hours of operation) [187]. In addition, harsh cleaning can decrease membrane life and may not be tolerated by other components of the membrane module (e.g. glue seals, gaskets and O-rings). Consequently, the main goals of cleaning optimisation are to reduce water and chemical consumption as well as operational downtime without compromising membrane cleanliness or membrane longevity. These goals can be achieved by simplifying cleaning protocols (e.g. utilising cleaning synergies), optimising cleaning blend formulation or optimising cleaning conditions. Optimisation of chemical cleaning requires an understanding of fouling, and conversely, an analysis of chemical cleaning effectiveness can provide information about fouling mechanisms. For this, it is first necessary to understand the function of the different cleaning chemicals and their observed cleaning effectiveness.

The action of chemicals used for membrane cleaning in the dairy industry have been discussed in a number of literature sources (e.g. [8,188,189]), and are summarised in Table 6 below. Briefly, alkalis, oxidants and enzymes are used for digesting organic material. Strong oxidants (e.g. chlorine) can accelerate membrane degradation ageing, and are more commonly used for disinfection purposes. Acids and sequesterants are used for dissolving mineral precipitates and chelating mineral ions, respectively. Surfactants decrease interfacial energy, thus weakening hydrophobic interactions and aiding desorption. Lower interfacial energies have also been associated with better cleaning [187,190,191].

Table 6: Categories and functions of membrane cleaning agents [188,189].

Cleaning agents	Examples	Major Function
Alkali / caustic	NaOH	Hydrolysis, solubilisation of organics; alteration of surface charge
Acids	HCl, HNO ₃ , H ₃ PO ₄ , citric acid	Dissolving inorganic salts
Sequesterants	Citric acid, EDTA	Chelation of minerals
Oxidants	NaOCl, Cl ₂ , H ₂ O ₂	Oxidation of organics; disinfection
Surfactants	SDS	Reducing interfacial energy, desorption
Enzymes	Proteases, lipases	Hydrolysis of proteins and lipids

Given the prevalence of protein and lack of minerals in the irreversible fouling layer of skim milk UF (see Section 3.1), it would be expected that cleaning agents that target organics would be most effective. However, several studies have found that cleaning with sodium hydroxide (NaOH) alone, which is typically done at pH 11-12, only provides relatively minor flux improvements after water rinsing, and is no more effective than acids [103,192–194]. Wemtsy Diagne et al. [103] cited a previous study by Begoin that showed NaOH alone did not hydrolyse

1 milk proteins at pH 11.5 and 50 °C in 1 hour. It has also been suggested that caustic-induced
2 gelation occurs during alkaline cleaning, of which the gelation rate was found to slowest at
3 around pH 12 [195].
4

5 Sodium hypochlorite (NaOCl, an oxidant) and sodium dodecyl sulfate (SDS, a surfactant) have
6 been found to be more effective, particularly when applied under alkaline conditions [53,192].
7 The synergistic effect between NaOCl and NaOH may be explained by the protein deposit
8 having a more open structure under alkaline conditions due to stronger electrostatic repulsions,
9 facilitating better solid-liquid contact between adsorbed proteins and cleaning fluids [188,193].
10 The combination of SDS and NaOH allows simultaneous weakening of hydrophobic interactions
11 and strengthening of electrostatic repulsion, both of which are conducive to protein desorption.
12

13 The effectiveness of SDS over NaOH and HCl has also been observed with membranes fouled
14 by sweet whey or model BSA/β-LG solutions [196]. This highlights the importance of reducing
15 interfacial energy in cleaning, and is consistent with the dominant contribution of hydrophobic
16 interactions over electrostatic interactions in adsorptive fouling. To this end, Rabiller-Baudry et
17 al. [187,190,191] characterised a range of basic and industrial chemical cleaning blends and
18 found a linear positive correlation between interfacial energy of the cleaning solution and
19 residual protein. They suggested that an optimum cleaning solution should have an interfacial
20 energy of less than 30 mJ/m² with a non-polar character.
21

22 In accordance with the apparent lack of minerals in skim milk fouling layers (see Section 3.1),
23 acids and chelatants alone have been shown to be quite ineffective [59,192,194]. In addition, acid
24 cleaning was found to increase flux despite little or no removal of protein [193,194]. The flux
25 increase was observed with nitric (HNO₃), phosphoric (H₃PO₄) and citric acids, but not with
26 sulphuric (H₂SO₄) or hydrochloric (HCl) acids. In the case of H₃PO₄ and citric acid [194] 20-
27 30% protein removal was reported to correspond to full flux recoveries. Furthermore, the
28 magnitude of flux recovery after nitric acid cleaning was observed to increase with the amount of
29 residual protein present prior to the acid cleaning step (no additional protein was removed during
30 nitric acid cleaning). The authors attributed this to the adsorption of oxoanions to the residual
31 proteins, resulting in a more hydrophilic protein deposit. Preceding NaOH cleaning with a nitric
32 acid step also did not result in any improvements in flux or protein removal [193,194],
33 suggesting either the absence of salt bridges, or the ineffectiveness of acid cleaning in removing
34 them. Furthermore, as will be discussed below, sequesterants such as EDTA can achieve the
35 same purpose and are already present in most commercial alkaline cleaning blends, suggesting
36 there is no real need for an acid cleaning step. These studies also demonstrate that flux may not
37 always be a reliable indicator of membrane cleanliness.
38

39 Although EDTA (a sequesterant) and NaOH were not found to be effective in isolation, when
40 used together they resulted in significant flux recovery for skim milk UF [192]. This suggests the

importance of even small amounts of salts in stabilising the protein deposit. This could be via salt bridging or co-adsorption with the protein in order to maintain charge neutrality [65]. The greatest effect has been observed with NaOH/NaOCl [197] and NaOH/SDS/EDTA blends [192], with ~100% flux recoveries being reported. Daufin et al. [59,198] reported full flux recovery of ceramic zirconium dioxide (ZrO₂) membranes fouled by fresh skim milk after cleaning with NaOH/NaOCl or a blend of NaOH, EDTA and anionic surfactants. Kazemimoghadam & Mohammadi [192] observed full flux recovery after UF (PSf) of pasteurised skim milk using a blend of NaOH/SDS/EDTA. They also reported successful applications on industrial plants, achieving 100% flux recovery while significantly reducing water usage. In a contrasting study, Wemsy Diagne et al. [103] reported only a 60% flux recovery of PES UF membranes fouled by UHT skim milk (50°C) and cleaned using Ultrasil-10 (a commercial blend of NaOH/SDS/EDTA). The discrepancies here could be due to difference in milk used (UHT vs pasteurised), a lower cleaning temperature (46°C vs 50-60 °C), or application of a TMP during cleaning (vs no TMP). Nevertheless, a NaOH/SDS/EDTA (alkaline/surfactant/sequesterant) blend appears to be capable of achieving full flux recovery in one cleaning cycle, given the correct cleaning conditions.

Overall, the cleaning studies above also show that almost complete membrane rejuvenation can be achieved using NaOH/SDS/EDTA or NaOH/NaOCl blends alone, provided the cleaning conditions are optimised. Moreover, as the acid cleaning step is ineffective (at least for organic membranes), omission of this step from the current alkaline-acid-alkaline cleaning protocol commonly used in the industry would result in significant reductions in cleaning costs and operational downtime.

Table 7: Details of experimental conditions employed by the cleaning studies discussed in this review.

Milk Source	Membrane	Cleaning Agents	Fouling Conditions	Cleaning Conditions	Ref.
Pasteurised Whole Milk	PSf (30kDa)	HCl, HNO ₃ , NaOH, NaOCl, EDTA, SDS, EDTA+NaOH, EDTA+SDS+NaOH	50 °C, TMP=3bar, 30min	30 °C, no TMP, 30min, pH 9.2-12.7 for alkaline cleaning	[192]
UHT Skim Milk	PES (5-10kDa)	NaOH (pH11.5), P3-Ultrasil10 (pH12.0), P3-Ultrasil53 (1wt%) + HNO ₃ /HCl (pH2.5)	46 °C, TMP=1-4bar, CFV=0.3m/s, VRR=1, 3hr	TMP=2bar, CFV=0.3m/s, VRR=1	[103]
Skim Milk	PES (5-10kDa)	HCl (pH1.6), NaOH (pH11.5), NaOH (pH11.5) + NaOCl (200ppm active Cl ₂)	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1, 2.5hrs	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1, 1hr	[193]
UHT Skim Milk	PES (5-10kDa)	NaNO ₃ (8.5g/L, pH6.6) HCl (pH1.6), HNO ₃	50 °C, TMP=2bar, CFV=0.3m/s,	50 °C, TMP=2bar, CFV=0.3m/s,	[194]

		(pH1.6) H ₃ PO ₄ (pH1.6), citric acid (pH1.8), H ₂ SO ₄ (pH1.6), NaOH (pH11.5), NaOH (pH11.5) + NaOCl (200ppm active Cl ₂), P3-Ultrasil53 (1wt%) +HNO ₃ /HCl (pH2.5)	VRR=1	VRR=1	
Skim Milk (diluted 1/3)	PES (5-10kDa)	NaOH (pH11.0), Tween20 (pH9.2), Ultrasil10 (pH11.4)	20 °C, TMP=2bar, CFV=2m/s, VRR=1, 2.5hrs	NaOH (pH11.0): 40-50 °C, 1hr Tween20 (pH9.2): Room temp., 0.5hr Ultrasil10 (pH11.4): 40 °C, 1hr	[53]
BSA+β-LG	PSf (100kDa)	HCl (0.1M),NaOH (0.1M), SLS (0.75wt%), TAZ (Terg-A-Zyme) (1wt%)	n/a	n/a	
Whey	PES (10kDa)	HCl (0.05M), NaOH (0.1M) SLS (1wt%), Protease A (0.0005wt%)	n/a	n/a	[196]
UHT Skim Milk	PES (5-10kDa)	NaOH (pH11.5) SDS (3.5mmol/L, pH11.5) Tween 20 (0.06mmol/L, pH11.5) P3-Ultrasil10 (0.1-0.4wt%, pH11.5-12.0) Ultraclean II (0.3vol%, pH11.5)	50 °C, TMP=1 or 2bar, CFV=0.5 or 2m/s 2.5hrs, VRR=1	n/a	[187]
	PES (5-10kDa)	Ultraclean II Ultrasil10 SDS+Tween20 SDS (3.5mmol/L) Tween20 (0.06mmol/L)	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1	[190]
UHT Skim Milk	PES (5-10kDa)	NaOH (pH11.5) Tween20 (0.06mmol/L) SDS (3.5mmol/L) SDS+Tween20 Ultrasil10 (pH11.5-12.0)	50 °C, TMP/CFV =2bar & 2m/s or 1bar&0.5m/s, VRR=1, 2.5hrs	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1	[191]

		Ultraclean II (pH11.5)			
Skim Milk	ZrO ₂ (10kDa)	NaOCl (1000mg/L active Cl ₂ , pH11) HNO ₃ (0.036mol/L) HNO ₃ /NaOCl NaOCl/HNO ₃	50 °C, Constant flux= 60LMH, CFV=4m/s, 175min	TMP=3bar, CFV=6m/s 5min HNO ₃ , 10min NaOCl	[59]
UHT Skim Milk	PES (5-10kDa)	NaOH (pH11.5) NaOH (pH11.5)+NaOCl (200ppm active Cl ₂) Ultrasil10 (pH11.5)	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1, 2.5hrs	50 °C, TMP=2bar, CFV=0.5m/s, VRR=1	[197]

1

2 8. Conclusions

3 Recent advances in our understanding of milk chemistry, membrane science, and mathematical
4 modelling can yield new insights into the mechanisms of flux decline in skim milk UF. In
5 particular, the past decade has seen significant advances towards characterisation of the CP layer
6 in skim milk UF. The quantification of the intrinsic dewatering properties of casein micelles via
7 osmotic stressing, and the ability to directly observe the behaviour of CMs during filtration have
8 revealed the importance of CM compressibility and permeability on UF flux. This constitutes a
9 good basis for understanding CP in skim milk UF, though much remains to be explored. For
10 instance, there are still discrepancies between expectations from osmotic stressing experiments
11 and the actual filtration behaviour which have not been completely resolved. Filtration forces
12 clearly do influence both CM packing and gel formation, however the precise interactions are
13 still not fully understood. This is important when taking into consideration the hydrodynamic
14 forces during cross-flow filtration, as the operating TMPs in UF and MF are of a magnitude
15 higher than the gel point of CMs. This means that although gel formation is inevitable in skim
16 milk UF, it may be mechanically disrupted by hydrodynamic forces. In this regard, mechanical
17 properties of the gels formed could be relevant. Furthermore, CM composition and size can be
18 influenced by processing conditions, but the impact on its dewatering properties is yet to be full
19 described in skim milk UF. Moving from model CM dispersions to skim milk applications, a
20 comprehensive characterisation of the impact of WPs on CM dewaterability is also necessary.
21 Mathematical modelling is a powerful tool for studying CP, and there has been success with
22 using osmotic pressure data to model dead-end filtration of CMs. However, the models and
23 accompanying physical data developed thus far have not taken into account the effects of whey
24 proteins on particle polydispersity and their influence on CP during cross-flow filtration.

25

26 Meanwhile, membrane fouling by skim milk has been consistently shown to be caused primarily
27 by proteins, with some mineral fouling only seen on ceramic membranes, and the underlying
28 mechanisms of fouling have been discussed. However, these observations are based on a limited
29 number of independent fouling studies which employ different experimental parameters

(operating conditions, milk source, membrane characteristics), and despite the consistency in results, ought to be validated with more controlled experiments. In particular, a better understanding of the influence of milk sources (e.g. fresh, reconstituted, UHT) on filtration behaviour would be valuable for reconciling the results obtained with different milk sources in the currently available literature and any future works.

Nevertheless, the prevalence of protein fouling and its discussed mechanisms are also consistent with observations from cleaning studies. An important result here is the ineffectiveness of the acid cleaning step, as its omission from the current industrial cleaning practice (alkaline / acid / alkaline with disinfection) will result in significant reductions in chemical and water consumption and also operational downtime. Adding to this, NaOH/SDS/EDTA or NaOH/NaOCl blends alone have been demonstrated to be capable of achieving complete membrane rejuvenation, provided the cleaning conditions are optimised for. These are promising avenues for optimising skim milk UF as a whole, but still require more rigorous testing to ensure complete fouling removal.

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Graphical Abstract