THE FUNCTIONAL PROPERTIES OF MILK PROTEIN CONCENTRATES

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Abstract

The aim of this thesis was to explore aspects of the functional properties of MPC85 (milk protein concentrate, 85% protein). A rheological study of milk protein concentrate (52°C) prior to spray drying showed a slight age-thinning behaviour which lasted about one hour, after which the apparent viscosity of the concentrate remained constant. This result is the opposite of skim milk concentrate which age-thickens at evaporator temperatures. The flow behaviour of the concentrate was adequately described by a Power Law rheological model.

The rheological properties of reconstituted commercial MPC85 were studied at various temperatures and concentrations. At low concentrations (<10% w/w total solids) MPC85 solutions were Bingham Plastics. The yield stress was found to increase with temperature and concentration. At high concentrations (>15% w/w total solids) the logarithm of apparent viscosity was found to increase linearly with protein concentration. These solutions were also found to be Bingham Plastics. At lower temperatures (< 35°C), however, these MPC85 solutions (>15% w/w total solids) were pseudoplastic and did not possess a yield stress.

The solubility of commercial MPC85 was found to be dependent on the temperature at which the solution was prepared, increasing from ≈59% at 20°C to 100% at 50°C. Homogenisation was shown to improve the solubility of MPC85 at 20°C. The rheological properties of MPC85 were profoundly influenced by the presence of any insoluble solids.

The effect of preheat treatments during the pilot-scale manufacture of MPC85 on functionality was investigated. Heat treatment had no effect on heat stability of reconstituted MPC85 solutions for whey protein denaturation (WDN) values up to 86%. Heat treatments resulting in ≥90% WDN produced a dramatic loss in heat stability. The variations in rheology and rennet coagulation properties among the pilot plant powders were found to be correlated with the apparent diameter of the casein micelles. In

reconstituted solutions the apparent diameter of the casein micelles increased gradually with heat treatments up to 86% WDN and dramatically at higher WDN levels.

The main effect of preheat treatments during manufacture on the rheology of MPC85 solutions was the linear increase in apparent viscosity with apparent diameter of casein micelles. The variation in apparent viscosity with apparent diameter of casein micelles was found to be greater at low shear rates. A schematic model was proposed to account for these observations.

A factorial design experiment was used to identify the components and interactions of components which play a significant part in determining the functionality of MPC85. This work demonstrated techniques for modelling heat stability - pH profiles and thereby allowing the quantitative comparison of the entire profiles of different solutions rather than comparisons at just single pH values or qualitative comparisons regarding the shape of the profile. The addition of divalent cations in the absence of added phosphate resulted in solutions that were completely unstable at 120°C.

Overall this work has provided a detailed characterisation of commercial MPC85, both of the rheology of the concentrate prior to spray drying and of the functional properties of the powder. The research presented here has implications for both processing and product formulation.

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1. Review of literature

1.1 Introduction

The conceptual focus of this thesis was to study the rheological properties of some of the newer milk protein products such as total milk protein, micellar casein and in particular milk protein concentrates. While there is much published literature available on the rheology of skim milk concentrates, whole milk concentrates and caseinates, there is considerably less information available about these newer products. The following literature review therefore covers: the chemistry of the main components of milk known to affect rheology, namely protein and salt components; the processing equipment and practices used in manufacturing milk protein products; the published literature on the rheology of the older milk protein products, especially skim milk concentrates which are the most similar in composition to the newer products; and a review of the information already published on the newer milk protein products.

The review of milk proteins will be limited to their basic chemical behaviour including their affinity for ions, temperature dependence and structural arrangement in milk products since these areas are of importance when considering the rheological behaviour of milk protein systems.

The objective of this review was to develop a plan for further research.

1.2 Chemistry and thermophysical properties of milk

Milk is composed of a complex mixture of lipids, proteins, carbohydrates, vitamins and salts. Of particular importance to the rheological properties of milk are the protein and salt components. Fox (1982), however, reports that the role of lactose in concentrated milk products may also be particularly significant and so a brief review of the chemistry and properties of lactose is also covered.

1.3 Milk proteins

There are seven major proteins present in milk at a total protein concentration of 30-35 g/litre. Milk proteins are classified as either caseins or whey proteins. Table 1-1 shows some of the structural and chemical characteristics of these major milk proteins.

Table 1-1 Some Structural and Chemical Characteristics of Milk Proteins (Kinsella *et al.*, 1989).

Property	Whey			Caseins			
	β-lg	α-la	BSA	α_{s1}-	α_{s2}-	β-	κ-
Molecular Weight	18362	14194	65000	23612	25228	23980	19005
Total No. residues	162	123	581	199	207	209	169
Apolar residues [%]	34.6	36	28	36	40	33	33
Isoionic point	5.2	4.2-4.5	5.3	4.96	5.27	5.2	5.54
No. proline residues	8	2	28	17	10	35	20
No. lysine residues	15	12	59	14	24	11	9
No. phosphoryl groups	0	0	0	8-9	10-13	5	1
No. disulphide bonds	2	4	17	0	1	0	1
No. thiol groups	1	0	1	0	0	0	0
Secondary structure (%)							
α-helix	15	26	54	-	-	9	23
β-sheet	50	14	18	-	-	25	31
β-turns	18	-	20	-	-	-	24
unordered	-	60	-	-	-	66	-
Native conformation	Globular			Extended			

Abbreviations used: β-lg = β-lactoglobulin; α-la = α-lactalbumin: BSA = bovine serum albumin : α_{s1}-casein: α_{s2}-casein: β-casein: κ-casein.

1.3.1 Casein proteins

Casein comprises the largest fraction of bovine milk proteins (80% total protein). The principal genetic variant of casein is α_{s1}-casein B (>40%). This variant possesses eight sites of post-translational phosphorylation; consequently, this protein exhibits interactions with calcium typical of the caseins. Another important characteristic is the clustering of polar and non-polar residues. These characteristics suggest a unique dipolar structure composed of a highly solvated, charged domain and a hydrophobic globular domain. Most likely the polar domain approaches random coil type behaviour

and the hydrophobic domain possesses a mixture of α-helix, β-structure, β-turns, and unordered structure. The flexible nature of the polar domain causes the molecular dimensions to be very sensitive to ionic strength and to binding of ions, particularly protons (H^+) and Ca^{2+}. In addition, intermolecular interactions between hydrophobic domains lead to self-association, or association with other caseins. These hydrophobic interactions become more important as the polar domain is discharged by binding of Ca^{2+} to the orthophosphate groups, since this binding greatly reduces the dimensions of the polar domain. The intermolecular interactions then result in precipitation of isolated α_{s1}-casein or formation of micelles by interaction with κ-casein (Swaisgood, 1985).

The general characteristics of α_{s1}-casein are shared by the other calcium-sensitive caseins i.e. α_{s2}-casein and β-casein. Structures of both α_{s2}- and β-casein are characterised by charged polar domains and hydrophobic domains. Like α_{s1}-casein, sequences in the polar domains, which may approach a random coil secondary and tertiary structure, are such that clusters of seryl residues are phosphorylated. α_{s2}-Casein contains several phosphoseryl clusters and thus is the most hydrophilic, whereas β-casein contains only a single phosphoseryl cluster in the N-terminal sequence and the remaining large C-terminal sequence is very hydrophobic.

Therefore, β-casein is the most hydrophobic of all the milk proteins and its characteristics are consequently the most temperature dependent. Predictably, the properties of α_{s2}-casein are more sensitive to ionic strength than to temperature (Swaisgood, 1985).

The amphipathic nature of the structure of κ-casein is a key factor in the unique ability of this protein to stabilise the milk casein micelle. κ-Casein does not contain clusters of phosphoseryl residues in its polar domain as do the calcium-sensitive caseins; hence it does not bind as much Ca^{2+} (1-2 versus 8-9 mol/mol for α_{s1}- and 4-5 mol/mol for β-casein (Swaisgood, 1985)), so the polar domain is not discharged or dehydrated by addition of this ion. Consequently, κ-casein is not precipitated by Ca^{2+}. In physiological buffers, isolated κ-casein exists in the form of large spherical aggregates resembling soap micelles, each held together by lateral interactions among the hydrophobic

domains. The hydrophobic domain of κ-casein interacts with similar domains of other caseins when they are present (Swaisgood, 1985).

1.3.1.1 Arrangement of casein in milk protein products

As a result of their relative predominance and tertiary and quarternary conformation the arrangement of caseins is the main determinant of the functional properties of milk and milk protein products.

In milk, and most milk protein products, the caseins, due to their phosphorylation and amphiphilic nature, interact with each other and with calcium phosphate to form micelles. The micelles range in size from about 30 nm to 300 nm. Typically, micelles are composed of 92% protein, with α_{s1}-, α_{s2}-, β-, and κ-casein in a ratio of 3:1:3:1, and 8% inorganic matter, composed primarily of calcium and phosphate (Swaisgood, 1985). While much work has been done on elucidating the nature of micelles their actual structure is still unclear.

Models purporting to represent structure of the casein micelle fall into two categories: sub-micelle models (Slattery and Evard, 1973; Schmidt, 1982; Walstra and Jenness, 1984; Walstra, 1990) and more recently non sub-micelle models (Visser, 1992; Holt, 1992; Horne, 1998).

The development of sub-micelle models is based on evidence from electron micrographs which show casein micelles composed of what appears to be sub-micelles about 10 nm in diameter (Schmidt and Buchheim, 1970; Knoop *et al.*, 1973; Kalab *et al.*, 1982). In the sub-micelle model of Schmidt (1982), refer Figure 1-1, sub-micelles of varying composition are linked by colloidal calcium phosphate. Sub-micelles enriched in κ-casein are located at the surface while κ-casein depleted sub-micelles are buried inside the micelle.

In recent years, sub-micelle models have been challenged on the basis that they do not explain changes in the micelle resulting from calcium sequestration; heating and subsequent cooling of milk; lowering the pH of skim milk. A full discussion of these

objections is given by Visser, (1992). Recently a non-sub-micelle model described as a dual-bonding model has been formulated (Horne, 1998) based on hydrophobic and electrostatic interactions. In this model, refer Figure 1-2, bonding occurs between the hydrophobic regions, depicted as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to colloidal calcium phosphate clusters (CCP). Molecules of κ-casein limit further growth and are labelled with the letter 'K'.

The debate over the micelle models is still continuing. At the time of writing, a paper by Walstra (1998) entitled "Casein sub-micelles: do they exist?" was cited by Horne (1998) as being accepted for publication. However, despite disagreement over the exact structure of the micelle, the concept of the casein micelle electrostatically and sterically stabilised by a 'hairy layer' coat of κ-casein appears to be universally accepted (Walstra, 1990; Swaisgood, 1992; Horne, 1998).

In the manufacture of caseinates the acidification and neutralisation treatments employed for precipitation and resolubilisation remove the colloidal calcium phosphate matrix that stabilises native casein micelles in milk. The resulting protein system consists of polymerised casein sub-units that are probably arranged in an ordered structure that allows maximum interaction through hydrophobic bonding and also retains the polar, acidic groups in an exposed position where they can be readily influenced by pH and ionic composition of the medium. Such reformed casein polymers bear little resemblance to native casein micelles in milk, even if produced under conditions that closely resemble those in milk with respect to pH, $[Ca^{2+}]$, [inorganic phosphate] and other compositional factors, since they do not contain the colloidal calcium phosphate structure of native micelles (Morr, 1982).

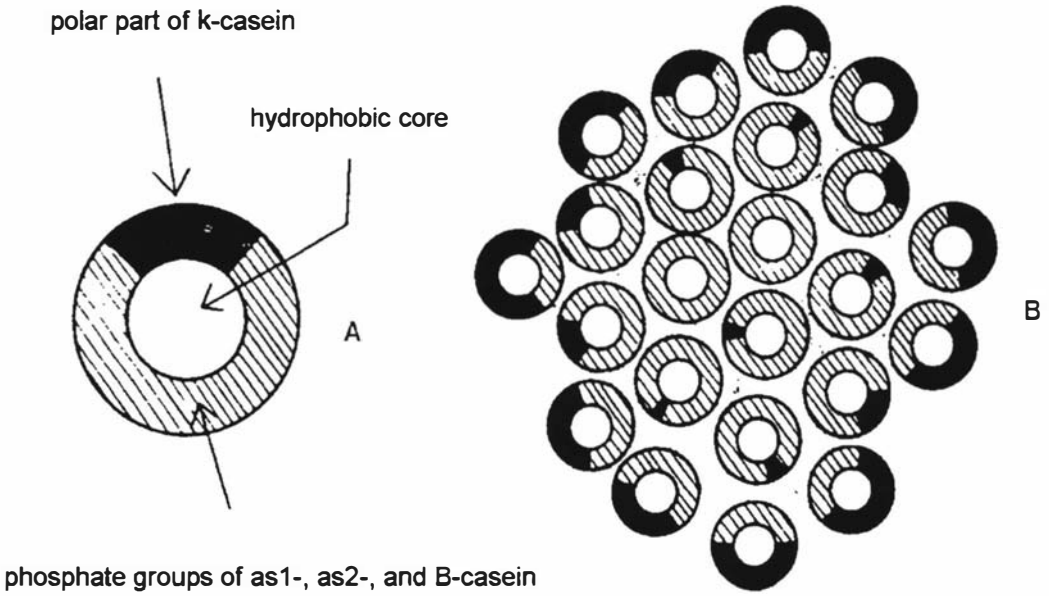

Figure 1-1 Casein sub-micelle model according to Schmidt (1982): A) sub-micelle; B) casein micelle.

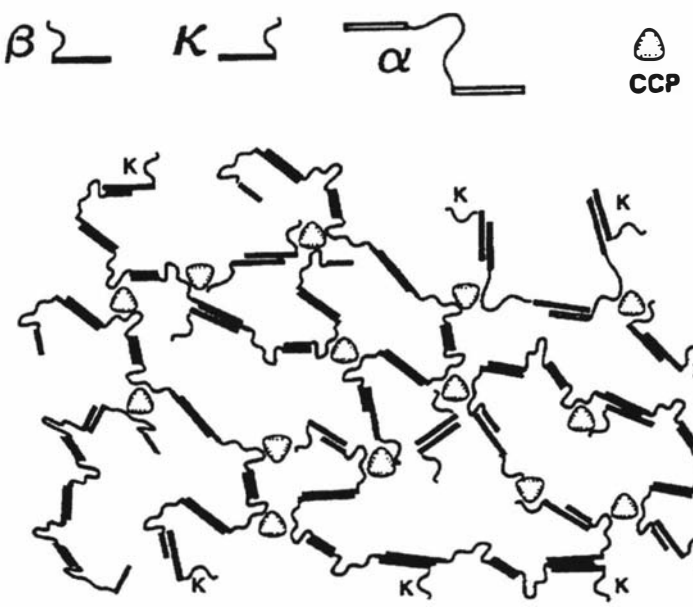

Figure 1-2 Dual bonding model of casein micelle structure (Horne, 1998).

1.3.2 Whey proteins

The structural and chemical characteristics of the three main whey proteins are shown in Table 1-1. The whey proteins possess a fairly uniform distribution of proline, polar and non-polar amino acid residues along their respective polypeptide chains and so exhibit a compact, globular conformation with substantial helical content in their native state (Morr, 1983). Hence, these proteins fold intramolecularly, burying most of their hydrophobic residues so that extensive self-association or interaction with other proteins does not occur (Swaisgood, 1985). The whey proteins are, however, susceptible to heat processing that causes denaturation and protein-protein interactions involving disulphide interchange and calcium-mediated aggregation. In addition, they undergo intermolecular interactions with κ-casein during heat processing of milk through disulphide interchange. Whey proteins therefore play a key role in determining the heat stability of milk concentrates (Morr, 1983).

α-Lactalbumin normally occurs as a monomer, the hydrodynamic properties of which indicate a nearly spherical, very compact globular protein. α-Lactalbumin is a calcium-binding metalloprotein, which is also capable of binding zinc and probably other metals. The heat stability of α-lactalbumin is reduced by removal of Ca^{2+} (Fox, 1989).

β-Lactoglobulin, however, does undergo limited self-association; at the pH of milk a dimer is formed with a geometry resembling two impinging spheres. The structure of β-lactoglobulin is dependent on pH; thus, below pH 3.5 the dimer dissociates to a slightly expanded monomer, between pH 3.5 and 5.2 the dimer tetramerizes to give an octamer, and above pH 7.5 the dimer dissociates and undergoes a conformational change giving an expanded monomer. The functionality of β-lactoglobulin is greatly influenced by the presence of both a sulphydryl group and disulphide bonds. The relative importance of the sulphydryl group is influenced by conformational changes since this determines the availability of the sulphydryl group for reaction. Thus, under appropriate conditions, β-lactoglobulin readily participates in sulphydryl-disulphide interchange reactions and this affects many of its characteristics, such as solubility (Swaisgood, 1985).

BSA contains numerous disulphides which impose conformational restrictions, but since the molecule contains no long-distance disulphide bonds, it is relatively flexible. BSA binds several ligands, at different sites. Binding of hydrophobic molecules, such as fatty acids, apparently occurs in hydrophobic pockets that can open and close to admit large insoluble hydrophobic molecules. Cations, especially Cu^{2+} and Ni^{2+}, are bound on the surface (Fox, 1989).

1.4 Milk salts

The milk salts include all the components that are present as ions or are in equilibrium with ions and are mainly distributed between the soluble and colloidal phases (Walstra and Jenness, 1984) with a limited amount bound to the fat globules (Walstra and Jenness, 1984). Milk is supersaturated with calcium and phosphate allowing the formation of insoluble colloidal calcium phosphate (CCP) complexes which stabilise the casein micelle. The micellar system also contains magnesium and citrate which interact and form part of the CCP structure. The complexity of the milk salt system is illustrated by the diagram, refer Figure 1-3, devised by Jenness and Patton (1969) which shows the probable equilibrium between calcium and other components. Further details of the chemistry of milk salts are given in review articles by Fox and McSweeney (1998), Holt (1997) and Holt (1985).

The salt balance between the colloidal and soluble phases largely determines the physico-chemical state of milk and hence the functionality of the proteins. The milk salts are of particular interest in this review as they have been shown to have a major effect on the rheology of milk protein solutions. This aspect of the effect of milk salts is discussed in more detail in section 1.7.5.

The concentration of the salts varies among milk protein products depending on their method of manufacture and their immediate environment. Factors such as temperature, concentration and change in pH, can cause major shifts in the balance of milk salts between the colloidal and serum phases of milk (Jenness and Patton, 1969). Shifts in the equilibrium of the salts during manufacturing, particularly of calcium and phosphate, can

alter the stability of the micelle and in extreme cases micelle structure is lost, as in the manufacture of caseinates.

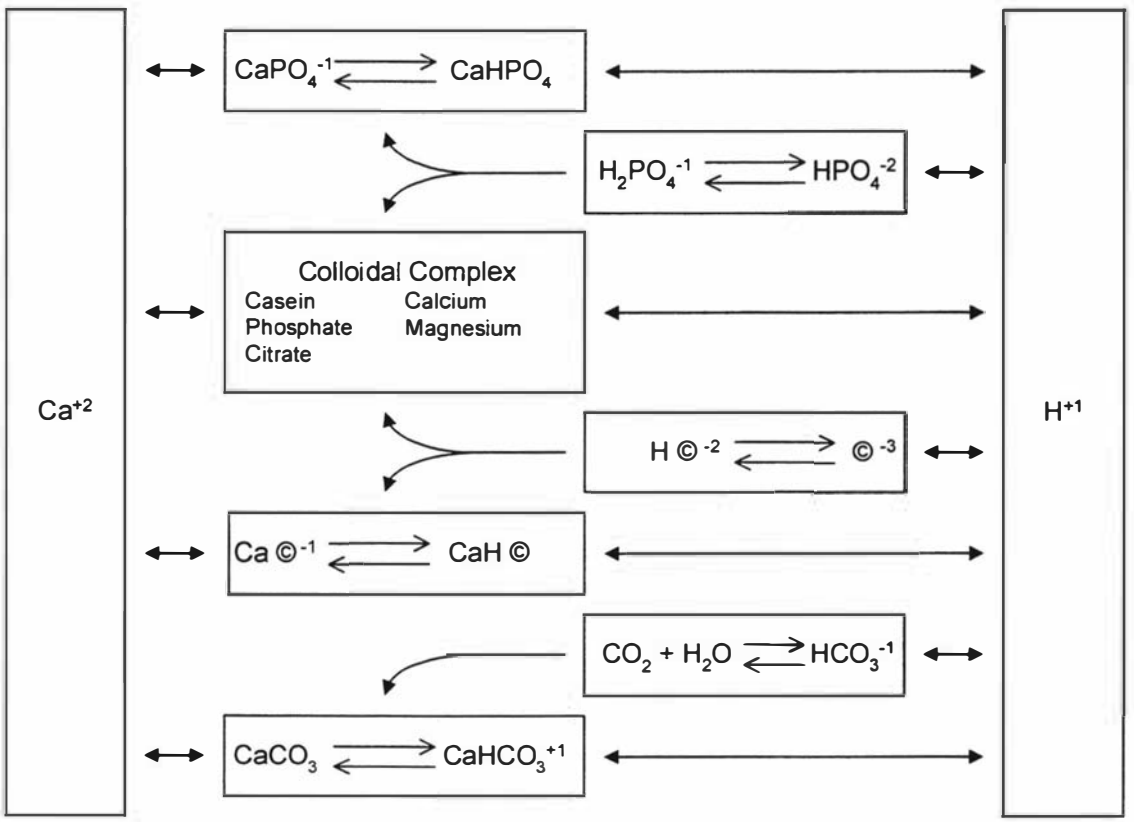

Figure 1-3 Equilibrium among the milk salts. © = citrate. Magnesium can be substituted for calcium in this diagram (Jenness and Patton, 1969).

1.5 Lactose

Lactose is the predominant carbohydrate in bovine milk, and accounts for over 50% of the solids in skim milk.

Lactose occurs in both α and β crystalline forms, with an equilibrium ratio of $\beta/\alpha = 1.68$ at 20°C (Swaisgood, 1985). The β form is far more soluble than the α form, and the rate of mutarotation is rapid at room temperature but very slow at 0°C. The α-hydrate crystal form, which crystallises under ordinary conditions, occurs in a number of shapes, but the

most familiar is the "tomahawk" shape, which imparts a "sandy" mouth feel to dairy products, such as sandy ice cream (Swaisgood, 1985).

The very low solubility of the α-lactose anomer of lactose results in its crystallisation in frozen products. Crystallisation of α-lactose in frozen milk is typically accompanied by prompt destabilisation and precipitation of casein, the reasons for which are not fully understood. Soluble lactose may have a direct stabilising effect on casein, and if so, this is lost with lactose crystallisation. Lactose crystallisation may also have an indirect effect on casein stability, since its removal from the unfrozen phase decreases solute concentration resulting in additional ice formation. The effect is similar to that observed during the concentration of milk. Thus, calcium ion concentration in the unfrozen phase increases and more tertiary calcium phosphate precipitates, yielding a decline in pH and casein instability (Swaisgood, 1985).

β-Lactose, which is always formed in any solution containing lactose, has been shown to retard the crystallisation process greatly, and incidentally is responsible for a much slower crystallisation of lactose than sucrose, which does not have an isomeric form to interfere with the crystallisation process. The retarding action of β-lactose during the crystallisation of α-hydrate lactose is ascribed to the fact that the β-galactosyl part of its molecule is the same as in α-lactose. The β-lactose molecules, along with α-molecules, become attached to certain crystal faces which are acceptors of β-galactosyl groups. Once the β-lactose molecules are incorporated on the crystal, they impede further growth, because of their β-glucose group, which is foreign to the crystal structure (Nickerson, 1974).

1.6 Review of processing operations involved in the production of powdered milk protein products

Understanding the effect of process variables on concentrate rheology is of great importance if the processing and quality of milk protein powders are to be optimised. The benefits of process optimisation have long been understood for traditional powdered milk protein products and consequently there is a large body of information available surrounding the unit operations involved in these processes. There is not however similar information available surrounding the unit operations peculiar to the newer powdered milk protein products, nor is there much information available on how the new products are affected by traditional practices and operations. The relationship between the rheology of skim and whole milk concentrates and the optimisation of their manufacture will be outlined first followed by a more detailed discussion surrounding the individual unit operations. Parallels between the rheology and unit operations of these traditional milk concentrates and newer products will then be discussed.

The optimisation of milk powder manufacturing centres around reducing the overall production cost by removing as much water as possible via evaporation rather than by the more expensive spray drying operation. However the degree of water that may be removed by evaporation is limited. As the concentration of milk solutions increases there is a concomitant increase in viscosity which may lead to a decrease in the functionality of the spray dried product.

A concentrated milk of high viscosity can lead to the formation of larger spray particles which, because of their lower ratio of surface area to volume, require a longer time to dry (Beeby, 1966). Powders from such high viscosity milks have high levels of moisture, poor solubility, undesirable flavours and may contain burnt particles (Beeby, 1966). Hence controlling the viscosity of milk concentrates enables a low cost product of high quality to be produced.

Snoeren *et al.*, (1981) described three variables which controlled the viscosity of skim milk concentrate: preheat treatment of the milk, mass concentration and holding time of

the concentrate prior to spray drying. The viscosity of whole milk concentrates is affected by a fourth unit operation, that of homogenisation (de Vilder and Moermans, 1983; Reuter and Randhahn, 1978; Snoeren *et al.*, 1984b).

1.6.1 Preheat treatments

In the manufacture of skim milk powder the milk is heated prior to concentrating. The reason for preheat treatment is to pasteurise the product and also to produce desired functionality and/or storage stability in the final milk powder. Pasteurisation can be performed at 74°C for 30 s without easily measurable damage to the whey proteins (Kessler, 1981), however 72°C for 15 s is the standard in New Zealand. High temperature treatment (85°C, 20 min) results in denatured whey proteins, and is used when producing milk powders for the bakery trade (provides higher volumes in baked products) (Kessler, 1981).

Beeby (1966) reported that increasing the preheat treatment of milk from 60°C/30 min to 90°C/30 min increases the viscosity of the concentrated milk and its susceptibility to heat-induced gelation and that the effect of preheat treatment increased with the degree of concentration. Beeby found that high preheat treated milk (30 min at 90°C) is unstable, with regard to age-thickening and eventual gelation, at quite low temperatures (50°C).

Bloore and Boag (1981) reported that a high-temperature-short-time preheat treatment (10s at 113°C) gave a lower initial concentrate viscosity and a slower rate of viscosity increase with holding time than a low temperature, long time treatment (120s at 80°C) giving a similar degree of denaturation as measured by the Whey Protein Nitrogen Index.

1.6.2 The effects of concentration, concentrate preheating and concentrate holding on the rheology of milk protein concentrates

During the manufacture of skim and whole milk powders, the milk is concentrated in an evaporator. The degree that the milk can be concentrated is limited by the viscosity of the concentrate. Beeby (1966) reported that increases in viscosity reduce the rate of heat transfer through the milk during the evaporation stage. This leads to local overheating and

consequently to the quicker build up of solids in the calandria of the evaporator, thus reducing the effective operating time of this equipment.

Following concentration in the evaporator, the concentrated milk is held for a period of time in a balance tank prior to concentrate preheating and atomisation in the spray drier. It is well known that the viscosity of milk concentrates increases during this holding period. This phenomenon is called age-thickening.

Heating of the concentrated milk (50°C to 80°C) just before atomisation has the double purpose of enabling a final product of good bacterial quality to be obtained and achieves greater efficiency and easier atomisation during the spray drying process (de Vilder *et al.*, 1979). Skim milk is typically heated to 80°C .

The viscosity of milk increases exponentially with increasing total solids, in the range 44 to 50% total solids (Hayashi and Kudo, 1989; Bloore and Boag, 1981). The relationship between the concentration of both whole and skim milk, and their viscosity depends on the volume fraction of the proteinaceous material and the fat present in milk and on the viscosity of the medium (Snoeren *et al.*, 1983). The interdependence of these factors can be described by Eilers' relation shown below :

1-1
$$\eta = \eta_{ref}\left(1 + \frac{1.25\,\phi}{1 - \phi/\phi_{max}}\right)^2$$

where η = viscosity of the suspension, η_{ref} = viscosity of the medium, ϕ = volume fraction of the dispersed particles, and ϕ_{max} = maximum attainable volume fraction. The value of ϕ_{max} in a system containing spheres of the same size is 0.74, whereas in a system with spheres of different sizes it may be higher.

A relationship for the viscosity of concentrated skim milk has also been adequately described by a regression equation relating the viscosity (measured at a shear rate of $356s^{-1}$) of the concentrate to total solids (43.9% to 51.6% w/w), temperature (17.8°C to

80.7°C) and the protein content of the skim milk (38% to 42% w/w dry basis) (Bloore and Boag, 1981). The regression equation of Bloore and Boag is shown below:

1-2 $$\ln \eta = 3.765 + 0.207 \times T_S - 0.207 \times T_C + 0.044 \times T_C^2 + 0.075 \times P$$

where P = (protein content - 39.74)

T_S = (concentrate total solids (%) - 47.7)/2

T_C = (concentrate temperature (°C) - 45.3)/10

and η = viscosity (cP)

This regression equation is only applicable for predicting apparent viscosity at a shear rate of 356 s^{-1}. This limitation is due to the pseudoplastic behaviour of concentrated skim milk. The equation of Bloore and Boag does not describe any relationship between apparent viscosity and shear rate.

A further limitation to the regression equation of Bloore and Boag that it is based on data collected within 14 seconds of the concentrate being at rest. This equation is therefore not applicable to concentrates that have been held for a longer period as they will be affected by the phenomenon of age-thickening. At any one temperature (>50°C) the viscosity of concentrates on holding has been found to decrease to a minimum and then increase (Beeby, 1966). Further, at any one concentration the increase in viscosity begins earlier the higher the temperature of holding. Beeby (1966) concluded that the initial decrease in concentrate viscosity on holding at a particular temperature was due to relatively weak intermolecular forces that are broken by thermal agitation. The subsequent increase in viscosity may be explained by the aggregation of protein. As the concentration of milk solids increases, the solubility product of many of the salts in milk is exceeded and the salts may precipitate on the surface of the casein micelles. This destabilises the micelles to heating and aggregation occurs. In its early stages, aggregation is accompanied by an increase in viscosity but as the process continues gelation eventually occurs (Muir, 1980). In their review on changes to milk components during evaporation, Singh and Newstead

(1992) note that it is likely that this type of aggregation and gelation is caused by the precipitation of calcium phosphate on the surface of the casein micelles. Snoeren *et al.*, (1984a) suggest that age thickening is due to the loosening of casein micelles. As a consequence of concentration, the pH drops and the ionic strength increases, both of which favour the solubility of β-casein, which results in an increased voluminosity of the casein. Snoeren *et al.*, (1983) believe that the mechanism controlling the thickening of whole milk concentrates is also linked to denaturation of whey proteins. According to Snoeren *et al.*, (1982) heat induced viscosity changes in skim milk concentrates can be traced back to denaturing of the whey proteins, and consequently to a highly increased hydration and bulkiness, in comparison with undenatured whey protein.

There is some debate over the importance of age-thickening in concentrated milks with regard to the quality of the spray dried powder. Snoeren *et al.*, (1981) showed that properties of spray dried milk were only related to the initial basic viscosity of the concentrate and not the structural viscosity, caused by holding (50°C) of the concentrate ("age thickening"). The basic viscosity was affected by the preheat treatment of the milk and by raising the dry matter content of the concentrate. Snoeren *et al.*, (1981) therefore concluded that the increase in apparent viscosity, observed on holding, must be completely or almost completely disrupted by the disk in the spray-drying plant.

However, it is not known whether the decrease in apparent viscosity due to the high shear rates encountered during spray drying are reversible on decreasing the shear rate, i.e. would a concentrate that had undergone age-thickening have the same apparent viscosity at low shear rates as it did prior to being subjected to high shear rates or the same apparent viscosity as it did prior to holding. The question of what minimum shear rate is required during spray drying to enable the breakdown of the structural viscosity is also not answered. It is clear from this discussion that it is important to understand the pseudoplastic behaviour of milk concentrates with regard to the degree of age-thickening. It is also clear that the viscosity of the concentrate should ideally be determined at a rate of shear which is equal to that exerted by the disk in the spray-drier, but this is not practical because of high atomisation shear rates. Baldwin *et al.*, (1980) report that the thickening of the concentrate during holding can cause problems during pumping. The shear rates resulting from pumping are probably not sufficient to disrupt the structural viscosity

resulting from holding. If the shear rates resulting in the disruption of structural viscosity during spray drying could be simulated, perhaps by homogenisation, then problems associated with age-thickening may be reduced.

Reddy and Datta (1993) fitted power law models to viscosity data of whole milk between concentrations of 40% and 70% and temperatures of 35°C and 65°C. The consistency coefficient was found to be dependent on both temperature and concentration. The flow behaviour index was found to be independent of temperature.

In the manufacture of UHT milk concentrates most of the interesting rheological developments take place after production is completed. The post-production rheological changes possess a long time constant. The rheological changes involved are not believed to be the result of a diffusion process (Prentice, 1984). Although the mean free path of any particle is necessarily small because of the close packing, movement is not so restricted that it would account for changes on a time-scale of weeks or months. What appears to happen is that slow changes in the protein take place, whereby it gradually unfolds somewhat and complexes of fat and protein appear, whilst the soluble whey proteins form a net-like structure which tends to enmesh the casein. The end product of the reactions is for the net-like structure to pervade the whole milk, giving rise to gelation (Prentice, 1984). Age thickening has also been associated with the crystallisation of lactose in the concentrate (Baucke and Sanderson, 1970)

In conclusion the apparent viscosity of milk concentrate entering the spray drier appears to be dependent on the degree of concentration, the holding temperature, especially at temperatures above about 40°C (Bloore and Boag, 1981), period of holding, and variations in the salt balance.

1.6.3 Spray drying

Snoeren *et al.*, (1981) reported that if the basic viscosity of the milk concentrate was increased by intensifying the preheat treatment of the milk or by raising the dry matter content of the concentrate, the milk powder produced showed increased moisture content, particle density and bulk density, and decreased vacuole volume. It was also found that the

particle size distribution was affected by the viscosity of the concentrate (Snoeren *et al.*,1981; Hayashi and Kudo, 1989). Particle size of powdered milk (skim and whole) increases with increasing viscosity. Increasing particle size has a negative effect on product solubility (Hayashi and Kudo, 1989; de Vilder *et al.*, 1979). Baldwin *et al.*, (1980) studied the influence of the viscosity of the concentrate on the characteristics of spray dried milk powder. From this work it was concluded that water evaporation from the drying droplet of a high viscosity concentrate is difficult and protein denaturation is possible.

Hayashi and Kudo, (1989) give a solution to spray drying problems caused by high viscosity milk concentrates. They found that particle size of powdered skim milk can be decreased by increasing atomisation pressure (to 25 MPa). This is probably related to the disruption of structural viscosity, caused by holding, and/or a decrease in basic viscosity in keeping with the pseudoplastic nature of the concentrate due to the increase in shear associated with increasing the atomisation pressure.

1.6.4 Homogenisation

The production of whole milk powder and concentrates involves an additional step, that of homogenisation. Homogenisation is necessary to reduce the free fat content of the final powder. High free fat contents in powders result in poor reconstitution due to bad wetting (Kessler, 1981). However, it is well documented that when whole milk concentrate is homogenised, its viscosity increases (de Vilder and Moermans, 1983; Reuter and Randhahn, 1978; Snoeren *et al.*, 1984b), thus resulting in difficulties during spray drying.

The increase in viscosity of homogenised whole milk concentrate may be accounted for by the rise in apparent volume fraction (Snoeren *et al.*, 1984b). It would appear as if the serum in the space between the casein micelles adhering to the fat globules had become part of the fat-protein complex.

Increasing homogenisation pressure, results in an increase in the moisture content of the powder and a decrease in the free fat content (Snoeren *et al.*, 1984b). The ADMI-solubility index is in direct proportion to the homogenisation pressure (Snoeren *et al.*, 1984b). The

changes in milk powder properties that occur when the milk concentrate is homogenised are explained by the increased viscosity of the concentrate, resulting in bigger droplets being formed at the atomising wheel. The larger droplets experience unequal drying and local overheating. As the heavy drops are insufficiently stopped by the air flow, some of them are precipitated against the wall before they are completely dry, thus causing wall deposit around the atomiser (de Vilder *et al.*, 1979). The effects of increased wetting due to a decreased free fat content are overcome by the deleterious effects resulting from poor drying. Hence the ADMI solubility index of milk powder produced from homogenised milk is higher than that of a non-homogenised milk powder.

de Vilder *et al.*, (1979) carried out a comprehensive investigation of homogenisation. They studied the influence of the dry matter content of the milk concentrate, homogenisation and the heating of the concentrate on the physical characteristics of whole milk powder. They found that, regardless of whether the milk concentrate was homogenised or not, a marked increase in the ADMI solubility index was always observed when the dry matter content of the concentrate exceeded 50%.

The viscosity of the concentrate itself was also observed to increase with concentration. The viscosity of the non-homogenised concentrate increased from 3.1 to 23.0 Pa.s with increasing dry matter content (43.2 to 54.7%). Following one-stage homogenisation viscosity increased from 6.9 to 780.0 Pa.s; following two-stage homogenisation it increased from 6.2 to 440.0 Pa.s. Mulder and Walstra (1974) ascribed the increase in viscosity following one-stage homogenisation of milk to the appearance of fat clusters. During two-stage homogenisation these clusters were largely broken in the second stage, so that a lower viscosity was observed although the homogenisation level was virtually unchanged.

de Vilder *et al.*, (1979) showed that the spray drier itself has an homogenising effect which increases with the dry matter content of the milk concentrate. If the dry matter content is low (e.g. 42.3%), the homogenising effect is low. They ascribed this to the fact that the viscosity and, consequently, the frictional forces in the liquid flow on passing through the atomiser are low. Concentrates with high dry matter contents are viscous: they are subject

to many inner frictional forces on passing through the atomiser, which result in homogenisation of the fat globules.

The homogenising effect of the spray drier can explain why a powder manufactured from a non-homogenised concentrate has a high viscosity after re-dissolution (approximately an order of magnitude greater than the concentrate). If the product has previously been subjected to one stage or two stage homogenisation, a second or third homogenisation takes place during the atomising process itself, so that fat clusters present are broken up. This causes the re-dissolved powder to have a lower viscosity than that of a solution of the same concentration made from the powder of a non-homogenised concentrate.

When the viscosity of a milk concentrate, subjected to two-stage homogenisation, was compared with the viscosity of a solution of the same concentration prepared from the spray dried powder, de Vilder *et al.*, (1979) found that the milk powder viscosity was an order of magnitude higher even though the degree of homogenisation that occurred in the spray drier was slight. This led de Vilder *et al.*, (1979) to the conclusion that homogenisation of the fat during spray-drying is not in itself the only reason for the increase in viscosity. They proposed that the denaturation of proteins at increasing concentrations is likely to be more important in this respect.

Increasing the homogenisation pressure (14 to 20 MPa) for a 46% concentrate was observed to only affect the free fat content of the powder. The other characteristics of the powder (i.e. viscosity, ADMI-solubility index and N_2 penetration) were not significantly affected. The viscosity and free fat content in the concentrate were observed to increase (de Vilder, *et al.*, 1979).

Snoeren *et al.*, (1984b) found that the thickening of the concentrate (approximately 55%) expressed as "viscosity increase with time" was in proportion to the degree of homogenisation.

There does not appear to be any information in the literature regarding the effect of homogenisation on the apparent viscosity of skim milk concentrates. In light of the work of Snoeren *et al.*, (1981) and Hayashi and Kudo (1989) this would be an interesting area

for research. The incorporation of a homogenisation step may assist the handling of skim milk concentrates which suffer from high levels of age-thickening.

1.6.5 Summary of processing operations involved in powdered milk protein products

The processing operations involved in the manufacture of powdered milk protein products, apart from preheat treatments and homogenisation, are designed to minimise the cost of production. The increase in apparent viscosity of the concentrate, due to both basic and structural viscosity, limits the degree of concentration possible by evaporation before quality problems are observed in the powder. The basic viscosity is influenced by preheat treatments, concentration, temperature and, in the case of whole milk, homogenisation. The structural viscosity appears to be influenced by concentration, length of holding, and holding temperature. The actual effect of structural viscosity on the quality of the powder seems to be dependent on the shear regime imposed by the spray drying operation. High apparent viscosity is also associated with pumping problems.

1.7 Milk protein concentrate

As a result of developments in recent years, milk protein concentrates with different protein contents have been produced by ultrafiltration/diafiltration for various end uses. There is great diversity of terms used in the literature dealing with these products; "retentate powders", "native milk protein concentrate", "ultrafiltered milk protein concentrate", "milk powder from ultrafiltered skim milk", "skim milk retentate powder", and "high-protein lactose-free milk powder". The casein in these products is in a similar, micellar, form to that found in milk while the whey proteins are also reported to be in their native form. The products have relatively high ash and calcium contents, since protein bound minerals, such as micellar calcium phosphate, are retained.

1.7.1 MPC manufacturing process

A general process flow diagram for the manufacture of MPC is shown in Figure 1-4. MPC of 50-90% protein content (dry basis) can be produced by this process with the original

casein/whey protein ratio, depending on the ultrafiltration and diafiltration parameters chosen.

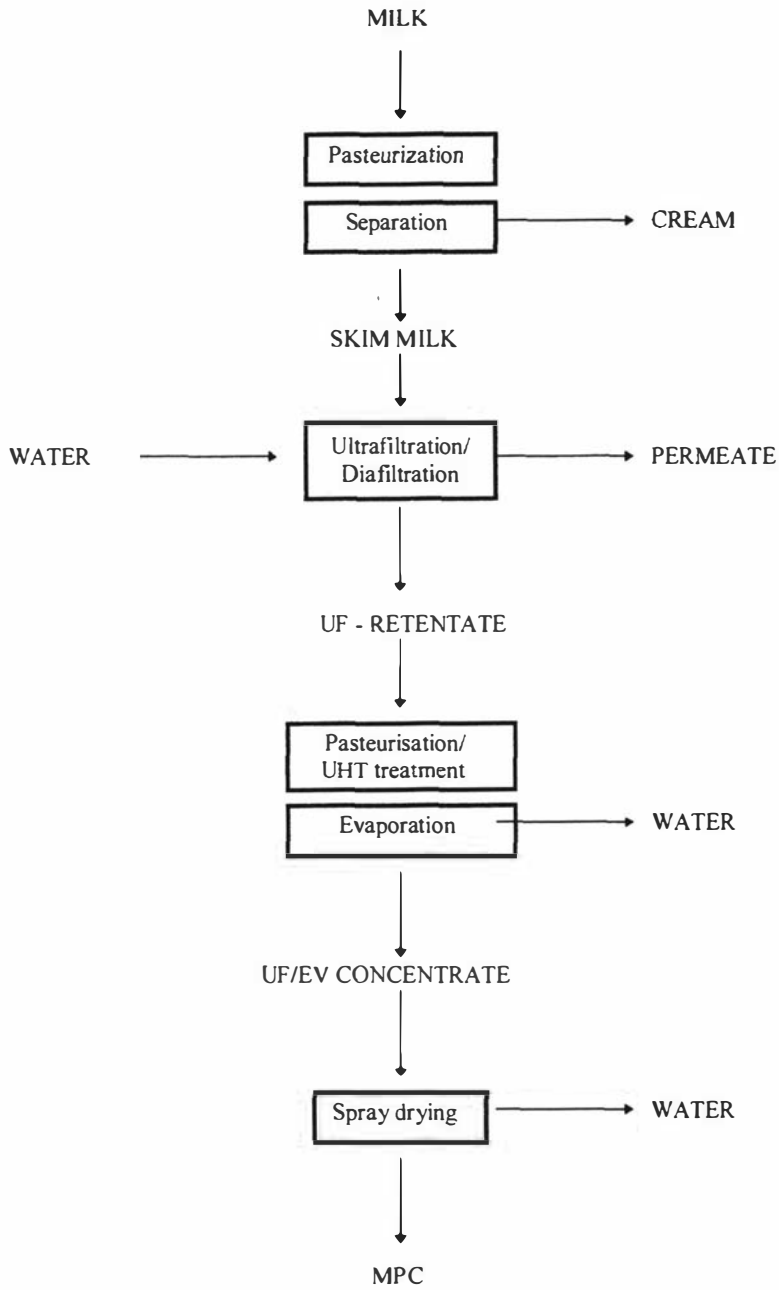

Figure 1-4 Schematic diagram of the MPC manufacturing process (Novak, 1991)

1.7.1.1 Membrane processing

In its simplest form ultrafiltration consists merely of pumping the feed solution under pressure over the surface of a suitably chosen membrane. In the UF process the pressure gradient across the membrane would force solvent and smaller species through the pores of the membrane, while the larger molecules would be retained. The retained phase, or "retentate" or "concentrate" stream as it is referred to, will be enriched in the retained macromolecules, while the permeate stream will be depleted of the macromolecules. The retentate will of course contain some of the permeable solutes as well. In fact it may be the very same or higher concentration than in the permeate stream, depending on that species' rejection by the membrane. However, since the retentate now forms a much smaller volume than the feed, there has in effect been a "purification" of the retained species.

The degree to which the retained species has been purified is normally presented in terms of the volume concentration ratio (VCR):

1-3
$$VCR = \frac{\text{Initial feed volume } (V_o)}{\text{Retentate volume } (V_R)}$$

VCR is also sometimes referred to as "concentration factor" (CF)

The main advantages of membrane concentration over concentration by evaporation are:

1. the food is not heated, and there is therefore negligible loss of nutritional or eating quality and particularly less loss of volatiles, and

2. in contrast with boiling, membrane concentration does not involve a change in phase and therefore uses energy more efficiently.

3. by manipulating the membrane pore size, and pressure drop over the membrane a controlled separation of particles from the process fluid can be achieved in parallel with concentration.

An essential factor affecting the process of separation during ultrafiltration is the change in flow properties with increasing solids content, since these determine the flow conditions in ultrafiltration plants.

Although a considerable purification of the protein can be done by direct ultrafiltration, the flux will drop to uneconomically low values and the pumping power required will rise due to increase in viscosity of the retentate. Thus in order to effect a further purification, it is necessary to resort to "diafiltration". Diafiltration refers to the process of adding water to the retentate and continuing the elimination of membrane permeating species along with the water during ultrafiltration. Diafiltration can be conducted as either one of two modes: discontinuous or continuous diafiltration. A schematic of the two modes is shown in Figure 1-5.

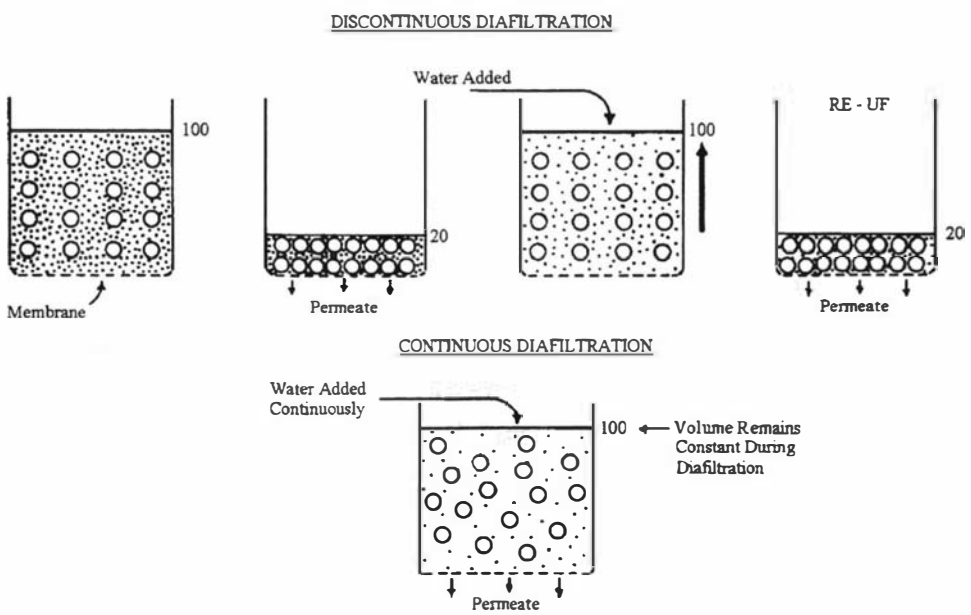

Figure 1-5 Schematic illustration of discontinuous (top) and continuous diafiltration (bottom) (Cheryan, 1986).

Discontinuous diafiltration, also termed "UF/Re - UF", refers to operations where permeable solutes are cleared from the retentate by volume reduction, followed by re-dilution with water and re-ultrafiltration in repetitive steps.

In the process of continuous diafiltration water at the appropriate pH and temperature is added to the feed tank at the same rate as the permeate flux, thus keeping the feed volume constant during processing.

1.7.2 Composition and structure of milk protein concentrates

Typical compositions of commercially produced milk protein concentrates are shown in Table 1-2

Table 1-2 Composition of commercially produced Milk Protein Concentrates

	MPC85	MPC80	MPC75	MPC56
Energy [kJ/100g]	1543			
Protein [N x 6.38] % Dry Basis	85.4			56.0
True Protein		78.4	73.5	
Non Protein Nitrogen		1.6	1.5	
Moisture [%]	4.1	5.0	5.0	3.8
Fat [%]	1.7	1.7	1.5	1.2
Lactose [%]	4.6	5.5	10.9	31.0
Ash [%]	7.3	7.8	7.6	8.0
Calcium [mg/100g]	2100	2450-2550	2300-2400	
Potassium [mg/100g]		250-300	450-550	
Phosphorous [mg/100g]		1500-1700	1450-1650	
Sodium [mg/100g]	120	120-150	130-180	
pH [5% at 20°]	7.0			6.8

Note: The data on MPC85 and MPC56 powders are typical compositions from New Zealand (New Zealand Dairy Board product sheet) and the data on the MPC80 and MPC75 powders are Hungarian (Novak, 1991).

Mistry and Hassan (1991b) made a study of the microstructure of MPC[1], skim milk, and commercial caseinate powders. Electron micrographs from this study are shown in Figure 1-6, Figure 1-7, and Figure 1-8. The surface of MPC powder particles, shown in Figure 1-6, was always smooth with large dents. The interior of the particles was usually hollow

[1] Mistry and Hassan referred to MPC as HMPP (delactosed, high milk protein powder)

with a wall thickness of approximately 2μ. The particles of skim milk powder produced under drying conditions identical to those of MPC were considerably different (Figure 1-7). There was less variation in size of particles and, unlike MPC, these particles had a wrinkly surface. The skim milk powder particles were also characterised by dents on the surface. Mistry and Hassan (1991a) reported that the difference in surface morphology between MPC and skim milk powder was probably due to differences in the protein composition of the two powders; skim milk powder contains approximately 35% protein, whereas MPC contained 84%). However, Warburton and Pixton (1978) reported that as the moisture content of dried milk particles increases up to 7 % the morphology of the surface is wrinkled with the number of wrinkled particles greater the higher the moisture content. The cause of surface wrinkles has been related to the presence of anhydrous lactose in a glassy form (Saito, 1985). The presence of anhydrous lactose in spray-dried milk powders contributes to their hygroscopicity (Kalab *et al.*, 1989). Above 7 % moisture content, the lactose glass becomes sufficiently dilute for crystallisation to occur (Warburton and Pixton, 1978). The tendency to form wrinkles is greatest at high inlet temperatures and also when large temperature differences occur between the hot air and the milk particles (Caric and Kalab, 1987).

The microstructure of commercial caseinate powders (Figure 1-8) was similar to that of MPC. Caseinate powder particles were characterised by a smooth surface and the presence of dents. The microstructure of MPC and caseinate powders were similar to the microstructure of other dried protein powders, based on soy milk and with different functional properties (Mistry and Hassan, 1991b). Based on the previous discussion on surface morphology it is likely that the similarity between these powders is due to the absence of high levels of lactose. Mistry and Hassan (1991b) conclude that the similarity of microstructure of MPC suggests that the composition will influence the structural properties of protein powders, but microstructure may not directly affect functional properties.

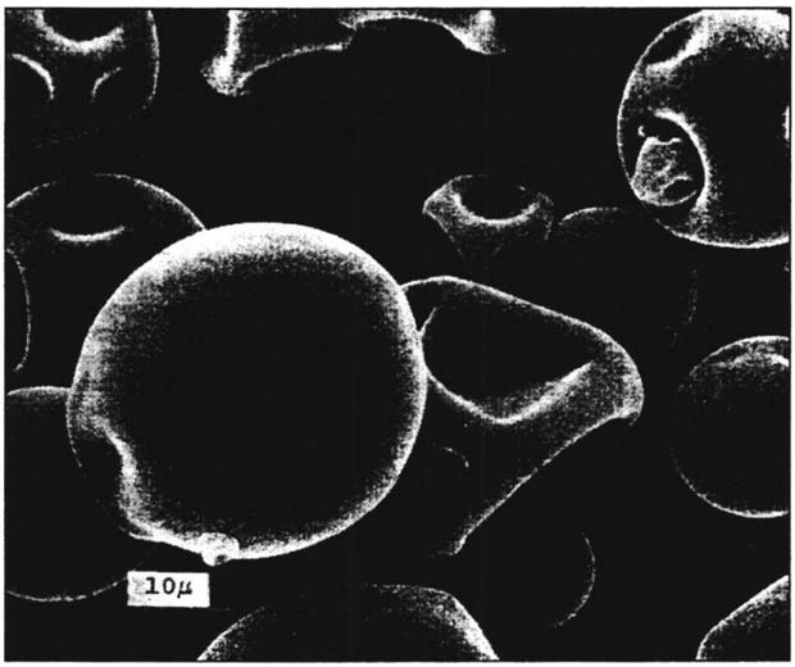

Figure 1-6 Scanning electron micrograph of MPC85 powder showing smooth surface and dents on the particles. A wide range of particle sizes are evident. (Mistry and Hassan, 1991a).

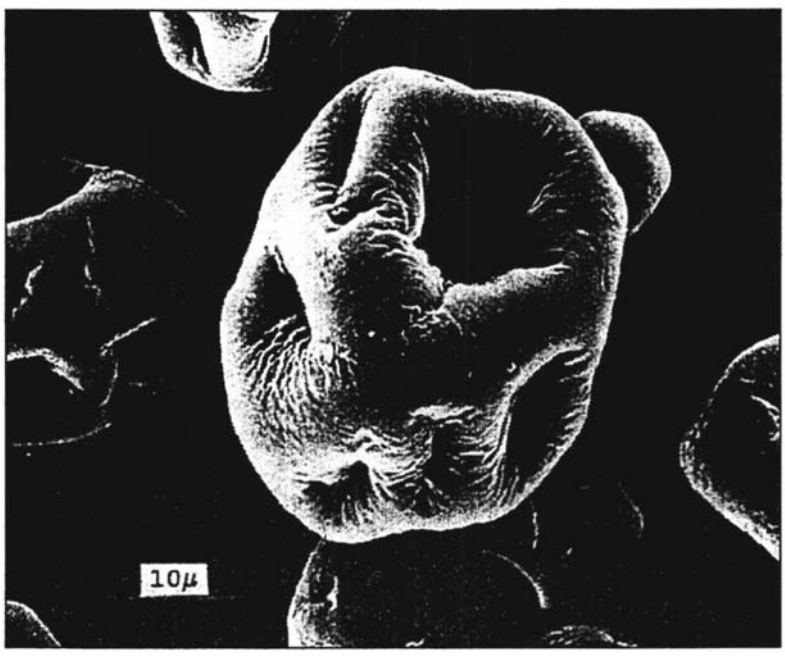

Figure 1-7 Scanning electron micrograph of spray-dried skim milk powder. Powder particles are characterised by a wrinkled surface with dents. A narrow range of particle sizes are evident. (Mistry and Hassan, 1991a).

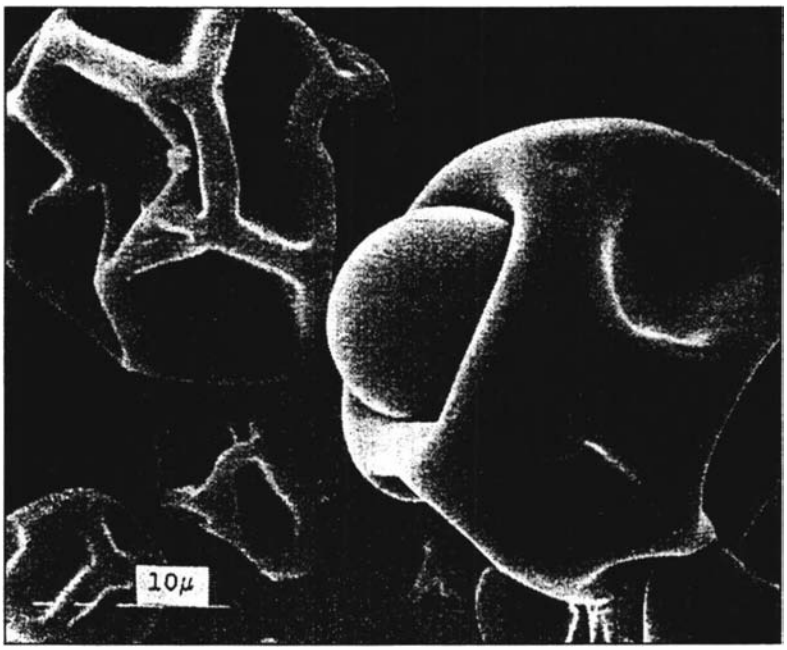

Figure 1-8 Scanning electron micrograph of commercial caseinate powder. Powder particles have a smooth surface and possess dents. A wide range of particle sizes are evident. (Mistry and Hassan, 1991a).

1.7.3 Effect of heat treatments on MPC85 manufacture

The UF flux of reconstituted skim milk can be related to the heat treatment received during processing of the skim milk powder used. High heat treatments gives the highest UF flux which decreases in descending order from medium to low heat skim milk powder. The increase in flux is attributed to the heat denaturation of whey proteins which are considered to be potent foulants in UF in their native form (Abd El-Salam and Shahein, 1989).

Randhahn (1976), in a study of the flow properties of skim milk concentrates obtained by UF, reported that prolonged storage (particularly at low temperatures and high solids contents) gave rise to solidification of the concentrates.

1.7.4 Functional properties of MPC

The main functional properties of MPC of interest for this work are the rheology and solubility of MPC.

The presence of non-dissolved particles are known to significantly affect rheology. Zwijgers (1992) reported that MPC80 is hardly soluble at 16°C (>40g sedimentible material/100g solution) with the best results (< 0.5g sedimentible material/100g solution) when dissolved at temperatures of 40 - 60°C. Details of the experimental procedure employed by Zwijgers were not given. Pierre *et al.*, (1992) observed that raising the reconstitution temperature to 50°C instead of 24°C brought the solubility index from 74 to 97.2%. Schuck *et al.*, (1994) showed that the insoluble matter in MPC solutions was not the result of denaturation, but of the slowing of water transferral towards the interior of the grain of powder. These authors suggested that the transfer of water to the interior took place as if contact with water created a high surface viscosity, slowing internal hydration. The suggested increase in surface viscosity was related to the high protein content of the particles. This hypothesis was confirmed by Davenel *et al.*, (1997) who used NMR techniques for following the reconstitution of native micellar phosphocaseins (NMC). They showed that two phenomena were occurring during the NMC rehydration: the strong and almost instantaneous absorption by powder particles followed by a further slow absorption leading to an acceleration in the solubilisation of particles.

There is hardly any published data on the rheology of MPC. Zwijgers (1992) compared MPC80 with Na- and Ca-caseinate solution showing that on a protein basis [%] MPC80 had the least viscosity. No details of the viscosity measurements were given. The author concluded that the lower viscosity was due to more compact protein structures in MPC80. Low viscosity is an important feature for high protein energy drinks and clinical formulas with a high caloric density. The viscosity of protein solutions is related to water-binding capacity. Babella (1989) established that the water binding capacity increased with increases in the degree of heat treatment.

Other functional properties that are mentioned in the literature include emulsion and heat stability; and nutritional and organoleptic properties.

In Hungary the most common application for MPC is in the meat and canning industry. MPC's most important functional property with respect to the canning and meat industry is the emulsifying capacity (EC). Typically the EC of MPC is in the range 7-10 g oil/g protein, which is comparable to the EC of caseinates. The EC has been found to decrease with increases in the degree of heat treatment (Babella, 1989). Increasing the inlet air temperature to the spray drier resulted in a product with a lower moisture content and a decrease in EC. The EC increased with increases in the speed of the atomising disc of the spray drier (i.e. reducing the drop size).

Increasing the calcium ratio (calcium:total mineral) within the mineral salt content has been found to decrease the solubility and EC of MPC (Babella, 1989). Changing the calcium ratio does not appear to affect the water-binding capacity. When the potassium or sodium ratio was increased within the mineral salt content, the water-binding capacity increased considerably, the EC decreased and the heat stability as well as solubility did not change significantly (Babella, 1989).

The organoleptic properties of MPC have been described (Zwijgers, 1992) as possessing a characteristic 'milky' flavour. MPC has a higher nutritional value than soy protein isolate (Zwijgers, 1992). MPC based model emulsions were found to be heat stable up to 13 minutes at 130°C and 20 minutes at 120°C. Ca-caseinate based nutritional formulas destabilise by then (Zwijgers, 1992). The MPC80 based emulsions were monitored during storage of 6 months at ambient temperature and found to be stable.

1.7.5 Effect of milk salts on the rheology of milk protein concentrate solutions

During the ultrafiltration and diafiltration stages of MPC85 manufacture, the salt and protein concentration of the process fluid changes as skim milk is transformed into MPC85 concentrate. One of the main end uses of MPC85 is in nutritional and enteral formulations where salts are an important ingredient. Therefore understanding how salts affect the rheological properties of MPC85 would be of importance.

Much of the work concerning the effect of salts on the rheological properties of milk protein solutions has been conducted on caseinate or whey systems (Carr, 1994;

Hermansson, 1972, 1975; Konstance and Strange, 1991; Korolczuk, 1981, 1982a, 1982b, 1982c; Rha and Pradipasena, 1984; Towler, 1971, 1972, 1974). Caseinates and whey protein concentrates are used as functional ingredients in technical and edible applications where they contribute to the overall rheological characteristics. Salts are often a common ingredient in these applications. Research into the rheological effect of salts on actual micellar systems, apart from calcium caseinate, has been limited to identifying natural variations in salt concentrations as contributing to rheological problems such as age thickening in concentrated milks prior to spray drying and over long term storage in the case of UHT concentrates.

In general, it is expected that the changes in the rheological properties of MPC85 solutions on salt addition will follow the trends reported for other milk protein systems. It is, however, likely that salt addition to MPC85 will act in a slightly different manner than salt addition to either caseinate or WPC solution due to its retention of the micelle structure. Towler (1972) observed that if micellar calcium was retained during precipitation of casein, the retained calcium is much more effective in causing viscosity increase of caseinate than calcium added subsequently. Konstance and Strange (1991) reported that phosphate addition to Ca-caseinate resulted in a larger increase in apparent viscosity compared to Na-caseinate.

1.8 Planning research direction from literature review

The literature indicates that high viscosity is the limiting factor in milk powder production due to its effect on the spray drying operation and subsequent quality of the milk powder. The viscosity of concentrated milks may be controlled by the time-temperature scheme employed in the preheat operation, the operating parameters of the homogenisation step, the degree of concentration in the evaporators, concentrate heating, holding time before spray drying, and the operating parameters of the spray drying operation.

There is also evidence in the literature to suggest that the introduction of a homogenisation step prior to spray drying for skim milk concentrates may enable the reversal of structural viscosity resulting from age thickening.

Due to the many parallels in the production (i.e. skimming of milk, evaporation, and spray drying) and composition (i.e. milk proteins in native form and same ratio of whey to casein) of skim milk powder and milk protein concentrate it is envisaged that milk protein concentrate will behave in a manner similar to skim milk concentrate and skim milk powder.

The initial thrust of this research will therefore study the rheology of both the milk protein concentrate during production and the reconstituted powder. In particular the rheological study will focus on:

a) determination of the degree of time-dependency of MPC85 concentrate ex evaporator to enable optimisation of the evaporator and spray drying step.

b) investigation of the effect of high shear rates on the age thickening of MPC85.

c) investigation of the basic rheology of reconstituted MPC85 with respect to added salts and lactose; concentration, temperature, and shear.

d) application of the acquired rheological knowledge to improving product functionality.

It was hoped that through this approach insights would be gained into the development and control of time dependent rheological behaviour. It was also hoped to relate the rheological properties of the concentrate with cold solubility since rapid and complete reconstitution of dry powders in water is essential for practical use. It was thought that the information gained through the rheological characterisation of reconstituted MPC85 would be useful for predicting how the rheology of skim milk changes during the ultrafiltration and diafiltration operations. The ability to predict the rheology during these operations would be useful for process optimisation. During these operations the process fluid is being concentrated with concomitant changes in the milk salt equilibrium and hence the viscosity of the fluid. It is envisaged that the rheological characterisation of MPC85 and its relationship to salt and lactose addition will enable optimisation of product processing and formulations in which MPC85 is an ingredient.

Salts are a common ingredient to formulations of both technical and edible applications and are known to have a significant effect on the rheology of protein solutions.

Overall an understanding of the effects of rheology throughout processing and subsequent product formulation will contribute to the current understanding of the functional properties of milk proteins and thus provide a basis for product improvement.

2. Rheology of commercial MPC85 concentrates during processing

The rheology of milk concentrates is known to be of importance in the performance and design of spray dryer operations and the properties of the powders produced. Age thickening of concentrates becomes a serious problem since it is attempted to operate the spray dryers at as high a total solids content as possible to maximise thermal economy, and it is therefore desirable to be able to predict age-thickening. This has been discussed in section 1.6. The objective of the work described here is to determine the rheological behaviour of MPC85 concentrate prior to spray drying. This knowledge will assist in the optimisation of spray drying during MPC85 manufacture. This work was conducted at Anchor Products - Hautapu.

2.1 Materials and methods

2.1.1 Methodology of rheological experiments

For each of the rheological tests performed at Anchor Products, Hautapu, samples of MPC85 concentrate were collected from a sample tap at the exit of the evaporator in the MPC85 plant. The sample was placed in a beaker, which was immersed in a water bath set to the exit temperature of the evaporator, 52 - 53 °C. The spindle of the viscometer was then lowered into the beaker and the concentrate was covered with paraffin oil to prevent evaporation and skin formation. The viscometer was then started and measurements collected and stored electronically.

The viscometer and waterbath were situated on the outer side of the door to the process hall, but still within the red line area. Due to the positive pressure in the process hall, whenever the door was opened a considerable breeze blew over the viscometer which contributed to the noise present in the data. The time from the start of sample collection to start of the viscosity measurements was about 30 s.

The shear rate range was limited from $1.10s^{-1}$ to $54.76s^{-1}$ due to the minimum and maximum speed of spindle rotation of the Brookfield RVDVIII (5 to 250 rpm) and the

geometry of the LV spindle and the stainless steel beaker used to hold the sample. The shear rate was calculated using equation 2-1.

2-1 $$\dot{\gamma} = \frac{2\omega R_c^2 R_b^2}{x^2\left[R_c^2 - R_b^2\right]}$$

where $\dot{\gamma}$ = shear rate (s^{-1})

ω = angular velocity of spindle ($rad.s^{-1}$)

R_c = radius of container (m)

R_b = radius of spindle (m)

x = radius at which shear is being calculated (here $x = R_b$)

$$\omega = \left(\frac{2\pi}{60}\right) N \qquad N = \text{rpm}$$

In these experiments R_c and R_b were 4×10^{-2} m and 9.421×10^{-3} m respectively. Ideally R_c should not exceed $2R_b$ for well defined shear rates. A container of this geometry however, was not available.

The Brookfield RVT outputs results as "display units" which must then be converted to shear stress by the following equation:

2-2 $$F = \frac{M}{2\pi R_b^2 L}$$

where: F = shear stress (Pa)

L = effective length of spindle (m)

M = torque input by instrument (N.m)

The torque (M) input by the instrument is calculated by multiplying the display unit reading by the full scale torque divided by 100. The full scale torque of the RV calibration spring is 7.187×10^{-4} N.m. The effective length of the spindle used here was 7.493×10^{-2} m.

2.1.2 Total protein analysis

The total protein contents of the MPC85 concentrates were determined using the Kjeltec 1026 System (Tecator, Sweden). Approximately 0.2 g of sample was accurately weighed into a digestion tube. Two Kjeltabs (each containing 3.5g K_2SO_4 and 0.0035 g Se) were added to the tube followed by 15 ml of concentrated H_2SO_4. The digestion tubes were placed in a block digestor unit and digested at 420°C until clear. Digestion was continued for a further 10 min. After digestion the tubes were allowed to cool until they could be touched, at which point ≈70 ml of hot milli Q water was added. The ammonia in the diluted digested sample was distilled into a 25 ml solution of 4% boric acid which was then titrated against 0.1 N HCl to a grey-mauve end point. The % nitrogen was calculated by equation 2-3.

2-3
$$N = \frac{1.4 \times T \times A \times 100}{W}$$

where N = nitrogen content [%]

A = exact molarity (normality) of HCl

W = weight of original sample [g]

T = volume of HCl [ml]

The percentage of nitrogen in each sample was converted to percentage protein by multiplying the nitrogen value by a conversion factor of 6.38. A blank sample was run to determine the amount of non-sample nitrogen being measured.

2.2 Results and discussion

2.2.1 Investigation of the time dependency in MPC85 concentrate

To determine the degree of time dependent rheological behaviour exhibited by MPC85 concentrate the effect of continuous constant shear on the apparent viscosity of concentrate held at the evaporation temperature was investigated. Seven samples were taken to obtain apparent viscosity data with time over one hour at seven constant shear rates: 1.10 s^{-1}, 2.19 s^{-1}, 8.76 s^{-1}, 17.52 s^{-1}, 26.28 s^{-1}, 39.43 s^{-1}, and 54.76 s^{-1}. The order of measurement was randomised. Originally it was envisaged that by combining this data together flow curves at different time intervals could be obtained and fitted with rheological models. However, due to the variation between the data sets this analysis was not possible.

The plot of the raw data, shown in Figure 2-1, shows two clear trends: a) with the exception of the 2.19 s^{-1} data, the apparent viscosity decreases with increasing shear rate; and b) the apparent viscosity of MPC concentrate decreases with holding time to reach a constant apparent viscosity at all shear rates. This observation suggests that, MPC concentrate, like skim milk concentrate, is a pseudoplastic fluid and shows a decrease in apparent viscosity with initial holding. However, unlike skim milk concentrate (Beeby, 1966), MPC concentrate does not show an increase in apparent viscosity after the initial decrease. Age-thickening in skim milk concentrates has been observed at temperatures as low as 44°C (Buckingham, 1978).

The large variation among the low shear rate data is due to the decreased sensitivity of the viscometer at low shear rates. However despite the increased noise at low shear rates it is still apparent that the viscosity decreases with holding time.

By inspection, the apparent viscosity of the sample tested at 2.19 s^{-1} is considerably lower than what one would expect based on the viscosity values of the other six samples. The low viscosity value is extremely unlikely to be the result of a real shear rate effect. The most likely reason for the discrepancy was thought to be a variation in the protein content. However, a protein analysis of all the samples (Table 2-1) revealed that while the 2.19 s^{-1} sample had a slightly lower total protein content, 20.70 % w/w, than the other samples this did not in itself account for the lower viscosity.

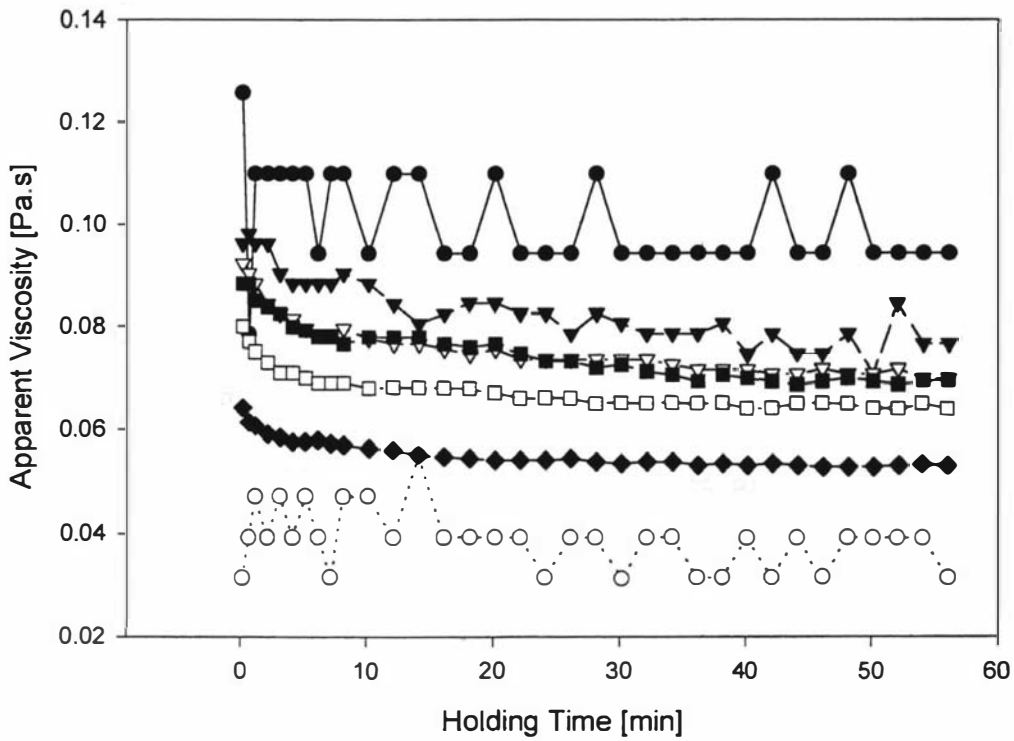

Figure 2-1 The change in apparent viscosity with holding time (52°C) of ex-evaporator MPC concentrate subjected to a continuous shear rate of 1.10 s^{-1} (●), 2.19 s^{-1} (○), 8.76 s^{-1} (▼), 17.52 s^{-1} (▽), 26.28 s^{-1} (■), 39.43 s^{-1} (□), and 54.76 s^{-1} (◆)

The 54.76 s^{-1} sample had a total protein content of 20.78 % w/w. The actual reason for the discrepancy of the 2.19 s^{-1} data was not able to be traced. On inspection of the process quality control data it was found that the collection time of the sample coincided to a change in the raw milk silo.

Table 2-1 Protein content of MPC85 ex-evaporator concentrate samples used in constant shear experiments

Shear Rate [s^{-1}]	Total Protein Dry Basis [% 6.38xN]	Total Solids [% w/w]	Total Protein Wet Basis [% w/w]
1.10	85.25	25.24	21.52
2.19	85.08	24.33	20.70
8.76	85.76	25.1	21.53
17.52	88.00	24.92	21.93
26.28	87.83	24.96	21.92
39.43	86.14	24.81	21.37
54.76	85.93	24.18	20.78

The results of the constant shear experiments were in contrast to those of Beeby (1966) who observed that the apparent viscosity of skim milk concentrate on holding at any one temperature (60, 70, 80, and 90 °C) decreased to a minimum and then increased. At any one concentration Beeby (1966) observed that the increase in apparent viscosity began earlier the higher the temperature.

Due to the occurrence of apparent age-thinning (i.e. decrease in apparent viscosity with holding time) in the MPC85 concentrates, it was necessary to determine whether the time-dependent decrease in viscosity was influenced by the method of measurement i.e. continuous shearing of the samples. To determine the validity of the method of measurement, two samples were taken and the viscosity, at 26.28s^{-1}, was measured at 0, 6, 10, 15, 20, 30, 40, 50, 60 and 77 minutes for the replicate set 1 and at 0, 6, 11, 15, 26, 31, 36, 43, 51, and 62 minutes for replicate set 2. Each measurement took 10 seconds with the solution at rest between measurements.

The results of this experiment confirm the decrease in apparent viscosity with holding time observed with the continuous shear experiments (Figure 2-2). However, the apparent viscosities of the replicates are quite different, with replicate 2 having a viscosity of less than half of replicate 1.

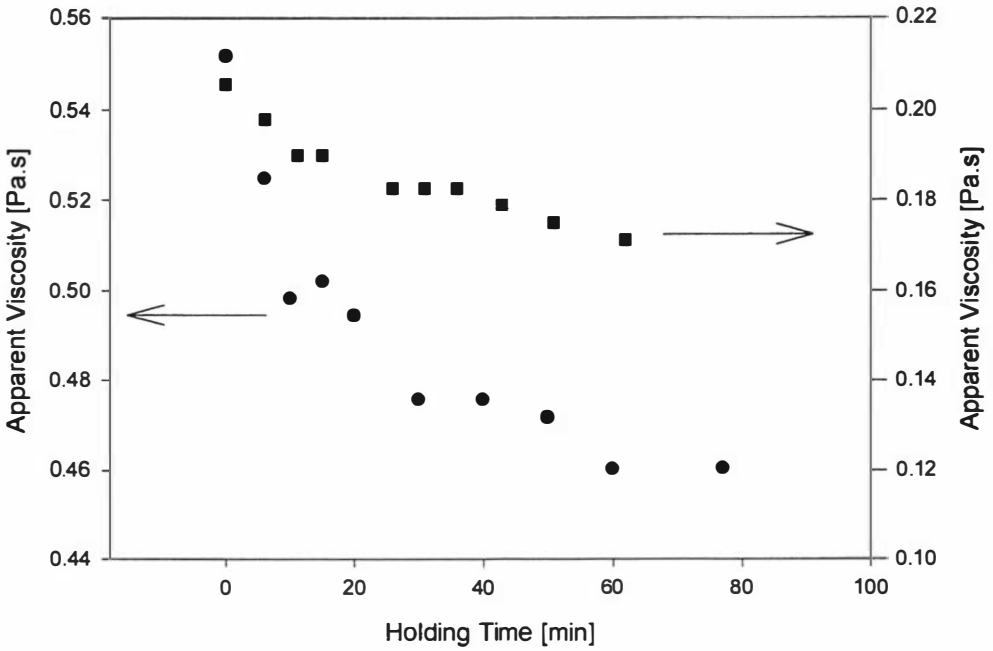

Figure 2-2 Change in apparent viscosity with time of MPC85 ex-evaporator concentrate subjected to a non-continuous shear rate of 26.28s^{-1} (Replicate 1, (●); Replicate 2 (■)) Each data point was based on measurement for 10 s at 26.28s^{-1} with the solution at rest between measurements.

Further to this the apparent viscosities of both solutions are up to ≈6 times greater than the viscosity of the 26.28s^{-1} sample tested under continuous shear conditions. The total solids and protein contents of replicate 1 were 25.17% and 21.63% respectively, while replicate 2 had total solids and protein contents of 23.16% and 19.63% respectively. Based on the protein contents one would expect that the order of viscosity of solutions would be: the continuous shear sample (21.92% protein) > replicate 1 (21.63 %) > replicate 2 (19.63 %). The actual order based on apparent viscosity for these samples at t = 0 min is: replicate 1 (0.55 Pa.s) > replicate 2 (0.21 Pa.s) > continuous shear 26.28 s^{-1} (0.088 Pa.s). The fact that these differences in viscosity are present at a holding time of t=0 min suggests that the variation in viscosity cannot be fully explained by the method of viscosity measurement and the protein content of the solutions. It can be concluded from this experiment that the method of measurement is valid and that there is considerable variation in the quality, with regard to viscosity, of the MPC85 produced over the time of these experiments. In addition to the variation in magnitude of apparent viscosity there is also a difference in the rate of decrease in viscosity of the solutions. This difference in the rate of decrease in apparent viscosity will be expanded on later in the discussion.

Having confirmed the observation that MPC85 concentrate ex-evaporator decreased in viscosity over the first hour of holding, it was thought possible that the MPC85 concentrate may possess a longer time constant for the mechanism resulting in the viscosity increase observed in skim milk concentrate. To explore this idea, another sample of concentrate was taken and held at 52°C for 5 hours at a continuous shear rate of 26.28s^{-1}. The results of this experiment, illustrated in Figure 2-3, showed the apparent viscosity of this sample to decrease markedly to 82% of the original value during the first 50 min with further holding resulting in only minor fluctuations, thus confirming the earlier results. This sample had total solids and total protein contents of 25.61 % w/w and 22.30 % w/w respectively.

In analysing these results it was noticed that the rate of decrease in apparent viscosity over the first hour for all the solutions seemed to be greater the higher the apparent viscosity of the solution at t=0 min. To analyse the relationship between these variables,

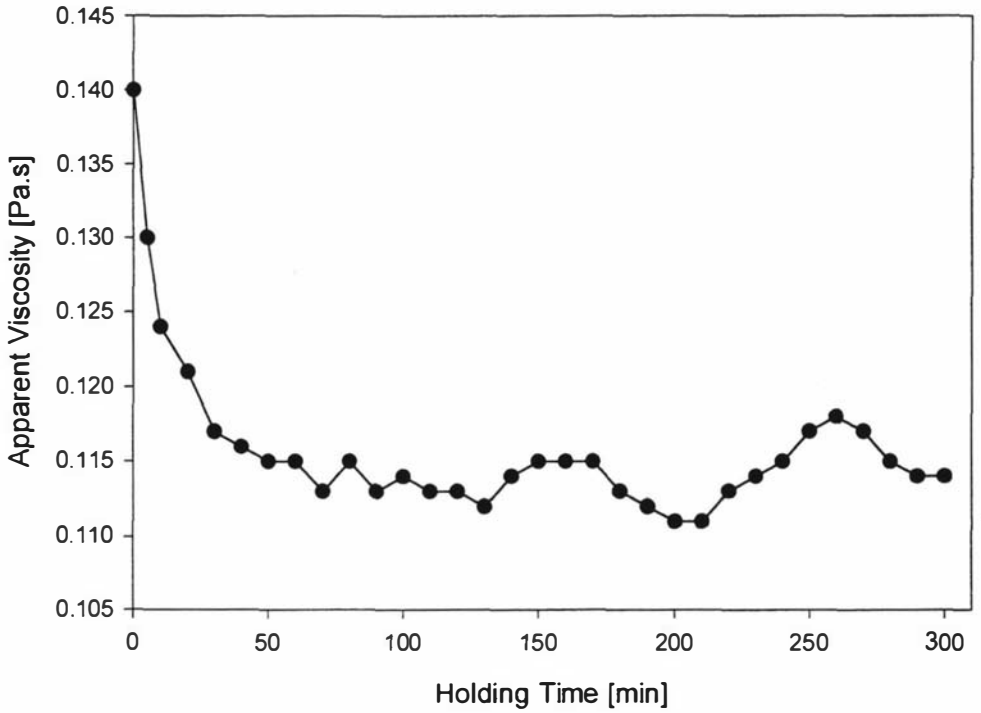

Figure 2-3 The change in apparent viscosity (continuous shear rate, 26.28 s^{-1}) of MPC concentrate ex-evaporator with holding at 52°C.

a linear regression model was fitted to the data to obtain, quantitatively, a rate of decrease in apparent viscosity over the first hour of holding. The actual shape of the apparent viscosity - time curves is however, non-linear. A linear model was chosen though, as a means of 'averaging out' the noise that was present in some of the curves. The relationship between the initial apparent viscosity (t=0 min) and the rate of decrease in apparent viscosity at a constant shear rate is shown in Figure 2-4 and may be adequately described (correlation coefficient = 0.976) by the following linear regression equation:

2-4 $$\Delta\eta = -0.0017 \times \eta_0 - 9 \times 10^{-5}$$

where: $\Delta\eta$ = Rate of decrease in apparent viscosity [Pa.s/min]

η_0 = Apparent viscosity at t = 0 min [Pa.s]

Variations in initial viscosity and rate of decrease in apparent viscosity over the first hour were not correlated with variations in total solids, shear rate, or protein content.

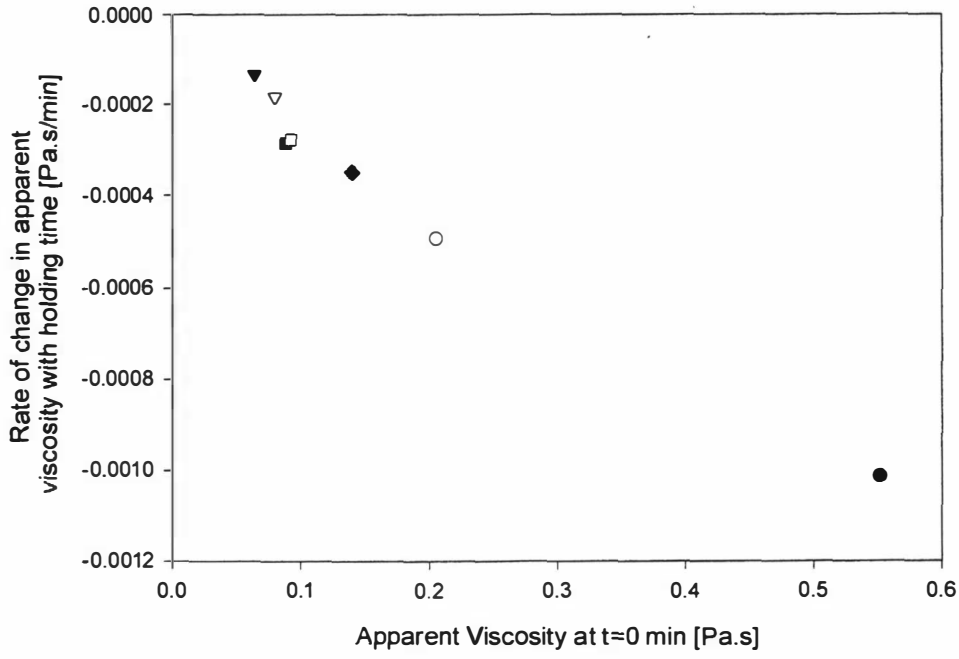

Figure 2-4 The rate of decrease in apparent viscosity over 1 hour as a function of the initial viscosity of the solution (non constant shear replicate 1, (●); non constant shear replicate 2, (○); continuous shear 54.76 s^{-1}, (▼); continuous shear 39.43 s^{-1}, (▽); continuous shear 26.28 s^{-1}, (■); continuous shear 17.52 s^{-1}, (□); continuous shear over 5 hours 26.28 s^{-1}, (◆).

2.2.2 Effect of holding time on the flow properties of MPC85 concentrate

To determine how the rheological properties of the concentrate changed throughout the decrease in apparent viscosity, an up/down shear sweep experiment was conducted on the same sample held at 52°C after 0 min, 20 min, 30 min, 45 min, and 60 min. The advantage of conducting a shear sweep experiment over the continuous constant shear experiments was that the same sample could be used for all measurements, thus the problems relating to variation in the quality of the concentrate could be eliminated. The increase in consistency of the data would enable rheological models to be fitted and the relationship between holding time and the rheological coefficients to be elucidated. The concentrates were at rest in-between shear sweeps.

By inspection of the raw data in Figure 2-5, it can be seen that the apparent viscosity of the concentrate decreased with holding time. However, there was no noticeable hysteresis observed in any of the flow curves. This indicates that the time constant for the decrease in apparent viscosity is so small that decreases in apparent viscosity were not measurable over the length of a given shear sweep experiment i.e. 2 min 30 s.

The Power Law and Herschel Bulkley models were fitted to all the data by using the *Solver* function in *Excel 95* to minimise the residual sum of the squares by changing the cells containing the flow behaviour index, consistency index, and in the Herschel Bulkley model the yield stress variable. The only constraint of the model was that the yield stress was ≥ 0. In all cases the yield stress variable had an optimised value of 0, indicating that MPC85 concentrates do not possess a yield stress. All of the models had correlation coefficients of >0.99. Therefore it can be concluded that MPC85 concentrates are adequately described by the Power Law model. The changes in value of the Power Law coefficients with holding time at the evaporator temperature, 52°C, are shown in Figure 2-6.

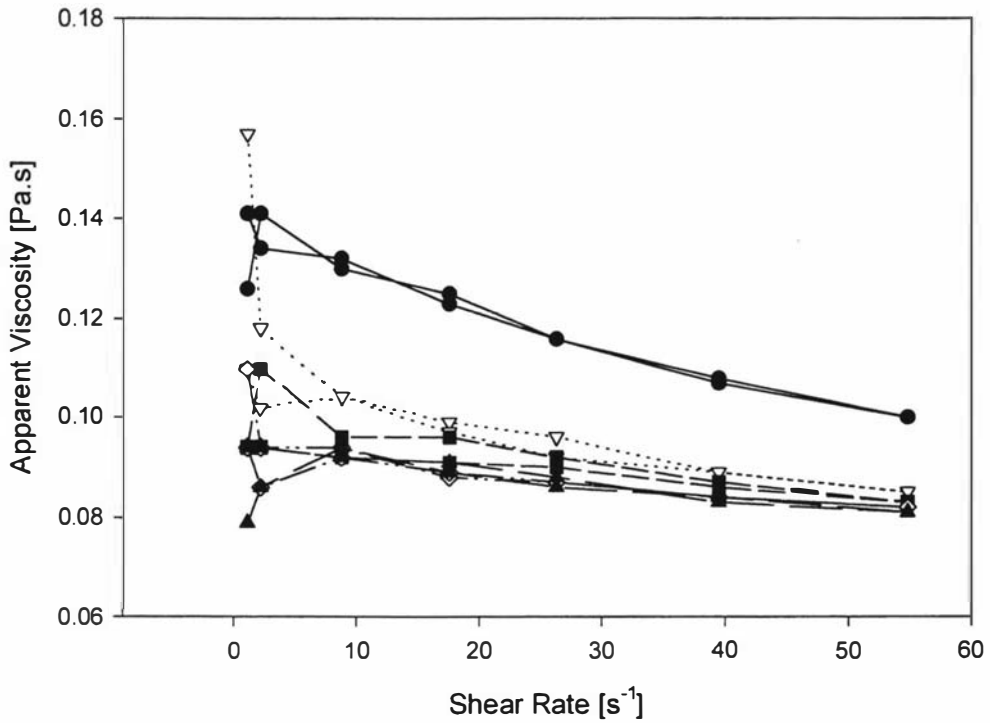

Figure 2-5 Shear Sweep curves (1 to 55 s^{-1}) of MPC85 concentrate ex-evaporator at holding times of 0 min (●), 20 min (▽), 30 min (■), 45 min (◊), and 60 min (▲)

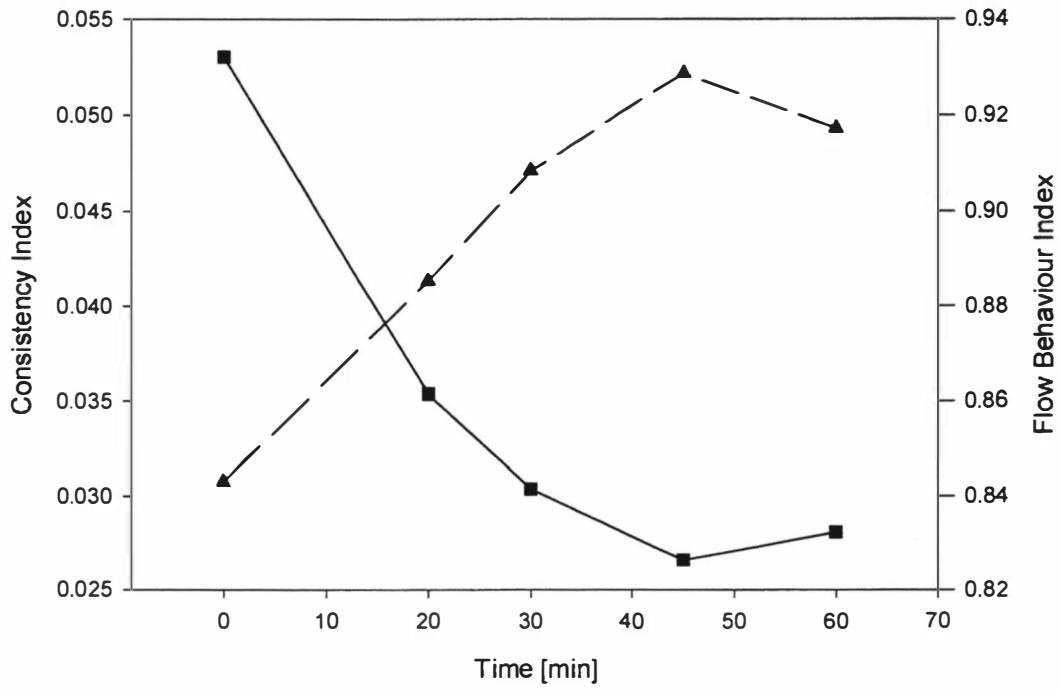

Figure 2-6 Variation in the consistency index [■] and flow behaviour index [▲] with holding time for milk protein concentrate ex evaporator, at 52°C.

As indicated by the consistency index, Figure 2-6 shows quite clearly that MPC concentrates decrease in viscosity with holding time. The consistency index decreases sharply on initial holding and levels out after about 45 min. The flow behaviour index, however, shows a sharp increase in value which then levels out after about 45 min, i.e. MPC85 concentrates become more Newtonian on holding. Due to the increase in Newtonian behaviour, decreases in apparent viscosity on holding are more marked at low shear rates.

The increasing Newtonian behaviour of the concentrate with holding in part explains why the concentrates with high initial viscosities, in the earlier experiments, were observed to show a greater absolute rate of decrease in viscosity. Due to the pseudoplastic nature of MPC85 concentrate, for a given solution the viscosity will be higher at lower shear rates. On holding the concentrate becomes more Newtonian i.e. the difference between the viscosity of the concentrate at low shear rates and high shear rates decreases. So one would expect the viscosity of a concentrate subjected to a low constant shear rate to decrease more than if the same concentrate was subjected to a higher constant shear rate.

The initial decrease in apparent viscosity observed over the first hour may be mediated by the same mechanisms responsible for the initial decrease observed in skim milk concentrates described in section 1.6.2, i.e. perhaps due to relatively weak intermolecular forces being broken by thermal agitation.

It is clear from these results that MPC85 concentrate does not age-thicken with holding at evaporation temperatures. The lack of age-thickening is contrary to initial assumptions that MPC85 concentrate would behave like skim milk concentrate and hence exhibit age-thickening behaviour. Buckingham (1978) observed appreciable age-thickening at 44°C in all skim milk concentrates containing more than 40% w/v total solids (TS). Assuming the skim milk had a dry weight protein content of 37% then 40% TS would be equivalent to a 14.8% protein solution. The results of Buckingham were confirmed by Baldwin *et al.*, (1980) who reported typical figures for a doubling in viscosity at 40°C, due to age-thickening, after 4h at 44% TS, 3h at 48% TS, and after 1h

at 51% TS. Snoeren *et al.*, (1981) observed an increase in apparent viscosity (46% TS, $392s^{-1}$) of 154% on holding at 50°C for 2h. The apparent viscosity of skim milk concentrate ($392s^{-1}$) preheated for 5 min at 95°C has been reported to increase by 205% at total solids of 40% at 50°C over 2h (Snoeren *et al.*, 1981).

A possible explanation for the difference in the behaviour of MPC85 and skim milk concentrate may lie with the lower salt content of MPC85 concentrate. As discussed in section 1.6.2 the mechanism believed to be responsible for age-thickening in skim milk concentrate is that as the concentration of milk solids increases, the solubility product of many of the salts in milk is exceeded and the salts, particularly calcium phosphate (Singh and Newstead, 1992), may precipitate on the surface of the casein micelles. This destabilises the micelles to heating and aggregation occurs resulting in an increase in viscosity (Muir, 1980; Singh and Newstead, 1992). During the processing of MPC85 most of the soluble salts are removed during the ultrafiltration and diafiltration steps. The respective decreases in total calcium, magnesium, sodium and inorganic phosphate are 24%, 72%, 94%, and 47%. These values were calculated from the data given in Table 5-1, on page 151. Therefore, it is likely, due to the low salt concentration in MPC85, that the mechanisms responsible for age-thickening in skim milk do not operate in MPC85 systems. The lower salt concentrations probably would not affect the mechanisms thought to be responsible for the initial decrease in apparent viscosity (section 1.6.2).

2.3 Conclusions

The main conclusions of this work are that MPC85 concentrate ex-evaporator in contrast to skim milk concentrate shows age-thinning behaviour on holding at evaporator temperatures (52°C) rather than age thickening. The age thinning was found to last for about an hour after which the apparent viscosity of the concentrate remained at a constant value. The rate at which a given concentrate, subjected to a constant shear, decreases in viscosity was found to be dependent only on the initial apparent viscosity of the concentrate. The higher the initial apparent viscosity of the concentrate the greater the decrease in viscosity with time. Variations in initial viscosity and rate of decrease

were not correlated with variations in total solids, protein content or shear rate. A model predicting the rate of decrease in apparent viscosity with time was obtained.

The concentrate did not possess a detectable yield stress. The flow behaviour of the concentrate was found to be adequately described by the power law model. The coefficients of the power law model were found to change with holding. The consistency index decreased resulting in the observed decrease in viscosity. The flow behaviour index increased with holding. The increasing Newtonian behaviour of the concentrate with holding in part explains why the concentrates with high initial viscosities show a greater absolute rate of decrease in viscosity.

It was proposed that the lack of age thickening behaviour in MPC85 concentrate compared with skim milk concentrate is a result of the salt concentrations being at levels below the solubility constant. If the salt concentrations are below the respective solubility constants then the salts, in particular calcium phosphate, will not precipitate on the outside of the casein micelle with the resulting destabilisation and increase in apparent viscosity.

3. Rheology of reconstituted commercial MPC85 powder

Understanding the rheological behaviour of reconstituted MPC85 powder is vital if the manufacturing processes for formulations incorporating MPC85 are to be optimised. The purpose of this chapter is to characterise the rheology of reconstituted MPC85 at concentrations and temperatures that may be used in manufacturing processes. This information will also be important in understanding how the rheology changes in the ultrafiltration and diafiltration operations during the manufacture of MPC85 itself. Preliminary experiments were conducted to provide guidelines for later experimental work. This preliminary work included: characterising the powder with respect to composition, investigating the stability of reconstituted MPC85 to holding, developing a suitable method of reconstitution, and determining the effect of solubility on the rheology of the reconstituted powder.

3.1 Materials and methods

3.1.1 Composition of commercial powder

MPC85 powder from a 20kg bag of commercial MPC85 powder manufactured by Anchor Products Hautapu (manufactured on 19th October 1995, Batch Cipher CF19) was used in all of the experiments described in this thesis where the term "commercial MPC85" is used. The results of the routine factory conducted microbial analysis for the bag of MPC85 were within specifications. Sharma (1998) showed that MPC85 was stable with regard to changes in colour, solubility, viscosity and heat stability at 7°C and recommended that to control Maillard reactions, MPC85 should be stored at low temperatures. The bag of MPC85 was stored at -18°C throughout the length of the work.

The basic composition of the commercial MPC85 powder used in these experiments is shown in Table 3-1.

Table 3-1 Anchor Products Hautapu Compositional determination of MPC85 Batch Cipher CF19.

Component	[% w/w]
Total Protein (dry basis)	86.1
Fat	1.40
Lactose	4.6
Ash	7.4
Moisture	3.70

3.1.2 Composition of low heat skim milk

A low heat skim powder (LHS) was used as a benchmark for comparison in some of the experiments. The LHS was obtained from Kiwi Milk Products Ltd, Hawera. The product name and batch number were non fat/spray dried/low heat milk powder (LHS), and J4627 respectively.

Table 3-2 Specification for Low Heat Skim Milk powder (Source: Ingredient Specification sheet; Kiwi Milk Products Ltd, Hawera).

	Typical	Maximum	Minimum
Fat (Rose Gottlieb)	0.80 %	1.25 %	
Moisture (101-103°C, 2 hrs)	3.80 %	4.00 %	
Protein	37.80 %		
Lactose	49.80 %		
Ash (minerals)	7.80 %		
WPNI (undenatured whey protein)	6.5 mg/g		6.0 mg/g

3.1.3 Preparation of solutions of reconstituted MPC85

The method of reconstituting the MPC85 powder, unless otherwise stated, was as follows: The mass of water and sodium azide (0.02 % w/w) required to give a final solution with the desired concentration was calculated. The water/azide solution was heated to 50°C in a pre-weighed stainless steel beaker (0.08 m internal diameter) containing a stirrer (three blades; blade diameter 0.06 m) of known weight. The stirrer was attached to an overhead motor and set at a speed which would create an adequate vortex in the solution. The MPC85 powder was added a teaspoon at a time. Each successive addition was made when the previous addition had been fully wetted. The speed of the stirrer was adjusted to maintain a vortex. Periodically the stirrer was turned

off for about 20 seconds to allow any entrained air to escape. Allowing air to escape sped up the wetting of further MPC85. Once the MPC85 powder was fully wetted, the stirrer speed was decreased to a point where there was no vortex but there was still movement of the liquid on the surface (typically 500 to 700 rpm). The MPC85 solution was then stirred for 1 hour. The speed of rotation was decreased to minimise the amount of entrained air as solutions containing air bubbles are known to give erroneous rheological results. During stirring, the beaker was covered with tin foil to minimise evaporation loss. A second reason for covering the beakers was that solutions of high MPC85 concentration formed a surface skin if they were not covered. At the end of stirring the beaker containing the solution and the stirrer was weighed. Milli-Q water was added if necessary to reach the required mass.

For solutions which were homogenised, the solution was fed into a bench-top homogeniser (Niro Soavi Omogeneizzatori; model Panda Number 2638, Parina - Italy) and homogenised at 150 bar in a single stage process.

Before homogenisation the solution was allowed to settle (typically 2 minutes) to allow entrained air to rise to the surface. Foam, if present, was largely removed with a spoon. The beaker was then recovered with a new piece of tin foil containing two holes at opposite sides. By tipping the beaker on a sufficient angle the solution was able to be siphoned off leaving any residual foam behind. The solutions were then checked for air bubbles by looking at a thin film of each solution through glass held up to a light. A further check for entrained air was made by examining the bob from the viscometer after measurements and examination of the flow curves. Typically solutions with entrained air show hysteresis and give non-reproducible results.

3.1.4 Determination of MPC85 solubility

The solubility of MPC85 was determined by preparing a solution of 3.5% protein using the method outlined in section 3.1.3 with the following exceptions. The temperature at which the solution was mixed was set at either 20, 30, 40, 50, or 60°C. After 1 hour of stirring at the required temperature the speed of the stirrer was increased to achieve a vortex, thus ensuring a homogeneous solution, and two samples were removed by

Pasteur pipette and placed in pre-weighed moisture dishes. The weight of the moisture dishes containing the bulk solution sample were then recorded and the dishes placed in an oven set to 108°C to dry overnight. The presence of a vortex ensured that the bulk solution was homogeneous when the samples for total solids analysis were removed. The remaining solution was centrifuged at 700g for ten minutes in a 50ml centrifuge tube. Two samples of the serum were removed by Pasteur pipette and analysed for their total solids content in the manner described above.

After drying the moisture dishes were removed from the oven and placed in a desiccator for ≥ 30 minutes. The dishes were then weighed and the total solids calculated. The solubility was then calculated from equation 3-1.

3-1
$$\text{Solubility} = \frac{\text{Total Solids of Supernatant}}{\text{Total Solids of Bulk Solution}} \times 100\%$$

3.1.5 Rheological measurements

The rheological measurements in this chapter were conducted on two rheometers: the Bohlin VOR (Bohlin Rheologi AB, Lund, Sweden) controlled strain rheometer linked to a computer running the software program Bohlin Rheometer System Version 3.10 and the Haake VT500 Viscotester (Haake Mess-Technik GmbH Co. Karlsruhe, Germany) viscometer linked to a computer running the software program VT500 version 1.4. The temperature of the measuring system for the Haake VT500 was controlled by a Grant waterbath fitted with a P21 temperature programmer control and cooling system. The Bohlin VOR rheometer was initially the only instrument that was to be used throughout this work; however due to continual malfunctions and time delays in repairs it became necessary to use the Haake VT500. The advantages of using the Bohlin VOR are that it can control temperature to ± 0.1 °C (compared to the Haake VT500 ± 0.5°C), and can measure very low viscosity fluids accurately at low shear rates which would allow the detection of yield stresses if present. A further advantage of the Bohlin was that it was capable of performing oscillation experiments. The main disadvantage of the Bohlin was that the range of the applied shear rate was limited by the torsion bar. If a wider range of shear rates was desired, this necessitated the fitting of a lighter or heavier bar

and re-calibration of the machine as well as preparing a new sample for measurement. Due to time constraints caused by heavy Bohlin usage and malfunctions it was not always possible to fit a new torsion bar to ensure that all samples were measured over the same shear rate range. The Haake VT500, while not capable of measuring as accurately as the Bohlin, did not have the same limitations with regard to shear rate range. This enabled comparison of samples over a wide range of shear rates and hence allowed the use of rheological models for comparison.

The main viscometry test used was the shear sweep experiment. The viscosity of the fluid was measured over a range of shear rates. By plotting shear stress against shear rate one can obtain a flow curve. From a flow curve, structure build up or breakdown in the sample with increasing shear rate (i.e. dilatancy or pseudoplasticity respectively) can be detected. If an up/down shear sweep is performed, time dependency can also be detected. If time dependent behaviour is present torque values obtained on the "down curve" may be lower or higher than those obtained on the "up curve", depending on whether the fluid is thixotropic or rheopectic respectively, and consequently the flow curve is in the form of a "hysteresis loop" (Dinsdale and Moore, 1961).

The shear rate ranges mentioned below were those planned but not necessarily those that were achieved for every solution studied.

3.1.5.1 Rheological measurements using the Haake VT500

Due to the low accuracy of the Haake VT500 with low viscosity fluids all experiments, unless stated otherwise, were conducted at 15°C. This temperature was chosen as a compromise between being able to handle the highly viscous solutions and being able to measure the lower viscosity solutions. It is well known that the viscosity of protein solutions increases with decreases in temperature. The solutions were placed in the Haake VT500 rheometer set to 15°C and left to equilibrate for 10 minutes. The NV and MV1 geometry concentric cylinders were used depending on the viscosity of the solution. Following equilibration the solution was subjected to increases in shear rate from 10 to 1000s^{-1} at steps of 20s^{-1} over a period of 3 minutes immediately followed by a corresponding decrease in shear rate.

3.1.5.2 Rheological measurements using the Bohlin VOR Rheometer

The temperature at which shear sweep experiments were performed on the Bohlin varied from 15°C to 65°C. To prevent drying out a thin layer of paraffin oil was placed on top of the solutions. A temperature equilibration time of 10 minutes was used. The shear sweep experiments were performed over the shear rate range 1.16 to 116s^{-1}. The C14 and C25 geometry cup and bob sets in conjunction with a 4g and 0.3g torsion bar were used for the viscometry tests depending on the viscosity of the solutions. The shear rate was set to increase logarithmically so that the flow curve at low shear rates would be more accurately described, enabling the existence of a yield stress to be more readily identified. Each sweep took 8.41 minutes with measurements taken every 25s.

3.2 Effect of method of reconstitution on solubility

The main structural elements of a solution that can affect the rheology and depend on the method of reconstitution are: the presence of air bubbles (covered briefly in sections 3.1.3 and 3.3) and, the degree of solubility of the powder, covered in this section. The problem of air incorporation in a solution generally occurs in solutions of high viscosity and causes the solution to possess an even higher viscosity. The presence of insoluble particles in a solution leads to a higher viscosity, compared to a solution which does not contain insoluble particles. The higher viscosity, caused by the presence of insoluble particles, may lead to air incorporation. Therefore the effect of the method of reconstitution on solubilisation was investigated first. The main variables thought to affect solubilisation of MPC85 are: temperature (Pierre *et al.,* 1992) and, the flow velocity of water during reconstitution (Schuck *et al*., 1994).

The effect of reconstitution temperature on the solubility of commercial MPC85 was investigated over the temperature range 20 to 60°C following the method outlined in section 3.1.4. The results of these experiments, shown in Figure 3-1, clearly show that the solubility of MPC85 decreases as the reconstitution temperature decreases below 50°C. An attempt to model the relationship between reconstitution temperature and solubility with the Arrhenius Equation, and Power Law models did not arrive at an adequate solution (R^2's of 0.76 and 0.85 respectively). Log plots of the data did not

show linearity. From this analysis the relationship can only be described qualitatively: solubility increases rapidly with temperature and asymptotes to almost 100% solubility at about 50°C. The effect of temperature of reconstitution on the solubility of MPC85 reported here is comparable to that of Pierre *et al.*, (1992) who found that increasing the reconstitution water temperature from 24°C to 50°C increased the solubility of native micellar casein from 74.0% to 97.2%.

Schuck *et al.*, (1994) concluded that increasing the flow velocity of water during reconstitution of MPC85 facilitated solubility. These authors found that the solubility increased when the period of agitation was extended to 900s or if agitation is increased to 10000 rpm. However, when this method was applied to the concentrations required for viscosity measurements in this work (up to 17.5% protein) excessive foaming occurred. It was thought that homogenisation would provide the increase in flow velocity of water necessary to facilitate solubility without excessive foaming. It is recognised though, that homogenisation is a complex process involving effects such as intense shear and cavitation as well as increasing the flow velocity of water.

To investigate the effect of homogenisation on solubility, the solution reconstituted at 20°C was homogenised at 150 bar and its solubility determined. This homogenisation step increased the solubility of the solution from 58.85% to 88.75%.

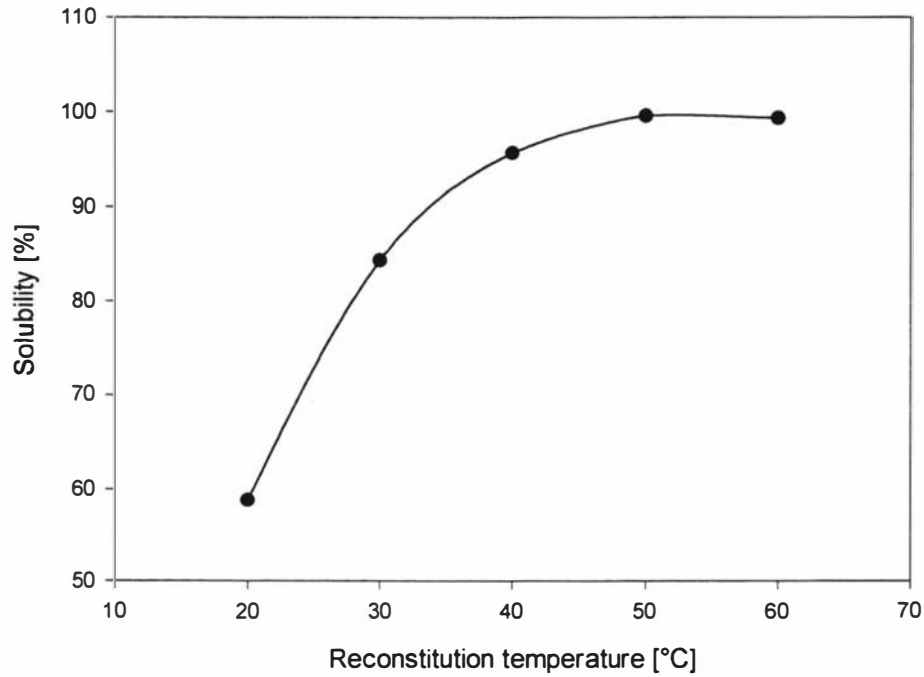

Figure 3-1 The effect of reconstitution temperature on the solubility of commercial MPC85 in MilliQ water.

3.3 Effect of solubility on apparent viscosity

Having established the effect of the reconstitution temperature on the solubility of MPC85 the question arose of how sensitive rheological measurements of reconstituted MPC85 solutions are to insoluble solids. To answer this question a series of 20% w/w MPC85 solutions were prepared following the procedure outlined in section 3.1.3 with the following exceptions. The temperature at which each solution was mixed was set at either 20, 30, 40, 50, or 60°C. A sixth solution was prepared with reconstitution water set to a temperature of 20°C. Following mixing this solution was homogenised at 150 bar in a single stage homogeniser. The rheology of each of these solutions was examined by a shear sweep experiment at 15°C conducted on the Haake VT500 rheometer. The details of these rheology experiments are described in sections 3.1.5 and 3.1.5.1.

The solutions showed no signs of air incorporation: no air bubbles could be seen on either the cup or the bob surface at the end of the experiment once the solutions were carefully poured out of the cup; and the flow curves showed no signs of hysteresis. Typically the flow curve of a solution with entrained air bubbles will show a much higher viscosity on the downward sweep (in extreme cases the solution may show no decrease in viscosity with decreasing shear) than the upward sweep and both flow curves show considerable "noise".

The flow curves of the reconstituted MPC85 solutions are shown in Figure 3-2. The scale of the apparent viscosity axis of this figure is logarithmic to show more clearly the differences between all of the flow curves on one graph. By inspection one can see that: the pseudoplastic nature of the solutions, indicated by the decrease in apparent viscosity with increasing shear rate, increased with the degree of insoluble matter; and that the apparent viscosity of the solutions also increased with the degree of insoluble matter. It should be noted that the solubility and apparent viscosity data were obtained from solutions comprised of 3.5% protein and 20% total solids respectively. It is likely that the solubility of MPC85 at 20% total solids is slightly different than at 3.5% protein due to differences in ionic strength. However, despite the difference in protein

concentrations the general trends reported here are thought to be valid. The relationship between flow properties of the solutions and their solubility was explored further through the fitting of rheological models.

The solutions with lower solubility (58.85%, and 84.42%) possessed, at high shear rates, viscosities that exceeded the measuring capabilities of the Haake VT500 rheometer. The highest shear rate at which measurements were able to be taken for these solutions was $300s^{-1}$. Therefore only the shear rate range of 10 to $300s^{-1}$ has been plotted and only this data was used for model fitting. A comparison of rheological models is only valid if the shear rate range over which the models were fitted is the same. The viscosity data at low shear rates for solutions with high solubility showed a certain degree of noise. This noise is due to the limited sensitivity of the Haake VT500 at low viscosity. The downward sweep flow curves showed less noise than the flow curves obtained on the upward sweep. The presence of more noise on the upward sweeps is probably an artefact of the rheometer resulting from the overcoming of inertia when the bob is first rotated. Only the data obtained on the downward curves has been used for the fitting of models.

The Herschel Bulkley and Power Law models were fitted to each set of data in the manner described in section 2.2.2. This analysis revealed that no yield stress was present in any of the solutions and that their flow behaviour was adequately described by the Power Law model. The correlation coefficient in all cases was >0.99. The relationship between the Power Law coefficients and the solubility of the suspension is illustrated in Figure 3-3.

The data contributed from the solution which had been homogenised (88.75% solubility) is in line with what one would expect based on the other data points indicating that the homogenisation does not change the rheological properties of the solution i.e. the only effect of homogenisation is to facilitate solubility.

The implications of this set of experiments is that one must have complete solubility to obtain reliable rheological data. Hence all solutions prepared in this work were dissolved at 50°C unless otherwise stated.

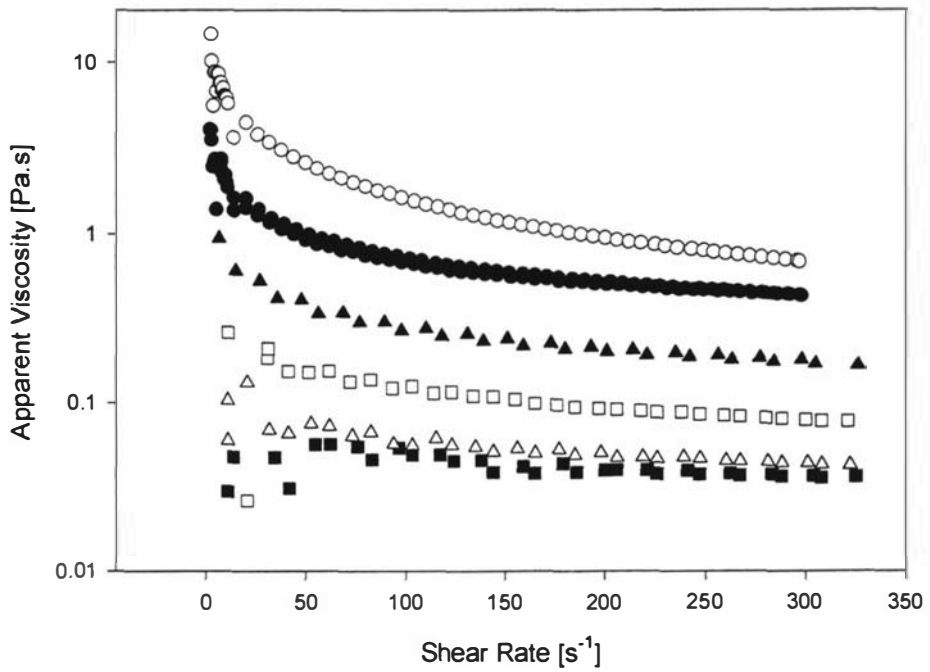

Figure 3-2 The flow curves of reconstituted MPC85 with solubility's of 58.85% (○), 84.42% (●), 88.75% (▲), 95.62% (□), 99.34% (△), 99.60% (■). Note: the solution with 88.75% solubility was created by homogenisation of a slurry at 20°C.

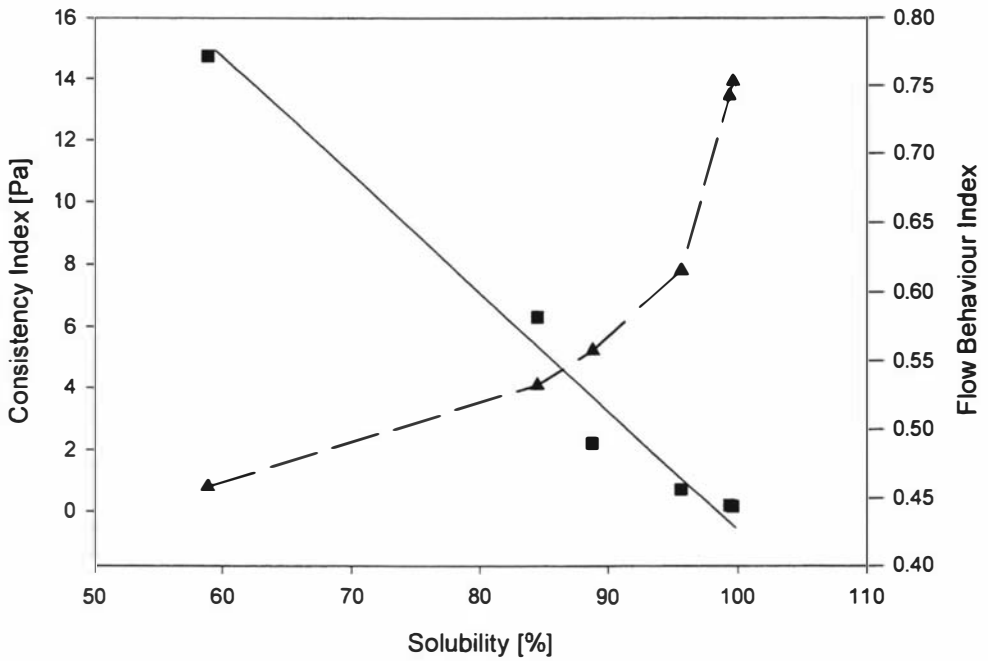

Figure 3-3 The relationship between the consistency index (■) and flow behaviour index (▲) with solubility for reconstituted MPC85 (20%w/w; 15°C; 10 to 300s^{-1}). Note: the solution with 88.75% solubility was created by homogenisation of a slurry at 20°C.

3.4 Effect of cold storage on rheology

At the start of the experimental work it was thought that it would be convenient to make up several reconstituted solutions one day, store them overnight at 5°C, and examine their rheology the next. To determine whether overnight storage would have an effect on rheology a 20% w/w solution of reconstituted commercial MPC85 was prepared, separated into four sealed beakers, stored at 5°C, and its rheology measured over a period of three days. A solution was removed from the cold storage on successive days and allowed to equilibrate at room temperature for 1 hour before being placed in the Haake VT500 rheometer for testing. The experimental set-up for the Haake VT500 was that described in section 3.1.5.1. Over the course of this experiment, it became apparent that the viscosity of the solution was increasing during storage. It was thought that the increase in viscosity might be due to the dissociation of β-casein from the casein micelle. This contributes to an increase in viscosity because dissociated protein molecules have higher hydrodynamic volumes (Singh *et al.*, 1997). In addition, the decrease in the density of the casein micelle due to the dissociation of β-casein may also contribute to the increase in apparent viscosity (see section 4.3.4.).

A study by Larsson *et al.*, (1995) found that heating a suspension of artificial micelles to 30°C for 30 min minimised the problem of the β-casein molecules leaving the micelle during long-term storage at low temperatures by allowing the loosened β-casein molecules to be re-incorporated into the micelles. To investigate this hypothesis the solution held for the longest period of time (3 days) was, after the shear sweep at 15°C, heated in the Haake VT500 to 50°C for half an hour then cooled back to 15°C and left to equilibrate for ten minutes. Following equilibration an upward and downward shear sweep was performed on the solution. A thin layer of soy oil was placed on top of the MPC85 solution to prevent it drying out.

The flow curves of all of these experiments are shown in Figure 3-4. The main features of this figure are that the apparent viscosity increased with storage at 5°C, and that the flow curve after storage for three days at 5°C and heating at 50°C for 30 min coincided

with the flow curve of the solution at t = 0 days. There is some noise at low shear rates on the upward shear sweeps. The reason for this noise has been discussed in section 3.3.

Both the Power Law and Herschel Bulkley rheological models were fitted to the flow curves obtained from the downward shear sweep. As with earlier modelling no evidence was found for the presence of a yield stress and the flow curves were found to be adequately described by the Power Law model (all fits possessed correlation coefficients of >0.99). The relationships between the consistency and flow behaviour indices and cold storage time are shown in Figures 3-5 and 3-6 respectively.

It is apparent from these figures that the consistency index (and pseudoplastic behaviour) of solutions of reconstituted MPC85 increase approximately linearly with storage at 5°C. The data also show that the rheological changes resulting from cold storage may be largely reversed by incubation at 50°C for half an hour. This finding lends credence to the hypothesis that the rheological changes are due to an increase in the hydrodynamic volume of the micelle due to dissociation of β-casein. The increase in viscosity with cold storage observed with solutions of reconstituted MPC85 agree with observations made by Randhahn (1976) who found that skim milk concentrated by ultrafiltration at prolonged storage times (particularly at lower temperatures and higher solids contents) gave rise to solidification of the concentrates.

The implication from this experiment was that rheology of reconstituted MPC85 solutions should be measured on the day they are prepared. This guideline was adhered to throughout the research project.

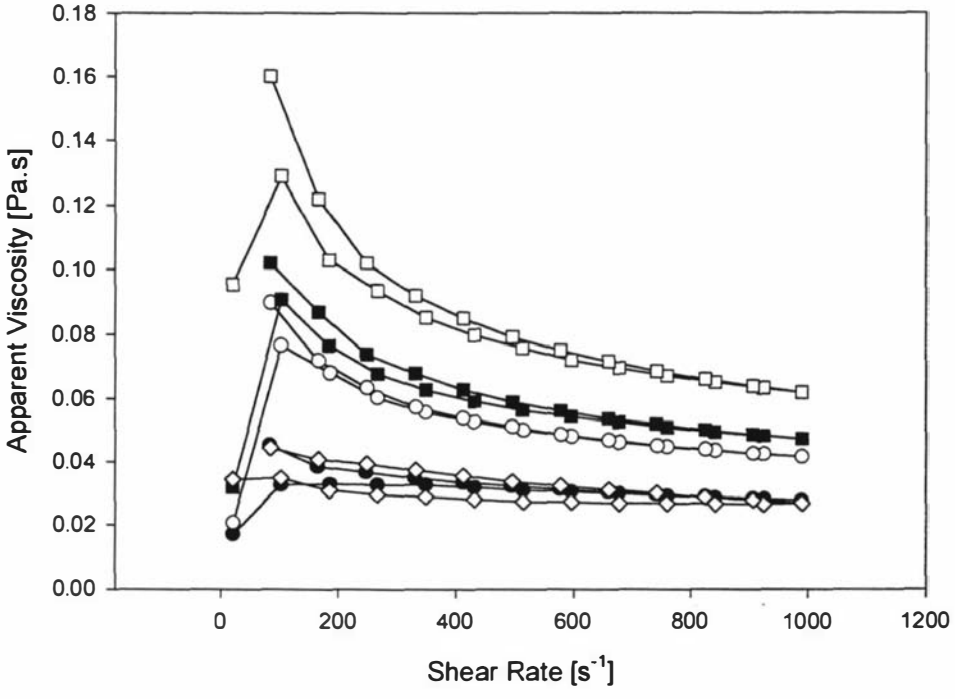

Figure 3-4 The effect of storage at 5°C on the apparent viscosity (15°C, 10 to 1000s^{-1}) of reconstituted 20% w/w MPC85 held for 0 days (●), 1 day (○), 2 days (■), 3 days (□), and held for 3 days heated to 50°C for 30min and then cooled to 15°C (◊).

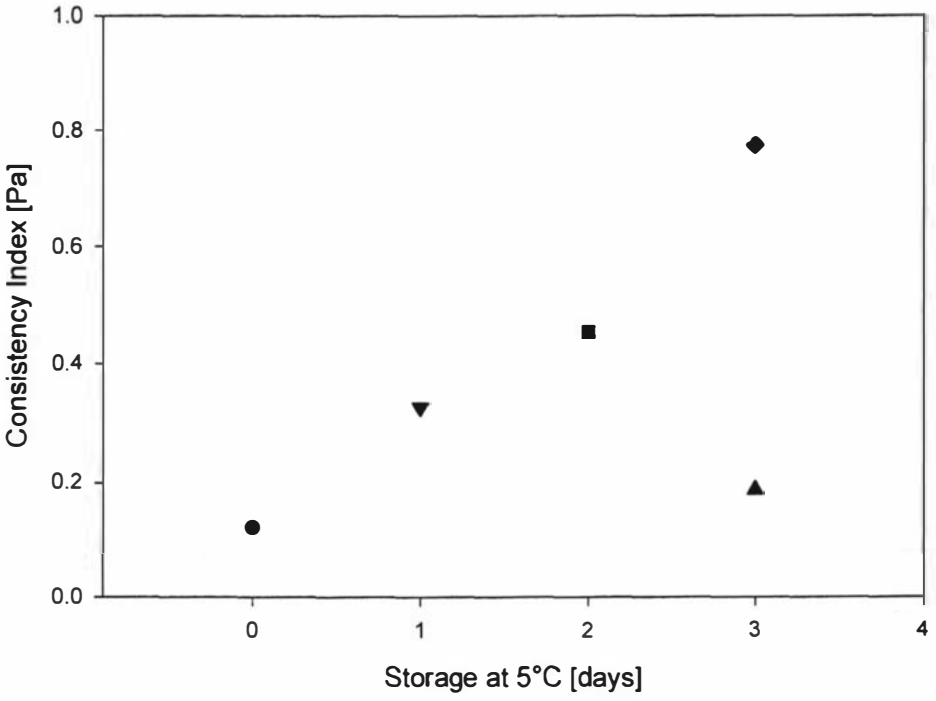

Figure 3-5 The effect of storage at 5°C on the consistency index (15°C, 10 to 1000s^{-1}) of reconstituted 20% w/w MPC85 stored for 0 days (●), 1 day (▼), 2 days (■), 3 days (◆), and stored for 3 days heated to 50°C for 30min and then cooled to 15°C (▲).

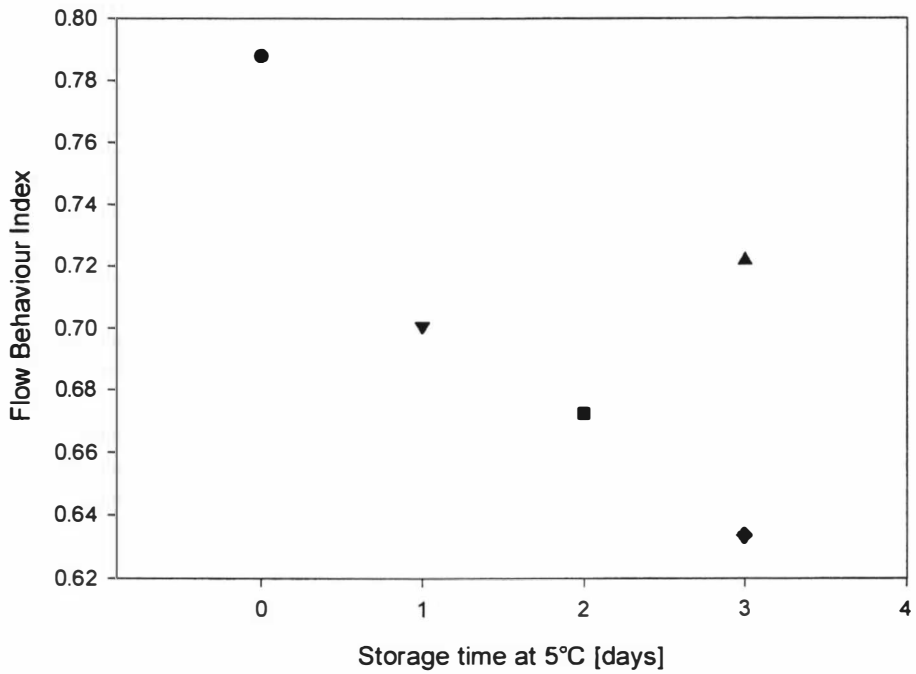

Figure 3-6 The effect of storage at 5°C on the flow behaviour index (15°C, 10 to 1000 s^{-1}) of reconstituted 20% w/w MPC85 stored for 0 days (●), 1 day (▼), 2 days (■), 3 days (◆), and stored for 3 days heated to 50°C for 30min and then cooled to 15°C (▲).

3.5 Effect of holding at 52°C on rheology

One of the main findings from the experiments described in chapter 2 was that MPC85 concentrate ex-evaporator, contrary to skim milk concentrate, did not age-thicken and actually thinned with time held at evaporator temperatures. The objective of the following experiment was to determine whether the powder produced from the concentrate exhibited the same rheological properties when reconstituted.

To this end a 17.5% w/w total protein solution of MPC85 was prepared and placed in the NV geometry of the Haake VT500 rheometer which was set to a temperature of 52°C. A thin layer of soy oil was placed on top of the solution to prevent drying out. The actual experiment was the same as that described in section 2.2.2 with the exception that the shear rate range was 10 to 1000s^{-1} and the shear sweeps were conducted at 0min, 10min, 20min, 32min, 40min, 50min, and 60min. The solution was allowed to equilibrate for two minutes before measurements were taken. The total solids of the reconstituted solution was not able to be as high as that of the concentrate studied in section 2.2.2 due to handling problems during preparation at higher concentrations.

The flow curves of this experiment, shown in Figure 3-7, contain a high degree of noise due to the low viscosity of the solutions and the limitations of the Haake VT500. However, apart from the flow curve of the shear sweep conducted at 0 min all the flow curves appear to coincide i.e there does not appear to be any change in the rheological properties of the solution with holding at 52°C. The 0 min flow curve possessed a hysteresis in which the downward sweep curve had a lower apparent viscosity. This may be the result of a real decrease in apparent viscosity at 52°C with time which occurs within the first 10 minutes or an artefact - the equilibration time may have been insufficient for the entire solution to reach 52°C. A more detailed analysis, fitting Power Law models to each downward flow curve and looking at the change in the coefficients with holding time did not yield any statistically significant trends at a 95% level. The coefficients from the model fitting are given in Table 3-3. A regression analysis of the consistency index did yield an equation which indicated that apparent viscosity decreased with holding time but the level of significance for the equation was only 84%. Therefore there is no evidence to suggest that holding at 52°C has any affect on the rheology of 20% w/w reconstituted MPC85 solutions.

Table 3-3 Power Law Coefficients describing the change in flow properties of a 20% w/w MPC85 solution held at 52°C.

Holding Time [min]	Consistency Index [Pa]	Flow Behaviour Index	Correlation Coefficient
0	0.0063	1.005	0.711
10	0.0058	0.992	0.997
20	0.0045	1.026	0.997
32	0.0030	1.082	0.988
40	0.0057	0.990	0.921
50	0.0049	1.011	0.997
60	0.0033	1.065	0.967

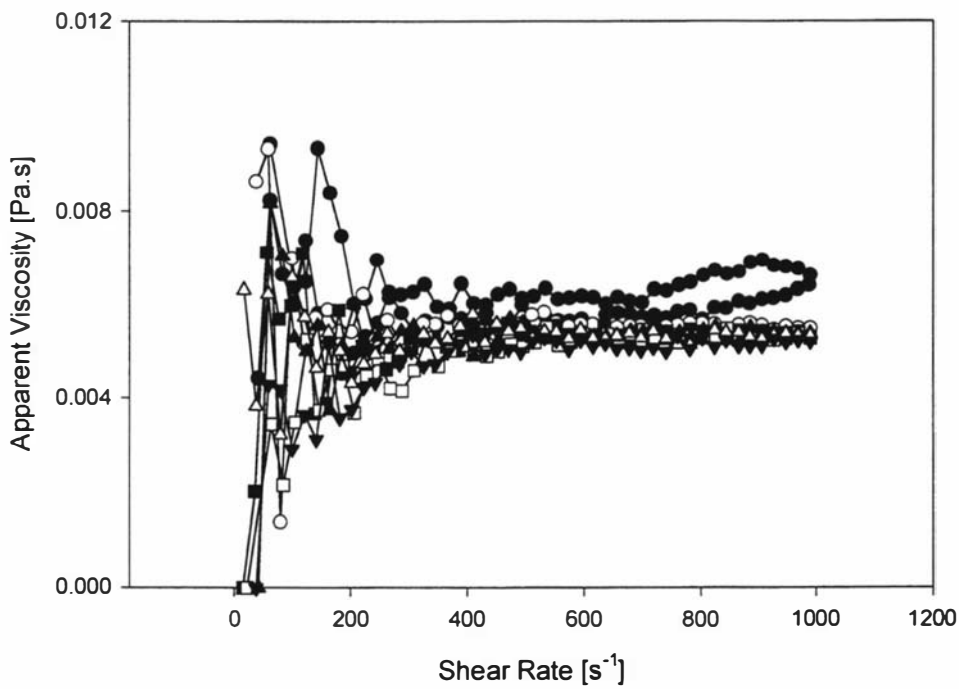

Figure 3-7 The effect of holding at 52°C on the flow curves of reconstituted MPC85 20% w/w solutions: 0min, (●); 10min, (○); 20min, (■); 32min, (□); 40min, (▲); 50min, (△); 60min, (▼).

3.6 Comparison of the effects of concentration on apparent viscosity for reconstituted commercial MPC85 and low heat skim milk powder

To determine the sensitivity of rheological measurements to MPC85 concentration a shear sweep experiment (10 to 1000s^{-1}, 15°C) was conducted on a series of solutions covering the total solids concentration range of 0 to 19% dry weight. A similar study was also carried out using a commercial low heat skim milk powder, LHS, as a substrate to provide a benchmark for comparison. The composition of the LHS powder is given in section 3.1.2. The total solids concentration range of LHS solutions, calculated to give an equivalent range based on protein concentration, was 0 to 44% dry weight. The raw results of these experiments are shown in Figures 3-8 and 3-9 for MPC85 and LHS respectively. The data show no sign of hysteresis indicating that time dependent behaviour was not present in the solutions over the time scale of the experiments (3 minutes per shear sweep).

The sensitivity of apparent viscosity (291s^{-1}) of both LHS and MPC85 to protein concentration is illustrated in Figure 3-10 (a). A shear rate of 291s^{-1} was chosen to enable comparisons to be made with other workers (refer Figure 3-11) and because at this shear rate the data is more stable than at lower shear rates.

From Figure 3-10 (a) it appears that LHS has a higher apparent viscosity than MPC85 at a given protein concentration. Generally, the viscosity of a dispersion is a function of the volume fraction of the dispersed particles and of the viscosity of the solvent. The viscosity of skim milk concentrate is a function of the volume fraction of the proteins present and the viscosity of the milk serum. The volume fraction depends on the hydration of the protein, the protein composition and the protein content (Hallström and Dejmek, 1988). The calculation of protein concentration however, as protein mass divided by total solution mass, indirectly includes the mass of non-protein solids such as salts and lactose. These components do not directly affect apparent viscosity. The inclusion of these components can mislead the interpretation concerning the apparent viscosity of LHS and MPC85 solutions. By plotting apparent viscosity against the protein:water ratio the potentially misleading effect of the non-protein solids components may be removed. If the logarithm of apparent viscosity is plotted against

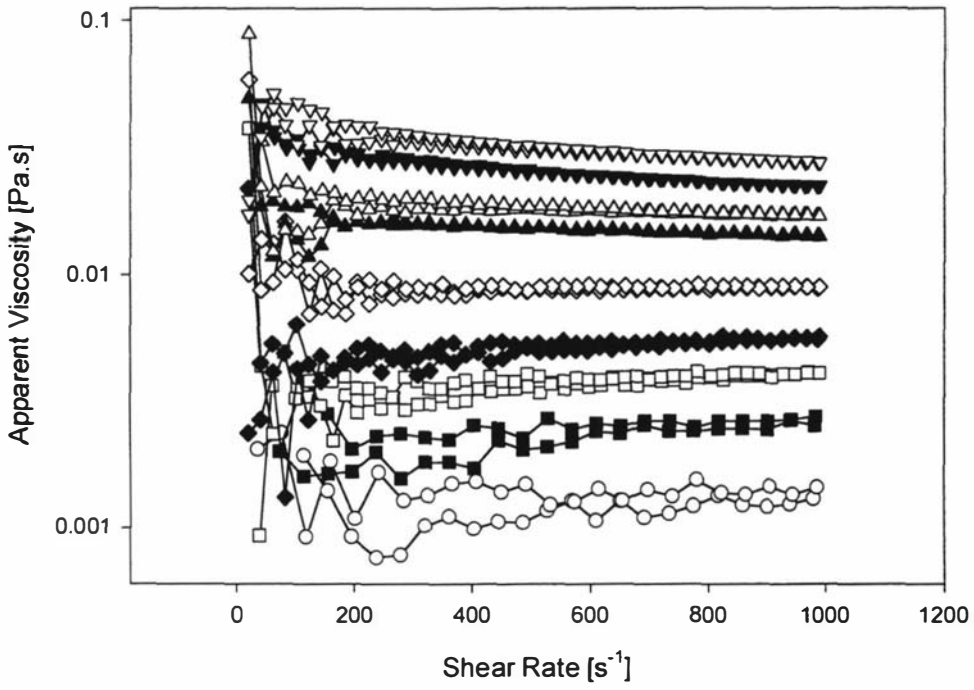

Figure 3-8 The shear sweep curves for reconstituted MPC85 solutions of varying total solids concentration: (○), 4.81%; (■), 8.69%; (□),10.49%; (◆),12.42%; (◇),14.37%; (▲),16.22%; (△),17.09%; (▼),17.99%; (▽), 18.89%.

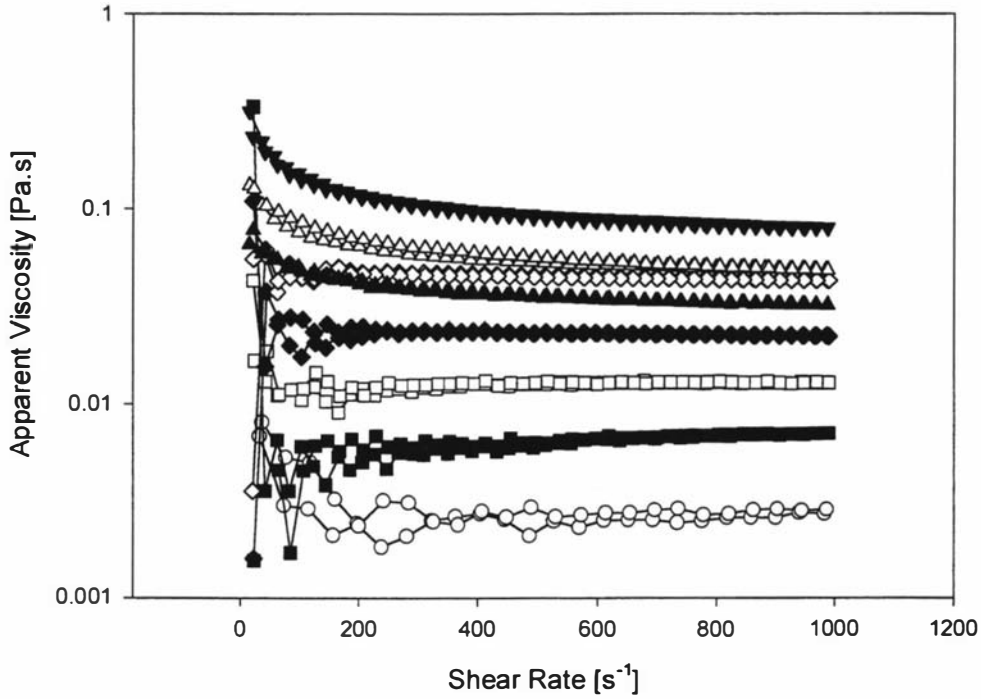

Figure 3-9 The shear sweep curves for reconstituted LHS solutions of varying total solids concentration: (○),14.00%; (■), 22.96%; (□), 27.30%; (◆), 32.36%; (◇), 35.45%; (▲), 39.09%; (△), 41.54%; (▼), 43.15%.

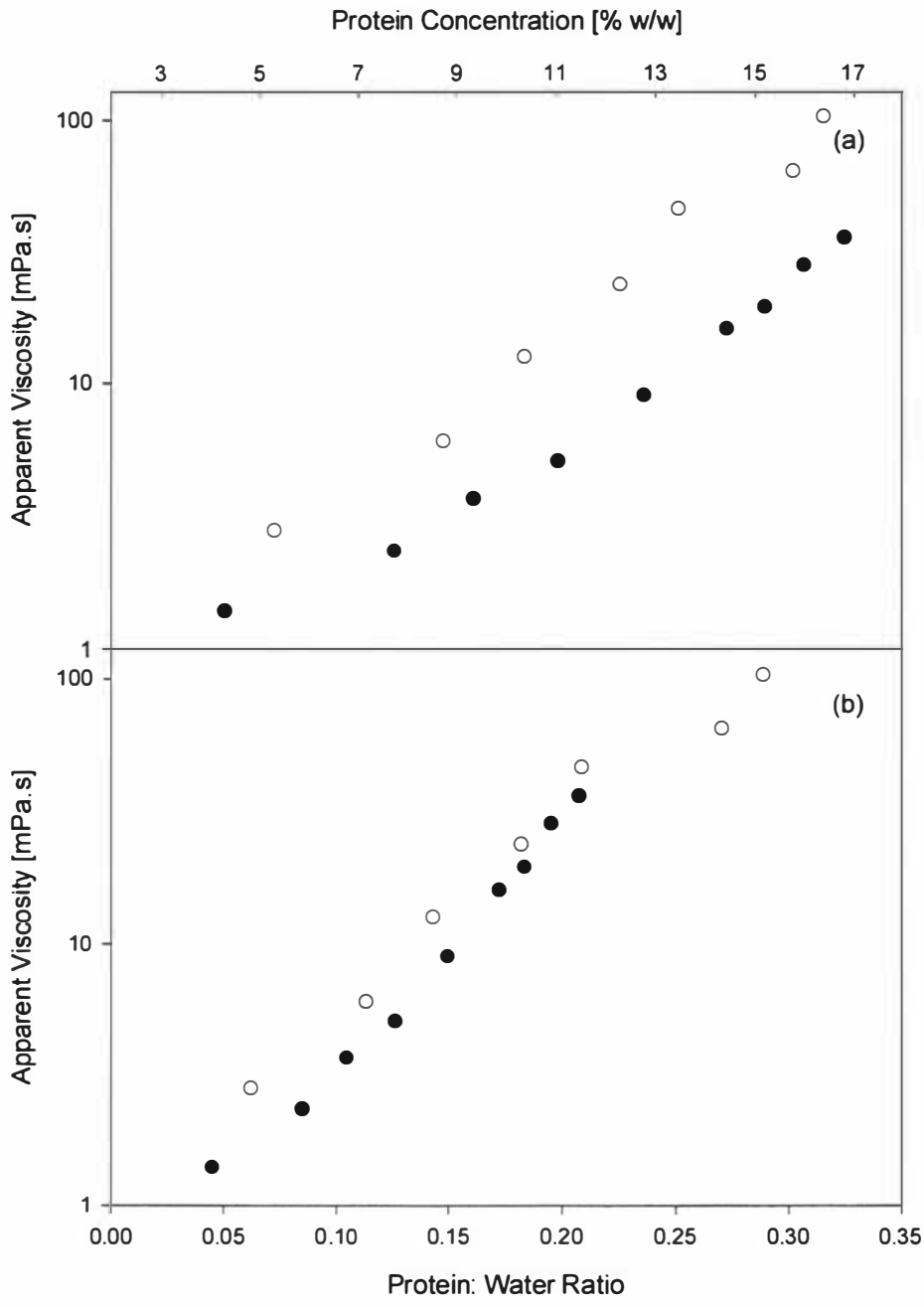

Figure 3-10 The apparent viscosity of MPC85 (●) and Low Heat Skim (○) at 15°C, 291s^{-1}, as a function of protein concentration (a) and protein:water ratio (b).

the ratio of protein to water, refer Figure 3-10 (b), little difference is observed between MPC85 and LHS. The corollary of this result is that the other components have little effect on the overall apparent viscosity.

The apparent viscosity of the MPC85 and LHS solutions are compared, on the basis of the protein:water ratio, with viscosity data for sodium caseinate, and whey protein concentrate in Figure 3-11. The sodium caseinate and whey protein concentrate data were obtained from Carr (1994) and Tang et al. (1993). The pH of the sodium caseinate solutions (pH 6.70) is lower than that of the other solutions (pH 7.0). The difference in pH would not, however, affect the validity of comparisons as sodium caseinate solutions show little variation in apparent viscosity (± 26%) over the pH range 6.50 to 7.40 (Carr, 1994). The difference in temperature between the solutions (Na-caseinate, 25°C; WPC, 22°C; LHS and MPC85, 15°C) would result in a slight exaggeration of the viscosity difference between MPC85 and WPC and a slight underestimate of the difference between Na-caseinate and MPC85. The lower shear rate of the Na-caseinate data would exaggerate viscosity differences.

For all of these products the logarithm of apparent viscosity appears to increase linearly with the protein:water ratio. Linear relationships between the logarithm of apparent viscosity and concentration have been reported for caseinate (Towler, 1974) and WPC (Tang et al., 1993) systems. The results of fitting a linear regression model to this data, for each product, are shown in Table 3-4.

Table 3-4 Linear regression coefficients describing the viscosity / protein:water ratio relationship for various dairy products.

Product	Slope	Intercept	Correlation Coefficient
Sodium caseinate	26.51	-0.9046	0.98
MPC85	9.04	-.3572	0.98
LHS	6.83	0.0766	0.97
WPC	4.89	-0.0005	1.00

By inspection of Figure 3-11 and Table 3-4 one can see that up until a protein:water ratio of about 0.10 there is little variation between the log viscosity data of the MPC85, LHS and WPC solutions. At higher ratios WPC has a lower viscosity while LHS and

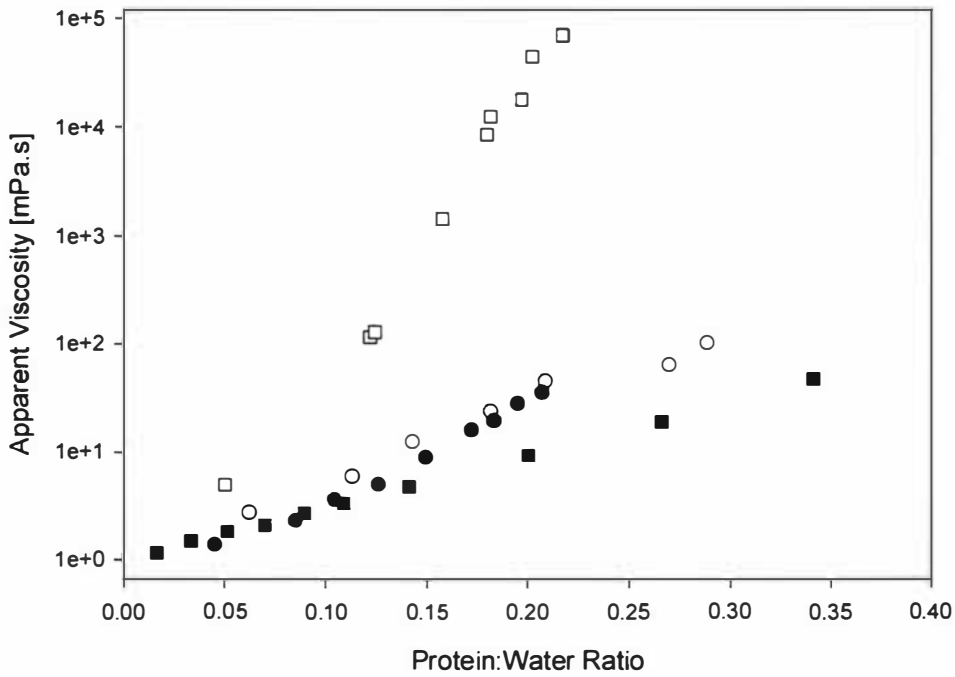

Figure 3-11 The apparent viscosity of MPC85 (●) and Low Heat Skim (○) at 15°C, pH 7.0, 291 s^{-1}; sodium caseinate (□), 25°C, pH 6.7, 100s^{-1}; whey protein concentrate (■), 22°C, pH 7.0, 291s^{-1} as a function of protein to water ratio. The sodium caseinate and whey protein concentrate data was obtained from Carr (1994) and Tang et al. (1993) respectively.

MPC85 are still very similar. It is not possible to compare the viscosities on the basis of the protein:water ratio coefficient (slope) alone as the actual log viscosity is also affected by the constant (intercept). However, the protein:water ratio coefficient (slope) indicates the effect of additional protein on viscosity increase. This is obviously much higher for sodium caseinate than for the other products.

The viscosity of proteins in solution is governed by the size and shape of the molecules, the association of the protein with the solvent and protein-protein interactions. Globular proteins, such as whey, are compact and therefore have only a small effect on viscosity (Rha, 1978, 1979). Most globular proteins are biologically active and their shape ensures their mobility is not hindered and the dynamics of the system are not greatly disturbed. Generally globular proteins bind approximately 50 g water per 100g of protein (Kinsella *et al.*, 1989). This is in contrast to casein micelles which bind larger amounts of water (2-4 g/g protein). Mechanical entrapment of water in the micellar matrix is partially responsible for this large water-holding capacity. The κ-casein 'hairs' that protrude from the surface of the micelles also contribute to the large amount of water associated with the casein micelle. The high viscosity of MPC85 and LHS compared to WPC is probably due to the presence of micelles and their associated high water binding capacity.

In contrast to WPC, sodium caseinates, possess an extended random conformation. The random conformation would result in a large viscous drag when the solution is sheared. Any distortion away from the random conformation, such as would occur due to viscous drag in a flowing liquid, gives a decrease in entropy and hence generates an elastic force (Hearle, 1982). Caseinates may therefore be expected to be more viscous than whey proteins or micellar systems.

To gain an understanding of the flow properties of MPC85 with concentration (protein:water ratio) rheological models were fitted and variation of the coefficients with concentration was studied. The LHS viscosity data was also modelled to provide a comparison. To this end, Power Law and Herschel Bulkley models were fitted to the decreasing shear sweep of each shear sweep data set in the manner detailed in section

2.2.2. As with earlier modelling no evidence was found for the presence of a yield stress and the flow curves were found to be adequately described by the Power Law model (all fits possessed correlation coefficients of >0.99). In line with the correlation observed between the logarithm of apparent viscosity and protein:water ratio the Power Law coefficients have been plotted in the form they appear when the Power Law model has undergone a logarithmic transformation i.e. log K and n.

The relationship between the logarithm of consistency index and protein:water ratio (Figure 3-12) appears to be similar for both MPC85 and LHS. As with the relationship between log apparent viscosity and protein:water ratio, the relationship seems to be linear. A regression of the consistency index on protein:water ratio, C, yielded the following equations for MPC85 and LHS respectively:

3-2 $$Log\ K = 12.41 \times C - 3.639 \qquad (R^2 = 0.96)$$

3-3 $$Log\ K = 9.65 \times C - 3.234 \qquad (R^2 = 0.99)$$

When the flow behaviour index is plotted against protein:water ratio (Figure 3-13), two distinct patterns of rheological behaviour can be observed. Up until a protein:water ratio of approximately 0.11, MPC85 solutions exhibit Newtonian behaviour. Increasing the protein:water ratio above this results in increasingly pseudoplastic behaviour. The concentration at which LHS deviates from Newtonian behaviour appears to be slightly higher than that exhibited by MPC85. This difference may be an indication of a slightly larger micelle size and hence increased water-binding due to physical entrapment within the micelle.

The cause of the change in rheological behaviour observed at a protein:water ratio of 0.11 is probably related to a change in the concentration regime. When considering the effect of concentration on the rheology of protein solutions, Hearle (1982) proposed that three regimes of behaviour may be envisaged: a) for very dilute solutions there is virtually no direct interaction between protein molecules; b) as the protein solution becomes more concentrated the protein molecules may still be considered as separate entities, but their interactions must be taken into account; c) and for very concentrated

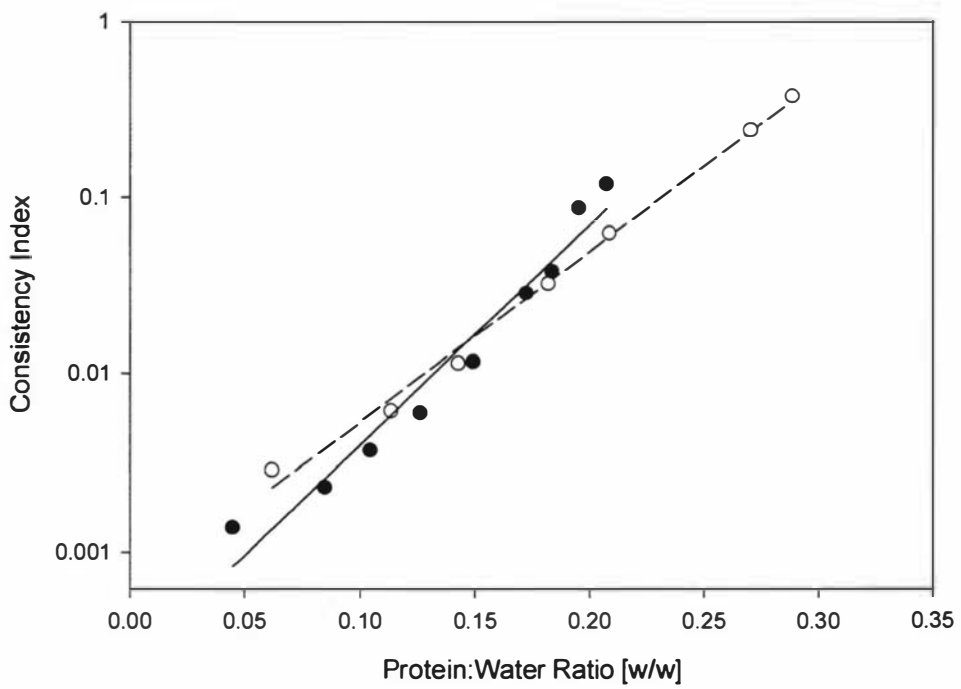

Figure 3-12 The relationship between consistency index (10 to 1000s^{-1}, 15°C) and protein:water ratio for MPC85 (observed data (●); regression line (—)) and LHS (observed data (○); regression line (---)).

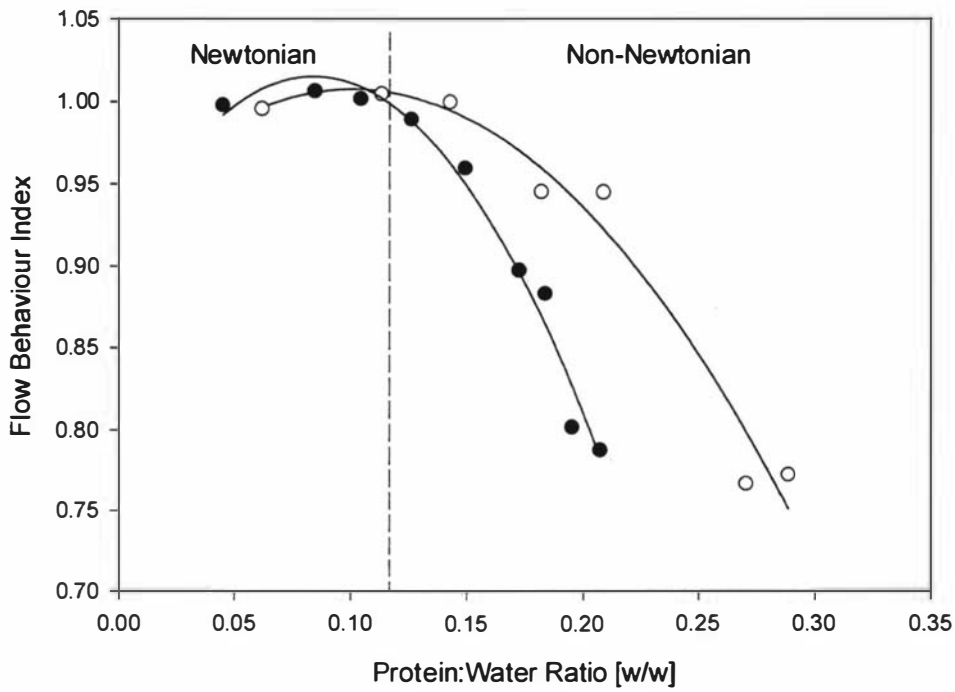

Figure 3-13 The relationship between protein:water ratio and flow behaviour index (10 to 1000s^{-1}, 15°C) for MPC85 (●) and LHS (○).

solutions the molecules are in direct contact, and flow would be dominated by the movement of molecules past one another. When undisturbed, dispersed molecules in solutions of concentration regimes b) and c) influence several layers of adjacent solvent molecules so forming a solvated layer. At very low shear rates there is little effect on the layered structure and interactions would be constant as the aggregates are of constant size. However, higher shear rates would progressively remove the solvated layers, giving a reduced aggregate size, and hence a lower apparent viscosity through the intermediate shear range. At some high shear rate the solvated layers would be completely removed, resulting in a constant apparent viscosity at very high shear rates (Tung, 1978). It is proposed that the change from Newtonian to progressively more pseudoplastic behaviour above a protein:water ratio of 0.11 is due to increased interaction between micelles resulting from a change in the concentration regime. Tang et al. (1993) reported that whey protein concentrate started to show signs of pseudoplastic behaviour at 15% total solids concentration. This is equivalent to a protein:water ratio of 0.14. The higher protein:water ratio at which WPC exhibits non-Newtonian behaviour, compared to LHS and MPC85 is probably a reflection of its lower water binding capacity.

One of the key aspects affecting the viscosity of milk concentrates is the hydration level of the protein. Korolczuk (1982a,b,c) asserts that one can calculate the hydration level of proteins by analysing the viscosity data of concentrated protein solutions (1.5 to 14 % w/w). Application of the equations of Korolczuk to the LHS and MPC85 viscosities, over the entire concentration range, resulted in calculated hydration levels of 1.75 and 3.07 g water /g protein respectively. This result is in line with the findings of Creamer and Yamashita (1976) and de la Fuente and Alais (1974) who showed that increasing the Ca concentration decreases hydration level. However, the equation of Korolczuk does not take into account changes in hydration with concentration. When the experimental data for MPC85 is analysed in two concentration ranges the calculated hydration level is quite different: 4.48g/g for 4 to 12 % w/w; and 2.50 g/g for 12 to 17 % w/w. The 'hydration level' was also found to be dependent on the shear rate at which it was calculated. Due to the large variation in the hydration levels one cannot be confident that these hydration values, while in the correct order of magnitude, are

correct. All other equations for viscometrically determining hydration levels are only valid for very dilute solutions.

3.7 Effect of temperature and MPC85 concentration on viscosity

To fully characterise the rheological properties of a protein solution it is necessary to understand how the rheological properties of the solution are affected by variation in temperature as well as concentration. In this study the effects of temperature, 15 to 65°C in steps of 10°C, on the rheological properties of reconstituted MPC85 solutions with concentrations of 4.47, 8.37, 12.75 and 18.30 % (w/w) protein were examined. The solutions were prepared by the method described in section 3.1.3 and were homogenised at 200 bar. A fresh solution was prepared for each temperature and concentration combination. The rheological properties of each solution were measured using an upwards shear sweep followed by a downwards shear sweep on the Bohlin VOR Rheometer as described in section 3.1.5.2 . Due to the limitations imposed by the torsion bar not all of the solutions were able to be measured over the shear rate range of 1 to 116s^{-1}. The shear rate range that was common to all of the solutions was 1 to 18s^{-1}. To enable valid comparisons to be made all of the data presented here is from this shear rate range.

The raw data for these experiments was very similar and is typified by the data collected for the 18.30 % protein solutions (Figure 3-14). The features common to all flow curves of the same concentration were that: the decrease in the logarithm of apparent viscosity with shear rate increased with temperature; the apparent viscosity at shear rates >5s^{-1} decreased with increasing temperature; and there was no hysteresis present in any of the flow curves. The relative change in the logarithm of apparent viscosity with temperature decreased with decreasing concentration.

Figure 3-15 summarises the overall change in apparent viscosity, at 18s^{-1}, with temperature and protein concentration. This figure clearly shows that apparent viscosity increases with decreasing temperature and/or increasing protein concentration. It is also clear that effect of temperature on apparent viscosity increases with protein concentration.

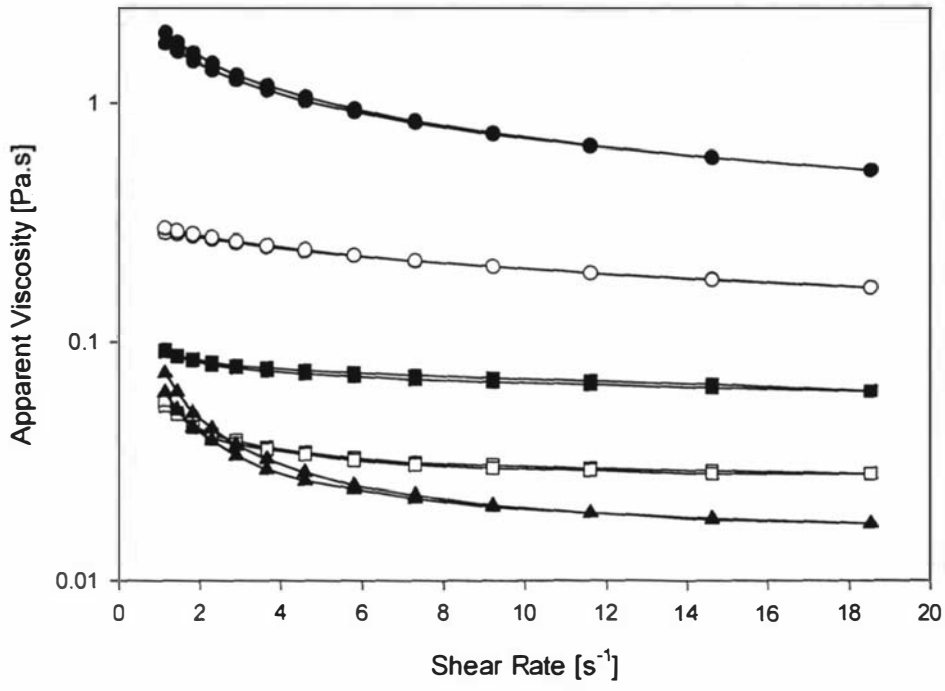

Figure 3-14 The flow curves of reconstituted MPC85, 18.3% protein concentration, at 15°C (●), 25°C (○), 35°C (■), 45°C (□), and 55°C (▲).

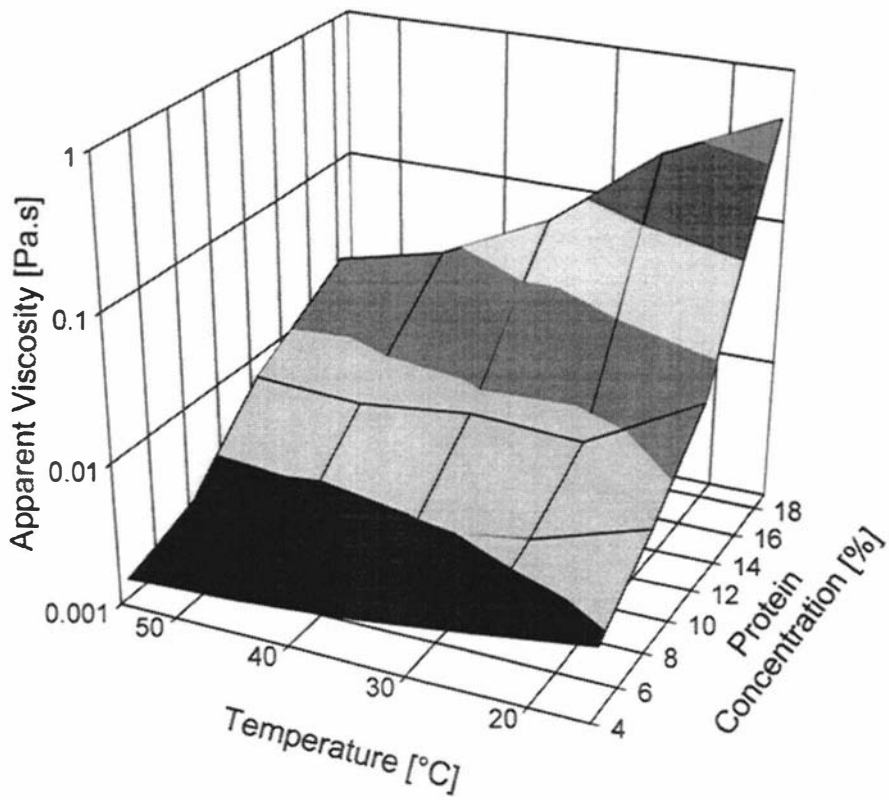

Figure 3-15 Apparent viscosity at 18.30 s^{-1} of reconstituted MPC85 as a function of protein concentration and temperature.

To quantify these trends the flow curves were modelled with both the Power Law and Herschel Bulkley rheological models. The rheological models were fitted to each flow curve by the method described in section 2.2.2 and the results are shown in Table 3-5. The modelling analysis showed evidence of the presence of a yield stress term, especially at higher temperatures. To determine whether the detected yield stress significantly reduced the sum of the square of the residuals a partial F test was performed. The partial F-test analysis showed that the yield stress was significant at a 95% level in all temperature/concentration combinations except at 18.30 % w/w, < 55°C and at 12.40% w/w, < 35°C. The detection of a yield stress in this work at low concentrations at 15°C seems to contradict the findings presented in section 3.6. However, a comparison with those results is not valid as the shear rate range over which the rheological models were fitted in section 3.6 differs from the range used here; 10 to 1000s^{-1} compared to 1 to 18.3s^{-1} respectively. The presence of a yield stress is an important flow characteristic in food materials as it imparts some sensory attributes (Ramana and Ramanathan, 1992). Table 3-5 shows that the yield stresses are all very low.

Table 3-5 Coefficients of Power Law and Herschel Bulkley rheological models

Solution Parameters		Power Law Model			Herschel Bulkley Model				Partial F-Test	
Protein Concentration	Temperature	K [Pa.s^n]	n	R^2	yield [Pa]	K [Pa.s^n]	n	R^2	Q ratio	F value
18.30	15	2.204	0.51	0.999	0.000	2.204	0.51	0.999	0.00	4.84
18.30	25	0.601	0.68	0.999	0.000	0.601	0.68	0.999	0.00	4.84
18.30	35	0.090	0.87	1.000	0.000	0.090	0.87	1.000	0.00	4.84
18.30	45	0.045	0.83	0.997	0.000	0.045	0.83	0.997	0.00	4.84
18.30	55	0.060	0.56	0.974	0.070	0.013	1.03	1.000	1617	4.84
12.40	15	0.022	0.95	1.000	0.002	0.021	0.96	1.000	1.49	4.84
12.40	25	0.004	1.16	0.995	0.000	0.004	1.16	0.995	0.00	4.84
12.40	35	0.029	0.53	0.966	0.035	0.005	1.06	0.999	279	4.84
12.40	45	0.036	0.38	0.939	0.042	0.003	1.07	0.994	88.8	4.84
12.40	55	0.064	0.20	0.843	0.070	0.002	1.10	0.992	198	4.84
8.37	15	0.026	0.51	0.973	0.030	0.005	0.98	1.000	694	4.84
8.37	25	0.006	0.81	0.994	0.006	0.003	1.02	0.999	81.3	4.84
8.37	35	0.007	0.70	0.990	0.007	0.003	0.98	0.999	156	4.84
8.37	45	0.007	0.59	0.975	0.008	0.002	1.03	0.998	90.8	4.84
8.37	55	0.009	0.46	0.952	0.011	0.001	1.00	0.996	124	4.84
4.47	15	0.005	0.82	0.991	0.005	0.002	1.07	0.998	28.2	4.84
4.47	25	0.005	0.73	0.980	0.006	0.001	1.13	0.999	143	4.84
4.47	35	0.005	0.59	0.974	0.006	0.001	1.01	0.995	43.0	4.84
4.47	45	0.006	0.49	0.947	0.007	0.001	1.00	0.995	87.9	4.84
4.47	55	0.007	0.41	0.949	0.008	0.001	1.03	0.992	52.3	4.84

The variation of the yield stress over all the temperature/concentration combinations studied is shown in Figure 3-16. The relative magnitude of the yield stress is plotted as the percentage contribution the yield stress term makes to the overall apparent viscosity at a shear rate of $18s^{-1}$. The main trend that is apparent in this figure is that the relative magnitude of the yield stress term increases with increases in temperature at a particular concentration. From these data it is difficult to distinguish a relationship between concentration and yield stress other than it appears that as the concentration increases the temperature required to detect a yield stress increases. In section 2.2.2 it was found that MPC85 concentrate ex-evaporator (24.8 ± 0.3 % w/w total solids, 52°C) did not appear to possess a yield stress.

The variation of the flow behaviour index with temperature and concentration, illustrated in Figure 3-17, shows that the solutions in which a yield stress were detected are essentially Newtonian with a mean flow behaviour index of 1.03 ± 0.02 (95% C.I.). The presence of a yield stress and a flow behaviour index of 1 is indicative of a Bingham Plastic.

The solutions with no detectable yield stress (20% w/w, < 55°C and at 15% w/w, < 35°C) are pseudoplastic with the degree of shear thinning increasing with increases in concentration and decreases in temperature.

The variation in the consistency index with temperature and concentration illustrated in Figure 3-18 shows a similar pattern to Figure 3-15 for apparent viscosity i.e. the logarithm of the consistency index increases with increases in concentration and decreases in temperature.

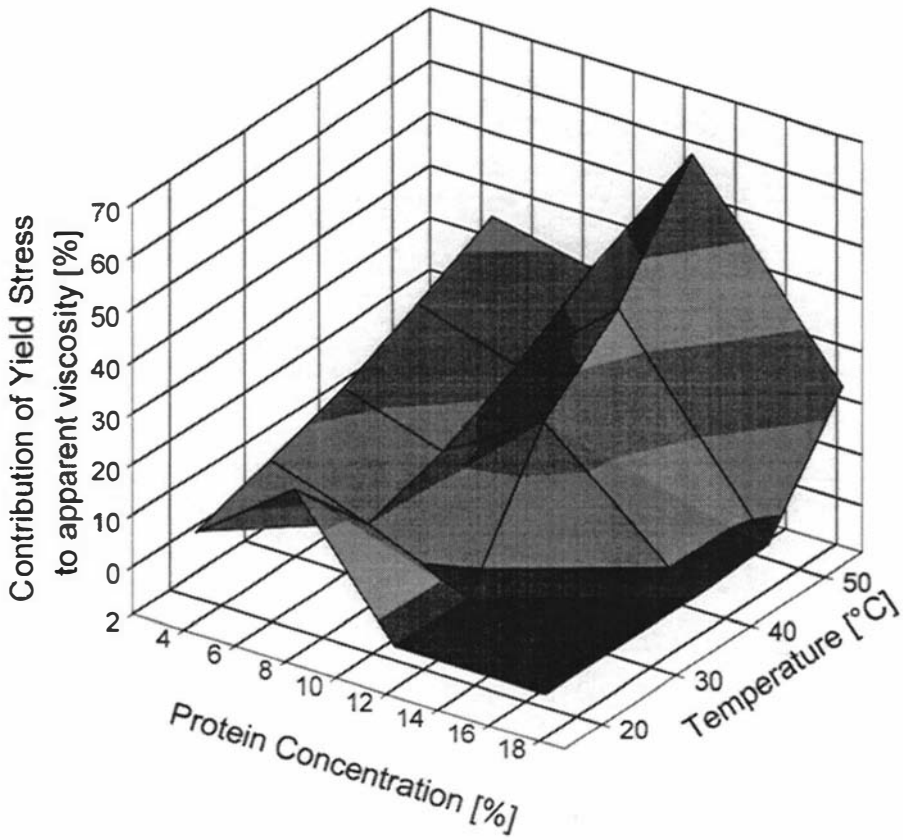

Figure 3-16 Relative importance of the yield stress shown as the percentage contributed by the yield stress to apparent viscosity at a shear rate of $18s^{-1}$ for MPC85 solutions at various temperature/concentration combinations.

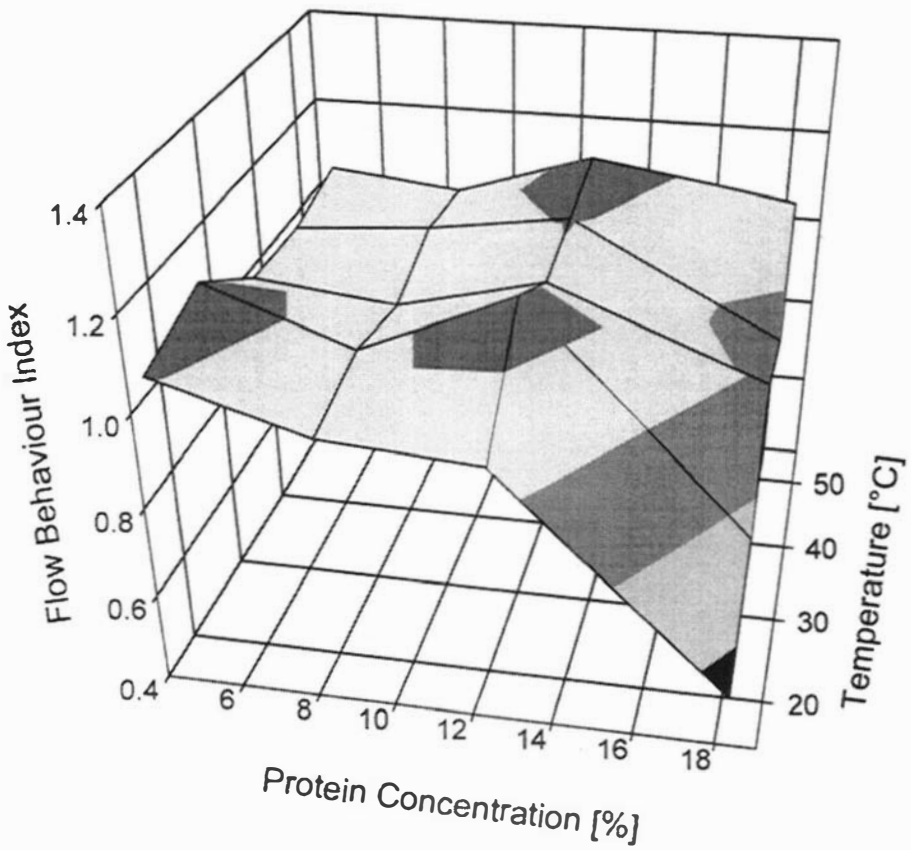

Figure 3-17 The flow behaviour index as a function of temperature and protein concentration.

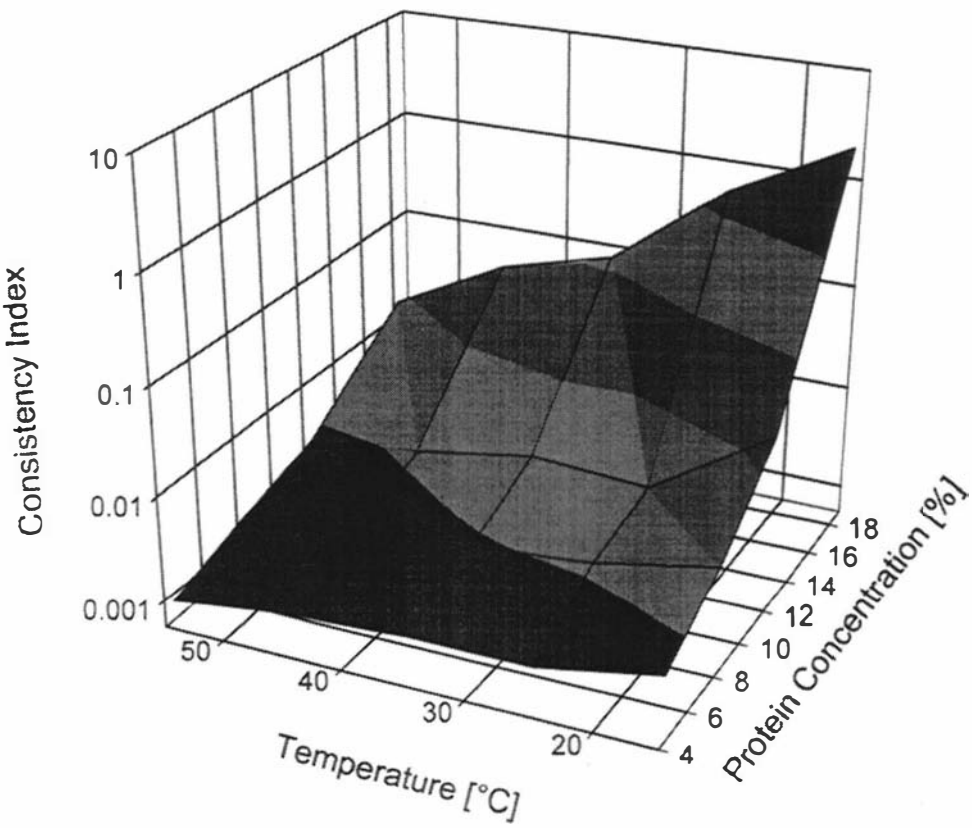

Figure 3-18 The consistency index as a function of temperature and protein concentration.

To quantify the trends observed with the effect of temperature on apparent viscosity at each concentration, the data was modelled with the Anrade equation (Anrade, 1930a): equation 3-4.

3-4 $$\eta_{app} = \beta e^{c/T}$$

where η_{app} = apparent viscosity (Pas), T = absolute temperature (K), β is a constant, and c = E/R where E = activation energy and R = gas constant.

This equation has been successfully applied to sodium caseinate (Towler, 1974; Fichtali *et al*, 1993) and rennet-tripolyphosphate caseinate (Towler, 1974) solutions. The Anrade equation was used here to model the variations in apparent viscosity at $18.30s^{-1}$ with temperature and concentration of MPC85 solutions. The Anrade equation was found to predict viscosity changes reasonably well as shown by the correlation coefficients, refer Table 3-6 and illustrated in Figure 3-19. The correlation coefficient for 12.40 % protein concentration is quite low although the general trend of the data (Figure 3-19) is in keeping with the data for the other concentrations. The apparent lack of fit at this concentration may be a result of the change in flow behaviour. At a protein concentration 12.40 % this solution is at a transitional concentration from solutions of lower concentration possessing a yield stress at all temperatures compared to the solution with 18.30% protein concentration which had a yield stress at only the highest measured temperature.

Table 3-6 Values of constants β [Pa.s] and c [dimensionless] in equation 3-4 relating apparent viscosity at $18.30s^{-1}$, pH 7.0 and temperature for reconstituted MPC85 solutions at different protein concentrations.

Protein Concentration	Ln β	c	Correlation coefficient
18.30	-30.33	8561	0.9805
12.40	-11.55	2085	0.5903
8.37	-14.66	2743	0.9304
4.47	-11.89	1722	0.9767

The Anrade coefficients, (Figure 3-20), remain almost constant at protein concentrations up to 12.40 % after which the C coefficient increases dramatically while the β coefficient decreases dramatically. Coefficient C is related to the activation energy for the flow process to occur therefore one would expect C to increase with concentration. At higher concentrations collisions between molecules caused by shear, and their subsequent temporary union during which the molecules acquire a common velocity of translation, would be more frequent (Anrade, 1930b). The interpretation of activation energies for the flow of liquids is difficult and there has been much discussion over whether it is a real physical property (Holdsworth, 1971). When a liquid is heated at constant pressure, two basic factors combine to reduce its viscosity. The first is that the thermal energy of the molecules increases and the second is that the intermolecular distances increase by the process of thermal expansion. In general, the higher the activation energy the greater the effect of temperature on the viscosity (Holdsworth, 1971). Horne (1998) suggested that as temperature is increased, the strength of the hydrophobic interactions increases causing the micelles to tighten up and become more compact allowing the suspension to flow more freely and so contributing to the decrease in apparent viscosity.

The protein concentration at which the Anrade coefficients increase/decrease dramatically is slightly higher, at 12.40% (0.14 P:W ratio), than that observed for the change in the flow behaviour index for the MPC85 solutions measured at 15°C reported in section 3.6 (0.11 P:W ratio). The difference observed in the protein concentration which marks a change in rheological properties is probably due to the lack of data points available to accurately define the transition in the temperature/concentration investigation.

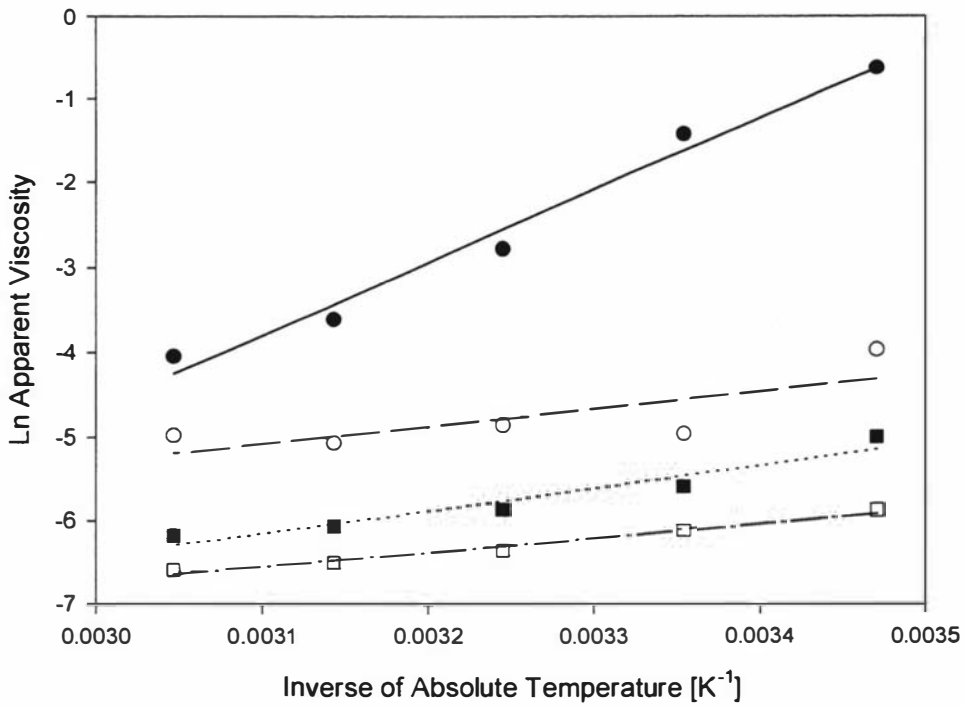

Figure 3-19 The natural logarithm of apparent viscosity of MPC85 solutions as a function of the inverse of absolute temperature at protein concentrations of 18.30 %, (●); 12.40 %, (○); 8.37 %, (■); and 4.47 %; (□) and as predicted by the Anrade Equation at 18.30 %, (—); 12.40 %, (– – –); 8.37 %, (···); and 4.47 %; (– •–).

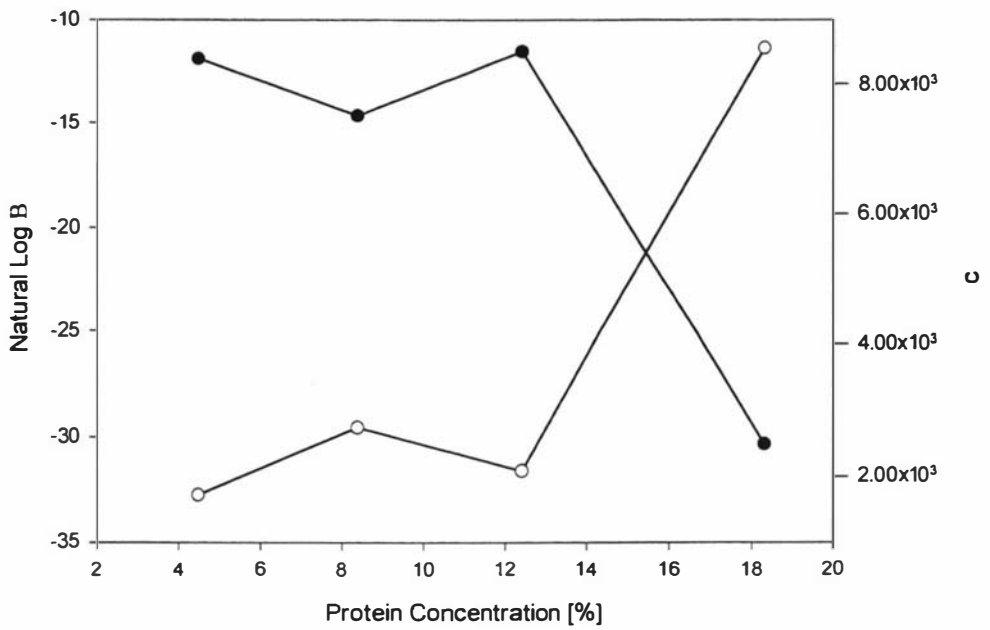

Figure 3-20 The variation of Anrade coefficients, B (●) and c (○), determined at 18.30 s^{-1} over the temperature range of 15 to 55°C, with protein concentration.

3.8 Conclusions

The rheological properties of reconstituted commercial MPC85 have been characterised. The rheological properties may be broadly classified into two protein concentration regimes: less than or greater than a protein to water ratio of about 0.11. At low concentrations MPC85 solutions may be described by a Bingham Plastic equation as their viscosity does not vary significantly with shear rate and a yield stress is detected. Overall the yield stress was found to increase with temperature and concentration. At high concentrations the logarithm of apparent viscosity was found to increase linearly with increases in protein concentration. The solutions were found to be adequately described by the Bingham Plastic model with a yield stress being detected at higher temperatures together with a constant apparent viscosity. The yield stresses were however low and only observed at low shear rates i.e. < 18.30 s^{-1}. At lower temperatures MPC85 solutions were pseudoplastic and adequately described by the Power Law model. The degree of pseudoplasticity was found to increase with higher concentrations and lower temperatures. The zone of transition between the two concentration regimes was found to be roughly the same as that for Low Heat Skim Milk powder i.e. about 0.11 P:W ratio.

The solubility of commercial MPC85 in water was found to be dependent on the temperature at which the solution was prepared, increasing from 58.85% at 20°C to 100% at 50°C. The solubility of MPC85 was found to be more sensitive to the preparation temperature at lower temperatures. The solubility of MPC85 solutions prepared at 20°C was found to increase from 58.85% to 88.75% after homogenisation at 150 bar. The rheological properties of MPC85 were profoundly influenced by the degree of solubility. The flow behaviour index (20 % total solids; 15 °C), decreased from 0.74 to 0.45 as the solubility decreased from 100% to 58%. Likewise the consistency index underwent an eight-fold increase as the solubility decreased.

The rheology of concentrated MPC85 solutions was found to be affected by cold storage (5°C, over four days) but not by holding at high temperatures (52°C, over an hour). The

apparent viscosity and degree of pseudoplasticity of MPC85 increased with storage at 5°C, however this effect was reversed by heating the solution to 50°C for half an hour.

Overall MPC85 is similar to low heat skim in that its rheological properties may be divided into two zones dependent on the protein to water ratio, the transition zone for each being about 0.11 P:W ratio. However, MPC85 differs from skim milk powder in that it does not exhibit time dependent thickening behaviour at high temperatures.

3.9 Impact of conclusions on the focus of the thesis

The conceptual focus of this thesis was to study the rheological properties of some of the newer milk protein products such as total milk protein, micellar casein and in particular milk protein concentrates. After reviewing the literature the conclusion was drawn, based largely on parallels with skim milk powder production (i.e. skimming of milk, evaporation, and spray drying) and composition (i.e. milk proteins in native form and same ratio of whey to casein), that milk protein concentrate would behave in a manner similar to skim milk concentrate and skim milk powder. One of the main problems highlighted by the literature review was the adverse affect of time-dependent thickening during the production and handling of skim milk concentrate. Therefore the initial thrust of this research centred on the rheology of the milk protein concentrate during production and of the reconstituted powder, with a particular focus on determining the degree of time-dependent thickening of MPC85 concentrate ex-evaporator and of the reconstituted powder. However the investigations into the rheological properties of MPC85 during production and of the subsequent powder did not reveal any evidence that MPC85 possessed time-dependent thickening properties. Indeed, the evidence suggested that the MPC85 concentrate ex-evaporator became less viscous with holding at evaporator temperatures. Despite the absence of age-thickening, MPC85 was found to possess similarities in rheological behaviour to skim milk: the presence of two protein concentration regimes characterised by different rheological properties; both exhibit complex flow behaviour at low concentration and high temperature with the presence of yield stresses observed at low shear rates. The distinct differences in rheological behaviour between MPC85 and skim milk lead to the question: what is the cause of the rheological differences? The answer to this question may lie with the difference in heat

treatment during processing and/or with compositional differences. An investigation into these two areas would, in addition to elucidating the reasons behind observed rheological differences, assist with the third objective outlined in section 1.8, that of applying the acquired rheological knowledge to improving product functionality. To further contribute to this third objective the opportunity was taken to broaden the functional properties under investigation to include heat stability and rennetability. It should be noted here that knowledge of rennetability and heat stability will be advantageous as two of the main end-uses of MPC85 are in cheese milk extension and enteral formulations requiring high temperature sterilisation.

The remaining sections of this thesis therefore explore the possible reasons for the differences observed between skim milk and MPC85 solutions. Chapters 4 and 5 examine the effect on functional properties of: heat treatment during processing; and the compositional differences between skim milk and MPC85 respectively. The last experimental chapter attempts to investigate the effects on functionality of the compositional differences between MPC85 and "simpler" dairy protein products such as sodium caseinate.

4. Effect of heating during pilot-scale manufacture on the functional properties of milk protein concentrate

As discussed in section 1.6.1 the functionality of a milk powder can be significantly modified by preheat treatments prior to concentration. In particular it is known that high heat treatments can result in an increase in the rate of age thickening at evaporator temperatures. The purpose of this section of work was to elucidate the effect of heat treatment during processing on the functional properties of reconstituted MPC85 powder. To achieve this objective it was decided to manufacture in a pilot plant MPC85 powders with different preheat treatments and then evaluate the functional properties of each powder with respect to rheology, heat stability and rennet coagulation properties.

4.1 Manufacture of pilot plant powders

Approximately 30 kg of commercial, "Tararua" brand, pasteurised non-homogenised whole milk was obtained from a local supermarket. The whole milk was separated at 45°C using a disc centrifuge. Following separation the skim milk (≈45°C) was subjected to a preheat treatment using a Spiraflo UHT plant (Alfa Laval, Hamilton, New Zealand) equipped with regenerative cooling and further water cooling which enabled an exit temperature of ≈ 25°C to be achieved. The preheat treatments applied to the skim milk were: no heat treatment (control), 72°C /30s, 80°C/30s, 90°C/30s, 100°C/30s, 110°C/30s, 120°C/45s, and 130°C/30s. The powder with the preheat treatment of 120°C/45s was the first powder made. The residence time of the skim milk, for this powder, in the UHT unit was initially calibrated to be 30s, but on rechecking the calculations was found to actually be 45s. The order in which the pilot plant powders were manufactured was randomised.

The heat treated skim milk was concentrated by ultrafiltration (UF) to a volume concentration ratio (VCR) of 5. The concentrated milk was diluted back to its original volume with de-ionised water, 20-25°C, to commence diafiltration (DF). The water diluted concentrate was ultrafiltered again to a VCR of 5. Diafiltration was conducted two more times. The VCR after the final DF was 5. The UF and DF operations took

approximately 10 hours in total. The concentrated DF milk was stored overnight at 5°C prior to spray drying. The UF operating parameters were: 20-25°C, 90 kPa pressure drop over filter, 90 to 130 ml/min flux. The ultrafiltration system was a Proto-Sep III Evaluator System (Koch Membrane Systems Inc., Wilmington, M.A., USA.) fitted with an HFK-131 Koch membrane with an area of 0.28 m^2.

After overnight storage at 5°C, the concentrated DF milk was warmed to 40°C in a water bath. The warm diafiltered milk was spray dried in an Anhydro Laboratory Spray Dryer No. 1 (Anhydro, Copenhagen, Denmark) fitted with a two fluid nozzle atomiser. The concentrate was pumped into the nozzle and dispersed into a mist by means of compressed air. The inlet air temperature was set to 190 to 200°C, and the feed rate was adjusted to attain an outlet temperature of 85 to 95°C. Powder collected from the spray drier was placed in a sealed plastic container and stored at 5°C. During drying the nozzle occasionally became blocked and was cleared by hand through the side hatch to the drier. Clearing the blockage on the nozzle took about 5-10s from time of opening the hatch till closing it. However, during this time the drier would lose pressure and cool slightly.

The pilot plant runs were conducted only once. However, because the runs were conducted in random order and there were runs under 8 systematically varying conditions, the trends observed in the powder properties are considered to be statistically significant.

4.2 Composition of pilot plant powders

4.2.1 Protein analysis

4.2.1.1 Total protein

The total protein of the pilot plant powders was determined in triplicate using the method described in section 2.1.2. All replicates agreed to ± 0.01 %. The total protein for each of these powders is shown in Table 4-1. There does not appear to be any

distinct trend between the preheat treatments and the total protein (dry or wet weight). The average total protein (dry weight) of the powders is 88.25 ± 1.03 %.

Table 4-1 The average total protein of the pilot plant powders

Sample	Total Protein (wet weight) [%]	Total Protein (dry weight) [%]
Control	83.44	86.43
72°C/30s	86.14	90.23
80°C/30s	85.55	89.12
90°C/30s	85.30	89.81
100°C/30s	84.24	87.09
110°C/30s	83.31	87.07
120°C/45s	83.48	86.90
130°C/30s	84.41	89.25

4.2.1.2 Whey protein denaturation

10 ml samples of reconstituted MPC85 (3.5% protein concentration) were diluted with 40 ml distilled water. Then 25 ml of these diluted solutions were adjusted to pH 4.6 by drop-wise addition of 0.1 N HCl and filtered. From each filtrate, 10 ml was used to determine the whey protein nitrogen (WPN) content by semi-micro Kjeldahl. The percentage of whey protein denaturation in each sample was calculated from equation 4-1.

4-1 $$\text{Denaturation}\,[\%] = \frac{(\text{WPN}_{\text{control}} - \text{WPN}_{\text{heated}})}{\text{WPN}_{\text{control}}} \times 100\,\%$$

Where the $WPN_{control}$ was taken as the control pilot plant powder, and the WPN_{heated} as the particular MPC85 powder under study. Thus the percentage of whey protein denaturation [WDN] relative to the control (sample which received no heat treatment) was determined. All samples were measured in triplicate and agreed to ± 0.05%.

The average WDN results are shown in Table 4-2. As expected the degree of whey protein denaturation increases with the severity of the preheat treatment. It should be noted that the control sample, because it has been produced from pasteurised milk, will

contain a low level of denatured whey protein. Hence the relative whey protein denaturation levels reported for these powders are slightly lower than their actual levels. At the pasteurisation temperature/time combination used in New Zealand (72°C for 15 s) denaturation is slight (≈ 6%) (Singh and Creamer, 1991).

Table 4-2 Average whey protein denaturation of MPC85 powders [%]

Sample	Whey Protein Denaturation [%]
Control	0.00
72°C/30s	1.30
80°C/30s	48.85
90°C/30s	71.52
100°C/30s	86.18
110°C/30s	90.42
120°C/45s	90.99
130°C/30s	92.30
Commercial	13.89

4.2.2 Elemental analysis

The elemental composition of all the MPC85 powders was determined using plasma emission spectrometry conducted by the ICP Facility at Grasslands Research Centre, Palmerston North. The full mineral composition is shown in Appendix B. The eight pilot plant powders plus the commercial powder samples were randomly coded. In addition to these samples, five extra samples of the pilot plant powders were sent for analysis with their own random codes as a check on the reproducibility of the analysis.

The Ca, K, Na, P and Mg mineral content (mmol/g powder) of the MPC85 powders are shown as a function of WDN (%) in Figures 4-1 to 4-5 respectively. The WDN axis is plotted with a category scale rather than a linear scale to enable the data from the high heat powders to be easily identified. From initial inspection the mineral content for Ca, K, and P appears to be independent of WDN and hence preheat treatment. However, for Na and Mg there appear to be two distinct populations: 0 to 86% WDN and 90 to 93% WDN. The Na and Mg contents seem to be independent of WDN within these two populations but not between them with the more severely heat treated population containing a lower Na content and a higher Mg content than the population of powders

with WDN values of ≤ 86 %. In order to confirm the existence of these two apparent populations the mean mineral contents for each element within the low heat and high heat powder populations were compared using a statistical test for comparing means of normal distributions with unknown variance (Appendix A).

The difference between the mean mineral content of the 'two' populations was only found to be significant, at a 95% level, for Na and Mg: i.e. the mineral content of MPC85 powders with respect to Al, As, B, Ca, Cd, Co, Cu, Fe, K, Mn, Mo, Ni, P, Pb, S, Se, Si, Sn, Sr, Zn was independent of preheat treatments prior to ultrafiltration. It should be noted though that many of the minor elements (As, Cd, Co, Mo, Ni, Pb, Se, Si and Sn) were out of range of the plasma emission spectrometer.

The statistically confirmed difference between mineral content, with respect to Na and Mg, of the low and high heat MPC85 powders has a further interesting feature: the absolute change in Na and Mg between the "low" and "high" heat powders is -0.026 and +0.011 mmol/g respectively. A statistical comparison of these absolute differences shows that the ratio of Na loss to Mg gain is 2:1 at a 95% level.

The absence of any variation of calcium and phosphate with preheat treatments is an unexpected result. It was thought that calcium and phosphate levels would increase with preheat treatments. Heating milk is thought to lower the amount of soluble and ionic calcium and phosphate concentrations by converting them to the colloidal state and precipitating on the outside of the casein micelle (Evenhuis and de Vries, 1956; Rose and Tessier, 1959; Davies and White, 1959; Kudo, 1980). The heat precipitated calcium phosphate does not sediment due to its association with casein micelles. Therefore one would expect that milk that had been heated would not lose calcium and phosphate in the permeate during ultrafiltration and diafiltration to the same extent as milk which had not been heated. There are several possible explanations as to why no increase was observed in calcium or phosphate levels in the MPC85 powders made with increasing preheat treatments.

Firstly there is doubt as to whether the heat precipitated calcium and phosphate actually associate with the micelle. Dalgleish *et al.*, (1987) found that the cation (Ca and Mg)

and total phosphate (organic and inorganic) contents of the sedimentible material (60,500g) after heating skim milk for various periods of time at 130°C remained constant. The constancy was found to be unaffected be either the original pH of the milk or the heating time. Dalgleish *et al.*, (1990) did however observe a decrease in the cation content of the supernatant which they ascribed to precipitation of minerals not onto the micelles but on to the walls of the heating vessel. The inorganic phosphorus content increased in the supernatant which was thought to be due to the dephosphorylation of the caseins. Dalgleish *et al.*, (1990) thought that a possible reason why they found that the cations deposited onto the walls of the heating vessel rather than casein micelles as suggested by other workers might be due to differences in the material of the vessel containing the milk during heating. They used a stainless steel vessel whereas in most of the other investigations glass tubes were used. It is probable that the heating of milk in glass containers, where deposition sites are much fewer, can cause the deposition of calcium phosphate on the micelles rather than on the walls of the heating system (Dalgleish, 1989). It is important to note that in the manufacture of the pilot plant MPC85 powders in this work heat was applied through stainless steel piping.

The second reason for the lack of variation in the calcium content of the pilot plant powders with preheat treatments may be related to the reversibility of the calcium and phosphate precipitation. Several workers have reported (Demott, 1968; Muldoon and Liska, 1969; Muldoon and Liska, 1972; Geerts *et al.*, 1983) that the Ca^{2+} activity of milk decreases strongly upon heating, the effect being more pronounced when longer times and higher temperatures are used. Further they all observed that on cooling a relaxation occurs over a period of two to three days, but that the starting value is not regained. Geerts *et al.*, (1983) reported that the recovery of Ca^{2+} activity at 20°C was linear with the logarithm of time after heat treatment for a period up to 24 hours. At that time 88% of the initial value had been regained. Measurements after 50 hours did not show any further recovery in activity. In the manufacturing of the pilot plant powders the heat treated skim milk at 20°C was ultrafiltered and diafiltered over a period of about 10 hours. From the work of Geerts *et al.*, (1983) one would expect that only some of the calcium and phosphate would have recovered and hence be lost from the system in the permeate. The remainder of the heat precipitated minerals would stay with the

concentrate and therefore be present in the final powder. The recovery of calcium and phosphate predicted by Geerts *et al.*, (1983) does not therefore account for the apparently complete recovery of calcium and phosphate. The reaction converting dissolved and ionic calcium and phosphate to the colloidal state is approximately:

4-2 $$3\,Ca^{2+} + 2\,HPO_4^{2-} \underset{cooling}{\overset{heating}{\rightleftharpoons}} Ca_3(PO_4)_2 \downarrow + 2\,H^+$$

When the milk is cooled, it becomes unsaturated with regard to calcium and phosphate and so the calcium phosphate complex dissolves. In the manufacture of the pilot plant powders, the Ca^{2+} and HPO_4^{2-} is removed from the system in the permeate, thus increasing the driving force for the dissolution of the calcium phosphate. The increase in the driving force may account for the relative speed of the dissolution reaction reaching equilibrium in the pilot plant (10 hours) compared to that reported in the literature (up to three days). It is possible that due to the increased driving force for dissolution created by ultrafiltration and diafiltration the equilibrium of the reaction is such that all of the heat precipitated calcium phosphate is dissolved. It is also likely that a portion of the native colloidal calcium phosphate may be dissolving from the casein micelles.

The preceding discussion does not account for the increase in magnesium and decrease in sodium levels observed for the three powders given the most severe preheat treatments. Indeed the experiments of Dalgleish *et al.*, (1987) showed that the total cation content as chelated by EDTA (i.e. Ca and Mg) remained constant irrespective of the degree of heat treatment. The implication from this experiment is that the magnesium content should be constant as well as the calcium content. However, Dalgleish *et al.*, (1987) determined the total cation content (Ca and Mg) by titration with EDTA, using a Ca electrode to define the end point. The rationale behind this method was that since EDTA complexes both Ca^{2+} and Mg^{2+}, the resultant titration produces the sum of both species. Dalgleish *et al.*, (1987) do not give any details of the accuracy of using a calcium ion electrode for measuring the content of both Ca^{2+} and Mg^{2+} concentration. Other workers, however, have commented on the usage of ion selective electrodes to determine Ca^{2+} concentrations in milk products. A study by Holt *et al.*, (1981) into the estimation of calcium ion concentration in milk diffusate by a selective

ion electrode found that no significant error resulted from varying magnesium within the normal range of milk diffusates. Geerts *et al.*, (1983) when establishing the cumulative interference of cations in milk on the measuring of Ca^{2+} concentration by ion electrode, replaced interfering ions with $MgCl_2$ to an identical ionic strength. $MgCl_2$ was chosen for its very low selectivity coefficient. In light of the accuracy presented by these workers it seems questionable that Dalgleish *et al.*, (1987) could accurately detect the changes in the Ca ion electrode readings contributed by Mg^{2+}. Evidence from research on fouling in heat exchangers suggests that calcium is preferentially adsorbed on to stainless steel. Foster and Green (1990) concluded that calcium phosphates appear to be deposited earlier than magnesium phosphates. This was expected from the very low solubility products of some forms of calcium phosphate (Lyster, 1981) and the tendency for calcium phosphates to have higher association constants than the corresponding magnesium salts (Holt *et al.*, 1981). Jeurnink and de Kruif (1995) found that the ash content of a fouling deposit in milk following pasteurisation consisted largely (90%) of calcium and phosphate with only minor contributions by magnesium and citrate. A decrease in the ratio of magnesium to calcium of 61% has been observed between the composition of skim milk and the deposited fouling layer (calculated from the data, p118, of Jeurnink, 1996). If the ratio of magnesium to calcium in skim milk is higher than the ratio measured in the fouling layer deposits then magnesium and calcium can not be precipitating on to metal surfaces at the same rate.

From the literature it seems that calcium and phosphate may precipitate preferentially onto metal surfaces rather than casein micelles. The affinity of calcium for phosphate is much greater than that of magnesium. Therefore one would expect that on heating the calcium would form a complex with phosphate and precipitate onto the walls of the heating vessel, thereby leaving a proportionately higher concentration of magnesium in the skim milk. It should be noted that this increase in magnesium concentration in the skim milk may not be observed in experiments conducted using glass. Further the relative increase in magnesium concentration is difficult to assess: the factors influencing this phenomena are not fully understood although precipitation of calcium onto stainless steel is thought to be partly increased by the temperature difference between the heating surface and the bulk milk temperature (Jeurnink, 1996).

The evidence of the higher magnesium content in the higher heat treated powders together with the evidence from the literature of a relatively higher magnesium concentration in the milk serum coupled with the deposition of calcium salts on the heating surface indicates that heating, in stainless steel piping, at temperatures of 110°C or higher might increase the driving force for the favourability of magnesium - protein interactions over calcium - protein interactions. It is interesting to note that the temperature of 110°C coincides with the temperature at which apparent disaggregation of casein with the formation of soluble (non-sedimentable) casein occurs (Singh and Fox, 1989) . It would also seem that the disaggregation of casein and/or the apparent magnesium-protein interactions may be coupled with a release of previously trapped sodium ions. The released sodium would be lost in the permeate in the subsequent UF and DF stages.

Pouliot *et al.*, (1989) observed that the amount of Mg solubilised on the cooling of heated milk was very low and seemed to be independent of temperature. This was in contrast to their observation that increasing amounts of Ca and P were transferred to the soluble phase as cooling temperatures were lowered. These observations support the argument that magnesium-protein interactions are irreversible in contrast to calcium and phosphate protein interactions.

Given the evidence that the ratio of sodium ions lost to magnesium ions gained is two, it would seem that the magnesium may be displacing the sodium ions. This ratio was reported by Clarke *et al.*, (1989), for Na loss to Ca gain, in an electrochemical study of calcium ion binding to sodium caseinate (15% w/w). Upon the addition of calcium chloride Clarke *et al.* (1989) found that a stage of ion exchange occurred (two sodium ions for one calcium ion) followed by more extensive calcium binding past the stage of complete desorption of the sodium ions. It is likely that Mg ions would behave in a similar manner to Ca ions. In a general review on the interactions of ions with proteins Barrow (1983) reported that alkaline metals (such as Na and K ions) react to only a limited extent with proteins, whereas alkaline earths, such as Ca and Mg are somewhat more reactive.

From the mineral analysis it is not possible to state where or how the Mg ion binding is occurring. The caseins, α_{s1}-, α_{s2}-, and β-caseins, bind divalent ions due to possessing a high concentration of phosphoseryl residues and a high net negative charge at neutral pH. β-lactoglobulin binds Na^+ ions to carboxyls and imidazoles and may form complexes with divalent ions, with one cation per free sulphydryl (Hambling *et al.*, 1992). α-la is capable of binding monovalent and divalent ions, the number and position of the binding sites appearing to be specific to the ion being bound (Brew and Grobler, 1992).

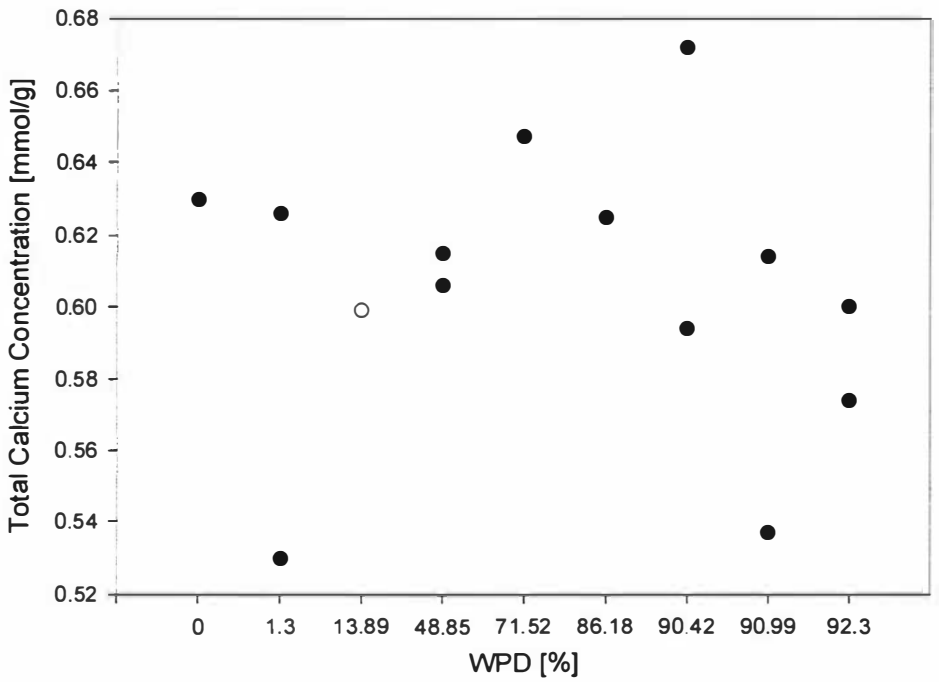

Figure 4-1 Total calcium concentration of pilot plant MPC85 powders (●) and commercial MPC85 powder (○)

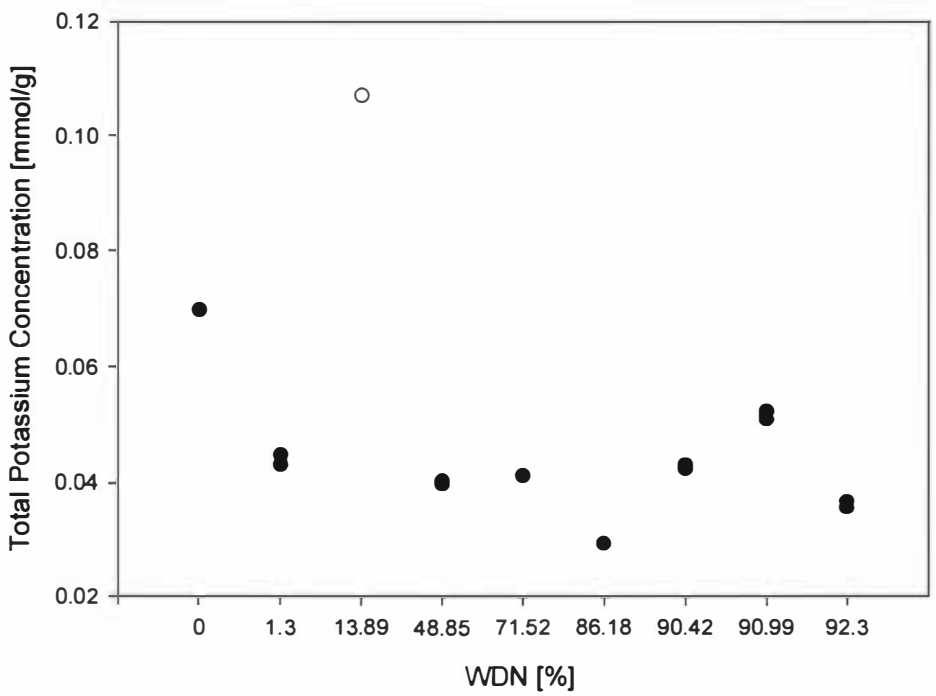

Figure 4-2 Total potassium Concentration of pilot plant MPC85 powders (●) and commercial MPC85 powder (○)

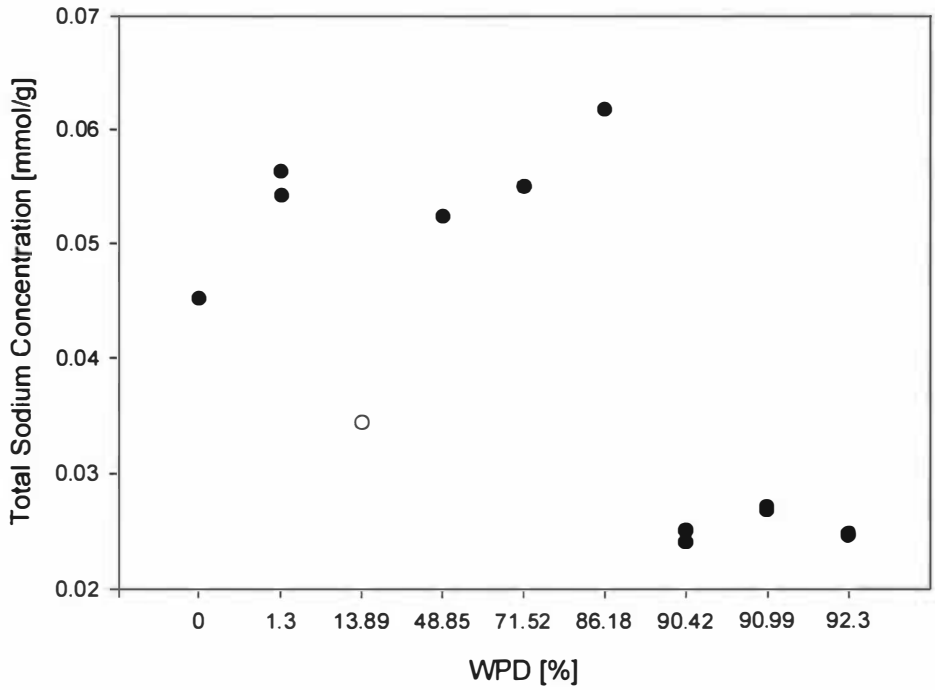

Figure 4-3 Total sodium concentration of pilot plant MPC85 powders (●) and commercial MPC85 powder (○)

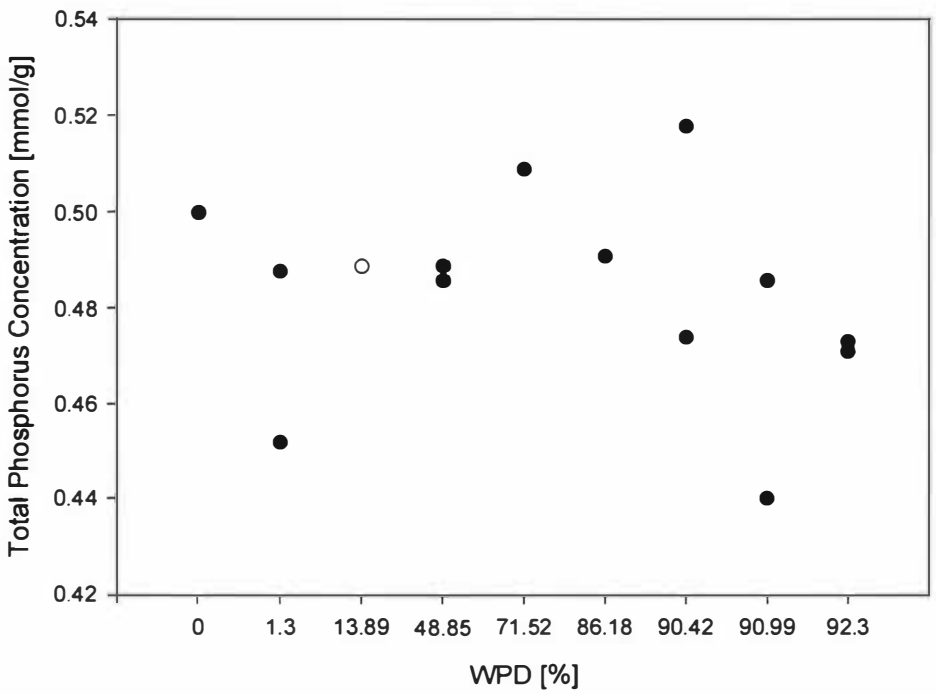

Figure 4-4 Total phosphorus concentration of pilot plant MPC85 powders (●) and commercial MPC85 powder (○)

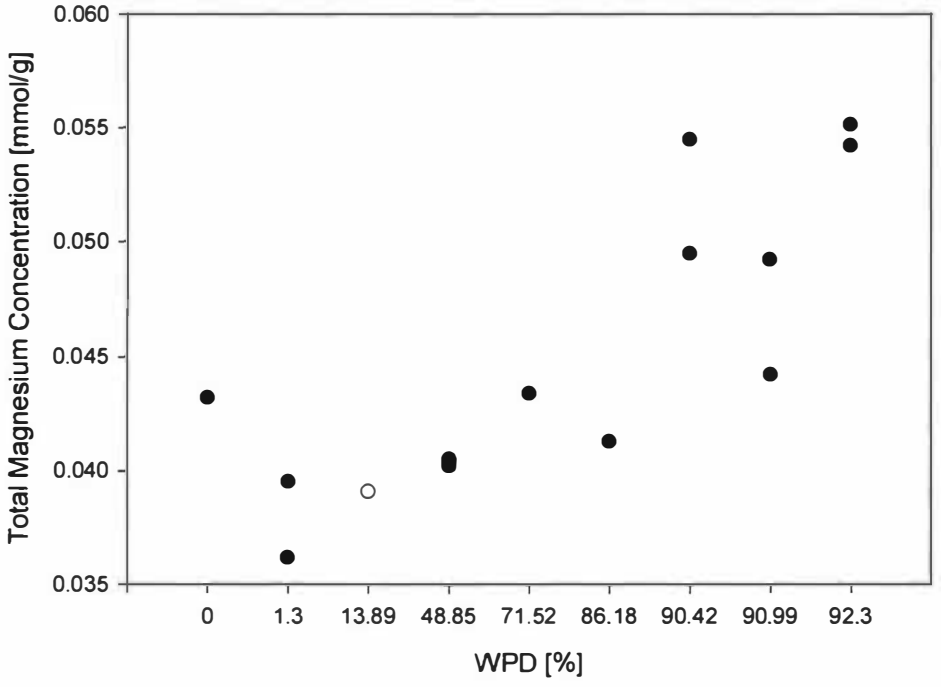

Figure 4-5 Total magnesium concentration of pilot plant MPC85 powders (●) and commercial MPC85 powder (○)

4.3 Functional properties

4.3.1 Solubility

The solubility of the pilot plant powders was determined using the method set out in section 3.1.4. The solubility was determined for each of the pilot plant powders by preparing the solutions at 50°C, 50°C followed by single stage homogenisation at 150 bar, and 50°C followed by single stage homogenisation at 200 bar.

The solubility results, shown in Table 4-3, show that not all of the MPC85 powders are fully dissolved either by preparation at 50°C or 50°C followed by single stage homogenisation at 150 bar. The immediate conclusion that can be drawn from this data is that MPC85 powders become increasingly more difficult to dissolve with increasing severity of preheat treatments. These experiments were only conducted once as the purpose was to find a suitable method for dissolving completely the pilot plant powders rather than accurately defining the solubility, or lack of, under a particular set of preparation conditions.

The commercial powder which had a whey protein denaturation level of 13.89% was, at ≈ 100%, more soluble than pilot plant powders with comparable levels of whey protein denaturation. The comparable pilot plant powders had a large variation in solubility (80 to 100%) at 50°C. The reason for the better solubility of the commercial powder may lie in the manufacturing process. One of the major differences between the manufacturing of the commercial and pilot plant powders is the evaporation step, which is not present in the pilot plant process. The evaporation step enables the commercial concentrate to be concentrated to about 28% total solids. The total solids of the pilot plant concentrate was limited by the ultrafiltration step to about 20%. The higher moisture content of the pilot plant concentrates would result in a longer drying period, and hence would increase the opportunity for heat damage, during the spray drying operation. It is therefore possible that the solubility variations among the pilot plant powders may be the result of damage sustained during drying. This is particularly so in light of the blockages that occurred during the drying stage of manufacture mentioned in section 4.1.

Table 4-3 Solubility of Commercial and Pilot Plant MPC85 powders at 5% total solids concentration

Sample	Solubility [%]		
	Dissolved at 50°C	Homogenised at 150 bar	Homogenised at 200 bar
Commercial	96.81	98.74	100.01
Control	79.08	82.18	99.86
72°C/30s	95.67	100.00	98.96
80°C/30s	81.24	82.04	98.79
90°C/30s	86.01	86.60	99.47
100°C/30s	81.11	68.10	98.04
110°C/30s	31.72	29.22	97.41
120°C/45s	22.45	52.38	98.23
130°C/30s	15.23	91.46	97.13

A second possible explanation for the insoluble nature of the pilot plant powders might be the formation of aggregates caused by ultrafiltration to 6x as reported by Hallstrom and Dejmek (1988). Hallstrom and Dejmek (1988) found that aggregates formed in concentrate which had been concentrated to 6x, did not disaggregate on dilution even during prolonged storage and could only be disrupted by homogenisation at pressures in excess of 200 bar.

The effect of homogenisation at 200 bar on solubility was determined using the method outlined above. The results of this trial showed that all the powders could be solubilised to a level > 97% through homogenisation at 200 bar. All solutions tested in the following sections were prepared by mixing at 50°C for an hour followed by homogenisation at 200 bar.

4.3.2 Particle size analysis of reconstituted MPC85 powders

The particle size analysis of the reconstituted MPC85 powders was determined by the method described by Anema and Klostermeyer (1996) by diluting approximately 150µl of 3.5% protein concentration solution with 10 ml of calcium imidazole buffer filtered through a 0.25µm filter. The diluted sample was allowed to equilibrate for ten minutes before an aliquot was placed in the measurement cell of the Malvern Zeta Sizer 4 System (Malvern Instruments Limited, Worcestershire, UK) . The Zeta Sizer was set up, in particle sizing mode, to take 5 measurements each lasting 180 seconds. The sample

was maintained at 25°C throughout the measurement by the waterbath surrounding the measurement cell.

The results show a slight increase in particle size with the level of whey protein denaturation up to 86% (Figure 4-6). Heat treatments causing whey protein denaturation levels greater than 86% result in a dramatic increase in the particle size. No model could be found that adequately described the relationship between particle size over the entire range of whey denaturation. If particle size is regressed against the whey protein denaturation level over the ranges 0 to 86 % and 86 to 92 % the slope of the regression analysis is 0.4383 and 24.02 nm/% WDN with correlation coefficients of 0.9244 and 0.9954 respectively. This equates to an increase in the rate of change in particle size with WDN of 5500 %. This sudden increase of particle size coincides with the changes in mineral composition with regard to Mg and Na. The coincidence of these two phenomena are probably indicative of a massive structural change. The reconstituted commercial powder has a significantly smaller particle size than all of the pilot plant powders at ≈188 nm.

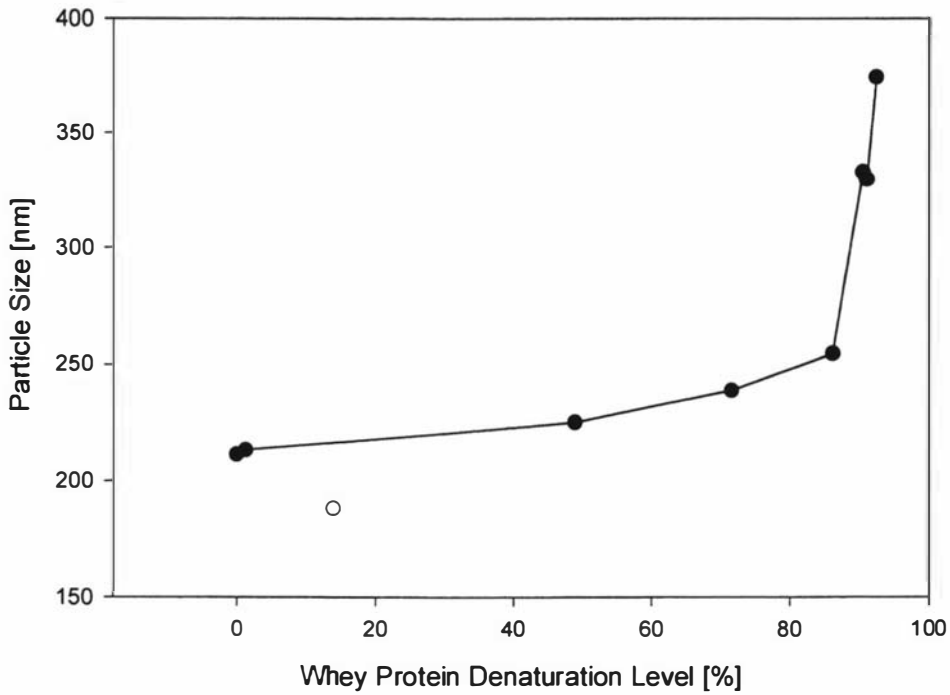

Figure 4-6 The particle size of reconstituted pilot plant MPC85 powders (●) and commercial MPC85 powder (○) as a function of whey protein denaturation level.

4.3.3 Rennet coagulation properties of reconstituted pilot plant powders

The rennet coagulation properties of reconstituted MPC85 powders (3.5% w/w, adjusted to pH 6.5-6.6) at 32°C upon addition of rennet were followed using an oscillatory rheological set-up on the Bohlin VOR Rheometer (frequency 0.1 Hz, amplitude 5%, measurement interval 60 s). An incubation temperature of 32°C was chosen as rennet curd produced at this temperature is known to be firm with minimal syneresis (Scott, 1986). The rheological parameters complex modulus, (G*); elastic, (G') and viscous, (G"), moduli; and phase angle were calculated using the analysis software of the Bohlin rheometer.

An important aspect of characterising the rennetability of a milk protein system is determining the time required for the system to gel, known as the rennet coagulation time. The rennet coagulation time (RCT), defined as the point at which the elastic or storage modulus (G') became greater than the viscous or loss modulus (G''), is shown in Figure 4-7 as a function of the degree of whey protein denaturation. The pilot plant powder with the highest whey protein denaturation level (92%) did not form a gel and so is not plotted in Figure 4-7. In general, the shape of this plot is curvilinear with the RCT increasing throughout the range of whey denaturation studied. The increase in RCT is greater for the powders with WDN values greater than 86%. This is in contrast to Dalgleish (1990) who, for a skim milk system, reported that a single plot of RCT against the extent of protein denaturation at various temperatures showed a curvilinear relationship at low extents of denaturation and an almost linear dependence when more than 40% whey protein was denatured.

While the rennet coagulation time is an important characteristic of a renneted system, to fully characterise the system the relationship between the development of the viscous and elastic components of the subsequent gel with time, and the development of the overall stiffness of the gel must be understood.

The viscoelastic nature of the gel may be followed by observing changes in G', G" and the phase angle (Figure 4-8, Figure 4-9 and Figure 4-10 respectively). The transition

from sol to gel is marked by rapid increases in both G' and G" and a concurrent sudden decrease in the phase angle from 90° to between 15 and 17° for gels formed from powders with 13% or less whey protein denaturation. These results are comparable with the work of Bohlin *et al.,* (1984) who observed the same trends, for skim milk, with regard to G', G", and a sudden drop in the phase angle from 90° to 15°. The G' and G" of these gels reach a plateau over a period of time.

Following the onset of gelation the phase angle in all of the gels remained reasonably constant (Figure 4-10). This observation is contrary to that observed by Bohlin *et al.*, (1984) who reported that the phase angle in the renneting of skim milk systems, although practically constant throughout the whole build up of gel strength, passed through a slight local maximum in the early stages (≈22 min). Biliaderis *et al.*, (1992) reported the presence of a similar but more pronounced maximum in a study of the rheological properties of yoghurt made from skim milk and ultrafiltered retentates. The presence of a local maximum in the phase angle is indicative of a partial loosening in the casein gel network.

The transition from sol to gel for the gels formed from powders with greater than 13% whey protein denaturation is characterised only by a sudden decrease in the phase angle (Figure 4-10). The G' and G" for these gels increase linearly over the entire time scale of the experiment (7200s) and do not reach a plateau value (Figures 4-8 and 4-9 respectively). However, during the course of the experiments the solution made from the pilot plant powder given a heat treatment of 90°C for 30s during processing was set running late at night and consequently not removed from the Bohlin until the next day. Hence although measurement had only been conducted over a 2 hour period the sol/gel had actually been at 32°C for about nine hours. This gave the opportunity to observe gel developments over a longer time period. Therefore, before this gel was removed the rheological parameters were measured over a period of ten minutes. The G', G" and phase angle measurements were constant at 86 Pa, 24 Pa, and 15.8° respectively. These values indicate that, given sufficient time, the final rheological properties of the gel may be independent of the degree of whey protein denaturation of the powder from which it is made. Unfortunately due to time constraints on the Bohlin, measurement of final gel rheology was not possible for all of the powders.

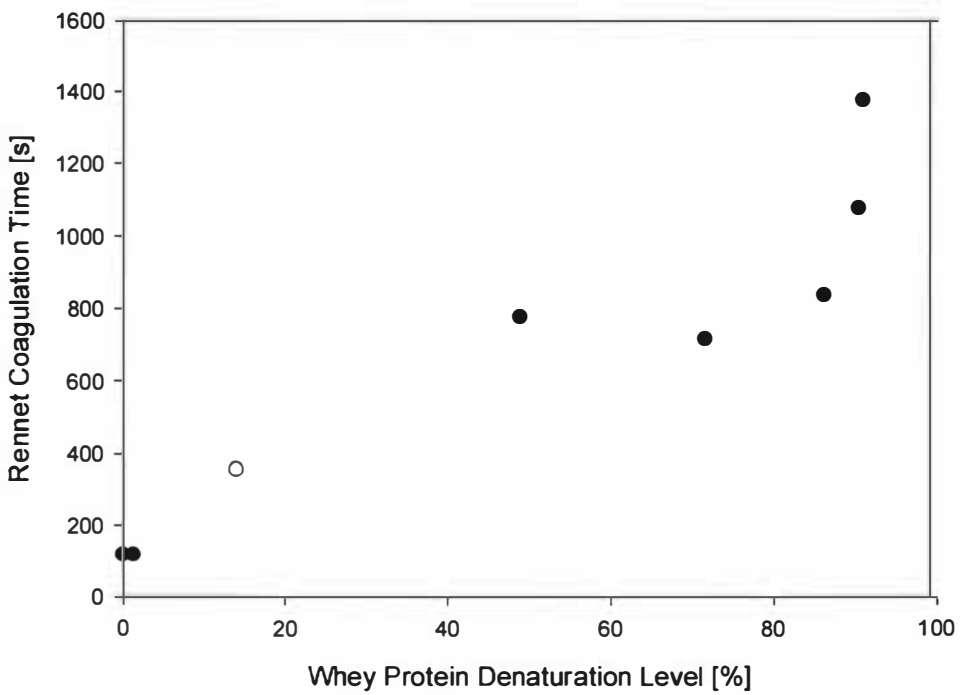

Figure 4-7 The time taken for a 3.5% protein solution reconstituted from pilot plant powders (●) and commercial MPC85 (○) to form a gel after addition of rennet at 32°C as a function of the whey protein denaturation level.

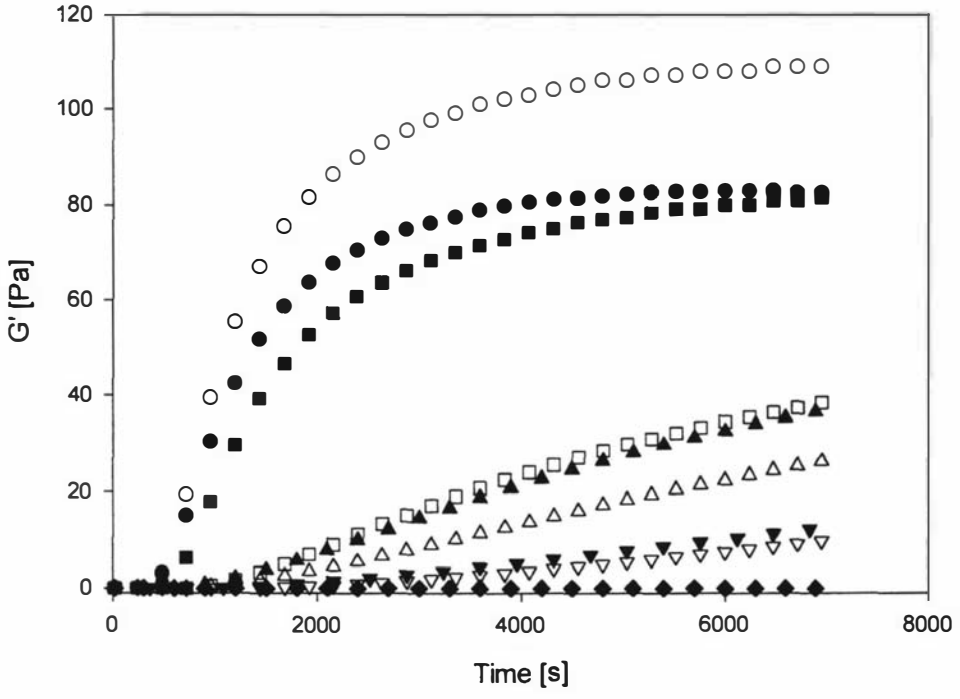

Figure 4-8 The development of G' with time following the addition of rennet at 32°C to solutions of MPC85 reconstituted from commercial powder (○), and pilot plant powders manufactured with the following heat treatments control (●), 72°C/30s (■),80°C/30s (□), 90°C/30s (▲), 100°C/30s (△), 110°C/30s (▼), 120°C/45s (▽), and 130°C/30s (◆)

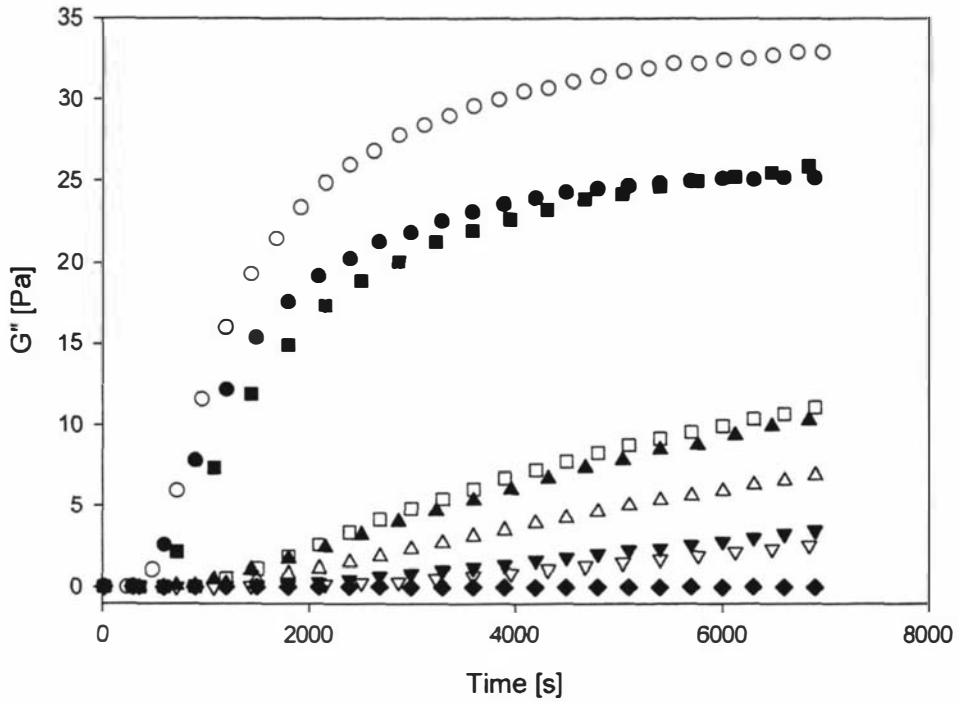

Figure 4-9 The development of G" with time following the addition of rennet to solutions of MPC85 reconstituted from commercial powder (○), and pilot plant powders manufactured with the following heat treatments control (●), 72°C/30s (■),80°C/30s (□), 90°C/30s (▲), 100°C/30s (△), 110°C/30s (▼), 120°C/45s (▽), and 130°C/30s (◆).

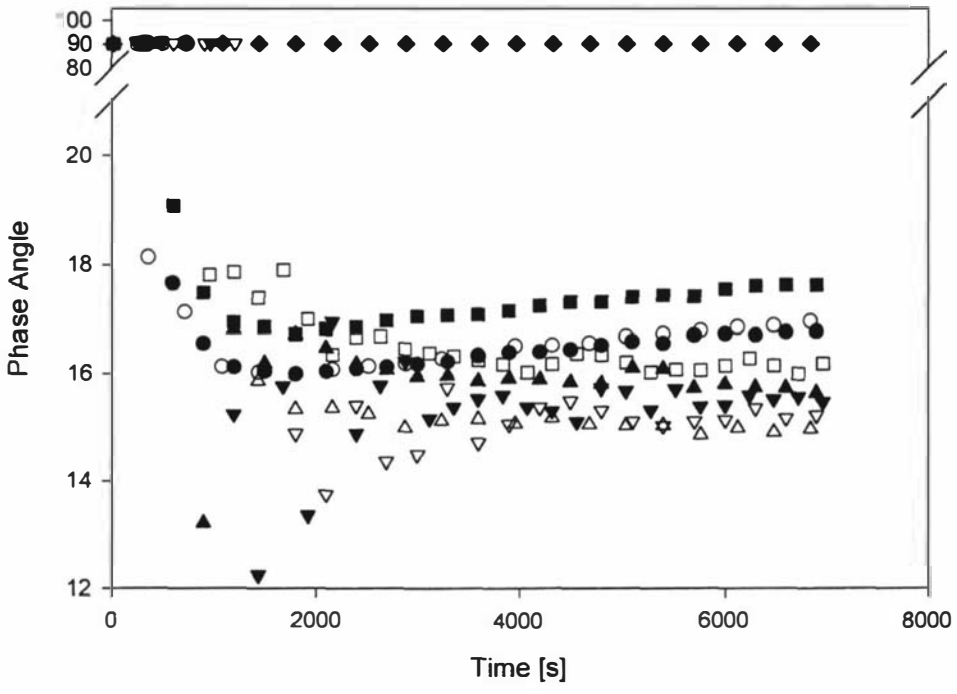

Figure 4-10 The development of the phase angle with time following the addition of rennet to solutions of MPC85 reconstituted from commercial powder (○), and pilot plant powders manufactured with the following heat treatments control (●), 72°C/30s (■),80°C/30s (□), 90°C/30s (▲), 100°C/30s (△), 110°C/30s (▼), 120°C/45s (▽), and 130°C/30s (◆)

By inspection of Figures 4.8 and 4.9, one can see that the rate of change in G' and G" with time increases linearly for each of the systems with high (> 13%) levels of denatured whey protein. The rate of change (i.e. slope) of G' and G" for these solutions (Figure 4-11) decreases with increases in the level of whey protein denaturation. Attempts to adequately model this relationship were unsuccessful. A linear relationship was observed, however, between the rate of change in G' and G" and particle size (Figure 4-11). This relationship is described in the following equations.

4-3 $$\frac{dG'}{dt} = 0.016 - 4.26 \times 10^{-5} \times \phi \qquad R^2 = 0.98$$

4-4 $$\frac{dG''}{dt} = 0.045 - 1.20 \times 10^{-5} \times \phi \qquad R^2 = 0.97$$

where dG'/dt = the change in the elastic modulus with time [$Pa.s^{-1}$]

dG''/dt = the change in the viscous modulus with time [$Pa.s^{-1}$]

ϕ = particle diameter [nm]

Forces from viscous flow are proportional to shear velocity, whereas forces from elastic deformation are proportional to the deformation only. This gives a resultant force, which is in general out of phase with the applied deformation. The phase angle therefore measures the relative contribution from the elastic, G', and viscous, G", flow in the gel. Figure 4-10 shows that from the point of gelation the value of the phase angle is very similar for all of the gels. Therefore despite the difference in the kinetics of gelation among the MPC85 powders there does not seem to be any difference in the relative contribution to the total shear stiffness of the gel arising from viscous flow and from elastic strains in the gel.

The development of the total shear stiffness of each of the gels as given by the complex modulus, G*, shown as a function of time from the onset of gelation in Figure 4-12. The G* curves resemble those of the development of G' and G" with time. By modelling the development of the complex modulus, information about the kinetics of the gel stiffness

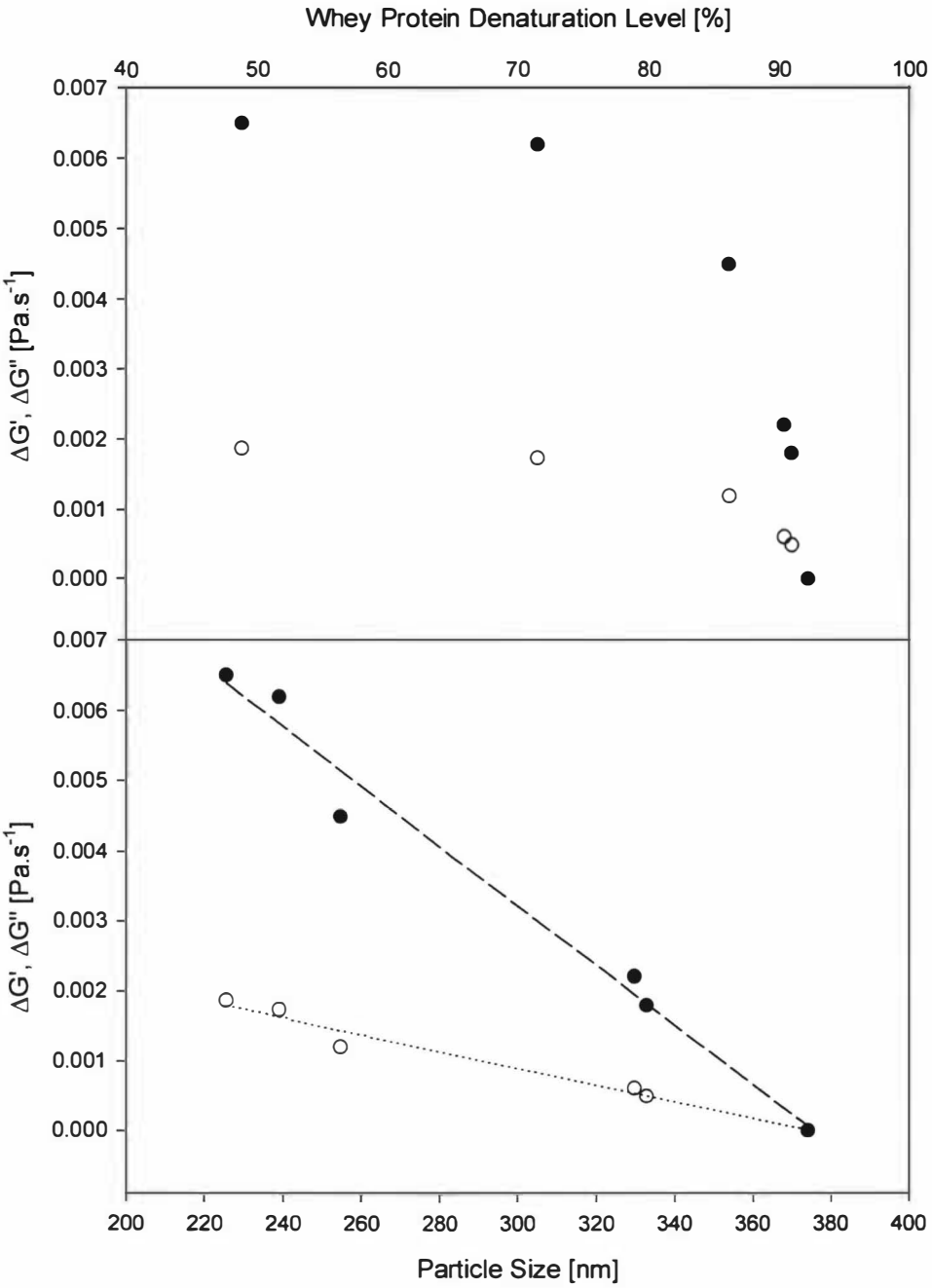

Figure 4-11 The rate of change in G' (observed data (●), regression line (---)) and G" (observed data (○), regression line (····) as a function of (a) the level of whey protein denaturation and (b) particle size for the pilot plant powders with greater than 48% whey protein denaturation.

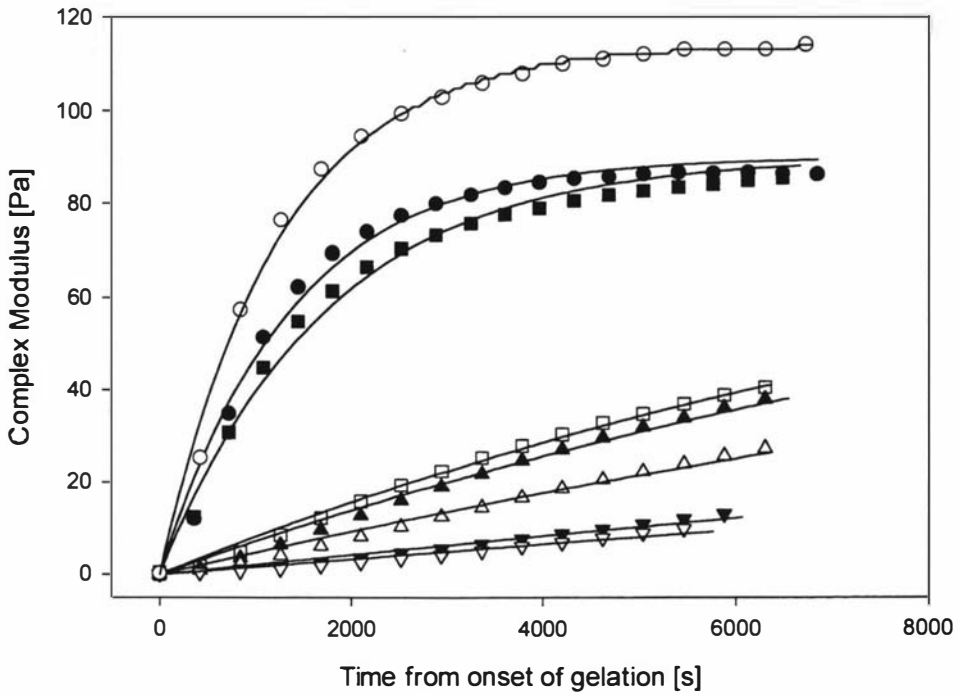

Figure 4-12 The development of the complex modulus with time from the onset of gelation in solutions of MPC85 reconstituted from commercial powder (○); and pilot plant powders manufactured with the following heat treatments control (●), 72°C/30s (■),80°C/30s (□), 90°C/30s (▲), 100°C/30s (△), 110°C/30s (▼), and 120°C/45s (▽); and as predicted by first order kinetics (—).

development can be obtained. The development of gel stiffness subsequent to the initial lag, has been shown to follow first-order kinetics for rennet coagulated systems (Scott-Blair, 1960; Olsen and Bottazzi, 1977) and fermented systems (Biliaderis *et al.*, 1992). The first order kinetic model reported by Biliaderis *et al.*, (1992), equation 4-5, has been used here to calculate an apparent rate constant for gelation.

4-5 $$k = \frac{2.303}{t} \times log\left(\frac{G^*_\infty - G^*_0}{G^*_\infty - G^*_t}\right)$$

where G^*_0, G^*_t, and G^*_∞ are the complex modulus values at time zero (onset of gelation), t, and the completion of the gelation process respectively, while k is the apparent rate constant (s^{-1}) and t is time in seconds.

Due to the limitations of the experiment, the gelation process did not continue to completion for the gels formed from powders with a level of whey protein denaturation greater than 13%. In these cases G^*_∞ was assumed to be 90 Pa, the plateau G* value of the gels formed from the pilot plant powders that completed the gelation process within the time limits of the experiment. The fact that the gel formed from the pilot plant powder given a heat treatment of 90°C for 30s (71.52 % WDN) during processing was found to have a plateau G* of ≈ 90Pa when measured about nine hours after the addition of rennet supports this assumption. The value of G^*_∞ for the commercial powder was 114Pa.

The value of k was determined for each gel by re-arranging equation 4-5 to make G^*_t the subject and then minimising the residual sum of the squares between the measured G^*_t and the predicted G^*_t by varying k using the solver function of the spreadsheet program *Excel 95*. The results of this analysis are given in Table 4-4. The kinetic model describes the rheological data well with correlation coefficients in the range of 0.95 to 0.99.

Table 4-4 Apparent Gelation Rate Constant of commercial and pilot plant MPC85 powder upon rennet coagulation at 32°C.

Sample	Apparent Gelation Rate Constant (s^{-1}) [$k \times 10^{-5}$]	Correlation coefficient
Commercial	81.8	0.99
Control	74.6	0.99
72°C/30s	58.5	0.99
80°C/30s	9.61	0.99
90°C/30s	8.42	0.99
100°C/30s	5.48	0.98
110°C/30s	2.44	0.97
120°C/45s	1.90	0.95

The main use of an understanding of the renneting ability of MPC85 is in cheese manufacturing. In cheese processing the key characteristic of the rennet curd is its stiffness at the time of curd cutting which normally takes place 30 to 35min after the addition of rennet. This corresponds to a gel stiffness of 30 to 40Pa (Bohlin *et al.*, 1984). The gel stiffness as measured by G* is shown for each of the MPC85 samples at 35 min, as a function of whey protein denaturation and particle size in Figure 4-13. It is apparent that the MPC85 powders with whey protein denaturation levels of 13% or less would reach the required curd firmness in 35 minutes or less. The graph shows that increases in curd firmness are correlated with lower levels of whey protein denaturation and smaller micelle size. A negative correlation with micelle size has also been reported for the renneting of whole milk systems (Niki and Arima, 1984).

Rennet coagulation of milk is a two stage reaction. The first stage is the enzymic hydrolysis of the Phe-Met bond of κ-casein. This stage has been described by Michaelis-Menten kinetics (Chaplin and Green, 1982), but van Hooydonk *et al.*, (1984) has suggested that first-order kinetics is followed. The second stage consists of the aggregation of the destabilised micelles, via von Smoluchowski kinetics (Payens, 1989). This theory for the aggregation of unstable colloids has the approximations that particles diffuse by Brownian motion and interact without activation energy, so that coagulation occurs spontaneously (Famelart, 1994). One would expect particles with a larger particle size to possess a slower rate of diffusion and therefore to have a slower coagulation rate. Pires *et al.*, (1997) also showed that the aggregation rate is reduced by increasing the

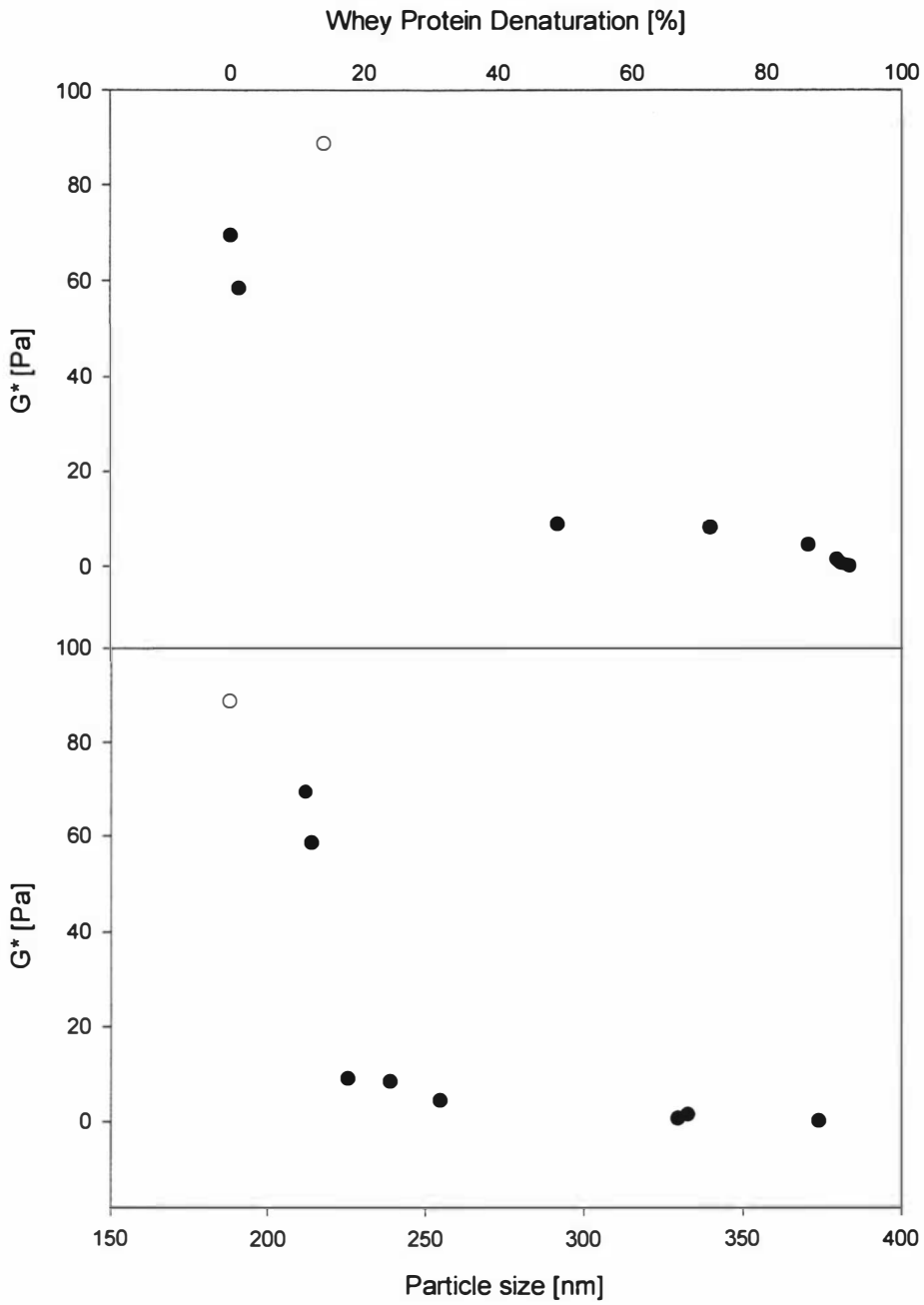

Figure 4-13 The complex modulus (●), 35 minutes after the addition of rennet for 3.5% protein MPC85 solutions made from commercial MPC85 (unfilled) and pilot plant powders (filled) as a function of (a) whey protein denaturation and (b) particle size.

viscosity of the medium. This may in part explain the decreasing gelation rate constant observed for the MPC85 powders when they are arranged in order of increasing particle size (Figure 4-11).

Extensive studies on the conversion of κ-casein by rennet in heated milk and in model systems containing casein micelles and β-lactoglobulin have been carried out (Hindle and Wheelock, 1970; Wilson and Wheelock, 1972; Wheelock and Kirk, 1974; and Shalabi and Wheelock, 1976). The results of these studies implied that the interaction between β-lactoglobulin and κ-casein inhibited the hydrolysis of κ-casein, because only about 75% of the substrate appeared to be accessible to rennet after the complete denaturation of the whey proteins (van Hooydonk *et al.*, 1986). Damicz and Dziuba (1975), however, found that not the extent but only the rate of conversion was affected in heated milk. The evidence that the pilot plant powder given a heat treatment of 90°C for 30s during processing did, after about nine hours, reach the same degree of curd firmness as those with less heat treatment supports the findings of Damicz and Dziuba.

4.3.4 Apparent viscosity

For this section of work the Bohlin rheometer was not available and the viscometer used was the Haake VT500 . The solutions studied here were reconstituted by mixing at 50°C for an hour followed by homogenisation at 200 bar. All solutions were reconstituted to give a final protein concentration of 17.5 %. The solutions were adjusted by the drop-wise addition of 0.1 N HCl so that they all had a pH of 7.00. The rheology of each solution was followed using an upwards shear sweep followed by a downwards shear sweep over the shear rate range of 20 to 900s^{-1} at 15°C.

By inspection of the raw results of these experiments (Figure 4-14), one can see that solutions of MPC85 increase in apparent viscosity and pseudoplasticity with increasing severity of heat treatment during processing. To quantify these observations both Power Law and Herschel Bulkley models were fitted to the downward shear sweep data of each flow curve. Yield stresses were only detected for the solutions made from the three pilot plant powders which had been given the most severe heat treatments during processing.

The addition of the yield stress term to these three powders significantly reduced the residual sum of the squares at a 95% level as determined by a partial F test. The flow parameters for each of the powders, along with the parameters for the commercial powder for comparison, are presented in Table 4-5. The models presented in this table fit the data very well with correlation coefficients > 0.996.

Table 4-5 Flow parameters for reconstituted MPC85 powders

Sample	Yield Stress [Pa]	Consistency Index [Pa.s^n]	Flow Behaviour Index [dimensionless]	Correlation Coefficient
Control	0	0.166	0.776	0.999
72°C/30s	0	0.244	0.736	0.999
80°C/30s	0	0.418	0.694	0.999
90°C/30s	0	0.942	0.553	1.000
100°C/30s	0	1.384	0.546	0.996
110°C/30s	12.34	2.343	0.560	0.999
120°C/45s	12.95	2.360	0.552	1.000
130°C/30s	13.40	2.329	0.573	1.000
Commercial	0	0.120	0.788	0.998

The consistency index and flow behaviour index for each solution are shown as a function of both the degree of whey protein denaturation and particle size in Figure 4-15. It is evident from this figure that in general as particle size/level of whey protein denaturation increases there is a concomitant increase in the consistency index and decrease in the flow behaviour index. The only anomaly to this trend is for the three solutions made from the powders with the highest heat treatment during processing. This anomaly may be attributable to the yield stress observed in these solutions.

To further explore trends in the data the apparent viscosity at 900s^{-1} was plotted against particle size and against the degree of whey protein denaturation, (Figure 4-16). The relationship between apparent viscosity and particle size and the level of whey protein denaturation was analysed by attempting to fit a variety of mathematical models. A linear relationship between particle size and apparent viscosity was observed, (equation 4-6), while no simple relationship could be found between apparent viscosity and the level of whey protein denaturation.

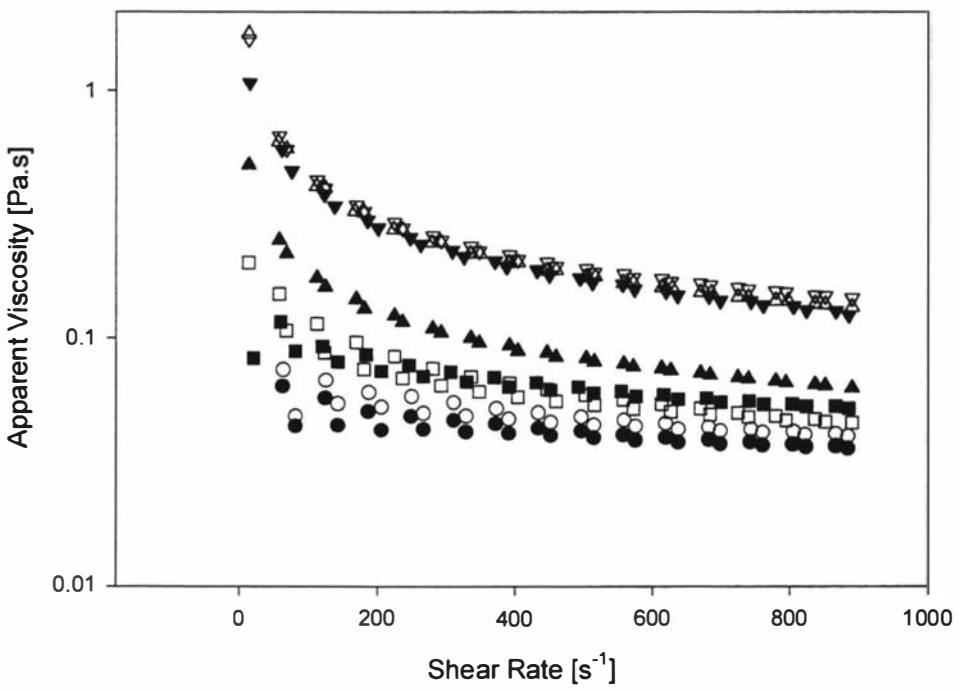

Figure 4-14 Flow curves at 15°C of pilot plant powders manufactured with the following heat treatments: Control, (●); 72°C/30s, (○); 80°C/30s, (■); 90°C/30s, (□); 100°C/30s, (▲); 110°C/30s, (△); 120°C/45s, (▼); 130°C/30s, (▽).

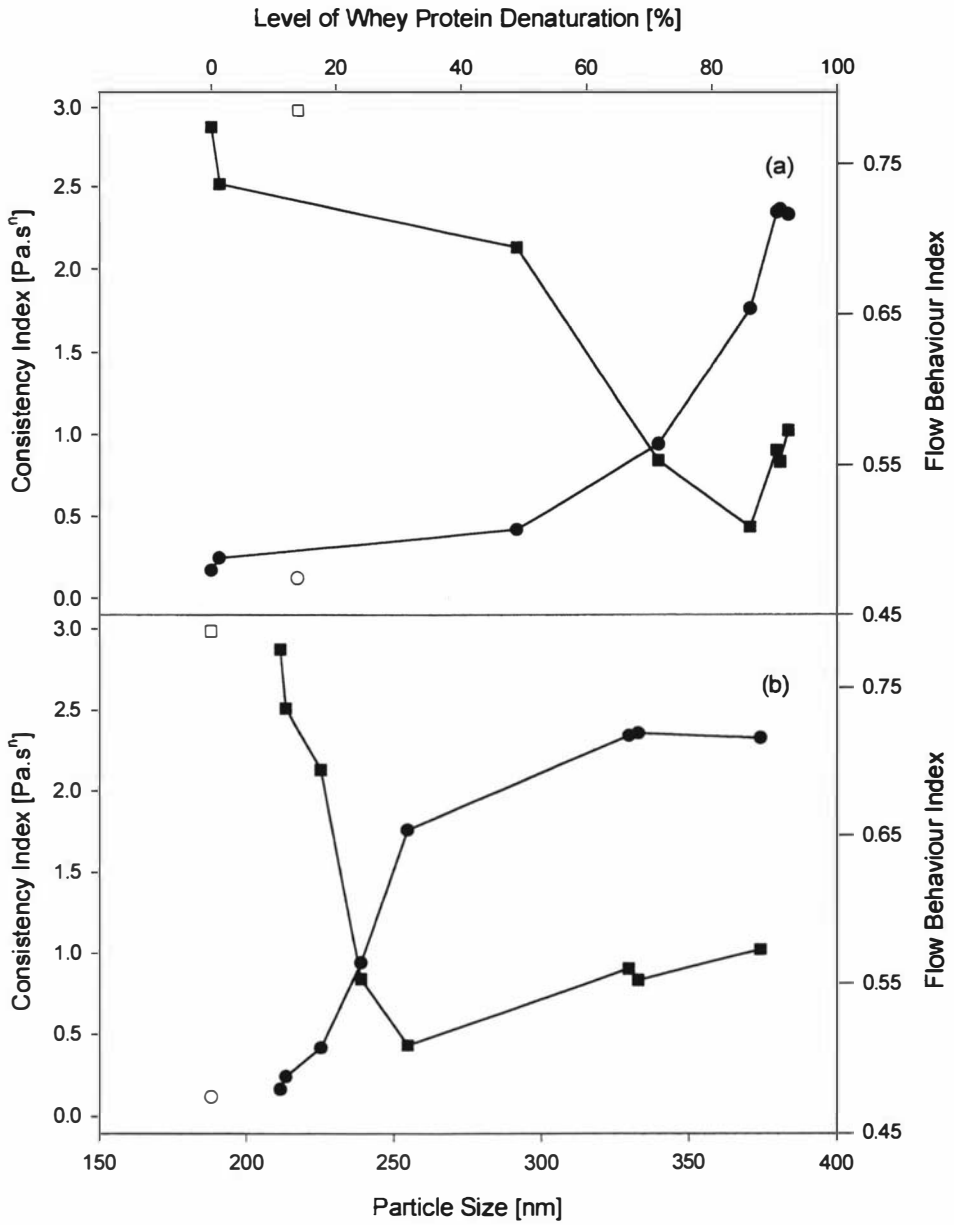

Figure 4-15 The consistency (●) and flow behaviour (■) indices as a function of the level of whey protein denaturation (a), and particle size (b) for 17.5% total protein MPC85 solutions reconstituted from pilot plant powders (filled symbol) and commercial MPC85 powder (unfilled symbol).

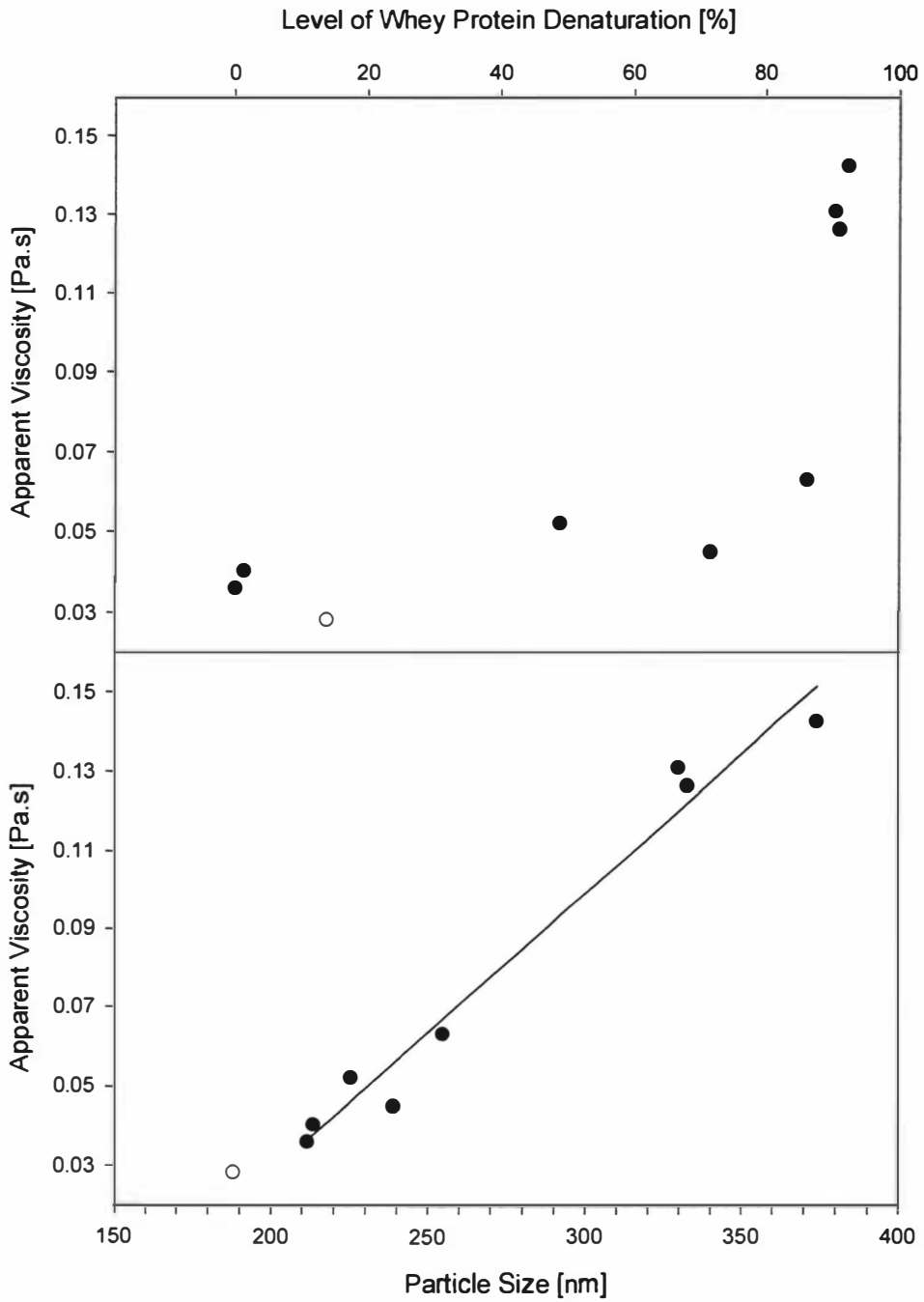

Figure 4-16 The apparent viscosity, at 15°C, $900s^{-1}$,of 20% MPC85 solutions reconstituted from pilot plant powders (●) and commercial powder (○) as a function of the level of whey protein denaturation and particle size.

4-6 $$\eta_{app} = m\,D + c$$

where η_{app} = apparent viscosity [Pa.s]

D = particle diameter [nm]

and m and c are constants representing the slope [Pa.s/nm] and intercept [Pa.s] respectively.

How this relationship varied with shear rate was then examined by performing a linear regression on plots of apparent viscosity against particle size for a range of shear rates. The linear model fitted well in all of these cases, (Figure 4-17), with the rate of change in apparent viscosity with particle size (m) decreasing with increasing shear rate.

By inspection of Figure 4-17 one can see that the sensitivity of apparent viscosity to particle size decreases at high shear rates. At high shear rates there is a difference of 350% in viscosity over the range of particle sizes compared to 2210% at low shear rates.

The "traditional" explanation for this observation centres on the layers of water molecules surrounding the dispersed molecules. Undisturbed dispersed molecules in a solution influence several layers of adjacent solvent molecules by reducing their mobility and so forming a solvated layer. At very low shear rates there is little effect on the layered structure and interactions would be constant as the aggregates are of constant size. However, higher shear rates would progressively remove the solvated layers, effectively reducing the "aggregate" size, and hence produce a lower apparent viscosity through the intermediate shear range. At some high shear rate the solvated layers would be completely removed, resulting in a constant apparent viscosity at very high shear rates (Tung, 1978). This view treats the dispersed particle, per se, as being of a fixed geometry with only the "aggregate" volume contributed by layers of structured solvent molecules changing.

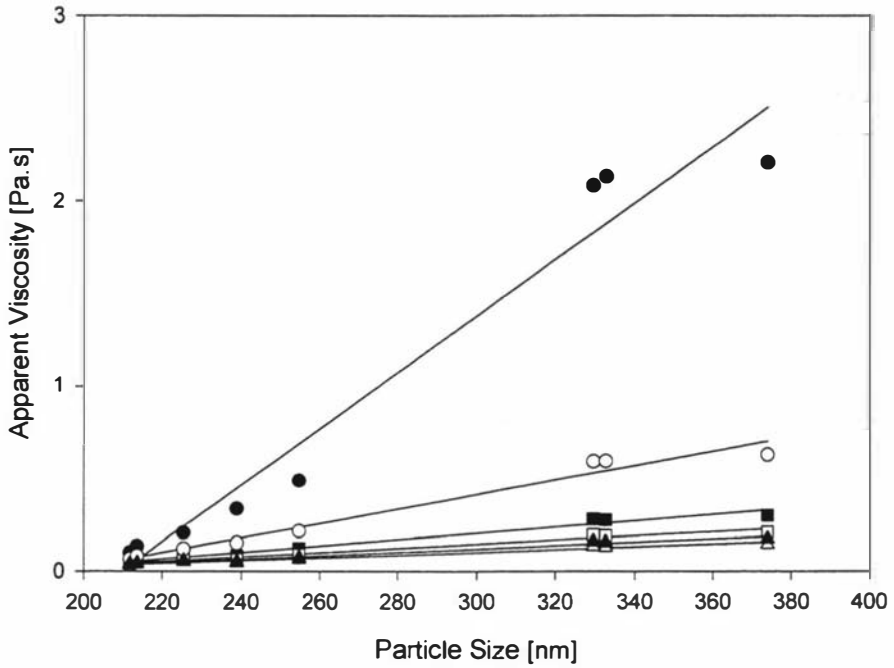

Figure 4-17 Apparent viscosity as a function of particle size for a range of shear rates: $20s^{-1}$, (●); $60s^{-1}$, (○); $210s^{-1}$, (■); $410s^{-1}$, (□); $610s^{-1}$, (▲); $900s^{-1}$, (△).

However, in the pilot plant system the high heat and low heat powders have almost the same apparent viscosity at high shear rates. This implies that the volume fraction of the dispersed particle must be almost identical. Therefore it is likely that some mechanism other than the removal of solvation layers may be occurring.

A possible mechanism explaining the larger than expected decrease in viscosity may be developed by borrowing concepts from the work on age-thickening by Snoeren *et al.*, (1984). Snoeren *et al.*, (1984) observed that the rise in viscosity of skim milk concentrates with ageing was more pronounced at lower shear rates. They proposed a mechanism based on the assumption that the casein micelles in the concentrate are spherical and that their tertiary and quarternary structures loosen during holding. This was thought to mean that at the surface some kind of hair-like protuberances were formed. The hairy structure was assumed to be partly free-drained, and the phenomenon was thought to cause, at low shear rates, a particle of a particular diameter. At high rates of shear the periphery of the particle was thought to be drained or deformed, which would effectively result in a decrease in particle diameter. The original particle size and hence viscosity would be observed upon reduction in the shear rate.

Evidence from the literature suggests that the increase in particle size with preheat treatments observed with the pilot plant powders may be due to an increase in the 'hairy layer' of the casein micelle. Oldfield (1996) proposed that upon heating skim milk, β-lg unfolds and may interact directly with κ-casein through disulphide bonds or aggregates via hydrophobic and disulphide bonds to form polymers. The polymers may also associate with κ-casein at the micelle surface (Oldfield, 1996). Therefore a heated casein micelle would be expected to increase in size due to the addition of chains of β-lg polymers attached to the diffuse hairy layer of κ-casein on the micelle surface. A model that recognises the denaturation/aggregation kinetics of β-lg and the reaction of aggregated β-lg with casein micelles, referred to as the "polymerisation model", has been developed by de Jong and van der Linden (1998). Electron micrographs of Mottar *et al.*, (1989) have shown the formation of filament-like structures on casein micelles resulting from the interaction between β-lg and κ-casein.

Therefore a mechanism similar to the one described by Snoeren *et al.*, (1984) seems reasonable for explaining the decreasing sensitivity of apparent viscosity to particle size with increasing shear. It is proposed that in a low shear rate environment the difference in apparent viscosity between the low and high heat powders is due to the increase in volume fraction resulting from the formation of hair-like structures arising from the aggregation of chains of β-lg polymers with κ-casein. As the shear rate increases, the hair-like structures would progressively drain until a point at high shear rate when they would collapse almost completely. The heat treated particle would then have a particle size, and hence a viscosity, similar to that of a solution of low heat powder. Upon a decrease in shear the chains of β-lg polymer-κ-casein would resume their original conformation and hence viscosity. This model is shown schematically in Figure 4-18.

The relationship between both slope and intercept of the plots in Figure 4-17 and shear rate were found to be adequately described by a power law relation ($R^2 > 0.995$).

4-7 $$M = a\,\dot{\gamma}^{-b}$$

4-8 $$C = c\,\dot{\gamma}^{-d}$$

where $\dot{\gamma}$ = shear rate [s^{-1}]

a,b,c and d = constants

By substituting equations 4-7 and 4-8 into equation 4-6 an equation predicting apparent viscosity based on the average particle diameter and shear rate can be derived.

4-9 $$\eta_{app} = (a\,\dot{\gamma}^{-b}) \times \phi + (c\,\dot{\gamma}^{-d})$$

The constants for equation 4-9 were optimised by minimising the sum of the relative values of the deviations between the predicted values and the observed values for apparent viscosity for all of the pilot plant powders over the shear rate range of 20 to 900s^{-1} using the *Solver* function in the spreadsheet program *Excel95*. The relative values of the deviations were minimised rather than the absolute values as this gave a better fit

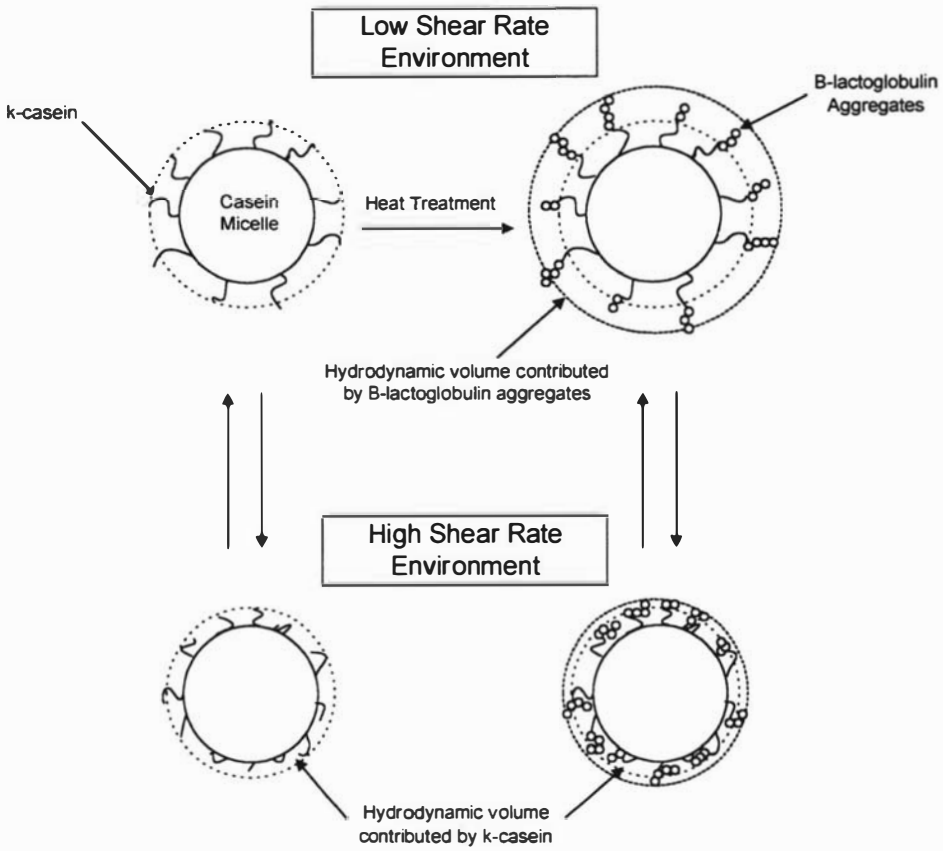

Figure 4-18 Highly schematic representation of the effect of heat treatment and shear on the hydrodynamic volume of casein micelles.

over the entire shear rate range. The optimised values for the constants a, b, c, and d were 4.18×10^{-2}, 6.02×10^{-1}, 10.7, and 6.68×10^{-1} respectively. The average deviation was 8.8 ± 0.7 % at a 95% level of confidence. The correlation coefficient for the model was 0.959 based on the absolute values of the deviations and 0.992 based on the relative values of the deviations. The predicted apparent viscosity and the experimental data are shown as a function of particle size and shear rate in Figure 4-19. The only deviations greater than 20% occur at the lowest shear rate ($20s^{-1}$). The deviations at this low shear rate are probably the result of the presence of a yield stress in the powders with levels of denatured whey protein greater than 90%. A plot of the residuals, (Figure 4-20), shows that there is systematic error which varies with particle size but not with shear rate.

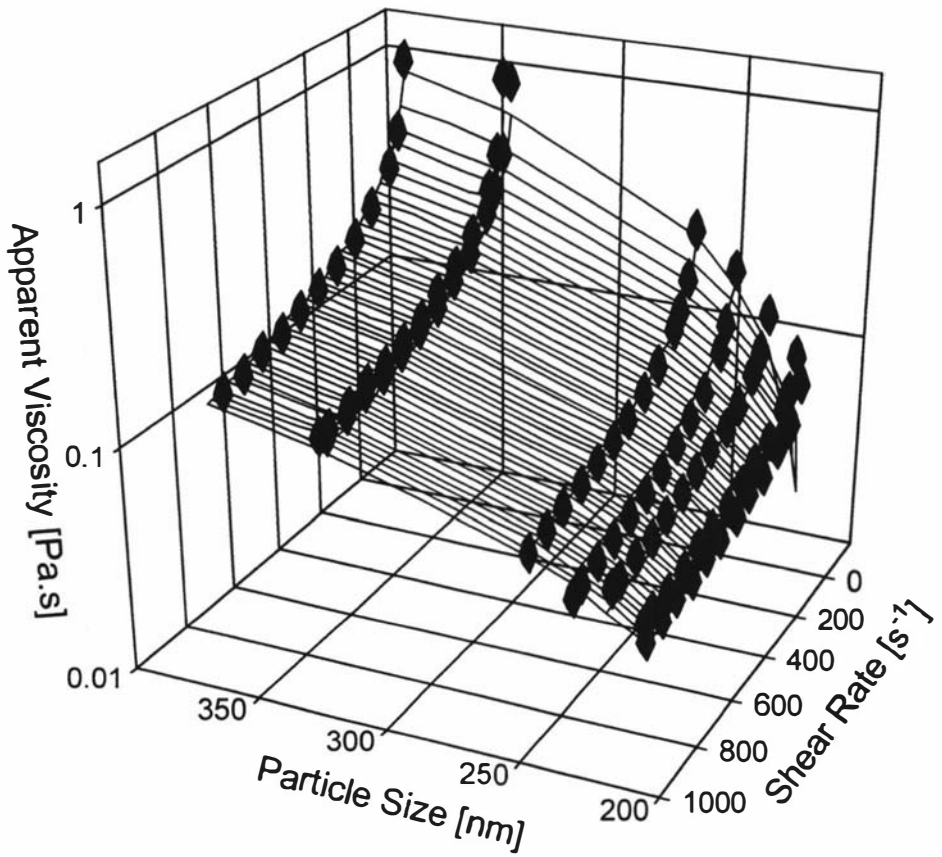

Figure 4-19 Apparent viscosity of MPC85 as a function of particle size and shear rate for observed data (●) and as predicted by equation (mesh).

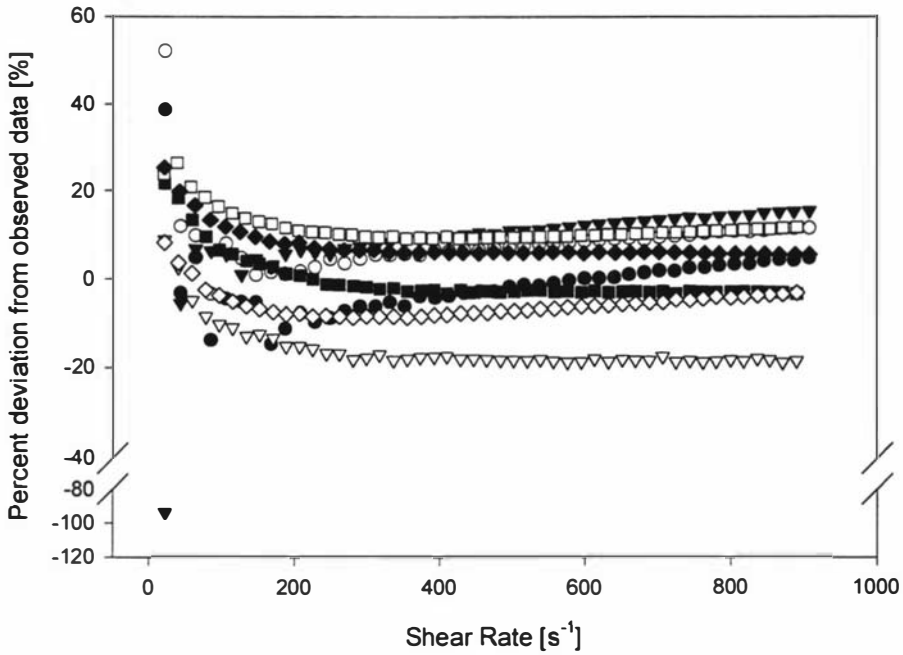

Figure 4-20 The percent deviation of the apparent viscosity predicted by equation 4-9 from the observed apparent viscosity data for the pilot plant powders: Control, (●); 72°C/30s, (○); 80°C/30s, (▼); 90°C/30s, (▽); 100°C/30s, (■); 110°C/30s, (□); 120°C/45s, (◆); 130°C/30s, (◇).

4.3.5 Apparent viscosity of reconstituted MPC85 held at 52°C

One of the main findings from the experiments described in section 2 was that MPC85 concentrate ex-evaporator, contrary to skim milk concentrate, did not age-thicken and actually thinned with time held at evaporator temperatures. In the previous chapter it was shown that reconstituted commercial MPC85 did not age-thicken at evaporator temperatures.

The objective of the following experiment was to determine the effect of heat treatment during processing on the rheology of reconstituted MPC85 when held at evaporator temperatures, 52°C. This objective was investigated by following the flow properties of the control and 130°C/30s pilot plant powders reconstituted to 17.5% total protein. The flow properties were followed by performing a series of shear sweep tests over time as described in section 3.5.

The apparent viscosities of the solutions were not found to vary significantly on holding as shown in Figure 4-21. An analysis of each shear sweep showed that both solutions could be modelled adequately by the power law model with no evidence of a yield stress. The variation in the power law coefficients with time seems to be largely random especially for the low heat pilot plant powder. Linear regression of the power law coefficients for the high heat pilot plant powder with time does show a slightly decreasing trend in the consistency index and a corresponding increasing trend in the flow behaviour index (Figure 4-22). The low correlation coefficients, (Table 4-6), indicate that there is a very poor correlation between variations in the regression coefficients with time.

Table 4-6 Flow properties, at 52°C of 17.5% total protein solutions reconstituted from low and high heat pilot plant MPC85 powders.

Sample	Consistency Index			Flow Behaviour Index		
	Slope	Intercept	Correlation Coefficient	Slope	Intercept	Correlation Coefficient
Control	2.2×10^{-4}	2.7×10^{-3}	0.30	-1.2×10^{-3}	1.12	0.37
130°C/30s	-5.2×10^{-4}	0.17	0.79	3.4×10^{-4}	0.75	0.54

As one would expect from the rheology studies conducted at 15°C the high heat powder has a higher apparent viscosity than the low heat powder and is also more pseudoplastic in behaviour. Overall this data does not provide any evidence of thickening with time. This contrasts with the findings of Snoeren *et al*., 1981 who found that increasing the severity of preheat treatments during the manufacture of skim milk powder increased the degree of age-thickening and decreased the total solids content at which age-thickening was observed.

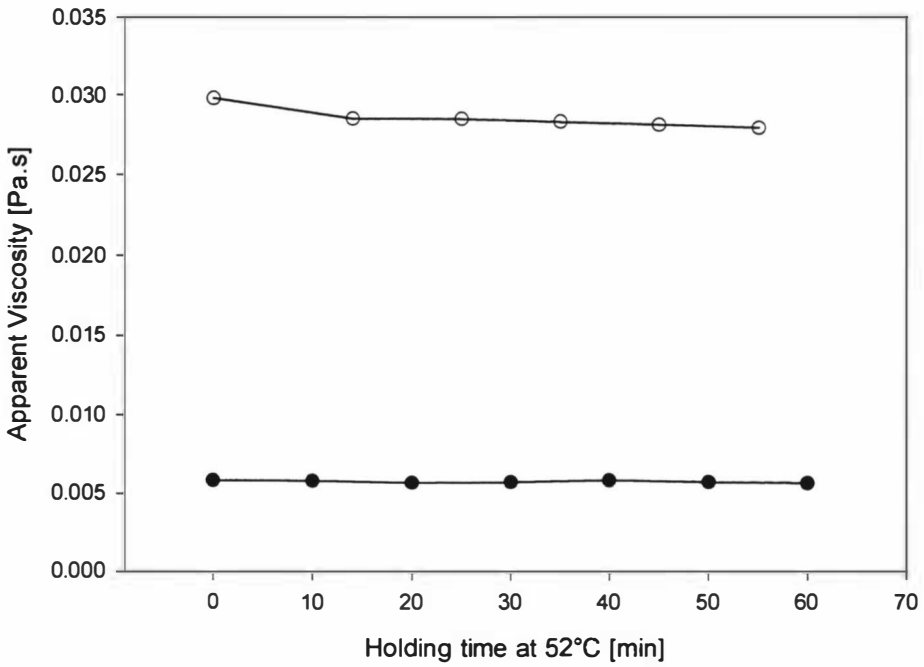

Figure 4-21 The relationship between apparent viscosity ($700s^{-1}$)and holding at 52°C for 17.50% total protein solutions reconstituted from pilot plant powders with whey protein denaturation levels of 0% (●) and 92% (○).

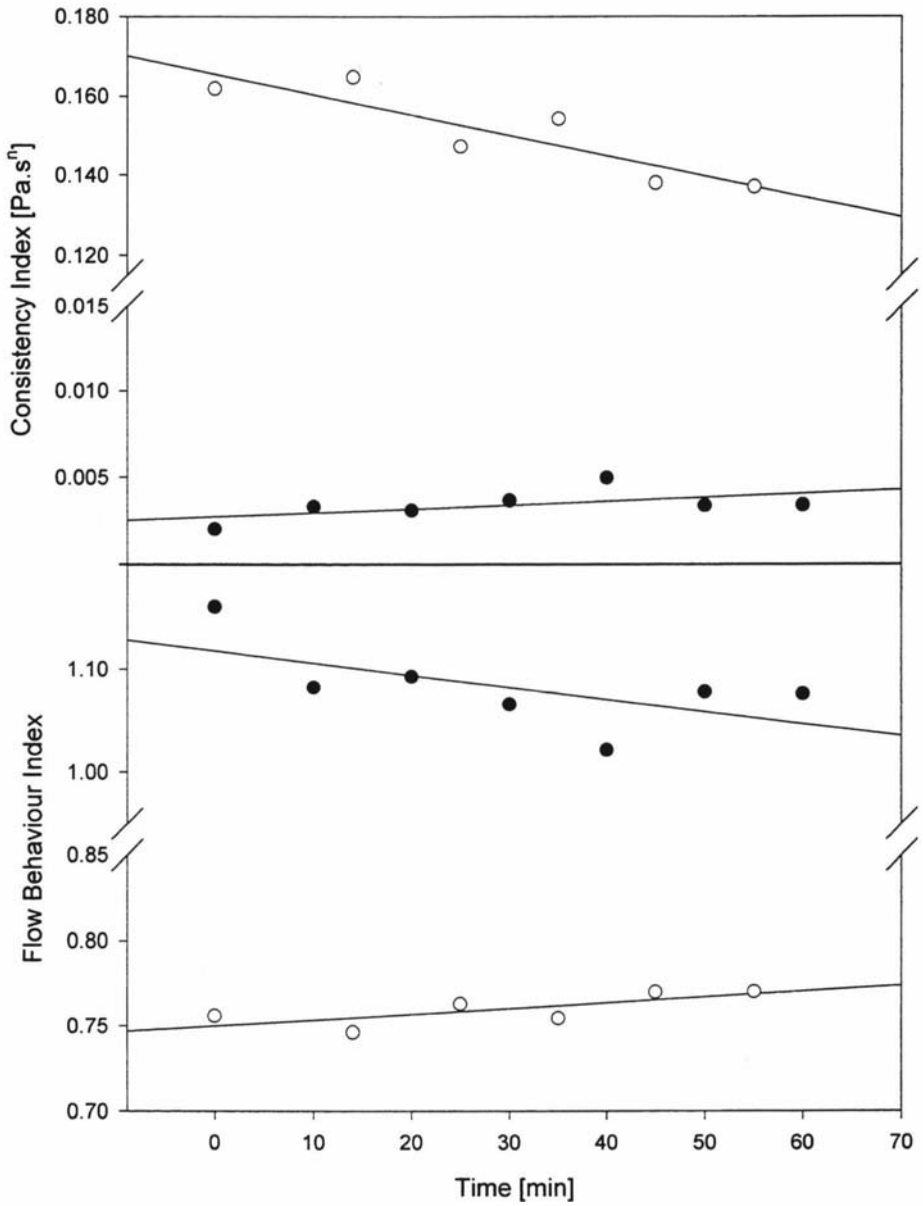

Figure 4-22 Power Law Coefficients and regression lines describing the change in flow properties (20 to 1000s^{-1}) of a 17.5% total protein solution at 52°C made from reconstituted Pilot Plant MPC85 (Control, filled; 130°C/30s, unfilled).

4.3.6 Heat stability of reconstituted pilot plant powders

In the USA, there is a growing interest in milk protein concentrates for incorporation into nutritional and enteral beverages. As these beverages are often can sterilised, an important requirement of the milk protein concentrate is good retort stability; i.e. the ability to withstand, at 5 or 10% total solids, heating at 121°C for 15 min without showing significant signs of destabilisation. Therefore, the heat stability of the MPC powders studied here was conducted at 120°C.

The heat stability of the reconstituted pilot plant powders was determined by the method described by Davies and White (1960). The pH of the MPC solutions (3.5 % w/w protein) was adjusted with 1 M HCl or 1 M NaOH to be within the range 6.1 to 7.3 at 20 °C and held for 20 min to equilibrate. After the equilibration time, the pH of the solutions was recorded. A 3 ml aliquot of each sample was transferred to a heat resistant Kimex screw cap test tube (internal diameter 10 mm, length 100 mm). The test tubes were fixed to a metal platform and immersed in an oil bath maintained at 120 °C.

The rocking mechanism attached to the platform and a stopwatch were immediately turned on. The heat coagulation time was recorded as the time taken from immersion of the tubes into the hot oil to the visual appearance of coagulation.

The heat coagulation times for the solutions reconstituted from the pilot plant powders and commercial MPC85 are shown as a function of pH in Figure 4-23. The pilot plant powders exhibit two types of heat stability behaviours. The powders subjected to preheat treatments of ≤ 100°C have similar heat stability/pH curves, with stability increasing markedly as pH is increased from pH 6.5 for commercial powder, pH 6.7 for the control, 72°C/30s, and 80°C/30s powders, and pH 6.8 for the 90°C/30s and 100°C/30s powders. All of these solutions had a maximum heat stability of about two hours. The three pilot plant powders subjected to preheat treatments > 100°C had similar heat stability/pH curves, with stability increasing gradually as pH is increased from pH 6.6. The maximum heat stability of these solutions was about ten minutes. It is interesting that no gradual transition in heat stability appears to exist as the preheat-treatment of the powders increases: powders with heat treatments of 100°C or less have

a maximum heat stability of two hours while those with more severe heat treatments have a maximum stability of 10 minutes.

The shape of the heat stability profiles is typical of a type B milk i.e. there is an absence of a maximum and minimum at around pH 6.6 and 6.9 respectively. Sweetsur and White (1974) showed that milks with a type A heat stability profile at 150°C could progressively become type B as the heating temperature was decreased to 130°C. However, based on the reduced calcium and phosphate levels in MPC85 one would expect to still see a type B curve at higher temperatures as it is known (van Boekel *et al.*, 1989) that for skim milk, below a critical level of soluble calcium and phosphate a type B curve is found. Conducting heat stability tests for MPC85 at higher temperatures would therefore be a useful future experiment and would provide valuable information for processes that have higher temperature processing steps.

When the HCT/pH curves are compared in terms of whey protein denaturation (%), refer section 4.2.1.2, it is found that the powders showing a marked increase in HCT with increases in pH have WDN values of between 0 and 86.18% whereas powders showing only a gradual increase in HCT have WDN values of >90.42%. These data suggest that the heat stability of MPC85 is relatively insensitive to preheat treatments resulting in WDN values of up to 86%. The data also suggest that a change occurs in MPC85, subjected to preheat treatments of between 86 and 90 %WDN, which causes a dramatic change in HCT (111 min at 86.18% compared to 11 min at 90.42% at a pH of 7.2). It is interesting to note that the sudden change in heat stability behaviour occurring between such a narrow range of whey protein denaturation coincides with the change in salt composition reported in section 4.2.2 and the increase in particle size.

These results contrast with the effect of preheat treatments on the heat stability of skim milk. van Mil and de Koning (1992) gave preheat treatments of 85 - 120°C for 1 - 3min prior to evaporation and spray drying during the manufacture of skim milk powder. They found that improvement in the heat coagulation time of reconstituted skim milk (20% total solids) at 120°C could be achieved by high heat treatment. These authors also reported that increasing the preheat temperature gave a better result than increasing the preheating time.

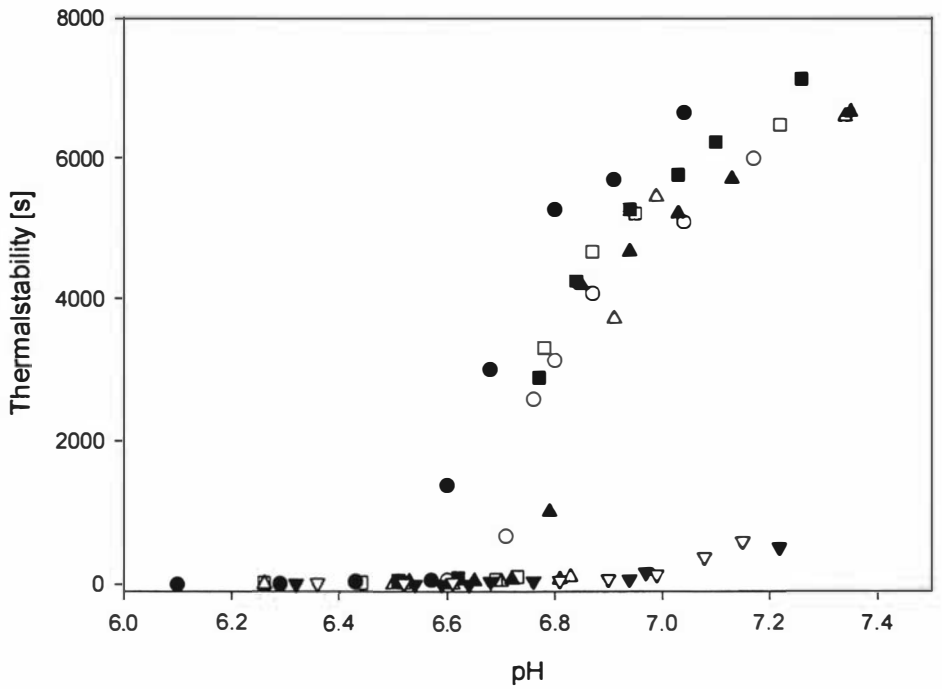

Figure 4-23 Effect of pH on the heat stability at 120°C of 3.5% protein solutions reconstituted from commercial MPC85 powder (●) and pilot plant powders: control, (○); 72°C/30s, (■); 80°C/30s, (□); 90°C/30s, (▲); 100°C/30s, (△); 110°C/30s, (▼); 120°C/45s, (▽); 130°C/30s, (◆).

4.4 Overall discussion and conclusions

The main observations of the effect of preheat treatments during processing on the functional properties of MPC85 may be summarised as follows: The study of the effect of heat treatment on heat stability showed that no loss in stability was observed for WDN values of up to 86%. Heat treatments resulting in 90% WDN or greater showed a dramatic loss in heat stability. The variation in rheology and ability to form a rennet gel among the pilot plant powders were found to be linearly correlated with particle size, while no simple equation could be found to explain the correlation with the degree of whey protein denaturation.

Previous studies have shown that when milk is heated, the whey proteins denature and associate with the casein micelles (Smits and van Brouwershaven, 1980). This has two different effects: first the micelle grows in size (Morr, 1969), and second the interaction between micelles changes. The denaturation and subsequent interaction of whey proteins with casein micelles in milk systems generally reduce the availability of their polar groups to interact with water.

The fact that the variation in functionality with regard to rennetability and apparent viscosity was found to be linearly correlated with particle size indicates that it is the effects of changes in particle size rather than the changes in the interaction between micelles in the MPC85 system that are dominant in these processes. This conclusion is supported by other workers who have shown the importance of size with regard to rennet gelation and rheology. Morr, (1989) found that heat treatments are responsible for the increase in viscosity in milk products primarily by increasing the amount of imbibed water retained within the heat-induced micelle microstructure. Jeurnik and de Kruif (1995), however, concluded that the observed increases in viscosity due to heating skim milk could not be explained by increases in micelle dimensions alone. They thought that the increases in viscosity were caused by clusters of micelles that were only temporary. The rationale behind this was that the attractions between micelles are weak and that heated milk is still a stable colloidal dispersion. Singh and Fox (1989) stated that

heating milk to 90°C produces only minor changes in the dimensions of the casein micelles.

The investigation into the effect of preheat treatment on heat stability was not found to be correlated with the geometry of the particles in solutions of reconstituted MPC85 powders. It may be deduced from this that the mechanism resulting in variation in heat stability is related to changes in the interaction between micelles rather than to changes in micelle dimensions.

However, while the functional properties of rheology and rennetability may be correlated with the dimensions of the particles in the reconstituted solutions, the actual change in particle size is a result of the heat treatments received during processing. The degree of whey protein denaturation is a measure of the extent of heating on the milk system. When the average particle size of the reconstituted solutions was plotted against the degree of whey protein denaturation a gradual linear increase in particle size was found with heat treatments resulting in up to 86% denaturation of the whey proteins. Heat treatments resulting in whey denaturation of 90% or more culminated in a dramatic 5500% increase in the slope of the particle size-whey denaturation curve.

The fact that this huge increase in particle size coincides with a change in the mineral content of the powders and a huge reduction in heat stability suggests that a massive structural change occurs in the casein micelle. It would also seem that magnesium and sodium play a crucial role in this structural change as sodium which was incorporated into the micelle (or whey protein) is displaced by magnesium and subsequently lost through the process of ultrafiltration and diafiltration. The ratio of the sodium displacement by magnesium is two moles to one.

Further research into the area of heating effects on the changes in mineral content of MPC85 powders would be of considerable value. Some interesting research projects include: establishing the type of calcium complexes deposited in the fouling layer and what part these complexes play in a milk system heated in a glass environment i.e. are the complexes which deposit in the fouling layer specific complexes that if formed in a glass environment are bound to the protein in an irreversible manner. It is clear from

other researchers that although the precipitation of calcium is high in glass environments almost all of the calcium re-dissolves on cooling. However, it is not clear what the calcium complexes are that do not re-dissolve. The observations reported here suggest that the calcium complexes that in the glass system are bound irreversibly to the protein are bound preferentially to the heating surface in a metal environment. If these complexes were bound to the protein in a stainless steel system one would expect to see a higher calcium content in the high heat treated pilot plant powders.

5. Effect of compositional differences between reconstituted milk protein concentrate and skim milk powder on their functional properties

5.1 Introduction

As discussed in section 3.9 one of the major findings of the this work is that, contrary to initial expectations, solutions of reconstituted MPC85 behave quite differently from reconstituted skim milk powder combined to the same protein concentration. It was thought that the reasons for the observed differences in behaviour lay, at least partly, with differences in heat treatment during processing and/or with compositional differences. The effect of heating during processing was examined in the preceding chapter. The aim of the work reported in this chapter was to investigate the effect that the compositional differences between skim milk and MPC85 have on the following functional properties; renneting, heat stability and apparent viscosity, with a view to identifying the components or interactions of components that significantly contribute towards the observed differences in functional properties.

It was decided that the best way to isolate the components or interactions of components that are responsible for differences in functional properties would be to conduct a full factorial design experiment using the commercial MPC85 as a base. Details on factorial design may be found in most text books on experimental statistics (e.g. Bhattacharyya and Johnson, 1977; Box *et al.*, 1978; Bethea *et al.*, 1995; Frigon and Mathews, 1997). The attraction of the factorial design experiment was that it would enable components and interactions of components to be identified that have a significant affect on functionality. However, due to the fact that each component is only examined at a high (concentration of component in skim milk) and a low level (concentration of component in MPC85) there is the potential to not pick up on significant interactions at intermediate component concentration levels. Consequently the results of the factorial design experiment will only identify whether a particular component or interaction of components has a positive or negative effect on the functional property in question. It will not determine how slight variations might affect the functional property.

In addition to elucidating the reasons for differences in functional properties between skim milk and MPC85 the information obtained from the factorial design experiment is likely to be important in a number of applications incorporating MPC85. The factors in the experiment are common ingredients in MPC applications.

5.2 Materials and methods

5.2.1 Materials

NaH_2PO_4, $CaCl_2.2H_2O$, $MgCl_2.6H_2O$, Na-azide, and NaCl (Analar grade)

Lactose monohydrate (Technical grade)

Commercial MPC85 (Batch Cypher CF19. Anchor Products - Hautapu)

Commercial Low Heat Skim Milk Powder (Batch J4627. Kiwi Milk Products Ltd - Hawera)

5.2.2 Experimental design

The results of the factorial analysis in this chapter have been analysed using a Yates Algorithm, refer Box *et al.*, (1978). The Yates Algorithm is one of the most important methods for analysing the data from a factorial design experiment allowing significant factors and interactions to be identified, and is well suited to using a spreadsheet software package. A letter is assigned to each component, known as a Yates Code, to enable the identification of the component in the Yates Analysis. Combinations of components are then written using each of the assigned letters for each of the components that are present in their "high" levels, that make up the combination in question; for example in this work a solution labelled as "ACE" would be commercial MPC85 with added phosphate (A), magnesium (C), and lactose (E). In the case where no components are added to the MPC85, the solution is labelled "1".

The main compositional differences, which are studied in this section, between MPC85 and skim milk are shown, with their assigned Yates Code in Table 5-1. In hindsight the

level of citrate should also have been included as citrate is known to be an important component in the chemistry of milk products.

Table 5-1 Composition of reconstituted (3.5 % protein) skim milk powder and MPC85 powder

Factor	Yates Code	Skim Milk	Commercial MPC85
Total inorganic P [mmole/kg]	A	19.70	10.40
Total Ca [mmole/kg]	B	32.44	24.65
Total Mg [mmole/kg]	C	3.49	0.98
Total Na [mmole/kg]	D	12.97	0.81
Lactose (% w/w)	E	5.10	0.22

The salt composition levels for skim milk and MPC85 were used as the "high" and "low" levels respectively for each factor. In the case of lactose the "high" level was taken as half the lactose concentration of skim milk. The reason for this was that, at the temperature apparent viscosity was measured at (15°C), lactose has a solubility limit of 19g/100g. To get sensible apparent viscosity measurements on the Haake VT500 it was necessary to have solutions with a protein concentration of 17.5%. On that basis an MPC85 solution reconstituted to have the same lactose level as an equivalent skim milk solution with a protein concentration of 17.5% would possess a lactose:water ratio of 31.2g/100g. Taking into account the water bound by the dissolved MPC and any salts the ratio of lactose to free water would likely be higher. Further to this while the rate of mutarotation from the α to the β form of lactose is rapid the actual growth of crystals is slow as it is impeded by β-lactose, refer section 1.5. Hence it would be difficult to gauge exactly what percentage of lactose was fully dissolved, and the α-lactose crystal size would vary with time. It is widely known that insoluble particles have a significant effect on the apparent viscosity of solutions. The relationship of insoluble particles to their effect on apparent viscosity is related to particle size. To isolate the effect which soluble lactose has on the rheology of MPC85 from the effects brought about by mutarotation and subsequent crystal formation, and therefore the introduction of time-temperature dependency, the "high" lactose level was taken as half the lactose level in skim milk i.e. a lactose:water ratio of 15.6g/100g. This lower lactose level would also facilitate the making of the solutions. Aliquots of a bulk lactose solution could be added

to an MPC85 solution which would contain a known amount of lactose. This would not be possible if a saturated lactose solution were used.

With five variables of interest and two possible levels for each variable the experiment is described as a 2^5 factorial design experiment. Consequently 32 solutions were made so that each of the possible combinations of variables could be examined for their functional properties.

5.2.3 MPC85 solution preparation

Commercial MPC85 (60 g) was dissolved in MilliQ water (190 g) at 50°C and mixed by overhead stirrer for 1 hr. The MilliQ water had enough Na-azide dissolved in it to give an azide concentration 0.02% w/w of the final MPC85/salt/lactose solution. For the solutions required to have lactose the MPC was dissolved in 200g of lactose solution (37.5g lactose, 162.5 MilliQ Na-azide solution).

The desired salt solutions were added drop-wise into the MPC solutions. The salt solutions were prepared by dissolving the required amount of salt in MilliQ water to give a total mass of 10g. The pH of the phosphate solution was adjusted to about pH 7.0 with 2 M NaOH.

Following the addition of the desired salt solutions the MPC solutions were weighed and MilliQ water was added to give a protein:water ratio of 0.22 and then homogenised at 200 bar using a single stage Niro benchtop homogenizer.

To eliminate the confounding effect of phosphate and sodium it was necessary to have a minimum base Na level determined by the amount of Na added when the phosphate is added. This, however, decreased the range of Na under study from 0.811 - 12.974 mmole/kg to 9.3 - 12.974 mmole/kg. Hence 9.3mmol/kg NaCl was added to every solution in which phosphate was not added, including the solution with no added components, i.e. Yates Code "1".

For completeness, solutions consisting of reconstituted commercial low heat skim milk powder and commercial MPC85 with no additives were made and tested. The results of these two solutions are presented in section 5.4.

5.2.4 Apparent viscosity

The pH values of the undiluted solutions prepared in section 5.2.3 were adjusted to pH 7.00 and left to equilibrate for half an hour. The solutions were then placed in a Haake VT500 viscometer set to 15°C and left to equilibrate for 10 minutes. NV and MV1 geometries were used depending on the viscosity of the solution. Following equilibration an up/down shear sweep experiment was conducted over the shear rate range of 10 to 450 s^{-1}. The apparent viscosity measurements were carried out at 15°C to ensure that the solutions were within the sensitivity of the viscometer. The viscosity profiles were determined in duplicate and agreed within ± 4 %.

5.2.5 Heat stability

The reconstituted MPC85/salt/lactose solutions prepared as outlined in section 5.2.3 were diluted with MilliQ water containing 0.02% Na-azide to a protein concentration of 3.5%. The heat stabilities of these solutions were determined by the method described in section 4.3.6. These heat stabilities were largely determined just once. However, in some circumstances it was deemed necessary to determine the heat stability at more pH values than the original six, to clearly define the heat stability pH profile. In some cases the glass tube exploded when immersed in the glycerol oil requiring the heat stability at that particular pH to be repeated.

5.2.6 Rennet coagulation properties

The reconstituted MPC85/salt/lactose solutions prepared as outlined in section 5.2.3 were diluted with MilliQ water containing 0.02% Na-azide to a protein concentration of 3.5%. The pH values of the diluted solutions were adjusted to 6.5 to 6.6. The solutions were left to equilibrate for 10 min and rechecked and had their pH adjusted if necessary.

10ml of each solution were placed in the wells of the Formagraph heating block. The samples were allowed to heat up to the set temperature (32°C) on the heating platform as measured by a thermocouple. 160 μl of 1:100 rennet solution was placed in the set of spoons and then the rennet on the spoons was added to the samples and stirred. The heating block was placed immediately into the Formagraph and measurements were begun. All samples were tested in duplicate and agreed within ± 5 %.

5.3 Results and discussion

5.3.1 Apparent viscosity

None of the shear sweep curves obtained from the viscosity experiments showed any sign of hysteresis indicating that time dependency was not significant. For some solutions, with low viscosities, there was a degree of noise observed at low shear rates. The noise was more apparent on the up curves than the down curves. The data from the down curves was used in the fitting of a power law model and the models had correlation coefficients in the range 0.994 to 1.000. The results of the power law model fitting are shown in Table 5-2.

A Yates analysis was conducted to determine which factors and interactions of factors significantly affect apparent viscosity. The apparent viscosity data for this analysis was calculated from the power law models shown in Table 5-2. The shear rate used in the calculations was $450s^{-1}$.

The significance of factors and interactions of factors was determined at a 95% level. The mean sum of the squares error for the analysis was estimated by pooling the sum of squares associated with all the four and five factor interactions on the assumption that these higher order interactions would measure differences arising principally from experimental error. These conditions were used for all the Yates analyses reported in this chapter. The factors and interactions that were identified as significantly affecting the apparent viscosity of MPC85 are shown in Table 5-3.

The data in Table 5-3 may be used to predict apparent viscosity by application of equation 5-1 (Box *et al.*, 1978. p334). Comparing the difference in the mean sum of the squares based on the application of equation 5-1 and the sum of the squares based on the difference between the average apparent viscosity and the observed apparent viscosity a correlation coefficient was calculated of 0.984.

5-1 $$\hat{Y} = \overline{E} + \left(\frac{E_1}{2}\right) x_1 + \left(\frac{E_2}{2}\right) x_2 + \left(\frac{E_3}{2}\right) x_3 + \ldots$$

where $\hat{Y}$ = estimate of parameter, $\overline{E}$ = average of all effects, E_n = the n^{th} significant effect, and x_n = -1 or +1 depending on whether the corresponding significant effect is at a low or high level respectively.

The factors having the greatest influence over the apparent viscosity of MPC85 are calcium (B), phosphate (A), and the calcium-phosphate interaction (AB). Together these three factors can explain 66.7% of the total variation observed in apparent viscosity among the solutions. The influence each of these factors has on apparent viscosity is in line with what one would expect from the literature. Roy and Yadav, (1978) reported that addition of phosphate to whole milk resulted in a progressive increase in apparent viscosity. They attributed this increase to the preferential binding of phosphate to the calcium and magnesium resulting in the removal of these cations from the casein micelles. Elimination of these cations from casein micelles is likely to lead to hydrogen bond formation and hydration of the micelles causing the increase in apparent viscosity (Roy and Yadav, 1978). The rationale of Roy and Yadav is supported by the evidence of Holt and Muir (1978) who found that variations in the average size of casein micelles in milk collected from creamery bulk silos over a period of 16 month period were positively correlated with the amount of inorganic phosphate and negatively with casein bound calcium. From the arguments developed in section 4.3.4 it appears that viscosity is positively correlated with micelle size.

The negative effect of the interaction of calcium and phosphate (AB) may be related to the binding properties of the micelle. Creamer and Yamashita (1976) studied the role of phosphate in casein micelle structure by looking at the effect of inorganic phosphate on

calcium caseinate aggregation. They concluded that phosphate binds to the calcium caseinate altering the net charge of the caseinate aggregates to favour micelle formation and to favour compact micelles with a low solvation. The more compact micelles would have a negative effect on apparent viscosity.

It is interesting that lactose (E) was shown to have a negative effect on apparent viscosity. This is contrary to Roy and Yadav (1976) who found that lactose addition to whole milk gave rise to a significant increase in apparent viscosity. The discrepancy in these findings is probably related to the higher lactose and mineral levels in the milk studied by Roy and Yadav coupled with the presence of fat. Indeed, due to the high initial level of calcium in whole milk compared with MPC85, the data of Roy and Yadav is more equivalent to the interaction of calcium and lactose in these experiments. When compared in this way the results are comparable. The positive influence of the interaction of lactose and calcium on apparent viscosity is probably due to the formation of a lactose-cation complex. Significant binding of Ca^{2+} by lactose is well established (Domovs and Freund, 1960; Herrington, 1934). The formation of such a complex would reduce the amount of calcium available to bind with the micelle. With less bound cations to hinder hydrogen bond formation and consequently hydration, the micelle is able to bind more water and so has a greater size than if only calcium were added to the system. The increase in size would contribute to an increase in apparent viscosity. In addition to the binding of calcium, lactose would be expected to favour association of moleculary dispersed caseins and presumably, the formation of large micelles over small ones (Arakawa and Timascheff, 1982). Mozersky *et al.*, (1991), however observed a decrease in micelle size with lactose addition to artificial micelles with a Ca concentration of 40mM. A decrease in particle size would result in a decrease in apparent viscosity. The mechanisms responsible for the negative effect of lactose on apparent viscosity are not known.

As stated in the methodology for this chapter the concentration of lactose was chosen so that it would be fully soluble. The rationale for this decision was that at higher concentrations some lactose would mutarotate into its α-form which is insoluble in water. It was thought that the presence of the crystallised α-lactose would increase

apparent viscosity as solid particles are known to disrupt the flow of liquid and so increase the resistance to shear. Baucke and Sanderson, (1970) showed that age-thickening with storage in concentrated UHT milk is associated with the crystallisation of lactose in the concentrate.

The observations from these experiments suggest that lactose may act in different ways depending on whether it is present in its α- (insoluble) or β- (soluble) form. To verify this hypothesis a second factorial design experiment would need to be performed with a higher level of lactose.

To determine how the factors and interactions of factors affect the rheology of MPC solutions in a more specific manner a Yates analysis was performed on each of the power law coefficients. The results of this analysis are shown in Table 5-4 and Table 5-5. The percentage of the total variation accounted for by the significant factors and interactions for the Yates analysis totals 87.1 and 91.4 % for the flow behaviour and consistency indices respectively.

The consistency index of a power law model is generally related to the apparent viscosity i.e. in general, if a solution has a higher viscosity than another solution it will possess a higher consistency index. Consequently one would expect factors and interactions that increase apparent viscosity to influence the consistency index in the same manner. This relation is exactly what is observed when the signs of the effects from Table 5-3 and Table 5-5 are compared. It would seem logical to assume that the mechanisms that increase apparent viscosity are the same as those affecting the consistency index.

It is interesting to note that the only significant effects influencing apparent viscosity that do not affect the consistency index, AC and ABD, explain 0.7 and 0.5 % respectively of the total variation in apparent viscosity. It is likely that the variation introduced by the power law modelling has resulted in a decrease in the significance of these effects.

When the consistency index of a power law model is plotted against the flow behaviour index for solutions differing in viscosity one generally finds that the flow behaviour index is negatively correlated with the consistency index. Extrapolating this generalisation to the factors affecting the power law coefficients one would expect a factor that has a positive effect on the consistency index to have a negative effect on the flow behaviour index. This relationship holds true for data determined from this experiment.

Table 5-2 Apparent viscosity ($450s^{-1}$) and Power Law coefficients calculated from non-linear regression analysis of the shear sweep curves (10 to 450 s^{-1}) at 0.22 P:W ratio, and 15°C.

Yates Code	Apparent Viscosity [mPa.s]	Flow behaviour Index	Consistency Index [$Pa.s^n$]	Correlation Coefficient
1	36.82	0.821	0.110	0.997
A	162.94	0.548	2.578	1.000
AB	26.27	0.944	0.037	0.998
ABC	21.71	0.896	0.041	0.996
ABCD	18.76	0.998	0.019	0.996
ABCDE	21.52	1.012	0.020	0.998
ABCE	17.20	1.002	0.017	0.997
ABD	27.66	0.876	0.059	0.998
ABDE	27.41	0.960	0.035	0.997
ABE	22.30	0.988	0.024	0.997
AC	158.46	0.591	1.928	0.998
ACD	44.01	0.420	1.522	0.998
ACDE	71.97	0.743	0.346	1.000
ACE	87.33	0.675	0.636	1.000
AD	134.32	0.607	1.482	0.999
ADE	20.68	0.888	0.041	0.968
AE	63.13	0.751	0.289	0.999
B	14.37	0.946	0.020	0.994
BC	14.44	0.964	0.018	0.995
BCD	14.74	0.901	0.027	0.994
BCDE	25.07	1.037	0.020	0.998
BCE	27.28	1.021	0.024	0.997
BD	19.48	0.895	0.037	0.996
BDE	17.53	1.049	0.013	0.998
BE	30.31	1.013	0.028	0.997
C	24.59	0.854	0.060	0.997
CD	21.90	0.928	0.034	0.997
CDE	16.91	1.031	0.014	0.997
CE	16.61	1.028	0.014	0.997
D	47.35	0.727	0.251	0.999
DE	18.92	0.948	0.026	0.996
E	24.57	0.831	0.069	0.998

Table 5-3 Yates analysis showing significant factors and interactions affecting apparent viscosity ($450s^{-1}$)

Yates Code	Effects	Contribution [%]	P
Average	0.044	---	---
A	0.042	21.8	< 0.0001
AB	-0.040	19.5	< 0.0001
ABC	-0.011	1.4	0.0065
ABE	0.017	3.7	0.0006
ABD	0.0066	0.5	0.0443
AC	0.008	0.7	0.0269
AE	-0.022	6.2	0.0001
B	-0.045	25.4	< 0.0001
BE	0.028	9.9	<0.0001
E	-0.026	8.4	< 0.0001
Unexplained Error		3.1	

Note: The term "Effect" refers to how a particular treatment affects the average effect. The term "Contribution" refers to the percentage error of the total variation about the average effect that is explained by a particular effect. Accordingly the "Unexplained Error" refers to the variation, as a percentage, in the data that is not accounted for by the significant effects. "P" is the probability that the effect could have occurred randomly.

Table 5-4 Yates analysis of factors and interactions affecting the flow behaviour index

Yates Code	Effects	Contribution [%]	P
Average	0.872	---	---
A	-0.131	17.0	0.0019
AB	0.112	12.5	0.002
ABC	0.062	3.8	0.0464
B	0.194	37.4	0.0041
E	0.129	16.4	0.0021
Unexplained Error		12.9	

Table 5-5 Yates analysis of factors and interactions affecting the consistency index

Yates Code	Effects	Contribution [%]	P
Average	0.354	---	---
A	0.620	16.4	0.0021
AB	-0.603	16.0	0.0022
ABE	0.456	9.1	0.0085
AE	-0.462	9.3	0.0081
B	-0.652	18.6	0.0015
BE	0.497	10.8	0.0058
E	-0.505	11.2	0.0053
Unexplained Error		8.6	

5.3.2 Heat stability

The heat stability-pH profiles of the MPC/salt/lactose solutions are shown in Figure 5-1 to Figure 5-3. To compare the heat stability of all the solutions with a view to determining the factors and interactions that significantly affect heat stability there are two possibilities: a) perform a Yates analysis on the heat stability at a fixed pH or b) find a model that adequately fits the heat stability profiles and perform a Yates analysis on the coefficients.

The main disadvantage with method a) was that the result would require interpolation between experimental data points and that the results would only be valid for a specific pH. Method b) had the advantage of being valid over the entire pH range and so making use of all of the experimental data. Therefore method b) was chosen for the analysis.

No reference could be found in the literature with regard to modelling the heat stability profile of milk protein solutions. However, by inspection it was thought that all of the heat stability profiles could be seen as sigmoidal or part of a sigmoidal curve. The modelling of this type of curve has been the subject of many studies in the fields of biology, forestry, and zoology where organisms and plants grow over time (Myer, 1990). Due to their application in the modelling of the growth of organisms and plants

the class of models that fit sigmoidal curves are known as "growth models". Growth models have also received attention in chemotherapy research (Carter *et al.*, 1983).

The most commonly applied growth models include the Gompertz, Logistic, Richards, Von Bertalanffy's, and Weibull models. The main disadvantage of the Richards, Von Bertalanffy's, and Weibull models is that they contain four parameters compared to three parameters for both the Logistic and Gompertz models. Due to the small number of data points in each heat stability curve (approximately six) the prediction of the extra parameter would unduly decrease the degrees of freedom in the predicted model. The main difference between the Logistic and Gompertz models is that the Logistic model is symmetrical about the inflection point whereas the Gompertz model is flexible. It is obvious that the heat stability curves are not symmetrical about their inflection point. Therefore the Gompertz model, equation 5-2, was chosen to model the heat stability data. Seber and Wild (1989) provide a more detailed discussion of the merits of each model.

5-2 $$Y = \alpha\, e^{-\beta e^{-\kappa X}}$$

where Y = heat stability [min] at pH X, and α, β, and κ are constants.

The relationship between the constants and the heat stability profiles are: α = the maximum possible heat stability, $\alpha \exp(-\beta)$ = the initial heat stability or level the curve starts at (so β is a measure of the growth from the start to the end: β = natural log (asymptote/start level)), and pH at inflection = $(1/\kappa)$ natural log (β). The relationships between the Gompertz coefficients and a generalised sigmoidal heat stability curve are illustrated in Figure 5-4.

The Gompertz coefficients for each Yates code solution are shown in Table 5-6. The Gompertz model adequately fitted all of the heat stability curves with correlation coefficients greater than 0.86. A Yates analysis was performed on each set of coefficients and the pH of inflection was estimated. The significant effects determined by this analysis are shown in Table 5-7.

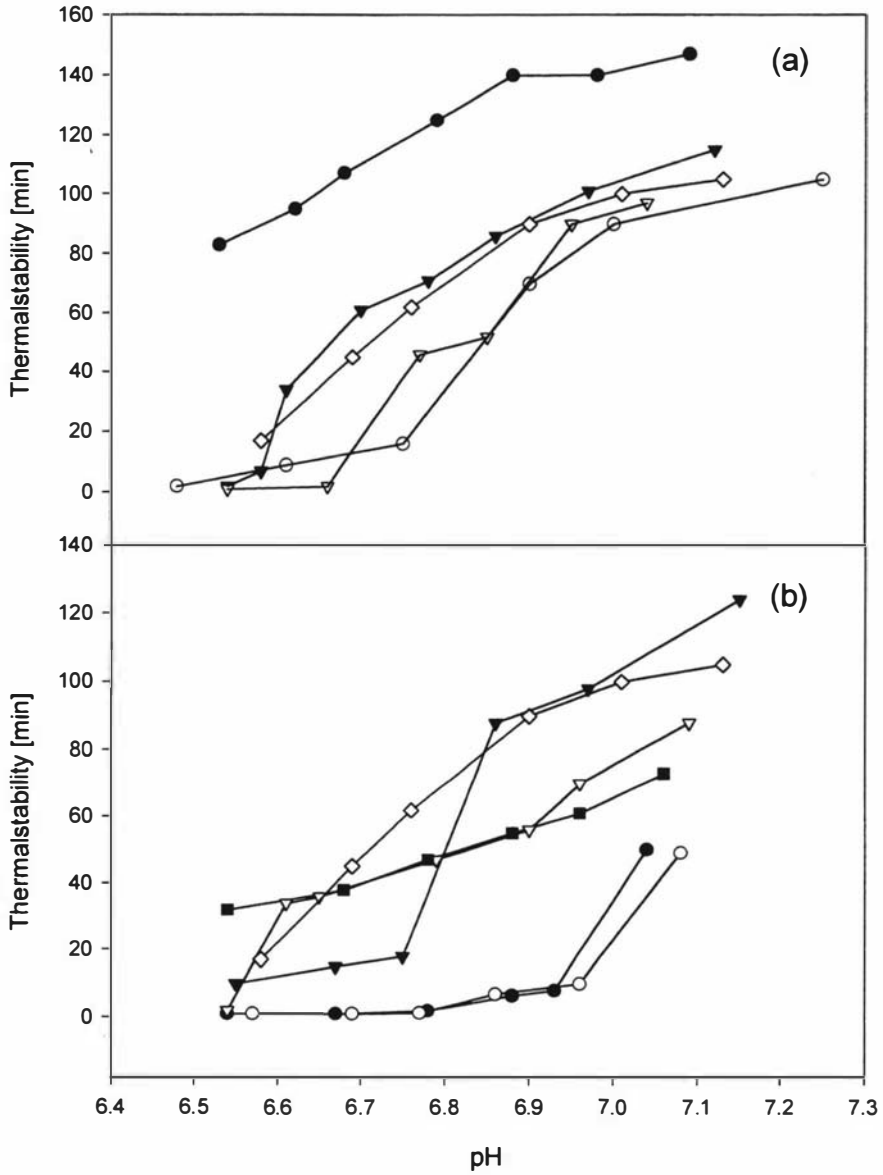

Figure 5-1 Heat stability curves at 120°C for solutions with the following Yates codes (a) "1", (◊); "A", (●); "AB", (○); "ABC", (▼); "ABCD", (▽); and (b) "1", (◊); "ABCDE", (●); "ABCE", (○); "ABD", (▼); "ABDE", (▽); "ABE", (■).

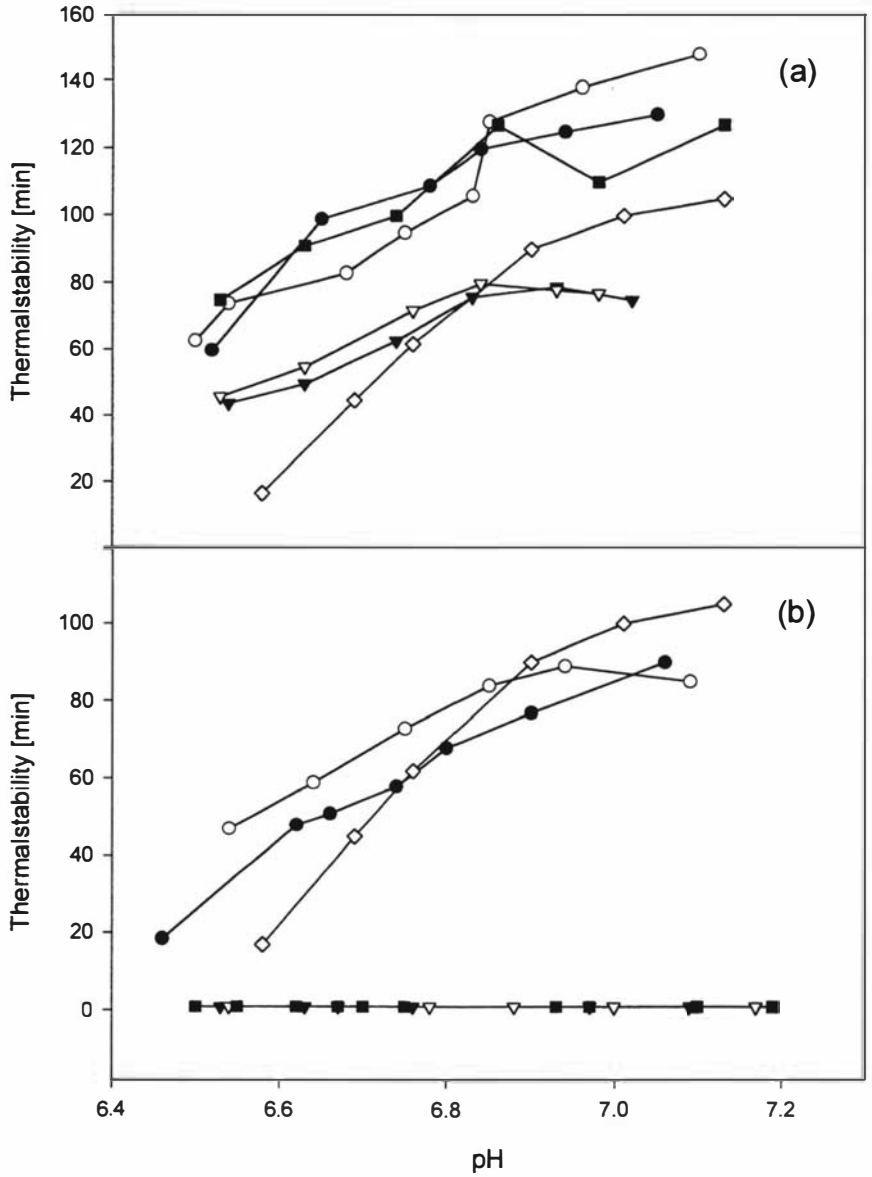

Figure 5-2 Heat stability curves at 120°C for solutions with the following Yates codes (a) "1", (◊); "AC", (●); "ACD", (O); "ACDE", (▼); "ACE", (▽); "AD", (■); and (b) "1", (◊); "ADE", (●); "AE", (O); "B", (▼); "BC", (▽); "BCD", (■).

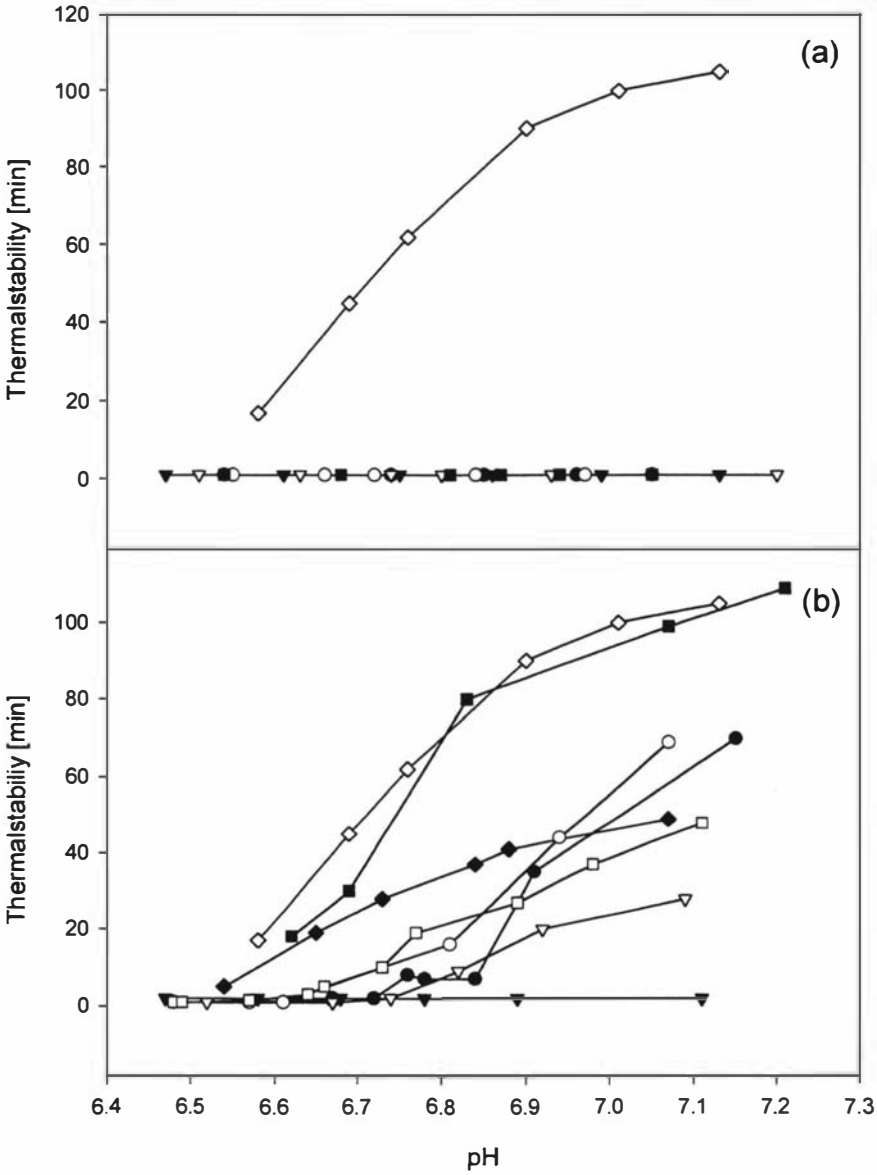

Figure 5-3 Heat stability curves at 120°C for solutions with the following Yates codes (a) "1", (◊); "BCDE", (●); "BCE", (○); "BD", (▼); "BDE", (▽); "BE", (■); and (b) "1", (◊); "C", (●); "CD", (○); "CDE", (▼); "CE", (▽); "D", (■); "DE", (□); "E", (◆).

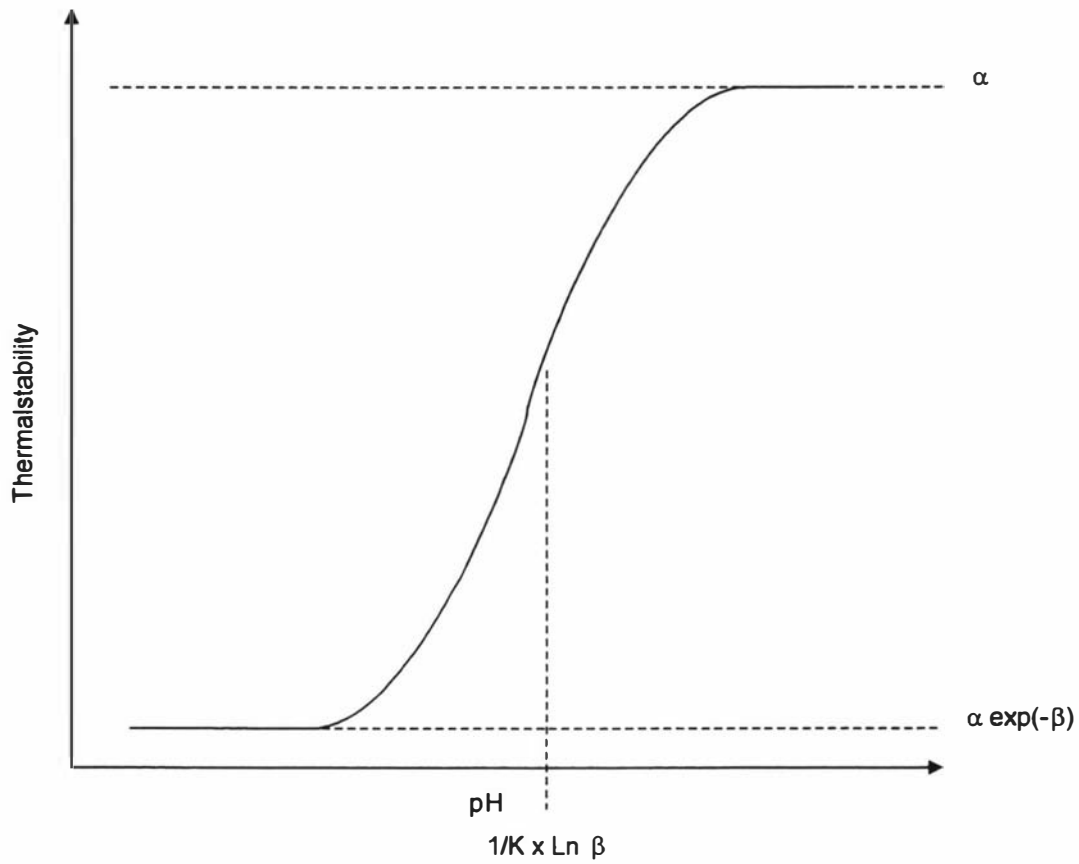

Figure 5-4 Generalised sigmoidal heat stability curve showing relationship with coefficients from Gompertz model.

From the analysis presented in Table 5-7 it appears that no factors or interactions are significant in determining changes in β. The reason for the apparent lack of significant factors or interactions lies with the metric in which the data was originally measured. The model determined by the Yates analysis, equation 5-1, is termed an *additive model.* The model is called an additive model because the effects due to each of the factors are added. However in some situations, particularly if the response covers a wide range (for β, y_{max}/y_{min} = 5.89 x 10^{32}), *non-additivity* occurs wherein the response relationship is multiplicative rather than additive. Non-additivity of this sort is termed transformable as the non-additivity may be eliminated by analysing, for example, the log, square root, or reciprocal of the original data. A full discussion of additivity is given by Box *et al.*, (1978) and Bhattacharyya and Johnson (1977). Taking the natural logarithm of the response data is a common transformation for removing non-additivity. Therefore a Yates analysis of ln β was performed. This analysis appears to be successful in removing the non-additivity and the significant factors affecting ln β are shown in Table 5-8.

The significant effects of the Yates analysis were used to predict the initial observed parameters, reported in Table 5-6, by substituting the values for the average and significant effects into equation 5-1. The predicted values for the parameters were compared with the observed values and found to be reasonably accurate having correlation coefficients of 0.88, 0.70, 0.69 and 1.00 for α, κ, ln β and the pH at inflection point respectively.

However on closer inspection, via a plot of the residuals (Figure 5-5), it was found that all the data points associated with MPC solutions which were unstable across the entire pH range were outliers. Using the residuals of the parameter α as an example these outliers had errors of 430% with the exception of the solution containing magnesium, sodium, and lactose (CDE) which had an error of 1660%. The average error for each predicted data point was 175 ± 116%. The errors quoted above are typical of the magnitude of errors found for the other parameters. The reason for the large apparent errors is the magnitude of the numbers in the unstable solutions; for example solution BDE had an observed α value of 1 and a predicted value of 5.3 and hence an error of

430%. However if the unstable solutions are excluded from the residual calculation then the average percentage error is 16±6% at a 95% level of confidence. The observed values for solution CDE go totally against the predictions and are out by an order of magnitude. Due to the apparently anomalous behaviour of solution CDE, this solution was replicated. The heat stability of this solution was identical to the earlier results. Predictions developed from an experimental design are based on all the possible combinations of the variables and therefore it seems that the combination of magnesium, sodium, and lactose in the absence of phosphate and calcium results in unique interactions that destabilise the MPC system.

The significant factors that influence all the Gompertz parameters are phosphate (A), calcium (B), and a calcium-phosphate (AB) interaction. Together these three factors explain 71.2, 63.1, and 63.3% of the variation observed for α, κ and ln β respectively.

In general all of the heat stability profiles show instability at low pH. As the pH of heating increases the micelles are more solvated (Creamer and Matheson, 1980) and all the proteins carry more negative charge thereby increasing the electrostatic repulsion between micelles and so they are are less likely to coagulate (Creamer and Matheson, 1980; van Boekel *et al.*, 1989). Further, lowering pH also causes Ca^{2+} activity to increase due to partial dissolution of the colloidal calcium phosphate (van Boekel *et al.*, 1989). Ca^{2+} ions screen the ester phosphate groups, so that the electrostatic repulsion diminishes. van Boekel *et al.*, (1989) also thought that the conformation of the C-terminal part of κ-casein is affected by an increase in Ca^{2+} activity resulting in reduced steric repulsion; these segments also impart a high surface charge to the micelles (-20 mV) (Singh and Fox, 1987). These effects of pH and calcium explain why the addition of calcium (B) was found to be detrimental to the heat stability of MPC85 and why, in general, the MPC85 solutions were found to be unstable at low pH.

However, while stability was observed to decrease in all solutions with decreasing pH, not all of the solutions were unstable. The solutions which were observed to be stable at low pH were A, ABE, AC, ACD, ACDE, ACE, and AD. The common compositional factor in all of these solutions is the addition of phosphate. The stabilising influence of phosphate (A) may be explained by its ability to bind calcium ions which leads to lower

levels of ionised calcium in solution (Horne, 1987). From the discussion in section 5.3.1 it was concluded that one of the reasons for the positive effect of phosphate on apparent viscosity was indirectly through increasing the average particle size of the micelles. It is well known that before particles can coagulate, they must encounter each other (Overbeek 1952). The frequency of these encounters is dependent on Brownian motion. Therefore as the average particle size of the micelles, and therefore the viscosity of the solution, increase the frequency of encounters decreases.

Due to the negative influence of calcium and phosphate addition on apparent viscosity and the research by Creamer and Yamashita (1976), (section 5.3.1), it was concluded that calcium and phosphate may result in a smaller, more compact micelle with a lower solvation. van Boekel *et al.*, (1989) noted that smaller micelles exhibit weaker van der Waals attraction. This would also contribute to a decrease in the number of effective collisions between micelles resulting in coagulation.

The results also show that lactose has a negative effect on the maximum stability of MPC solutions, as denoted by parameter α, while a calcium-lactose interaction has a positive effect on heat stability. In the absence of added calcium, the primary actions of lactose are probably its involvement with Maillard reactions with protein and thermal degradation. Singh and Latham (1993) concluded that the polymerisation of milk proteins during severe heating were likely to arise via the Maillard reaction. However, the effect of pH on Maillard browning is significant, with little, if any, browning occurring in solutions of pH 6 or less (Ellis, 1959). The thermal degradation of lactose yields 5-hydroxymethyl-2-furaldehyde and other products such as 2-hydroxyacetylfuran and isomaltol. Fragmentation of these primary degradation products leads to other chemical species, such as formic and lactic acid. The reduction of pH by the formation of acids, especially formic acid, is thought to be the primary reason for the heat coagulation of skim milk (Singh and Fox, 1989). Lactose has also been shown to stabilise β-lactoglobulin against thermal degradation (Park and Lund, 1984) by forming a browning complex which is believed to be antigenic (Otani *et al., 1985).* The heat stability patterns of milk are controlled, in part, by the proportions of surface κ-casein and soluble β-lactoglobulin. β-Lactoglobulin upon denaturation may complex with κ-

casein which stabilises casein micelles. The ability of the β-lactoglobulin-κ-casein complex to stabilise micelles appears to depend on the temperature, pH and Ca^{2+} concentration. At pH values ≥ 6.9 the formation of β-lactoglobulin-κ-casein complexes destabilises casein micelles by promoting the loss of micellar κ-casein, thereby reducing the charge, hydration and stability of the micelles (Singh and Fox, 1987).

To summarise, because lactose stabilises β-lactoglobulin against denaturation, lactose inhibits the formation of the β-lactoglobulin-κ-casein complex. Due to the ability of this complex to stabilise the micelle against coagulation at pH <6.9 and destabilise the micelle at higher pH, lactose addition therefore will destabilise the micelle at pH values <6.9 and promote stabilisation at pH ≥6.9. Any stabilisation of the micelle at pH ≥6.9, via the inhibition of β-lactoglobulin-κ-casein complexes would likely be masked by the destabilising effect of acid production due to the thermal degradation of lactose.

The positive effect of the calcium-lactose interaction on heat stability has been noted by previous authors. Kudo (1980) observed that lactose had a stabilising effect in artificial casein micelle systems, the maximum effect being observed at 20 g/litre lactose. Removal of lactose from milk by dialysis against a synthetic salts buffer reduced the heat stability (Shalabi & Fox, 1982a). It must be remembered that the systems under study by these authors contained calcium at levels equivalent to solutions in this study which had both calcium and lactose added. The stabilising effect of the calcium-lactose interaction is probably due to the ability of lactose to bind free Ca^{2+} (Domovs and Freund, 1960; Herrington, 1934). In this way lactose would act in a manner similar to phosphate addition. The presence of lactose during heating has also been shown to reduce the binding of calcium to whole casein with or without added calcium (Pappas and Rothwell, 1991). These authors suggested that the effect of lactose on decreasing the calcium binding to casein might be explained by a reduction of accessibility of calcium to binding sites as a result of Maillard reaction of lactose with lysine.

Lactose also appears to participate in a three factor interaction with phosphate and calcium resulting in a negative effect on κ and ln β. This interaction may be related to

the catalytic effect of calcium phosphate on the isomerisation of lactose and consequent decrease in the degradation of lactose (Martinez-Castro *et al.*, 1986).

The absence of magnesium from the factors that significantly affect the heat stability profiles of MPC85 is interesting in light of the findings of heat treatment on the mineral composition of MPC85 presented in chapter 4. From the discussion in section 4.2.2 concerning the effects of heat treatment on mineral composition one might have expected magnesium to play a role in determining the heat stability of MPC85. There are two potential explanations for the absence of magnesium:

a) These experiments were conducted in glass tubes which would mean that calcium would not be removed from the system through precipitation onto the heating surface. In this situation the calcium would then be available to interact with micelles more readily than magnesium.

b) The specific binding of magnesium that was shown to occur at temperatures ≥ 110°C in skim milk, (section 4.2.2), also takes place in the MPC85 solutions with added magnesium, but the binding does not have a direct effect on heat stability.

If explanation b) were responsible for the absence of magnesium then this would imply that the loss of functionality observed with the severely heat treated pilot plant powders while correlated with changes in mineral composition, the relationship was not causal.

Table 5-6 Estimated Gompertz coefficients for MPC/salt/lactose solutions

Yates Code	R-sq	α	β	κ	pH at inflection point
1	1	110.66	3.69E+19	6.75	6.67
a	0.983	160.62	5.93E+10	3.86	6.43
ab	0.992	106.37	9.17E+28	9.79	6.81
abc	0.971	113.28	2.67E+22	7.75	6.66
abcd	0.972	112.05	1.36E+23	7.85	6.78
abcde	0.986	149.22	1.48E+25	8.22	7.05
abce	0.975	155.39	1.25E+21	6.84	7.10
abd	0.959	122.55	5.89E+32	11.12	6.79
abde	0.911	131.13	2.82E+08	2.86	6.80
abe	0.941	86.99	5.11E+08	3.04	6.59
ac	0.985	131.5	1.82E+18	6.49	6.48
acd	0.948	237.7	1.79E+05	1.82	6.66
acde	0.937	84.28	9.02E+13	4.97	6.47
ace	0.952	83.28	4.62E+16	5.95	6.45
ad	0.861	128.24	1.86E+13	4.77	6.40
ade	0.999	102.25	3.75E+11	4.05	6.58
ae	0.966	91.28	2.52E+16	5.83	6.48
b	---	1	1.00E+00	1.00	14.00
bc	---	1	1.00E+00	1.00	14.00
bcd	---	1	1.00E+00	1.00	14.00
bcde	---	1	1.00E+00	1.00	14.00
bce	---	1	1.00E+00	1.00	14.00
bd	---	1	1.00E+00	1.00	14.00
bde	---	1	1.00E+00	1.00	14.00
be	---	1	1.00E+00	1.00	14.00
c	0.974	80.63	3.13E+24	8.17	6.90
cd	0.999	105.93	1.09E+17	5.67	6.92
cde	---	1	1.00E+00	1.00	14.00
ce	0.997	30.27	3.17E+30	10.27	6.84
d	0.989	108.09	2.73E+24	8.39	6.70
de	0.991	66.43	1.79E+13	4.45	6.86
e	0.997	52.54	1.77E+18	6.31	6.66

Note: Where the solutions, for a given Yates code, over the entire pH range coagulated almost immediately upon immersion in the oil bath the TSS (total sum of the squares) for the experimental data was zero. Therefore the correlation coefficient, R^2, was not able to be calculated, being indeterminate. The R^2 in these cases is denoted as "---". The pH at the inflection point was calculated from α and κ.

Table 5-7 Significant factors and interactions affecting Gompertz Model parameters

Coefficient	Significant factors and interactions	Effect	Variation explained by factor or interaction [%]	P
α	Average	80.02	---	---
	A	89.47	54.9	< 0.0001
	B	-36.92	9.4	0.0054
	AB	31.65	6.9	0.0108
	E	-30.16	6.2	0.0132
	BE	38.72	10.3	0.0043
	Unexplained Error		12.3	
β	---	---	---	---
	Unexplained Error		100	
κ	Average	4.54	---	---
	A	2.82	16.5	0.0048
	B	-1.89	7.4	0.0269
	AB	4.36	39.2	0.0005
	ABE	-1.71	6.0	0.0390
	Unexplained Error		30.9	
pH at inflection point	Average	4.82	---	---
	A	3.69	37.3	0.0001
	B	-2.81	21.7	0.0005
	AB	3.14	27	0.0003
	Unexplained Error		14	

Table 5-8 Yates analysis of the β data with non-additivity removed by a natural logarithm transformation

Significant factors and interactions	Effect	Variation explained by factor or interaction [%]	P
Average	30.54	---	---
A	18.61	15.4	0.0059
B	-11.94	6.4	0.0370
AB	30.52	41.5	0.0005
ABE	-11.29	5.7	0.0450
Unexplained Error		31	

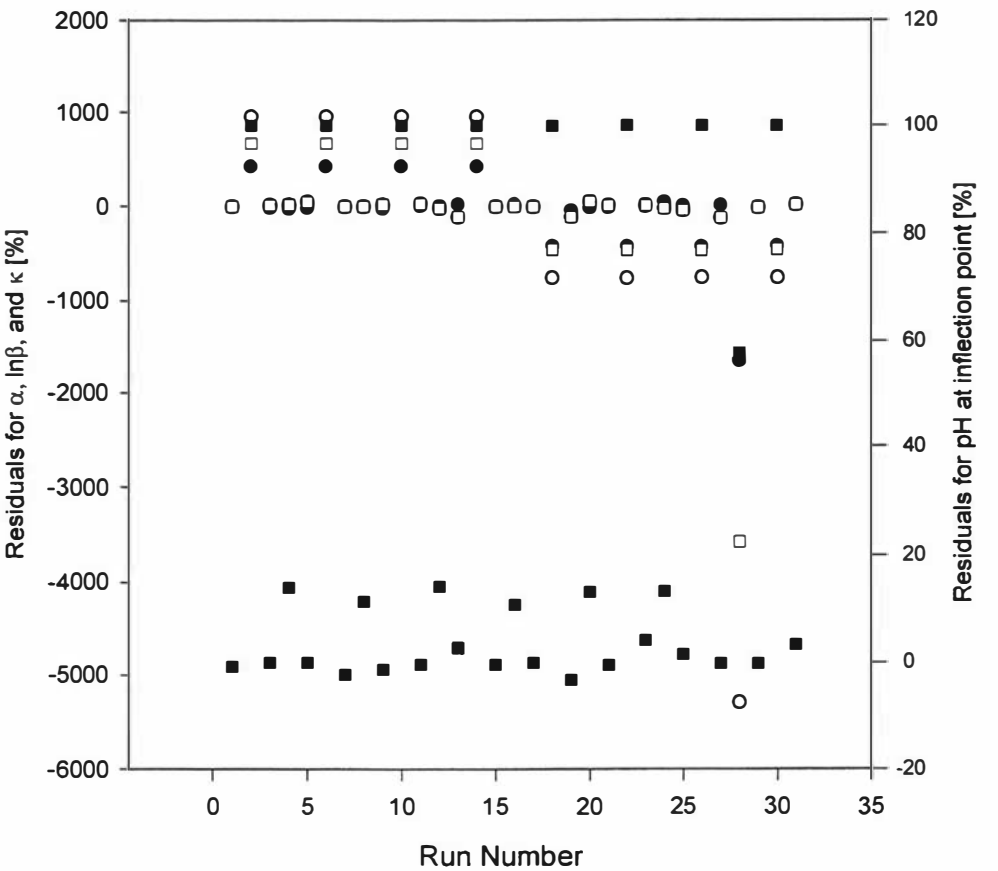

Figure 5-5 A plot of the percentage residuals for the Gompertz coefficients predicted from equation 5-1, for α, (●); κ, (○); ln β, (□), and pH at the point of inflection, (■).

5.3.3 Rennet coagulation properties

The Formagraph method described by McMahon and Brown (1982) measures milk clotting by the movement of small pendulums immersed in linearly oscillating samples of coagulating milk. Recording pendulum movements on photographic paper produces a diagram of firmness versus time. A typical output diagram is shown in Figure 5-6. The clotting time was measured in millimetres with 2mm equal to 1 min. The renneting and gelation times were measured from the Formagraph output and the firming rate calculated according to equation 5-3.

5-3 $$FR = \frac{1}{RT - GT}$$

Where FR = Firming Rate (min^{-1}); RT = Renneting Time (min); and GT = Gelation Time (min).

The results of the Formagraph renneting studies are shown in Table 5-9. By inspection the main feature of the raw data is that all the solutions that contained phosphate in the absence of calcium or magnesium (i.e. A, AD, ADE, AE) either did not form a gel or the gel was too weak for a renneting time to be measured and hence the firming rate to be determined. Of these solutions just the one containing only phosphate (i.e. A) formed a gel.

To perform a Yates analysis there must be data for each Yates code solution. To get around the problem of the absent data there were two possibilities: 1) treat the absent data as missing data and perform a conventional ANOVA or 2) estimate values for the absent data. The major drawback of method 1) was that in treating the data as "missing" one would be ignoring the fact that the combination of phosphate in the absence of calcium or magnesium had a major negative effect on the ability of MPC85 systems to form rennet gels. The potential problem with method 2) was how the estimate for the unknown values should be determined and the sensitivity of the Yates analysis to the estimated values. The "worst" case scenario for the non-gelling solutions was that they would not form a gel given an infinite length of time. By substituting a large number,

$1x10^{17}$, for the absent gelling and renneting data and performing a Yates analysis it was found that only certain effects were significant. The number 1×10^{17} was the largest value that would yield results from the Yates analysis i.e. the upper limit of the computer.

However, by substituting a large number into the analysis y_{max}/y_{min} became large and hence sensitivity was lost as the phenomenon of non-additivity occurred. To overcome the problems associated with non-additivity the substituted values would need to be smaller. It was assumed that all of the effects found to be significant with a large number substituted for the absent values were important. The minimum value that the substituted value could be was 45 min, as it was known that the solutions did not gel within this time frame. Therefore the substituted number was minimised with the constraints that the effects found to be significant with a large substituted number remained significant and that the substituted value was ≥ 45 min. The minimum number that could be substituted while fulfilling these constraints was 45.0 and 56.6 min for gelling time and renneting time respectively.

For analysis of the firming rate the extreme case is a firming rate of zero. To minimise y_{max}/y_{min} the substituted value was maximised with the constraint that all the effects found to be significant at the extreme case were to remain significant. The maximum value that the firming rate could possess based on these assumptions and constraints was 0.0113. The significant results of the Yates analysis based on the data and estimated data is shown in Table 5-10.

Predictions of the renneting properties of the MPC solutions based on the significant effects and equation 5-1 possessed correlation coefficients of 0.95, 0.93, and 0.86 and average errors of 16±5, 13±4, and 18±8 % for the gelation time, renneting time, and firming rate respectively.

The prediction for the gelation time of solution A, 40.11 min, is significantly different from the observed time, 23 min. The -71% error between the predicted and observed gelation time is a unique case. Based on all the combinations of the design experiment solution A should not gel (i.e. possess a gelation time of >45 min). As such the data for

this solution was replicated. The results of this experiment confirmed the earlier result. The observed gelation time of 23 min impacts through the Yates analysis on all the predicted effects of phosphate (A) resulting in predictions of 40.11 min for the solutions that did not gel rather than 45 min.

In the previous two sections the factors that were found to have the most significant effect on rheology and heat stability were phosphate (A), calcium (B), and a calcium - phosphate interaction (AB). These factors were also found to be significant in affecting the gelation properties, (Table 5-10), of MPC85. The importance of these factors in relation to renneting is expected. The coagulation of milk by the enzyme rennet is essentially a 2-stage process. First, the κ-casein which stabilises the casein micelles is hydrolysed. This stage is then followed with the rennet altered micelles being precipitated in the presence of Ca^{2+} ions. These two processes occur simultaneously during the later stages of the enzymatic reaction. This description of renneting is an over-simplification. A full treatment of the subject of the enzymatic coagulation of milk is given by Dalgleish (1992).

A minimum amount of calcium is needed to initiate the second stage of the gelation process (van Hooydonk *et al.,* 1986). The fact that the MPC85 solution with no additives (Yates code "1") formed a gel demonstrates that MPC85 does possess the minimum calcium concentration required for rennet gelation to occur.

The significant influence of calcium (B) on the renneting of MPC85 by reducing the renneting and gelation time and increasing the firming rate of the formed gel is consistent from observations concerning skim milk (van Hooydonk *et al.,* 1986; Marshall and Green, 1980). Dalgleish, (1983) established that the addition of Ca^{2+} to milk accelerates the overall clotting process, principally because of the effect on the aggregation stage of the reaction. The effect of calcium on the aggregation stage probably results from a neutralisation of negative charge within the micelles, diminishing the charge repulsion and allowing hydrophobic interactions to occur.

The negative influence of phosphate (A) is probably due to the binding of calcium by phosphate. The evidence that the solutions containing phosphate in the absence of added

calcium or magnesium did not gel suggests that the added phosphate may bind sufficient quantities of calcium to make the second stage of rennet coagulation unlikely.

The fact that solutions with added phosphate and calcium or magnesium gel implies that enough calcium (divalent ions) is unbound to allow the second stage of gelation to continue. A removal of calcium bound to the casein micelle increases the overall negative charge on the micelle and thus increases the electrostatic repulsion. The increase in the protein charge even if it does not lead to dissociation of the micelles would cause a weakening of the bonds, measurable as a decrease in gel strength (Horne, 1998). The somewhat surprising positive interactions, i.e. shorter times, of phosphate and calcium; and phosphate and magnesium will be discussed later.

Magnesium (C) is known to behave in a similar manner to calcium (van Hooydonk *et al.*, 1986; Green, 1982), with the ability to form complexes with both phosphate and casein. Magnesium would therefore be expected to be involved in similar interactions to calcium.

The magnitude of the effect of magnesium on gelation properties is less than that of calcium and calcium interactions. On the surface this is what one would expect as the concentration of magnesium (2.52mM/kg) added to the MPC85 solutions was less than added calcium (7.79mM/kg). One might also expect magnesium to have less effect than calcium due to the stronger affinity of calcium for casein and phosphate than magnesium. van Hooydonk *et al.* (1986) found that calcium and barium were more effective than magnesium and manganese in promoting the aggregation process of the converted micelles. Therefore, based on the relative concentration of added magnesium and its lower affinity for casein one could expect an effect which was at most one third (2.52/7.79) that of calcium. However the effect of magnesium is two thirds (-7.31/-11.25), and three quarters (-10.80/-14.30) that of calcium for gelling time and renneting time respectively. The effect on firming rate is almost identical to that of calcium (0.0471/0.0467).

The expectation of the behaviour of magnesium addition relative to calcium is based on the assumption that the beneficial effects of calcium addition increase linearly with

increases in calcium concentration. However Bringe and Kinsella (1986) found that increases in the addition of $CaCl_2$ produced progressively less effect on the aggregation of para-casein micelle. van Hooydonk (1986) reported that addition of $CaCl_2$ (up to 1.8 mM) to milk causes no change in the enzymatic rate. Bringe and Kinsella (1986) suggested that if specific ionic interactions, in addition to those involving calcium, are important in the coagulation of para-casein micelles as suggested by results from chemical modification studies (Hill, 1970; Kaye and Jolles, 1978) then one might expect $CaCl_2$ to inhibit para-casein micelle aggregation i.e. calcium would preferentially bind to sites that would otherwise form interactions with other species. Bringe and Kinsella (1986) also showed that the degree of hydrolysis of κ-casein at the gelation time markedly decreases with increases in $CaCl_2$ resulting in fewer sites on the para-casein micelles which can form crosslinks. These possible $CaCl_2$ effects may be counterbalancing the positive effect of $CaCl_2$ on para-casein micelle aggregation. The implication of the work of Bringe and Kinsella (1986) for this study is that if the beneficial effects of divalent cation addition increases non-linearly then it is plausible that the relatively small addition of magnesium would result in disproportionately large effects in the gelation parameters.

The negative effect of the calcium and magnesium interaction is surprising. The reason for this effect is not known but may be related to the additive nature of equation 5-1. In situations where calcium and magnesium are present together the equation adds the positive effect that each factor has on the gelation parameters. If the increases in the addition of divalent cations produces progressively less effect on the aggregation of para-casein micelles then equation 5-1 would overestimate the combined effect. The negative effect of the calcium-magnesium interaction counteracts this overestimate. The raw data in Table 5-9 supports this explanation.

The positive effect of the phosphate-calcium and the phosphate-magnesium interactions is an extension of this explanation. For example, in a solution with added calcium and phosphate the model subtracts the negative effect of phosphate and adds the positive effect of calcium. However following the non-linear effect of divalent cation addition the added calcium would have a larger effect than would be accounted for by merely adding and subtracting the effects of calcium and phosphate alone. The Yates analysis

identifies this "anomaly" and so the phosphate-calcium and phosphate-magnesium interactions are found to be positive.

The negative effect of lactose (E) and of the lactose-calcium interaction on the firming rate is interesting. The literature does not seem to deal with the effects of lactose on renneting. Work has however been carried out on the effects of sucrose on renneting and these studies bear parallels with the negative effect of lactose in this investigation. Famelart (1994) concluded that the addition of sucrose to skim milk results in a substantial retardation of both enzymatic and aggregation steps. These results are consistent with the involvement of diffusion-controlled steps in the sequence of reactions. Pearce (1976) reported that sucrose increased the time required for both enzymic and coagulation phases of rennet action. If lactose has a similar behaviour to sucrose then the above explanation would account for the negative effect of lactose.

Famelart (1994) also showed that sucrose decreased the amount of dissolved calcium. Lactose has also been shown to bind significant amounts of Ca^{2+} (Domovs and Freund, 1960; Herrington, 1934). The reduction of free calcium would retard the aggregation of para-kappa casein micelles for the reasons discussed earlier. However, it should be noted that the work of Geerts *et al.*, (1983) found that the addition of sucrose to skim milk resulted in an increase in the calcium ion activity.

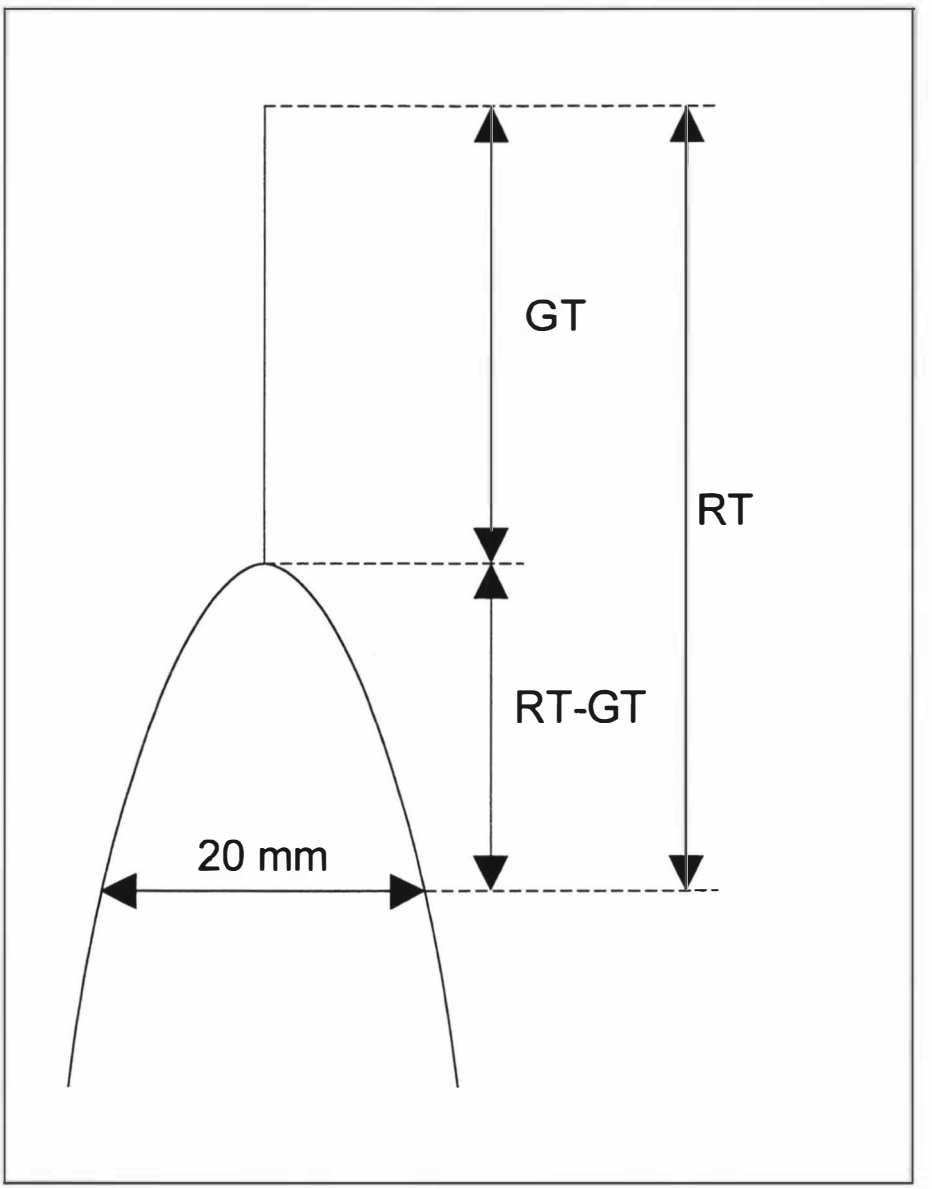

Figure 5-6 Diagram of coagulation and curd firmness as a function of time as recorded with the Formagraph. RT = Renneting time (min); GT = Gelation time (min) and 1/(RT-GT) = Firming rate.

Table 5-9 Raw data from Formagraph renneting studies

Yates Code	Gelation Time [min]	Renneting Time [min]	Firming Rate[min^{-1}]
1	17.375	25.75	0.1194
A	22.75	---	---
AB	11	17.5	0.1538
ABC	8.75	13.5	0.2105
ABCD	10.5	15	0.2222
ABCDE	9.25	16.25	0.1429
ABCE	8.5	16.25	0.1290
ABD	14.125	22.5	0.1194
ABDE	10.5	17	0.1538
ABE	19.5	30	0.0952
AC	17.75	25.625	0.1270
ACD	17.5	26.25	0.1143
ACDE	20.25	32.875	0.0792
ACE	21.75	33.625	0.0842
AD	---	---	---
ADE	---	---	---
AE	---	---	---
B	7.5	12.5	0.2000
BC	7.5	13	0.1818
BCD	9.25	14.833	0.1791
BCDE	10.5	19.25	0.1143
BCE	8.25	16	0.1290
BD	10.125	15.625	0.1818
BDE	8	16.75	0.1143
BE	11.25	22.75	0.0870
C	12.75	18.25	0.1818
CD	17	23.5	0.1538
CDE	11	18	0.1429
CE	11	33.625	0.0442
D	16.25	23.875	0.1311
DE	15.5	26.25	0.0930
E	10.625	16.5	0.1702

Note: Data labelled as "---" were not achieved.

Table 5-10 Significant results for Yates analysis of renneting parameters.

Parameter	Yates Code	Effect	Contribution [%]	P
Gelling Time	Average	15.78	---	---
	A	8.44	16.9	0.0006
	B	-11.25	30.0	0.0001
	AB	-6.13	8.9	0.0033
	C	-7.31	12.7	0.0013
	AC	-5.44	7.0	0.0058
	BC	4.75	5.3	0.0106
	ABC	4.13	4.0	0.0192
	Unexplained Error		15.2	
Renneting Time	Average	24.87	---	---
	A	11.74	19.0	0.0003
	B	-14.30	28.3	0.0001
	AB	-9.42	12.3	0.0011
	C	-10.80	16.1	0.0005
	AC	-7.55	7.9	0.0033
	BC	6.49	5.8	0.0067
	ABC	5.11	3.6	0.0188
	Unexplained Error		7.0	
Firming Rate	Average	0.1276	---	---
	A	-0.0391	11.9	0.0045
	B	0.0467	17.0	0.0019
	AB	0.0440	15.1	0.0025
	C	0.0471	17.3	0.0018
	AC	0.0271	5.7	0.0221
	BC	-0.0216	3.6	0.0500
	E	-0.0372	10.8	0.0056
	BE	-0.0232	4.2	0.0393
	Unexplained Error		14.3	

5.4 Functional properties of MPC85 and low heat skim

The aim of the work presented in this chapter was to identify the components or interactions of components that contribute towards differences in functionality between MPC85 and skim milk. To place the results of these experiments in the context of the original objective the Yates code solutions, "1" and "ABCDE", were compared qualitatively to their 'real world' equivalents: MPC85 and LHS respectively. The solutions tested here were made up to the same protein:water ratio. The renneting, rheological, and heat stability of these solutions are shown in Table 5-11, Table 5-12, and Figure 5-7 respectively.

Table 5-11 The renneting properties of milk protein solutions (0.0365 protein:water ratio; 32°C; pH 6.5-6.6)

Property	Yates Code "1"	MPC85	Yates Code "ABCDE"	LHS
Gelation Time [min]	17.38	12.5	9.25	21.75
Renneting Time [min]	25.75	17.25	16.25	34.63
Firming Rate [min^{-1}]	0.1194	0.2105	0.1429	0.0776

Table 5-12 The flow properties (20 to 450s^{-1}) and apparent viscosity (450s^{-1}) of milk protein solutions (0.2073 protein:water ratio; 15°C; pH 7.0)

Property	Yates Code "1"	MPC85	Yates Code "ABCDE"	LHS
Consistency Index [$Pa.s^n$]	0.110	0.120	0.028	0.063
Flow Behaviour Index	0.821	0.788	0.959	0.945
Correlation Coefficient	0.997	0.999	0.999	0.999
Viscosity [mPa.s]	36.20	32.10	21.38	44.70

By inspection the heat stability, apparent viscosity and flow behaviour of the Yates code "1" and MPC85 solutions are quite similar. This indicates that the 9.3 mmole/kg added sodium present in the Yates code "1" solution does not have a significant affect on these properties. From Table 5-11 it is apparent that the addition of sodium has a detrimental effect on the renneting properties of MPC85.

By comparing the functionality of LHS with the Yates solution "ABCDE" one can see that these solutions are very different. The cause of these observations may be either

purely compositional, in which case the higher citrate and lactose levels may account for the differences; and/or it maybe that the structural arrangement of minerals in LHS is different to that in MPC85 and hence simply adding minerals back to MPC85 will not result in a LHS-type functionality. The high stability of LHS above pH 6.9 compared to the Yates solution "ABCDE" indicates, based on the discussion in section 5.3.2, that the higher lactose level in LHS may be the cause of the increased stability. The higher citrate levels in LHS would also contribute to the increase in stability through the formation of complexes with calcium.

Overall these results show that sodium may be more important in controlling the renneting properties of MPC85 than indicated by the Yates analysis and that MPC85 can not be 'turned' into a product with LHS-type functionality by simply adding phosphate, calcium, magnesium, sodium to levels found in LHS and adding lactose to half the level found in LHS.

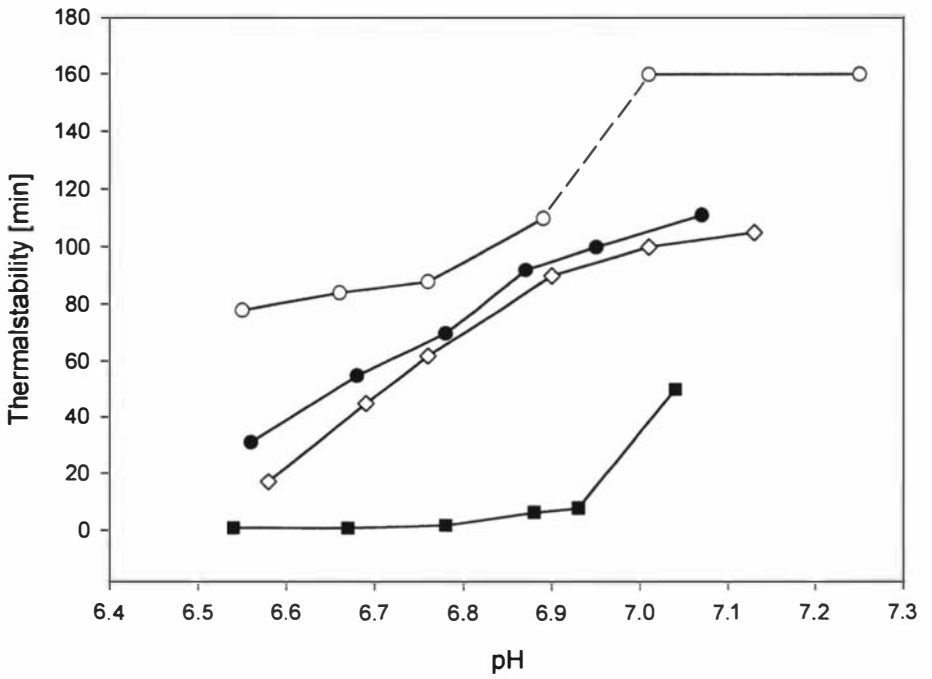

Figure 5-7 Heat stability curves at 120°C for solutions (0.0429 protein:water ratio) of MPC85, (●); LHS, (○); Yates code "1", (◊); and Yates code "ABCDE", (■). Note the solution labelled as Yates code "1" contains 9.3 mmole/kg added sodium. The LHS solutions with pH >6.9 were stable at the time the experiment was halted (160 min).

5.5 Conclusions

This work has identified the components and interactions of components which play a significant part in determining the functionality of MPC85. This information will be useful in optimising product formulations particularly with regard to clinical formulations. The key characteristics of this type of product are low viscosity; and heat stability at sterilisation time/temperature combinations (Zwijgers, 1992). Isocal® complete liquid diet tube-feeding formulation (Mead Johnson Nutritional Division, Indiana, USA) is typical of such products. Isocal® has calcium and sodium caseinates as its primary protein source and has the following mineral composition per 355ml can (product label of): calcium 220 mg, phosphorous 188 mg, iron 3.4 mg, magnesium 75 mg, copper 0.38 mg, zinc 3.8 mg, chloride 380 mg, potassium 470 mg, and sodium 188 mg. The results of the factorial design experiment would be helpful in the reformulation of this type of product with MPC85 as the primary protein source as they show how many of these minerals significantly affect viscosity and heat stability.

The factorial design experiment has also highlighted the complexity of the reactions that determine functional properties. In particular the design experiment has identified possible non-linear interactions between components. Therefore, to gain a full understanding with ability to make accurate predictions on functional behaviour it is necessary to determine the relationship between the identified significant components and interactions at levels intermediate to the simple "high" and "low" concentrations studied here.

The study of the effect of lactose addition at the level found in skim milk concentrates on the rheology of MPC85 concentrates would be an interesting area for further research. It is plausible that the rate of α-lactose crystal formation in concentrated MPC85 solutions would introduce a time-dependent quality to apparent viscosity. However, as stated in section 5.2, there would be difficulties in determining what the ratio of α to β lactose would be and also the rate of crystal formation.

As mentioned in the method section the effect of citrate was neglected in this study and its potential effect on functionality warrants examination.

Several of the discussions pointed towards affecting functional properties indirectly via affecting the size of the casein micelles. It would be interesting to measure the correlation between particle size and these interactions to confirm the suggested mechanisms.

The effect of storage and warming on the factors and interactions that significantly control the functional properties of MPC85 would also be of interest for further study. Roy and Yadav (1978) found that storage and forewarming altered the effect of salt and lactose addition to whole milk on apparent viscosity.

It is possible that sodium plays a more significant part in explaining the difference between MPC85 and skim milk and that this is masked by the narrow range of sodium being investigated. The effect of sodium may also be masked by variations in sodium levels due to pH adjustment. In particular it has been shown (section 5.4) that sodium may be more important in controlling the renneting properties of MPC85 than indicated by the Yates analysis.

In summary this work has demonstrated the usefulness of factorial design experiments in tackling the complexities of interactions influencing functional properties. Further to this MPC85 as a substrate has shown potential for facilitating the study of the casein micelle without the need for complex methods for producing artificial micelles. The study has also demonstrated techniques for modelling heat stability - pH profiles and thereby allowing comparison of the entire profiles of different solutions rather than comparisons at just single pH values.

In terms of the objectives of this chapter, while many of the components that control the functionality of MPC85 have been highlighted, it has also been shown that MPC85 cannot be 'turned' into a product with LHS-type functionality simply by adding the components focused on here to their levels found in LHS. The information gained will however be useful in the development of formulations which have MPC85 as an ingredient.

6. Effect of compositional differences between reconstituted milk protein concentrate and solutions consisting of sodium caseinate and whey protein concentrate on their functional properties

6.1 Introduction

Having examined the effects which compositional differences between MPC85 and skim milk have on the functionality of MPC85, the next logical step was to investigate the compositional differences between MPC85 and "simpler" milk protein products such as caseinate and whey protein concentrate. The major difference between products such as caseinate and total milk protein and the newer products such as MPC and micellar casein is that in the newer products the casein is complexed with calcium, phosphate and citrate in the casein micelle.

The nature of the micelle complex has been studied by manipulating the mineral environment of caseinate solutions so that micelles are formed. Such micelles are known as artificial micelles. Artificial micelles may be formed merely by the addition of calcium to sodium caseinate solutions. Micelle formation has been reported to occur at calcium concentrations of 7.3 mg/g (Carr, 1994) and 8 mg/g protein (Mulvihill and Fox, 1983). Inorganic phosphate (P_i) is contained in casein micelles in bovine milk as a constituent of micellar calcium phosphate, which plays an important role in maintaining the structure of casein micelles. Horne (1982) reported that P_i induced aggregation of α_{s1} casein below the normal critical calcium concentration. This suggests that P_i also affects the formation of casein micelles. Aoki (1989) reported that the incorporation rates of individual casein constituents into casein aggregates cross-linked by CCP were in the order α_{s2}- > α_{s1}- > β- casein.

In the main, studies of artificial micelle systems have been conducted with the aim of elucidating the structure of micelles *in-vivo.* There does not appear to be much published literature using the artificial micelle system as a means to examine functional properties of milk especially with regard to rheology and renneting. In the current work

the artificial micelle system has been used to examine the effect of mineral differences between MPC85 solutions and caseinate/WPC solutions on the functional properties of heat stability, rennetability and rheology. The variables tested in these experiments together with their levels in commercial MPC85 (3.5% protein) and an equivalent solution (casein:whey ratio) made from commercial sodium caseinate and whey protein concentrate (WPC) are shown in the table below.

Table 6-1 Composition of reconstituted (3.5% protein) MPC85 powder and an equivalent solution (casein:whey ratio) made from sodium caseinate and whey protein concentrate

Factor	Yates Code	Na-caseinate/WPC	Commercial MPC85
Total inorganic P [mmole/kg]	A	8.619	10.40
Total Ca [mmole/kg]	B	0.650	24.65
Total Mg [mmole/kg]	C	0.159	0.98
Total Lactose [mmole/kg]	E	1.704	6.106

Note: sodium was omitted from this design as the concentration of this species in a 3.5% protein caseinate/wpc solution (17 mmole/kg) was higher than in MPC85 (1.41 mmole/kg) i.e. it was not possible to have a "low" sodium level.

6.2 Methods

6.2.1 Solution preparation

The method of producing each of the protein solutions required was based on the methods of Schmidt *et al.* (1977) and Knoop *et al.* (1979). Both of these methods have become standards for preparing artificial micelles from sodium caseinate (Schmidt *et al.* (1977) method: Schmidt and Koops, 1977; Schmidt, 1979; Kudo, 1979; McLean and Schaar, 1989. Knoop *et al.* (1979) method: Zhang and Aoki, 1995a; Zhang and Aoki, 1995b). The method described by Knoop *et al.* 1979 is similar to the Schmidt *et al.*(1986) method with the main differences being that: the inorganic solutions were added directly to the caseinate solution rather than adding both the inorganic solutions and the caseinate solutions to distilled water; and the method of addition was drop-wise

by pipette rather than continuously at a fixed rate by pump; and the pH of the artificial micelle solution was adjusted to pH 6.7 after the solution had been made rather than by continuous adjustment throughout mixing.

In the method used in these experiments, a solution of artificial micelles was prepared by the drop-wise addition of a whey protein concentrate/sodium caseinate solution (6.3% w/v caseinate), and the various inorganic salt and lactose solutions (adjusted to pH 6.7) required to make up the desired composition of the final solution, to 200 ml of milli-Q water held at 37 °C. The minerals used in the experiment were all analytical grade except for the lactose which was technical grade. The additions were carried out with vigorous stirring (stirrer dimensions: three blades; blade diameter 0.06 m; motor speed: 500 rpm) and the pH of the solution was continuously monitored and maintained at pH 6.7. After the addition of all of the constituents required for a particular run, the solution was mixed for a further 30 min, with the addition of 0.1 N NaOH as necessary to maintain a pH of 6.7. This was to allow for the equilibration of the dissolved salts which tend to result in a decrease in pH (Schmidt *et al.*, 1977).

The solutions produced by the above method were investigated for their renneting and heat stability properties in the same manner as described in section 5.2.6.

The differing compositions of the solutions dictated by the experimental design meant that micelle formation would only occur in some of the solutions. Therefore, it was deemed necessary to measure the particle size distribution to determine in a quantitative manner the degree of micelle formation in each solution. The particle size analysis was performed using the method described in section 4.3.2. The only difference in this analysis was that not all of the solutions contained micelles. Only the solutions with added calcium had formed micelles. Therefore two buffers were used as diluents: a calcium imidazole buffer for micelle containing solutions and a sodium imidazole buffer for solutions where micelles had not formed. The possibility that micelles might form, disintegrate, or change size while in the diluted state was checked by performing five consecutive analyses every four minutes and determining if the average particle size varied with time. The average particle size for all solutions was found to be stable.

A sample of four solutions were diluted in pure water to see if it was possible to use water as a diluent for all the solutions. The initial readings from these measurements were within the standard deviation of the average particle sizes measured using the imidazole buffers but it was found that the particle size increased constantly with time. The presence or absence of micelles was determined by inspection: micelle forming solutions were white and solutions without micelles were translucent. Both buffers had an ionic strength of 0.03.

An attempt was made to manufacture concentrated artificial micelle solutions, 13.6% w/w casein (the equivalent casein concentration of a 20% w/w MPC85 solution), for use in the rheological study, by using the same method as described above with the exception that the concentration of the caseinate/WPC solution and salt solutions were increased to give the desired concentrations in the final solution. However, it was found that solutions produced in this manner were unstable and resulted in lumps of coagulated material

The problem of producing a concentrated solution of artificial micelles was solved by preparing a micelle solution as described above and concentrating it in a rotary evaporator. The rotary evaporator was operated at 30 °C and the vacuum maintained by a water vacuum. It was found that if the operating temperature exceeded 30 °C excessive foaming occurred. A major limitation of concentrating artificial micelle solutions by rotary evaporator was that as the solution was concentrated a point was reached where the system precipitated. The concentration point at which precipitation occurred varied depending on the composition of the solution being concentrated. For the purpose of producing concentrated solutions for the rheological study all the solutions needed to be of equal protein concentration. The maximum concentration that could be safely achieved for all the solutions was found to be 9.2% w/w casein.

The total solids of the concentrated solutions were determined gravimetrically and the solutions were then diluted so that they all had a concentration of 9.2% w/w casein. The rheological behaviour of the concentrated solutions was examined by an up-down shear sweep over the range of 0 to 1000 s^{-1} at a temperature of 15 °C. The shear sweep was conducted with a Haake VT500 Rheometer using an NV1 geometry.

6.3 Results

An analysis of the data from these experiments did not yield satisfactory results. The heat stability data showed that all the solutions with added calcium coagulated within a minute of immersion in the oil bath, whereas all solutions with no added calcium were stable for greater than 2 hours at 120 °C. It was expected that phosphate would have a stabilising effect on the system. The fact that phosphate did not stabilise the micelle system indicates that micelles resembling the structure of MPC85 micelles were not formed.

An analysis of the rheological data, including fitting a power law model and performing a Yates analysis on the coefficients, was conducted. No hysteresis was present in any of the flow curves and all the solutions were found to be Newtonian, which was probably due to the low protein content (section 3.6).

The Yates analysis only identified calcium as being a significant factor contributing to apparent viscosity. This was hardly surprising as calcium is responsible for micelle formation which is known to have a dramatic effect on the viscosity of milk protein solutions (Carr, 1994). The effect of calcium however only accounted for 39.5% of the variation. The Yates analysis of the particle size data showed that calcium was the only significant factor and accounted for 84% of the variation in particle size.

It was thought that perhaps the extreme effect that calcium was having on the system might have masked the effects of the other components. To test this hypothesis the effect of calcium was removed by adding the response of the calcium containing solutions to their corresponding solution which did not contain calcium i.e. solution "1" was added to "B", "A" was added to "AB" and so on. This process is known as *blocking* and results in the *confounding* of the calcium effect. Further details on blocking and confounding are given in Box *et al.*, (1978). However, the results of this analysis showed that none of the other components contributed to the variations in the response among the solutions.

It was plausible that the other components acted in different ways in the micelles than in the non-micelle system with the result that their effects were being cancelled out. To explore this option a Yates analysis was performed on only the solutions which contained added calcium. This analysis too did not show any of the other factors to contribute significantly to the rheological properties of the solutions.

Only solutions with added calcium formed a gel on the addition of rennet. This result was expected and confirms the report of Zittle (1970) that the addition of calcium to casein solutions caused micelle formation in the system, without which the solutions were water clear and rennet addition produced no visible changes. Of the solutions which did gel only those that also had phosphate present formed a gel strong enough for a rennet time and hence a firming rate to be measured. This suggests that a critical level of phosphate is required to enable milk proteins to form "strong" rennet gels. The increase in the renneting ability of artificial micelles with phosphate addition to the level found in MPC85 may be the result of low levels of phosphate stabilising the calcium-binding regions of the casein molecules so that the resulting protein-protein interactions favour micelle formation at a lower ionic calcium concentration (Creamer and Yamashita, 1976). This may allow more calcium to be available to participate in the coagulation of para-κ-casein micelles.

Due to the "missing" data for the solutions that did not gel, the Yates analysis was performed following the method described in section 5.3.3. The Yates analysis of the renneting data highlighted calcium, phosphate, and calcium-phosphate as being significant in reducing the renneting time and increasing the firming rate. Together these variables explained 99, and 98% of the variability associated with the renneting time, and firming rate respectively. Only calcium was found to contribute significantly to the variability of the gelation time, accounting for 79% of the variation. The significance of these factors may be explained by the mechanism discussed above.

As mentioned earlier, only the solutions with added calcium formed micelles. A particle size analysis of these solutions showed average micelle size in the range of 153 to 170 nm. A Yates analysis of only these solutions did not identify any factors that contributed significantly to this variation. The particle size of the solutions which did not form

micelles were found to have a range of 196 to 204 nm. It was believed that this result was probably an artefact resulting from extended casein monomers "touching" each other to give scattering equivalent to a 200 nm particle. This view is supported by a comparison of the polydispersity with that of a micelle forming solution (0.4 compared to 0.2). The polydispersity is an indication of the spread of the size distribution and may have a value of between 0 and 1.

6.4 Conclusions

The complete instability of all the solutions containing calcium and phosphate to heat suggest that "true" micelles might not actually be formed by the method used here. While the method of micelle formation was based on established methods (Schmidt *et al.* 1977 method: Schmidt and Koops, 1977; Schmidt, 1979; Kudo, 1979; McLean and Schaar, 1989. Knoop *et al.* 1979 method: Zhang and Aoki, 1995a; Zhang and Aoki, 1995b) the equipment available for this work meant that the addition of solutions and pH adjustment was conducted manually rather than with automated equipment. It is likely that the de-automation of the methodology for manufacturing artificial micelles is responsible for the questionable data gained from this study.

With this in mind it would be premature to make conclusions regarding the effects of these components in explaining the differences between caseinate-whey and MPC85 solutions.

7. Summary and conclusions

7.1 Introduction

The original aim of this thesis was to study the rheological properties of some of the newer milk protein products such as total milk protein, micellar casein and in particular milk protein concentrate. While there is much published literature available on the rheology of skim milk concentrates, whole milk concentrates and caseinates, there is considerably less information available about these newer products.

Initially it was assumed that milk protein concentrates would behave in a rheologically similar manner to skim milk concentrates, particularly with regard to thickening. In the manufacture of skim milk powder thickening of the concentrate prior to spray drying has been cited as a major cause of problems during pumping and spray drying (Baldwin *et al.*, 1980). High apparent viscosity of skim milk concentrate prior to spray drying has been correlated with poor solubility and functional properties (Hayashi and Kudo, 1989; de Vilder *et al.*, 1979).

On this basis a rheological study was conducted of milk protein concentrate (MPC85) prior to spray drying at Anchor Products - Hautapu, and on solutions of reconstituted MPC85 powder. These results showed that the earlier assumption of MPC85 possessing a similar rheological behaviour to skim milk was clearly wrong. The focus of the thesis then shifted to examining potential reasons for the observed differences in rheology: heat treatment during manufacture and compositional differences. For this part of the thesis other functional properties were investigated as well as rheology: heat stability and renneting.

The effect of heat treatment during manufacture was investigated by manufacturing MPC85 on a pilot plant scale with preheat treatments applied prior to UF and DF operations. The composition of the pilot plant powders was not affected by heat treatments resulting in up to 86% whey denaturation. Powders with higher levels of denatured whey proteins (≥90%) had a higher magnesium and a lower sodium content. The ratio of sodium loss to magnesium gain was 2:1.

The average apparent diameter of casein micelles of the reconstituted solutions increased gradually with the degree of whey protein denaturation (WDN) up to 86%. The rate of change in apparent diameter of casein micelles with WDN increased by 5500% for powders with greater than 90% WDN.

The compositional differences between MPC85 and skim milk were investigated by a factorial design experiment. The results of the factorial design experiment were shown to provide useful information to enable manufacturers and end users to manipulate their processes/formulations to improve product characteristics, particularly with regard to clinical formulations (refer section 5.5).

The remainder of this general summary is divided into three sections discussing each of the functional properties. Following the general summary the main conclusions and recommendations for future work are presented.

7.2 Functional Properties

7.2.1 Rheology

The rheological analysis of commercial milk protein concentrate (MPC85) (20-22% protein) prior to spray drying clearly showed that unlike skim milk concentrate no measurable thickening occurs at evaporator temperatures (52°C). Evidence was actually found to show that milk protein concentrate exhibits a slight age thinning behaviour which lasts about one hour, after which the apparent viscosity of the concentrate remains constant. Reconstituted concentrated commercial MPC85 powder (17.5% protein) and powders manufactured with preheat treatments resulting in up to 92.30% whey protein denaturation were also shown to have constant flow properties on holding at 52°C. It was thought that the absence of age-thickening in MPC85 might be due to the lower salt concentrations in MPC85 compared to skim milk as the mechanism believed to be responsible for age-thickening is related to the precipitation, on heating, of salts on the surface of the casein micelle resulting in destabilisation, aggregation and hence an increase in viscosity (Muir, 1980; Singh and Newstead, 1992).

The rheology of reconstituted concentrated MPC85 solutions (20% w/w) were however found to be affected by cold storage (5°C) over a period of days. The apparent viscosity and degree of pseudoplasticity of MPC85 increased with storage at 5°C, but this effect was reversed by heating the solution to 50°C for half an hour. Based on the work of Singh *et al.*, (1997) and Larsson *et al.*, (1995) it was concluded that the rheological changes resulting from cold storage were probably due to dissociation of β-casein from the micelle.

A comparison of the viscosity of reconstituted MPC85 with skim milk over a range of protein concentrations (0 to 18% w/w) showed that skim milk has a higher viscosity than MPC85. However, when compared on a basis of protein-to-water ratio no significant difference in viscosity was observed. This result implies that the other components that are included in the calculation of protein concentration i.e. salts and lactose, have little effect on the overall viscosity.

If the age-thickening data is interpreted on the basis of protein:water ratio rather than protein concentration then an MPC85 solution with a 17.5% protein concentration is equivalent to a skim milk solution with a total solids content of 37.2% rather than 47.3%. Concentrated skim milk with a total solids content of 37.2% would show minimal age-thickening at 52°C. The MPC concentrate prior to spray drying with a protein concentration of 20 to 22% would, however, still be expected to show significant age-thickening as these concentrations are equivalent to skim milk total solids of 41 to 44.5%. The absence of age-thickening behaviour in MPC85 is therefore, in addition to the lower salt content, also probably the result of the lower protein to water ratio in MPC85 solutions.

The rheological properties of MPC85 were profoundly influenced by the degree of solubility. The solubility of commercial MPC85 was found to be dependent on the temperature at which the solution was prepared, increasing from 58.85% at 20°C to 100% at 50°C. The solubility of solutions of MPC85 was more sensitive to temperature at lower temperatures. The solubility at 20°C was found to increase from 58.85% to 88.75% if homogenised at 150 bar. Increasing the severity of heat treatment during the manufacture of MPC85 was shown to decrease the solubility of the dried powder.

The rheological properties of reconstituted commercial MPC85 have been characterised. The rheological properties may be broadly classified into two concentration regimes: less than or greater than a protein to water ratio of about 0.11. At low concentrations MPC85 solutions may be described by a Bingham Plastic equation as their viscosity does not vary significantly with shear rate and a yield stress is detected. The yield stress was found to increase with temperature and concentration. At high concentrations the logarithm of apparent viscosity was found to increase linearly with increases in protein concentration. The solutions were found to be adequately described by the Bingham Plastic model with a yield stress being detected at higher temperatures together with a constant apparent viscosity. The yield stresses were however low and only observed at low shear rates i.e. $< 18.30\ s^{-1}$. At lower temperatures MPC85 solutions were pseudoplastic and adequately described by the Power Law model. The degree of pseudoplasticity was found to increase with higher concentrations and lower temperatures. The zone of transition between the two concentration regimes was found to be roughly the same as that for low heat skim milk powder i.e. about 0.11 P:W ratio.

The main effect of preheat treatments during manufacture on the rheology of MPC85 solutions was the increase in apparent viscosity which was found to increase linearly with casein micelle size. The apparent diameter of the casein micelles increased with the severity of the preheat treatment. The variation in apparent viscosity with apparent diameter of casein micelles was found to be greater at low shear rates. A model was proposed to account for these observations (refer Figure 4-18).

The major components significantly affecting the flow properties of MPC85 solutions were: phosphate, calcium, and lactose. Significant interactions between components were: phosphate-calcium, phosphate-lactose, calcium-lactose, phosphate-calcium-lactose, and phosphate-calcium-magnesium. The factors and interactions that significantly affected the consistency index had the opposite effect on the flow behaviour index.

7.2.2 Heat Stability

The main effect of preheat treatments during manufacture on heat stability showed that no loss in stability was observed for treatment resulting in whey protein denaturation values of up to 86% (maximum stability of ≈110min at 120°C). Heat treatments resulting in 90% whey protein denaturation or greater showed a dramatic loss in heat stability (maximum stability ≈10min). The fact that this huge reduction in heat stability coincides with a change in the mineral content of the powders and a huge increase in particle size suggests that a massive structural change occurs in the casein micelle.

The analysis of the factorial design experiment demonstrated techniques for modelling heat stability - pH profiles thereby allowing the quantitative comparison of the entire profiles of different solutions rather than comparisons at just single pH values or qualitative comparisons regarding the shape of the profile.

The major components significantly affecting the heat stability of MPC85 solutions were: phosphate, calcium, and lactose. Significant interactions between components were: phosphate-calcium, calcium-lactose, and phosphate-calcium-lactose.

7.2.3 Rennet coagulation properties

Increases in curd firmness were shown to be correlated with lower levels of whey protein denaturation and smaller micelle size. The development of the complex modulus with time from the onset of gelation was found to follow first order kinetics.

The major components significantly affecting the renneting properties of MPC85 solutions were: phosphate, calcium, magnesium and lactose. Significant interactions between components were: phosphate-calcium, phosphate-magnesium, calcium-magnesium, calcium-lactose, and phosphate-calcium-magnesium.

7.3 Main conclusions

1. MPC85 is rheologically similar to low heat skim in terms of apparent viscosity and concentration. However MPC85 differs from low heat skim in that it does not exhibit age-thickening at high temperatures.

2. Heat treatments prior to UF and DF operations during the manufacture of MPC85 resulting in up to 86% whey denaturation have no significant effect on the mineral composition of the spray dried powder. Powders manufactured with more severe heat treatments have higher magnesium and lower sodium levels.

3. This work has demonstrated the usefulness of factorial design experiments in tackling the complexities of interactions influencing functional properties.

4. The study has also demonstrated techniques for modelling heat stability - pH profiles and thereby allowing the quantitative comparison of the entire profiles of different solutions rather than comparisons at just single pH values or qualitative comparisons regarding the shape of the profile.

5. MPC85 was shown to possess type B heat stability profiles under all of the conditions studied in this work i.e. salt and lactose addition, and heat treatments during manufacture.

6. The study has also determined the sensitivity of the functional properties of MPC85 to variations in heat treatment during processing.

7. Investigations into the effect of compositional differences between MPC85 and skim milk successfully highlighted some of the components and interactions of components that significantly account for differences in functionality.

7.4 Recommendations for future work

1. The work on the rheology of fresh and reconstituted MPC85 concentrate was conducted at 52°C. It would be useful to characterise the rheology of the concentrate at higher temperatures (i.e. 60-70°C).

2. Preheat-treatments during the manufacture of the pilot plant MPC85 powders showed that only magnesium and sodium concentrations of the dried powders are altered. It would be interesting to determine the ion distribution between the serum and colloidal states immediately after preheat-treatment to isolate the effect of ultrafiltration and diafiltration on the mineral composition of the dried powder.

3. The factorial design experiment has highlighted the complexity of the reactions that determine functional properties. In particular the design experiment has identified possible non-linear interactions between components. Therefore, to gain a better understanding with ability to make accurate predictions on functional behaviour it is necessary to determine the relationship between the identified significant components and interactions at levels intermediate to the simple "high" and "low" concentrations studied here.

4. The effect of storage and warming on the factors and interactions that significantly affect the functional properties of MPC85 would also be of interest for further study. Roy and Yadav (1978) found that storage and forewarming significantly altered the effect of salt and lactose addition to whole milk on apparent viscosity. This information would be of particular value to end-users of MPC85 in the development of the control necessary for processing of products.

5. The effect of citrate was not studied in the factorial design experiments and its potential effect on functionality would be valuable future work.

6. Characterising the heat stability profile of MPC85 solutions at higher temperatures (140 to 150°C) would be useful for formulations which are UHT treated.

7. Several of the discussions during the analysis of the factorial design experiment indicated that many of the significant factors and interactions were resulting in changes to the functional properties by affecting the size of the casein micelles. It would be interesting to measure the correlation between particle size and these interactions to confirm the findings.

8. The rennet gelation properties of the solution made from the pilot plant powder with 71.52% denatured whey protein which was left overnight at 32°C was found to have the same curd firmness as powders with lower levels of denatured whey. This indicates that, given sufficient time, the final rheological properties of a gel may be independent of the degree of whey denaturation. Rheological measurements following the rennet gelation of all the powders over a long period of time would confirm this hypothesis.

Appendix A - Statistical Equations

The rheological models reported in this work were fitted using the *Solver* function in the *Microsoft Excel95* spreadsheet software. In all cases the models are fitted using a least squares method. The minimised quantity, the sum of the square of the residuals (*RSS*) is given with each type of model fit below.

The quantity minimised in fitting the mathematical models is given in equation 7-1.

7-1 $$RSS = \sum_{i=1}^{N} (y_i - \hat{y}_i)^2$$

Where RSS = the residual sum of the squares, y_i = the observed response value of condition i, $\hat{y}_i$ = the predicted response value of condition i.

The goodness of fit is measured by calculating correlation coefficient, R^2, defined in equation 7-2.

7-2 $$R^2 = 1 - \frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{N} (y_i - \bar{y})^2}$$

Where R^2 = the correlation coefficient and $\bar{y}$ = the average response value.

Test on mean of Normal Distribution, Variance Unknown (Montgomery, 1985)

Hypothesis:

7-3 $$H_o : \mu_1 = \mu_2$$

where H_0 = null hypothesis, H_1 = alternative hypothesis, μ_1 = mean of population 1, and μ_2 = mean of population 2

7-4 $$H_o : \mu_1 \neq \mu_2$$

Criteria for rejection

7-5 $$t_0 < -t_{\alpha,\nu}$$

where t_0 = calculated test statistic, $t_{\alpha,\nu}$ = tabulated test statistic at s level of confidence and ν degrees of freedom

Test Statistic

7-6 $$t_0 = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample from population 1, $\bar{x}_2$ = mean of sample from population 2, S_1 = standard deviation of sample from population 1, S_2 = standard deviation of sample from population 2, n_1 = size of the sample from population 1, n_2 = size of the sample from population 2

Number of degrees of freedom, V, for t_0

7-7 $$V = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{\left(S_1^2/n_1\right)^2}{n+1} + \frac{\left(S_2^2/n_2\right)}{n_2+1}} - 2$$

Appendix B - Mineral composition of MPC85 powders

Note:

- The mineral analysis was determined by Plasma Emission Spectrometry performed by W. Martin at the ICP Facility, Grasslands Research Centre, Palmerston North.
- The less than sign "<" indicates that the element in that sample had a concentration below the instruments limit of quantification therefore, the concentration given is the dilution corrected lower quantitative limit for that element.
- The samples labelled with the letter "r" were replicate measurements to check on the reproducibility of the changes observed in Na and Mg composition reported in section 4.2.2.
- Unit of measurement: µg / g

Sample	Control	72	72r	80	80	90	100	110	110r
WDN [%]	0	1.3	1.3	48.85	48.85	71.52	86.18	90.42	90.42
Al	3.67	4.69	<3.5	4.59	4.64	6.6	5.5	5.5	<3.5
As	<9.7	<7.8	<1.1	<8.7	<9.5	<9.0	<9.1	<8.0	<1.1
B	12.4	10	<1.1	10	10.9	11.3	10.3	9.7	<1.1
Ca	25250	25100	21262	24650	24300	25950	25050	26950	23797
Cd	<0.97	<0.78	<0.35	<0.87	<0.95	<0.90	<0.91	<0.80	<0.35
Co	<0.97	<0.78	<0.35	<0.87	<0.95	<0.90	<0.91	<0.80	<0.35
Cu	6	5.6	3.87	6.1	5.8	5	5.2	5.8	4.32
Fe	21.5	23.1	19.9	23.5	23.8	20.8	34.4	80	20.8
K	2735	1755	1689	1575	1560	1615	1155	1660	1686
Mg	1050	960	879	978	985	1055	1005	1325	1203
Mn	2.58	2.53	1.8	3.41	3.42	2.77	3.29	5.5	4.4
Mo	<0.97	<0.78	<0.35	<0.87	<0.95	<0.90	<0.91	<0.80	<0.35
Na	1040	1295	1246	1205	1205	1265	1420	554	577
Ni	<0.97	<0.78	<0.35	<0.87	<0.95	<0.90	<0.91	<0.80	<0.35
P	15500	15100	13991	15050	15150	15750	15200	16050	14694
Pb	<4.8	<3.8	<3.52	<4.3	<4.7	<4.5	<4.5	<4.0	<3.50
S	7995	8015	7041	8060	8185	8295	8020	8250	7138
Se	<9.7	<7.8	<3.52	<8.7	<9.5	<9.0	<9.1	<8.0	<3.50
Si	<9.7	<7.8	<148	<8.7	<9.5	<9.0	<9.1	<8.0	<147
Sn	<0.97	<0.78	<0.70	<0.87	<0.95	<0.90	<0.91	<0.80	<0.70
Sr	17.3	14.8	13.2	16.4	16.6	16.3	16.5	28	24.2
Zn	103	98	94.8	105	106	106	106	104	100

Sample	120	120r	130	130r	Commercial
WDN [%]	90.99	90.99	92.3	92.3	13.89
Al	3.71	<3.1	4.29	<3.2	3.3
As	<8.0	<0.93	<7.6	<0.96	<8.5
B	7.8	<0.93	8	<0.96	8.7
Ca	24600	21530	24050	22998	24000
Cd	<0.80	<0.31	<0.76	0.47	<0.85
Co	<0.80	<0.31	<0.76	<0.32	<0.85
Cu	4.9	4.17	6.6	5.58	1.2
Fe	46.2	60.3	23.3	23.2	5.6
K	2045	1994	1405	1438	4200
Mg	1195	1074	1340	1317	950
Mn	5.1	4.4	4.77	4.2	1.37
Mo	<0.80	<0.31	<0.76	<0.32	<0.85
Na	618	624	573	571	791
Ni	3.1	2.24	<0.76	<0.32	<0.85
P	15050	13615	14600	14654	15150
Pb	<4	<3.11	<3.8	<3.20	<4.3
S	7850	6649	7730	7129	7635
Se	<8.0	<3.11	<7.6	<3.20	<8.5
Si	<8.0	<131	<7.6	<135	<8.5
Sn	<0.80	<0.62	<0.76	<0.64	<0.85
Sr	23.5	20.9	20.2	18.2	7.8
Zn	100	96.7	97	99.7	92

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