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103
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Rheology of Sodium Caseinate Solutions

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Abstract

The effects of monovalent and divalent salts on the rheological properties of solutions of a commercially available sodium caseinate, Alanate 180, were studied by shear sweep and oscillatory rheological methods using a Bohlin rheometer at a caseinate concentration of 14 % w/w.

The logarithm of apparent viscosity of sodium caseinate solutions increased linearly with increases in added ionic strength (AIS) at both 25°C and 50°C. Linear regression models were calculated for each monovalent salt. The slope of the regression models for each salt, at a given temperature, followed the Hofmeister series. The increase in apparent viscosity between 0 and 0.2 AIS was proportionately larger than all increases caused by further salt additions.

The apparent viscosity of sodium caseinate solutions increased with divalent salt concentration until a maximum was reached. Increasing the salt concentration above that necessary for maximum apparent viscosity resulted in a decrease in apparent viscosity followed by precipitation. The apparent viscosity of a given solution with or without added divalent salt was an order of magnitude lower at 50°C than at 25°C.

Sodium caseinate solutions with added monovalent salts formed thermo-reversible gels at 5 °C. The effect of NaCl concentration on the gelation characteristics of sodium caseinate solutions was investigated. The gel-sol transition temperature was found to increase with increasing salt concentration.

The effects of salts on the rheological behaviour of sodium caseinate gels and solutions are discussed in terms of water activity, electric

double layers, protein-water interactions and current rheological theory.

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Table of Contents

1	Introduction	1
2	Literature Review	3
2.1	Chemistry of Milk Proteins	3
2.1.1	Casein Proteins	3
2.1.2	Whey Proteins	6
2.2	Rheology of Milk Protein Solutions	7
2.2.1	Rheology of Protein Dispersions	8
2.2.2	Shear Rate Thinning	9
2.2.3	Time Dependent Thinning	10
2.3	Variables Affecting Rheology of Casein Solutions	11
2.3.1	Effect of Protein Concentration	11
2.3.2	Effect of pH	17
2.3.3	Effect of Temperature	18
2.3.4	Effect of Shear Rate	20
2.3.5	Effect of Salt Addition	21
2.3.6	Effect of Chemical Addition	22
2.3.7	Effect of Endogenous Enzymes	23
2.3.8	Variation of Rheological Properties Between Casein Types	23
2.3.9	Discrepancies in Results Between Different Workers	24
2.4	Formulation of the Experimental Programme	24
3	Objectives and Scope	26
4	Experimental Methods	27
4.1	Introduction	27
4.2	Equipment and Materials	27

		v
	4.2.1 Equipment	27
	4.2.2 Materials	28
4.3	Preparation of Caseinate Solutions	28
4.4	Preparation of Caseinate - Salt Solutions	29
4.5	Moisture Analysis	31
4.6	Viscometry Tests	31
	4.6.1 Determination of the Equilibration Time	32
	4.6.2 Determination of the shear rate range	35
	4.6.3 Viscometry Test Set-up	37
4.7	Oscillation Tests	37
5	Characterization of Alanate 180 and Method Development	39
5.1	Effect of concentration on solutions of Alanate 180	39
5.2	Effect of pH on solutions of Alanate 180	41
5.3	The phenomenon of rod climbing in solutions of Alanate 180	45
5.4	Reproducibility of apparent viscosity measurements for solutions of Alanate 180	49
6	Effect of Monovalent Salts on the Rheology of Sodium Caseinate	54
6.1	Potassium Chloride	54
	6.1.1 The effect of potassium chloride on the shear sweep curves	54
	6.1.2 Effect of added potassium chloride on the apparent viscosity of sodium caseinate solutions	55
6.2	Sodium Chloride	61
	6.2.1 The effect of sodium chloride on the shear sweep curves	61
	6.2.2 Effect of added sodium chloride on the apparent viscosity of sodium caseinate solutions	61
6.3	Ammonium Chloride	65

6.3.1	The effect of ammonium chloride on the shear sweep curves	65
6.3.2	Effect of added ammonium chloride on the apparent viscosity of sodium caseinate solutions	65
6.4	Discussion	68
6.4.1	General protein interactions	68
6.4.2	Ionic strength and electrolytes	68
6.4.3	Effect of temperature	73
7	Effect of Divalent Salts on the Rheology of Sodium Caseinate	76
7.1	Calcium Chloride	76
7.1.1	The effect of calcium chloride on the shear sweep curves	76
7.1.2	Effect of added calcium chloride on the apparent viscosity of caseinate solutions	77
7.2	Zinc Chloride	78
7.2.1	The effect of zinc chloride on the shear sweep curves	78
7.2.2	Effect of added zinc chloride on the apparent viscosity of caseinate solutions	78
7.3	Magnesium Chloride	78
7.5	Discussion	82
8	Gelation of Sodium Caseinate Solutions with added Salt	85
8.1	Oscillation experiments	85
8.2	Results	89
8.3	Discussion	93
9	Conclusions and Recommendations	95
9.1	Effect of monovalent salts on the rheology of sodium	

		vii
	caseinate solutions	95
9.2	Effect of divalent salts on the rheology of sodium caseinate solutions	96
9.4	Application of findings to industry	99
9.5	Recommendations for future work	99
10	References	101
	Appendix	108

1 *Introduction*

Caseinates are widely used in various technical applications and formulated foods because of their physico-chemical, nutritional and functional properties. They are produced by neutralization of casein by an alkali such as sodium hydroxide (Southward, 1985). The solution is then generally spray dried. The major problem associated with sodium caseinate production and usage lies in understanding its viscous behaviour, which influences its dissolving time and limits the concentration for spray drying and use in technical and edible applications.

The impetus for this study results from an increasing movement among customers of technical casein to seek out cheaper alternative chemicals. At recent pricing levels, casein is always considered too expensive and is often a major cost component in the formulation (Haigh, 1992).

From a quality and functionality point of view there are still reasons casein is used ahead of other chemicals, although it is not known exactly why casein is better. An example of this is that for adhesives for bottle labelling, casein is the only ingredient that allows the glue to be used for high speed bottle labelling machines e.g. 90000 bottles per hour (bph). Cheap substitutes for casein are available but these lack some of the functional properties of casein. Casein is still therefore used in many applications e.g. a substitute formulation might be useful at a bottling speed up to 30,000 bph.

It is apparent from the literature that the rheological properties of protein solutions are highly dependent on the protein type and the environment of the solvent. An ingredient common to the formulation of both technical

and edible applications, which is known to have a significant effect on the rheology of protein solutions is salt.

The purpose of this study is to elucidate the effect of monovalent and divalent salts on the rheology of caseinate solutions. It is hoped that a fundamental rheological study of casein solutions will contribute to the current understanding of the functional properties of caseins in both technical and edible applications and thus provide a basis for product improvement.

2 *Literature Review*

2.1 Chemistry of 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 shows some of the structural and chemical characteristics of these major milk proteins.

2.1.1 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 leads 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 acidification and neutralization treatments employed for precipitation and resolubilization remove the colloidal phosphate matrix that stabilizes native casein micelles in milk. The resulting protein system consists of polymerized casein subunits 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 phosphate structure of native micelles (Morr CV (1982)).

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 characterized by charged polar domains and hydrophobic domains. Like α_{s1} -casein, sequences in the polar domains, which may approach 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.

Table 1 Some Structural and Chemical Characteristics of Milk Proteins

	Whey			Caseins			
	β -lg	α -la	BSA	α_{s1} -	α_{s2} -	β -	κ -
MW	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. Kinsella et al., (1989)

Thus, 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 stabilize the milk casein micelle. κ -casein does not contain clusters of phosphoserine 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), 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).

2.1.2 Whey Proteins

The structural and chemical characteristics of the three main whey proteins are shown in table 1. The primary structure of whey proteins is typical of compact globular proteins, with a rather uniform sequence distribution of non-polar, polar, and charged residues. 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).

α -la normally occurs as a monomer, the hydrodynamic properties of which indicate a nearly spherical, very compact globular protein. α -la is a calcium-binding metalloprotein, which is also capable of binding zinc and probably other metals. The heat stability of α -la is reduced by

removal of Ca^{2+} (Fox, 1989).

β -Lg, 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 -lg 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 -lg is greatly influenced by the presence of both a sulphhydryl group and disulphide bonds. The relative importance of the sulphhydryl group is influenced by conformational changes since this determines the availability of the sulphhydryl group for reaction. Thus, under appropriate conditions β -lg readily participates in sulphhydryl-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).

2.2 Rheology of Milk Protein Solutions

Rheology is the study of the deformation and flow of matter. Studies of the rheological properties of proteins are significant for the following reasons (Rha and Pradipasena, 1984):

1. They allow us to probe the conformation and interactions of the

molecules in solution.

2. They allow us to reduce some aspects of functional properties to a physical property described by fundamental units.
3. They provide us with a tool for process monitoring and control.

2.2.1 Rheology of Protein Dispersions

In most food and technical applications, proteins are present along with other components and, more importantly, are in a heterogenous state of aggregation and/or degree of denaturation. In such complex states, molecular properties exert a smaller direct influence, as they are overshadowed by aggregation and interaction effects.

The viscosity of proteins in dilute solutions is governed by the size and shape of the molecules. The intrinsic viscosity of a dilute protein solution is dependent on the shape factor (representing the axial ratio of the equivalent ellipsoid or rod) and the association of the protein with the solvent.

Globular proteins, such as whey, are compact and only have a small effect on the viscosity of water except at high concentrations (Rha, 1978, 1979). This is because 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.

Caseinates, however possess an extended random conformation. 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 viscoelastic than whey proteins.

Protein-water and protein-protein interactions in aqueous systems are vitally important in applications of milk proteins because these interactions control the more operational properties of wettability, sinkability, dispersibility, viscosity and solubility (Hermansson, 1972; Hermansson and Akesson, 1974; Kinsella et al., 1989). Dissolved protein is characterized by a certain degree of hydration, called also solvation or water binding capacity, defined as the number of grams of solvent bound per gram of dry protein (Korolczuk, 1981). Generally, globular proteins bind approximately 50 g water per 100 g of protein (Kinsella et al., 1989). This is in contrast to intact casein micelles which bind larger amounts of water (2-4 g/g protein). Mechanical entrapment of water in the micellar matrix (via colloidal calcium phosphate) 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.

2.2.2 Shear Rate Thinning

Most protein dispersions exhibit pseudoplastic flow behaviour in which the fluid exhibits shear thinning over a wide range of shear rates. In polymer dispersions, such as protein systems, molecules are randomly oriented and entangled when the fluid is at rest. This condition of random structure follows the tendency of systems in nature to maximise entropy. The material behaviour when sheared may be imagined to change over different shearing conditions. As the shear rate is increased, the asymmetric dispersed molecules tend to align themselves with the shear planes so that frictional resistance is reduced. In this manner the random structure at ultra-low shear rates gives way to shear-oriented structure at higher shear rates. The progressively decreasing resistance to flow at

higher shear rates is observed as a decreasing apparent viscosity. For any given pseudoplastic system it is reasonable to expect that at some high shear rate, the macromolecules will be fully aligned along the laminar shear planes and that no further streamlining will be possible; this will be the limit of shear thinning. As the shear rate is further increased, shear stress will be proportional to the shear rate.(Tung, 1978)

Pseudoplastic behaviour of highly solvated dispersed systems may be explained by a slightly different mechanism. When undisturbed, the dispersed molecules or particles influence several layers of adjacent solvent molecules by reducing their mobility and thus form a solvated layer structure. 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)

Shear thinning may also be explained by the rupture of hydrogen and other weak bonds resulting in dissociation of protein aggregates or networks. In all of these cases, the apparent diameter of molecules or particles in the direction of flow is reduced (Cheftel et al., 1985).

2.2.3 Time Dependent Thinning

A thixotropic system will experience a reversible decrease in shear stress and apparent viscosity at a constant shear rate and temperature (Tung, 1978). Fluids of this type are thought to consist of asymmetric dispersed particles or molecules that interact through adhesive or weak secondary bonding forces to form a network or aggregated structure at rest. When

continuous flow is imposed on the system at constant shear rate, some of the interparticle bonds break with time, thereby offering less resistance to flow, and the shear stress relaxes to some constant value over a period of time. When shearing treatment is stopped, the dispersed particles can once again form the network, or aggregated structure, as Brownian motion gradually restores the particles to positions where interparticle adhesion can occur. Over a sufficient period of time the original density of linkages is achieved, thus the process is reversible. Hermansson (1975) found that dispersions of whey protein concentrate were thixotropic. In some fluids, the time dependent loss of structure is not recoverable and fluids that experience a permanent loss of structure are called rheodestructive. Not all time dependent shear thinning systems fall into purely thixotropic or totally rheodestructive categories. In some materials a partial recovery of structure is noted.

2.3 Variables Affecting Rheology of Casein Solutions

Flow properties of protein dispersions are governed by composition, as well as molecular shape, size and charge, which are influenced by environmental conditions such as temperature, concentration, pH, ionic strength and previous processing or treatment history (Southward, 1985; Tung, 1978). Rheological parameters can be useful indices of structural changes in proteins. Moreover, information on the relationships between environmental conditions and flow properties of various proteins can be used to modify rheological behaviour so as to impart the desired functionality (Tung, 1978).

2.3.1 Effect of Protein Concentration

The effects of protein concentration on the flow properties of sodium

caseinate and WPC solutions at pH 7 in distilled water were studied by Hermansson (1975). She evaluated flow parameters for the power-law:

$$\tau = k\gamma^n \quad (2.1)$$

and power-law plastic:

$$\tau = \tau_o + k\gamma^n \quad (2.2)$$

constitutive equations, where τ is the shear stress, τ_o is the yield stress, γ is the shear rate, k is known as the consistency index, and n is referred to as the flow behaviour index. For Newtonian fluids $n=1$, and any other values indicate a deviation from Newtonian flow. k is the parameter corresponding to viscosity, but only when $n=1$ does k have the dimensions of viscosity.

Hermansson (1975) characterized WPC solutions as being of low viscosity over a broad concentration range (solutions increased in apparent viscosity from 0.018 to 0.399 Pa.s over the concentration range of 4 to 20 %). The flow was almost Newtonian in the range 4-12%, pseudoplastic in the range 14-16%, while at higher concentrations yield values could be estimated and the flow was plastic, with the flow behaviour index decreasing at >12% WPC.

Caseinate solutions possessed strikingly different properties. Yield values were not detected even at high concentrations and below 12% the dispersions were almost Newtonian and of low viscosity. Above 12%, they were slightly pseudoplastic and the consistency index increased greatly with increasing concentration (solutions increased in apparent viscosity from 0.02 to 0.55 Pa.s and 0.55 to 1434 Pa.s over the

concentration range of 4 to 12 % and 12 to 20 % respectively).

However, there is some disagreement in the literature over the flow properties of casein solutions. Korolczuk (1981) found that solutions of casein, dissolved in borax, at 4-17% concentration, at shear rates of 10-1000 s⁻¹, behaved similarly to Newtonian liquids. Konstance and Strange (1991) reported that the deviation from linearity, i.e. Newtonian behaviour, for calcium caseinate occurred at a lower concentration (7-8%) and resulted in a much higher viscosity than that found by Hermansson (1975).

Roeper et al., (1982) found that the viscosity of sodium caseinate solutions deviated from Newtonian behaviour at a concentration of approximately 22%. Towler (1974) reported that both rennet casein and sodium caseinate solutions (7.5-15%) exhibited pseudoplasticity over the entire concentration range, the degree of pseudoplasticity decreasing as the concentration of casein was decreased.

Fichtali et al. (1993) noted that the activation energy for flow tends to increase with the solid content of the sodium caseinate solutions, with values of 3.49, 7.32, and 9.09 kcal (gmol)⁻¹ for 10, 13, and 16% solids concentration, respectively. This implies that more energy is required to shear higher concentrations of sodium caseinate (Fichtali et al., (1993).

Despite the discrepancies over what concentration range the flow behaviour of casein solutions changes, all the researchers agree that at low concentrations casein solutions are essentially Newtonian and at higher concentrations casein solutions exhibit pseudoplasticity. Further, the viscosity of casein solutions increases exponentially with concentration and casein solutions do not appear to possess a yield stress.

When considering the effect of concentration on the apparent viscosity of protein solutions three regions of behaviour may be envisaged, refer figure 2.1.: (a) for very dilute solutions there will be virtually no direct interaction between protein molecules; (b) for more concentrated

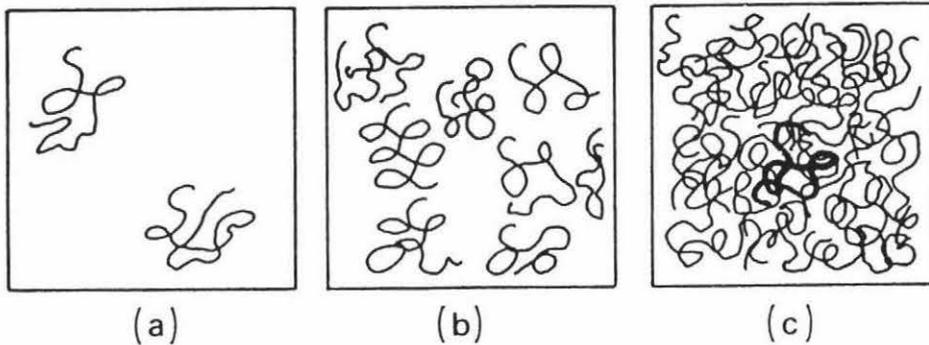


Figure 2.1 (a) Very dilute polymer solution. (b) Dilute polymer solution. (c) Concentrated polymer solution. (Hearle,1982)

solutions the protein molecules may be considered as separate entities, but their interactions must be taken into account; (c) for very concentrated solutions the molecules are extremely entangled, and flow would be dominated by the movement of molecular segments past one another.

The concentration regimes may be related to the concepts of swelling and solvation of protein molecules. Swelling can be defined as the spontaneous uptake of a solvent by a solid and is a phenomenon frequently observed as the first step in the solvation of proteins (Hermansson, 1972). In aqueous solutions the water molecules associated with a protein are said to solvate or hydrate the protein. The water associated with proteins can assume essentially three different states: vicinal, multilayer and bulk-phase (Cheftel et al, 1985).

Vicinal water is that which strongly interacts with specific hydrophilic sites of non-aqueous constituents by water-ion and water-dipole associations. When this type of water is at the maximum level, it is sufficient to provide single-layer coverage of accessible, strongly hydrophilic groups of non-aqueous constituents (Fennema, 1985).

Multilayer water occupies the remaining first-layer sites and forms several additional layers around hydrophilic groups of non-aqueous constituents; water-water and water-solute hydrogen bonds predominate (Fennema, 1985).

Bulk-phase water is water which occupies positions furthest removed from non-aqueous constituents; water-water hydrogen bonds predominate. Bulk-phase water may be either free (macroscopic flow is unimpeded) or entrapped (macroscopic flow is impeded) (Fennema, 1985).

Maximum solvation may therefore be said to occur in the infinitely dilute protein solution. As protein concentration increases maximum solvation may be prevented by the presence of various intermolecular forces in the swollen sample.

As swelling and viscosity are intercorrelated, Hermansson et al. (1975) proposed that the amount of swollen not fully solvated caseinate particles present is responsible for the enormous increase in viscosity with concentration >12 %. At high concentrations, the swollen particles will come closer together causing friction and weak interaction forces between the particles. For the purposes of rheological studies the protein may therefore be considered fully solvated throughout the very dilute concentration regime.

Korolczuk (1982) derived an equation expressing the logarithm of the

viscosity of a casein solution as a function of the concentration of casein in the solution and the level of hydration. The validity of the equation was proved experimentally for 68 solutions containing 2-16% casein, with hydration 0.7-3.8 g H₂O/g protein and with viscosities up to 104 cP.

The exponential rise of apparent viscosity in casein dispersions has been found to increase in line with rheological volume concentration theory (Frisch and Simha 1956)

The protein-protein interactions that occur in concentrated protein solutions can be broadly classified as hydrophobic, hydrogen-bonded, electrostatic and molecular entanglement (Towler et al., 1981).

The pseudoplasticity exhibited by concentrated solutions of casein and why may be explained by the theories outlined in section 2.2.2.

Several theories have been put forward to explain these phenomena: In extremely dilute dispersions the total viscosity effect is the sum of the effects caused by each of the individual suspended particles (Tung, 1978) . However, as their concentration is increased the disturbances of solvent flow produced by the suspended particles are no longer independent since flow patterns overlap, and aggregation and solvent immobilization occur.

Hermansson (1975) concluded that the increase in the apparent viscosity was more likely due to protein-water interactions (hydration) than to strong protein-protein interaction forces. This conclusion was probably based on the results of previous experiments. Hermansson (1972) and Hermansson and Akesson (1975) found that: a) Highly soluble non-swelling proteins, such as those in whey protein concentrate, had low viscosity; b) Soluble proteins with high initial swelling, such as caseinate, showed a highly concentration dependent viscosity, probably reflecting

the content of partially solvated particles, i.e. the swelling of the proteins is sterically limited at high protein concentrations.

Sorption isotherms of acidic caseins and sodium caseinate are similar up to water activity (A_w) values of 0.52. Between A_w values of 0.52 and 0.87 sodium caseinate shows a greater increase in water uptake than acidic caseins, while at A_w values of 0.87-0.97 water uptake of acidic caseins increases more sharply than that of sodium caseinate, uptake being in the order acidic casein pH 2.0 > acidic casein pH 2.5 > acidic casein pH 3.0 > sodium caseinate pH 7.0 (Mohanty et al., 1988).

2.3.2 Effect of pH

Studies investigating the effect of pH on the apparent viscosity of casein solutions are limited by the solubility of casein. Caseinates (Na, K, NH_3) are completely soluble $> \sim$ pH 5.5; solutions containing 10 - 15% protein can be readily prepared at pH > 6 . Casein is also soluble at $< \sim$ pH 3.5 (Mulvihill and Fox, 1989). Between pH 3.5 and 5.5, casein is very insoluble.

It is generally agreed that the apparent viscosity of casein solutions rises and then falls as the pH increases over the range 6.0 to 12.0. The pH of maximum viscosity varies among workers. Hermansson et al. (1975) reported that the viscosity of sodium caseinate both in distilled water and in 0.2 M NaCl increased from pH 6.0 to a maximum at pH 9.8-10.0, whereupon it rapidly decreased. Salzberg and Georgevits (1956), Hayes et al (1961), and Puri et al. (1972) observed similar maxima at pH 8.7, 9.5 and 10.8-11.0, respectively. Hayes et al., (1961) observed a broad minimum in apparent viscosity between pH 6.5 and 7.5. It should be noted that Hermansson et al. (1975) did not take measurements of solutions between pH 6.0 and pH 8.0.

Puri et al. (1972) suggest that the point of maximum viscosity occurs when complete neutralization is attained. The increase in apparent viscosity with pH increase is the result of formation of increasing amounts of largely hydrated caseinate ions which begin to aggregate to form hydrated casein micelles.

Korolczuk (1982) and Mohanty et al., (1988) observed that the viscosity and hydration of acidic casein solutions (< pH 3.5) was higher than those of neutral or basic solutions (pH 6-8), and that acidic casein solutions of acidified casein in water formed clear firm gels, without any opalescence, at protein concentrations above 9%. This characteristic property could be explained by intermolecular electrostatic interactions, as the net positive charge of $-NH_3$ groups per average casein monomer molecule at low pH levels is lower than the net negative charge of $-COOH^-$ and PO_4^{2-} groups at neutral and basic solutions. Thus, lower repulsive forces at low pH increase the probability of the formation of intermolecular aggregates, which causes the increase in the viscosity of protein solutions.

Hermansson (1975) found that the apparent viscosity of the WPC dispersions increased only slightly with pH and the viscosity remained low in the range studied (pH 6-10).

2.3.3 Effect of Temperature

The correlation between viscosity and the temperature of casein solutions has been well established (Sergeeva et al., 1973; Towler, 1974; Korolczuk, 1982; Roeper et al., 1982; Mohanty et al., 1988; Fichtali et al., 1993)

Over the limited range investigated (25°C to 60°C) Towler (1974) observed a linear relationship between the logarithm of the apparent

viscosity and the reciprocal of the absolute temperature for both rennet casein and sodium caseinate solutions. Fichtali et al., (1993) describe the effects of temperature (25-65°C) and concentration (10-16%) on sodium caseinate by a single equation:

$$\eta_{app} = \eta^* \exp \left[\frac{(K_1 + K_2 C)}{T} + K_3 C + K_4 C^2 \right] \quad (2.3)$$

where η_{app} = apparent viscosity, T = absolute temperature, C = concentration, and η^* , K_1 , K_2 , K_3 , K_4 are constants. Experimental data fitted to this equation yielded a coefficient of determination ($r^2 = 0.996$).

This relationship appears to hold true for all casein types. However, some casein solutions do deviate at high temperatures. Sergeeva et al. (1973) observed that while the viscosity of calcium caseinate solutions generally fell as the temperature rose, the solutions gelled reversibly around 88°C. Solutions of acidified casein also deviate from this relationship (i.e equation 2.3) at high temperatures. Korolczuk (1982) observed that between 25 and 80°C the viscosity of neutral caseinate solutions decreased as the temperature rose. Acidic casein solutions at pH 2.4-2.9 were characterized by a higher viscosity for the same protein concentration and the solution viscosity fell only in the temperature range 25-60°C; for higher temperatures the viscosity increased. Mohanty et al. (1988) found that the viscosity of acidic casein solutions decreased logarithmically with increasing temperature up to 70 °C. The increase in viscosity during heating, at high temperatures, of the acidic casein solutions could be interpreted as resulting from hydrophobic interactions (Korolczuk, 1982). Hydrophobic interactions are temperature-sensitive, being minimal at low temperatures and increasing with rise in temperature (Thompson and Farrel, 1973). Lower electrostatic repulsive forces at low pH values and higher hydrophobic interactions for the

higher temperatures could be responsible for the formation of intermolecular aggregates and thus the increase in viscosity and gel formation.

2.3.4 Effect of Shear Rate

The viscosity of casein solutions is dependent on shear rate (Towler, 1974). At low shear rates, e.g. $1-10 \text{ s}^{-1}$, the ratio of shear stress to shear rate (i.e. viscosity) is constant. This corresponds to a fluid exhibiting Newtonian properties. At higher shear rates ($>200 \text{ s}^{-1}$), however, the ratio of shear stress to shear rate decreases, indicating pseudoplastic behaviour of the casein solution. Under these conditions log shear rate and log shear stress are linearly related (Thompson and Hansen, 1953; Towler, 1974). It has been shown that pseudoplasticity is only exhibited at sufficiently high casein concentrations (Korolczuk, 1981; Konstance and Strange, 1991).

Continuous shear curves have shown whey protein concentrate (20%) and sodium caseinate (14%) dispersions to be thixotropic (Hermansson et al., 1975). Ammonium caseinate solutions appear more thixotropic than sodium caseinate solutions (Towler, 1972).

Casein solutions of 15% concentration exhibit thixotropy when subjected to sufficiently high shear. Solutions of casein which contain viscosity reducing agents also exhibit thixotropy under these conditions. When, however, the shear rate is reduced to a maximum of 1800 s^{-1} , the flow curves coincide and consequently the casein solutions exhibit virtually no thixotropy. Moreover, their pseudoplasticity is much less evident (Towler et al., 1981).

2.3.5 Effect of Salt Addition

Viscosity increase may be effected by incorporation of mono- and multi-valent cations. The addition of calcium chloride limits the solubilization of caseins. Under the conditions studied by Konstance and Strange (1991), in which the sample was centrifuged at 1000 x g for 20 min, a maximum of 60 mg/ml protein concentration was observed. The viscosity of these protein solutions was low and exhibited no deviation from Newtonian behaviour over the concentration range. Towler, (1972) observed that if micellar calcium is retained during precipitation of casein, the retained calcium is much more effective in causing viscosity increase than calcium added subsequently.

Konstance and Strange, (1991) studied the caseinate concentration - viscosity relationship in the presence of NaCl (0.15M to 0.45M). They found that the natural logarithm of apparent viscosity increased linearly with protein concentration up to 60 mg/ml. The flow behaviours of the NaCl-caseinate solutions were similar to those observed with calcium chloride. When the protein concentration of the NaCl solutions exceeded 60 mg/ml, the logarithm of apparent viscosity showed a ten-fold increase over that expected from the simple linear correlation between concentration and \ln viscosity observed over the concentration range 0 to 60 mg/ml. In a similar study with NaH_2PO_4 , the viscosity at casein concentrations > 70 mg/ml increased 50-fold over the expected linear response (Konstance and Strange, 1991).

Hermansson et al. (1975) observed that with sodium caseinate solutions (>10%) addition of salt (0.2, 0.5 and 1.0 M NaCl) caused considerable increase in viscosity, but no change in flow character; swelling and solubility were strongly reduced. Salt addition had little effect on solutions of whey protein concentrate.

Hermansson et al. (1975) suggests that the effect of salt may be simply a hydration effect or due to changes in the repulsive balance and the micellar structure by salt-protein interactions. Experiments with calcium caseinate indicate that extensive complexing may occur in a calcium polyphosphate-casein system (Towler, 1972).

Konstance and Strange (1991) found that both sodium and calcium caseinate showed remarkably similar behaviour with the effects of pH on viscosity being slightly greater for calcium caseinate.

2.3.6 Effect of Chemical Addition

Apparent viscosity of casein solutions may be reduced by minimizing the protein-protein interaction in solution. Reagents such as urea or guanidine hydrochloride interact with the solvent to alter protein hydrophobic and hydrogen-bonded interactions (Towler et al., 1981). Thus their action in decreasing viscosity is by the breaking of hydrophobic aggregates. Two of the casein components, α_{s1} - and β -casein, contribute to the viscosity of the solution in this way.

κ -Casein, however, interacts strongly with the other casein components through non-bonded interactions and κ -casein molecules are linked together by way of disulphide bridges, thus giving rise to relatively large aggregates in solution (Towler et al., 1981). The reduction of the disulphide bonds allows free movement of the κ -casein monomers, effecting a reduction in molecular weight, and consequently a lower viscosity for the whole system. Enzymatic cleavage of molecules will also reduce molecular weight (Towler, 1972). The α_{s2} -caseins also contain cysteine and could also be involved in disulphide bridge formation (Towler et al., 1981).

The effect of additives may be dependent on shear rate. At low shear rates ($<500 \text{ s}^{-1}$), Towler (1971) noted that dicyandiamide alters the viscosity of casein solutions quite markedly, but at high shear rates the difference is less marked.

2.3.7 Effect of Endogenous Enzymes

Milk contains endogenous proteinases such as plasmin (otherwise known as alkaline milk proteinase) and may contain proteinase produced by bacteria. Plasmin, derived from blood, is predominantly associated with the casein fraction in milk and has a pH optimum at 8.5 (Humbert and Alais, 1979). Plasmin is responsible for the hydrolysis of α_{s1} -, α_{s2} - and β -casein. The plasmin activity may therefore be an important factor in limiting the storage life of certain casein products. It has been observed that the viscosity of some casein solutions decreased rapidly to appear "water thin" after a period of three weeks (Richardson, 1982). This viscosity difference is attributed to proteolytic activity.

2.3.8 Variation of Rheological Properties Between Casein Types

Hermansson et al. (1975) tested six different commercially available caseinates in order to see whether the characteristic differences in flow properties were effects due to processing or origin. Although great differences were found in the consistency index, k , the characteristic properties defined for sodium caseinate are common to all the caseinates studied. Common features are the absence of yield value even in very thick dispersions, the almost Newtonian flow behaviour in distilled water ($n > 0.90$, concentration $< 12\%$), and the characteristic increase in viscosity on the addition of salt.

Sergeeva et al. (1973) observed that the caseinates of the alkali metals

gave more viscous solutions than those of the alkaline earths. Konstance and Strange (1991) reported similar results with an increase in viscosity for an increase in concentration being highest for sodium caseinate > calcium caseinate > rennet casein. Towler (1974) suggests that the reason for rennet casein having a much higher apparent viscosity than sodium caseinate, at equivalent concentrations, may be attributed to the higher concentration of calcium ions in rennet casein. Calcium ions are involved in cross-linking.

2.3.9 Discrepancies in Results Between Different Workers

Essentially all the workers agree on the general characteristics of caseinates: a) apparent viscosity increases exponentially with caseinate concentration, b) solutions are Newtonian at low caseinate concentrations, c) solutions exhibit pseudoplasticity at higher concentrations, d) apparent viscosity increases with increases in pH >6, e) apparent viscosity decreases with increases in temperature, f) apparent viscosity increases with salt concentration, g) pseudoplastic behaviour increases with shear rate.

The researchers, however, do not agree on exactly when these certain behaviours occur and the magnitude of the behaviours. The main reasons for such discrepancies probably lie in the methods used and types of casein studied.

2.4 Formulation of the Experimental Programme

There have been few studies on the effect of salt on the rheology of caseinate solutions. These studies have been concerned with sodium phosphate and sodium and calcium chloride (refer section 2.3.5). Hence, there is a need to investigate the effects of other salts used in both

technical and edible applications. Caseinates are generally used in alkaline solutions (i.e. $> \text{pH } 4.6$, the isoelectric point of casein), where the caseinate molecules have a net negative charge. The major influence of salts in applications can be expected to come from the cations. In this study, monovalent and divalent cations commonly used in caseinate applications were chosen: Na^+ , K^+ , NH_4^+ , Ca^{2+} , Mg^{2+} and Zn^{2+} . The concentration at which caseinates are used in applications is generally in the concentration region where the flow properties of caseinate solutions have been shown to deviate from Newtonian behaviour ($> 8\% \text{ w/w}$).

To obtain good characterisation of the rheological properties of a non-Newtonian liquid (such as concentrated caseinate solutions) it is necessary to run a viscosity measurement at several different shear rates. Therefore the solutions of sodium caseinate were studied using shear sweep experiments. Shear sweep experiments were conducted at 25 and 50°C to investigate the effect of temperature on the flow behaviour of caseinate solutions.

3 *Objectives and Scope*

The objectives of this study were to investigate the effect of monovalent (NaCl, KCl, NH₄Cl) and divalent (CaCl₂, MgCl₂, ZnCl₂) salts on the rheological properties of sodium caseinate solutions.

The effects of these salts were investigated over a range of added ionic strengths on solutions containing 14 % w/w sodium caseinate at 25 °C and 50 °C.

The effect of NaCl concentration, over the added ionic strength (AIS) range of 0 to 1.45, on the cold gelation of sodium caseinate solutions was also investigated.

A comparison of the gelation characteristics of caseinate solutions with added NaCl, KCl, NH₄Cl, and ZnCl₂ were made at 1.1 AIS

Preliminary experiments were carried out examining the effect of pH and caseinate concentration on rheological properties.

4 *Experimental Methods*

4.1 Introduction

The main objective of this study was to investigate the effect of salts on the rheological properties of sodium caseinate solutions. Before studying the effects of salt, preliminary experiments (section 5) were carried out in order to determine the rheological characteristics of the caseinate used for this study, Alanate 180, and hence to develop a suitable method.

4.2 Equipment and Materials

4.2.1 Equipment

Bohlin VOR Rheometer System, Bohlin Rheologi AB, Lund, Sweden

Sorvall SS-3 Automatic Centrifuge, Dupont Instruments, Instrument Products, Biomedical Division, Newtown, CT 06470, USA

Orion Benchtop pH meter Model 420A fitted with a Ross Sure-Flow electrode Model 81-65, Orion Research Incorporated, Laboratory Products Group, Boston, USA

Heidolph RZR 2050 Overhead Stirrer, Heidolph Elektro GmbH and Co. KG, Kelheim, Germany

4.2.2 Materials

Alanate 180 is a standard sodium caseinate for sale by the New Zealand Dairy Board. A typical composition of Alanate 180 is shown in table 1.

protein	91.1
ash	3.5
moisture	4.0
fat	1.1
lactose	0.1
	<hr/> <hr/>
	99.8 %

Ammonium chloride, calcium chloride, magnesium chloride, potassium chloride, and sodium chloride were of analytical reagent grade.

Sodium hydroxide, and zinc chloride were of technical reagent grade

Distilled Water

4.3 Preparation of Caseinate Solutions

Calculate the mass of water and caseinate needed in order to achieve the desired caseinate concentration.

Weigh the correct mass of water into a beaker, preferably stainless steel. Place the beaker in a water bath set to 60°C.

When the water in the beaker has reached 60°C, stir the water vigorously with the propeller-blade, attached to a Heidolph stirrer motor suspended over the water bath to create a vortex.

Add the caseinate powder slowly into the vortex of water in the beaker. The dispersion was then stirred at 60°C for 60 minutes to complete dissolution.

4.4 Preparation of Caseinate - Salt Solutions

Prepare a bulk concentrated (approx 20 %) caseinate solution as described in section 4.3.

Place the bulk caseinate solution in a refrigerator at 5°C, covered by parafilm, and determine total solids content by the method described in section 4.5. It is necessary to cool the solution to 5°C so that there will be minimal change in the solution moisture content by evaporation.

Remove the bulk solution from the refrigerator and divide the solution among the number of pre-weighed beakers necessary to study the range of AIS under investigation. Generally 70 to 100 g bulk solution was placed in each beaker.

Calculate the dilution necessary to achieve a 14 % caseinate solution and determine the weight of salt to add in order to achieve the desired AIS for each beaker.

Add approximately 3 ml of 1 N NaOH to the caseinate solution.

Weigh the required amount of salt into a beaker and add the required amount of water less 5 or 6 ml. Stir solution with a magnetic stirrer until

the salt is dissolved. Less water is added to the beaker than that calculated to allow for the mass of 1 N NaOH which has been added to the caseinate solutions.

Add the salt solution to the caseinate/NaOH solution and weigh. Add more water until the desired final weight is achieved.

Mix solution with an overhead stirrer until the solution appears homogeneous (approximately 5 minutes). Care should be taken to avoid splashing in the initial stages of stirring and the bottom of the beaker should be scraped with a metal spatula to ensure that all the caseinate solution is mixed in with the salt solution. Rod climbing is common when mixing the caseinate and salt solutions and can result in solids loss from the beaker. The problems of rod climbing can be overcome by scraping the stirrer shaft, with a spatula, to halt the solution climbing to levels which could cause loss of solution.

Allow the mixture to settle, to reduce the foaming caused by mixing. Then pour solution into a 50 ml centrifuge tube.

Cover the tube and place in a waterbath at 60°C for 30 minutes. This step reduces foaming and the amount of dissolved air in the solution. The solubility of air in water decreases with increased temperature. If this heating step was omitted, the dissolved air would come out of solution when the caseinate dispersions were tested at 50°C during the viscometry and oscillation experiments. As the air came out of solution, bubbles would form resulting in inaccurate measurement of the rheological parameters under investigation.

Following the heating step the tubes of solution were centrifuged at 6000 rpm for 10 minutes. This step assists the removal of air from the caseinate solutions and also destroys any remaining foam.

The deaerated solutions were then held in a refrigerator at 5°C until they were able to be tested on the Bohlin VOR Rheometer.

On the day that the caseinate solutions were to be tested all the tubes were removed from the refrigerator and allowed to equilibrate at room temperature, 22°C. This step was necessary in order to assist the handling of the solutions. Some solutions formed reversible gels at 5°C.

4.5 Moisture Analysis

The moisture analysis was based on 2 to 3 g of solution dried overnight in an oven set at 105 °C. All solutions were tested in duplicate

4.6 Viscometry Tests

For a non-Newtonian liquid it is necessary to run a viscosity measurement at several different shear rates to obtain a good characterisation of the rheological properties of the sample. A sweep in shear rate is used to provide a flow curve, i.e. a plot of shear stress against shear rate (Bohlin Reologi, 1991). 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 the shear sweep is up and down, i.e. by starting with the rested material, increasing the shear rate gradually to a maximum and then decreasing it again to zero, thixotropy can also be detected. Torque values obtained on the 'down-curve' are lower than those obtained on the 'up-curve' because of the gradual destruction of the 'thixotropic structure' by shear, and consequently the flow curve is in the form of a 'hysteresis loop' (Dinsdale and Moore, 1962).

The Bohlin VOR Rheometer was used to characterise the solutions using an up-down shear sweep experiment as described above at both 25 °C and 50 °C. The main parameters of a shear sweep experiment are those of defining the equilibration time, and the shear rate range to be covered.

4.6.1 Determination of the Equilibration Time

The equilibration time is the time between raising the temperature of the sample in the cup to the test temperature and beginning the experiment per se. The equilibration time allows the structure of the sample to equilibrate to the test temperature. The equilibration time is especially important for materials with a strong viscosity-temperature dependence such as sodium caseinate.

The equilibration time was determined at 25 °C. Presumably the larger the temperature difference between the solution temperature before testing and the test temperature the greater the solution's structural changes and hence a longer thermal equilibration time would be required. There were two worst case temperature history scenarios for a solution tested at 25 °C; that the solution had been tested directly after removal from the refrigerator (5 °C) or after the deaeration step (60 °C).

A solution of sodium caseinate at 15 % w/w was poured into the cup (C14 geometry), placed in the Bohlin and held at 5 °C for 30 minutes. After this holding time the temperature was raised to 25 °C. When the temperature of the solution, as measured by the Bohlin, had reached 25 °C rheological measurements were begun with the Bohlin in oscillation mode. The change in the dynamic viscosity of the solution over time at 25 °C is shown in figure 4.1. Figure 4.1 shows that the dynamic viscosity of the caseinate solution decreased from 13.4 Pas to 11 Pas over 450 seconds. The dynamic viscosity of the solution appeared to be constant

at 11 Pas after 450 seconds.

To simulate the situation where solutions were tested directly after deaeration, a sample was poured into the cup (C14 geometry) with a layer of glycerol on top to prevent moisture loss, placed in the Bohlin and held at 50 °C for 30 minutes. After this holding time the temperature was decreased to 25 °C. Again, when the solution had reached 25 °C, as measured by the Bohlin, rheological measurements were begun with the Bohlin in oscillation mode. Although the caseinate solutions were deaerated by holding in a water bath at 60 °C the temperature of the solutions after the centrifugation step was quite cool (approx 40°C). 50 °C was chosen as the 'worst case' hot temperature rather than 60 °C. Further, rheological testing at 60 °C resulted in the generation of bubbles from dissolved air thus distorting rheological measurements. Generation of bubbles from dissolved air was not observed at 50 °C. Figure 4 shows that the viscosity of the caseinate solution increased from 3.1 Pas to a constant value of 10 Pas after being held at 25 °C for 450 s.

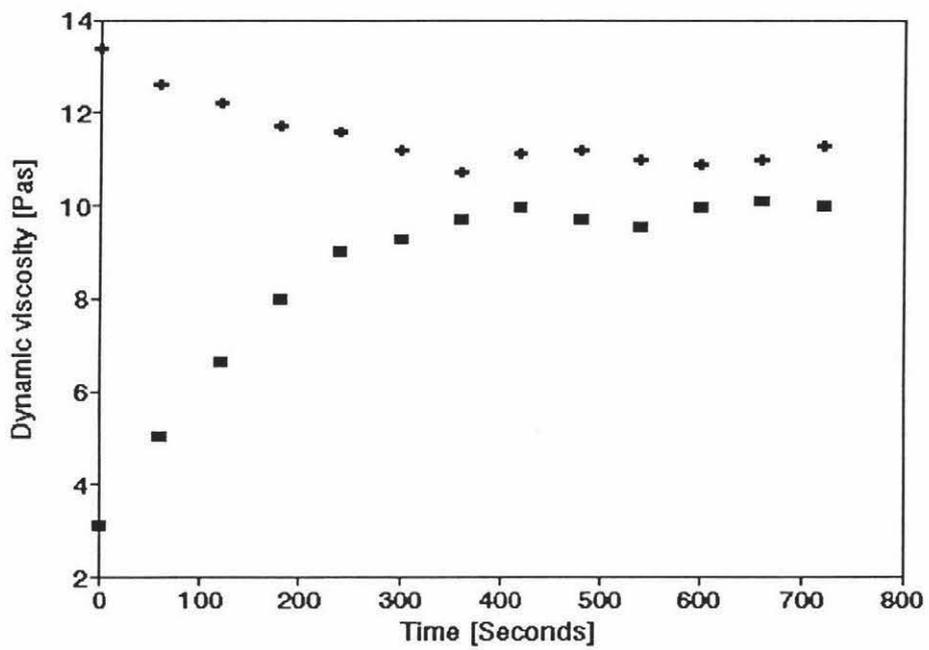


Figure 4.1 Thermal equilibration time for a 15 % Na caseinate solution at 25 °C with an initial temperature of 50 °C (■) and, 5 °C (+)

From these experiments it appeared that 450 seconds was the minimum equilibration time necessary. In order to ensure that the equilibration time was adequate, a safety factor was added to make the equilibration time 600 seconds. It was assumed that the equilibration time would be 'constant' within the limits of the safety factor for all future experiments both at 25 °C and 50 °C. It is not known why the final viscosities from the two equilibration tests are different. The solutions tested in each case were from the same original stock solution. It is possible that the solutions were still equilibrating and would have reached a similar viscosity after a longer equilibration period. The viscosity difference between the two tests (approximately 10%) is not significant when compared to the differences observed when salt additions are made to caseinate solutions (approximately 1300% for NaCl at 1.069 ionic strength).

4.6.2 Determination of the shear rate range

The viscosity of a solution may vary with the applied shear rate. Depending on how the solution behaves it may be classified as Newtonian, shear thickening, shear thinning or time dependent. The theory outlining these classifications has been detailed in section 2.2. As was discussed in section 2.2, it is known that solutions of caseinate may exhibit Newtonian or shear-thinning characteristics depending on the shear rate range over which the solution is tested.

Ideally, it would have been desirable for all the solutions to be tested over an identical shear rate range. If the solutions were tested over the same shear rate range it would have been possible to fit rheological models to the experimental data and hence characterize the test solutions in terms of the classifications given above.

Reliable rheological data from the Bohlin can only be obtained when the Bohlin is operated in the torsion bar range of 4 to 100%. Solutions of excessively high viscosity would apply too much torque to the torsion bar and hence would fall above the range of the bar. Conversely, a solution of excessively low viscosity would apply too little torque to the torsion bar and would fall below the range of the bar. There are two parameters which can be adjusted so that a solution can be tested over the reliable range of the torsion bar:

- a) The torsion bar itself can be changed. A heavier torsion bar can cope with a higher applied torque compared to a lighter bar.
- b) The geometry of the concentric cylinder can be changed. A geometry of smaller dimensions would decrease the torque applied to the torsion bar, whereas a larger geometry would increase the applied torque.

By manipulating these two parameters, it would be possible to test a range of solutions, of varying viscosities, over the same shear rate range. However, as a result of heavy usage of the Bohlin, and user restrictions, preventing general users from changing the torsion bar, this was not possible.

In order to make a valid comparison between the viscosities of different caseinate solutions it was necessary to compare them at the same shear rate. In their usage in industry, caseinate solutions are exposed to high rates of shear. A moderately high shear rate of 91.19 s^{-1} was therefore chosen as the standard rate of shear.

By manipulating both the torsion bar and the geometry of the concentric cylinder it was possible to test almost all the solutions over a shear rate

range that included 91.19 s^{-1} . One assumption that underlies the validity of comparing the viscosity of the solutions at only the same shear rate and not the same shear rate range is that the viscosity of the solutions are not dependent on previous shear history.

4.6.3 Viscometry Test Set-up

The C14 and C25 geometry cup and bob sets were used for the viscometry tests depending on the viscosity of the solutions. The bob was lowered into the cup until it was 3 mm from the bottom of the cup. Excess solution was removed from the cup so that the solution only just covered the bob. A thin layer of paraffin oil was placed on top of the caseinate solution for experiments carried out at $50 \text{ }^{\circ}\text{C}$. The paraffin was added to prevent the solutions from drying out.

4.7 Oscillation Tests

It was observed that some caseinate solutions with added salt formed gels while being stored in the refrigerator. As an adjunct to the viscometry tests, the solutions with the highest viscosities in each salt group, in which gelling occurred, were investigated for their gelling behaviour. The effect of increasing salt concentration on the gelation of caseinate solutions was investigated using NaCl. Gelling was monitored using the oscillation mode on the Bohlin.

The C14 geometry was used in all the oscillation experiments. The solutions were held at $50 \text{ }^{\circ}\text{C}$ for 1 hour, followed by cooling, at a rate of $0.7 \text{ }^{\circ}\text{C}/\text{min}$, to $5 \text{ }^{\circ}\text{C}$. After holding the solution at $5 \text{ }^{\circ}\text{C}$ for 1 hour the solution was heated at a rate of $0.7 \text{ }^{\circ}\text{C}/\text{min}$ to $50 \text{ }^{\circ}\text{C}$.

A thin layer of paraffin oil was placed on top of the caseinate solutions.

The frequency and the shear strain amplitude were set to 1 Hz and 5 % respectively for all the oscillatory tests.

5 *Characterization of Alanate 180 and Method Development*

According to the literature, (section 2.3), the major environmental conditions which influence the flow properties of protein dispersions are temperature, concentration, pH, ionic strength and previous processing or treatment history.

In the context of the work reported here it was deemed that temperature would not be a major influencing factor on rheological measurements as the Bohlin rheometer can control the temperature to ± 0.1 °C.

5.1 Effect of concentration on solutions of Alanate 180

The effects of concentration on the flow behaviour of caseinate solutions have been well documented, (section 2.3.1). At low concentrations casein solutions are essentially Newtonian and at higher concentrations casein solutions exhibit pseudoplasticity. Further the viscosity of casein solutions increases exponentially with concentration and casein solutions do not appear to possess a yield stress.

An initial experiment was conducted to quantify the relationship between viscosity and the concentration of Alanate 180. The outcome of this experiment would then enable a standard caseinate concentration to be chosen which would be high enough to show complex rheological behaviour and low enough to minimize the need to change the torsion bar in later experiments with salts. This information would also be useful in determining how precise the control of caseinate concentration should be in future experiments.

Four caseinate solutions were made up, in duplicate, using the procedures outlined in sections 4.3 and 4.4. The only deviations from these were in section 4.3 where no salt was added and the desired caseinate concentration was varied to achieve a caseinate concentration range of 12 to 20% w/w.

The rheology of each solution was examined by performing shear sweep experiments at 25 °C using the Bohlin VOR rheometer in viscometry mode. The range of the shear sweep differed between solutions because of the necessity of measuring the apparent viscosity over the reliable range of the torsion bar (4 to 100 %). The first replicate set was tested with a 42.9 g cm torsion bar while the second set was tested with a 315 g cm bar. In the absence of a common shear rate, the apparent viscosity of each solution was compared at the lowest shear rate which produced a reading over 5 % of the range of the torsion bar. The ratio of shear stress to shear rate (i.e. viscosity) is constant for sodium caseinate solutions (15 % w/w) at low shear rates e.g. 1 - 100 s⁻¹ (Towler et al., 1981). The solutions with a concentration between 14 and 20 % w/w had their lowest shear rates between 1.84 and 5.80 s⁻¹ whereas the two solutions of 11.83 % w/w concentration had their lowest shear rates at 58.1 and 367 s⁻¹ respectively.

As caseinate solutions increased in concentration the shear rate range that was possible for a given bar decreased. The weight of the torsion bar also affected the possible shear rate range, with the range increasing with the heavier bar at all caseinate concentrations.

The viscosity of sodium-caseinate solutions was shown to increase logarithmically with increases in caseinate concentration as shown in figure 5.1.

A linear regression of the data quantified the relationship as shown in equation (5.1).

$$\log[\eta_{app}] = 3.72 \times 10^{-1} C - 5.28 \quad (5.1)$$

Where η_{app} = apparent viscosity [Pas]
and C = the caseinate concentration [% w/w]

5.2 Effect of pH on solutions of Alanate 180

The effect of pH on the apparent viscosity of caseinate solutions has been studied before, but as discussed in section 2.3.2, the exact nature of the pH-viscosity relationship varies among authors. Hayes and Muller (1961) found that there was a flat minimum in the pH-viscosity curve at about pH 7, whereas Hermansson (1975) observed no minimum, with viscosity increasing with increasing pH over the range 6 to 10. An experiment was therefore conducted to determine the pH-viscosity relationship with the caseinate to be used in this work, Alanate 180.

Solutions of sodium-caseinate were made up using the method described in section 4.3 and 4.4. The only deviations from this method were that the desired caseinate concentration of the final solutions was 15.5% not 14% and the alkali, 1 N NaOH, was added in varying amounts to each solution, in order to achieve a pH range of 6.47 to 10.29. No salt was added to the solutions. The rheology of each solution was examined by performing shear sweep experiments at 25 °C using the Bohlin VOR rheometer in viscometry mode. The viscosity of each solution was

determined at a shear rate of 29 s^{-1} .

The relationship between viscosity and pH for sodium caseinate is shown in figure 5.2. The shape of this graph follows closely that observed by Hayes and Muller (1961) with a maximum viscosity at pH 9.5 and an apparent plateau over the pH range 6.5 to 7.45. A regression analysis on the data collected over the plateau range showed that apparent viscosity actually increased slightly with pH (0.94 Pas/pH unit). Further analysis, by treating the plateau data as if it were from the same population, showed a mean viscosity value of 3.53 Pas with a standard error, at a 95% level, of $\pm 26\%$.

It was clear from this experiment that if pH was to be minimized as a potential confounding variable then ensuring that the pH of the solutions was in the range of the plateau would be necessary. This plateau pH range is also the range in which caseinates are used in most food applications. The question which arises from this conclusion is how significant are the viscosity changes with pH over the plateau region when compared to the changes caused by the salts under investigation. The answer to this question determines how precise the control of pH must be for the salt study.

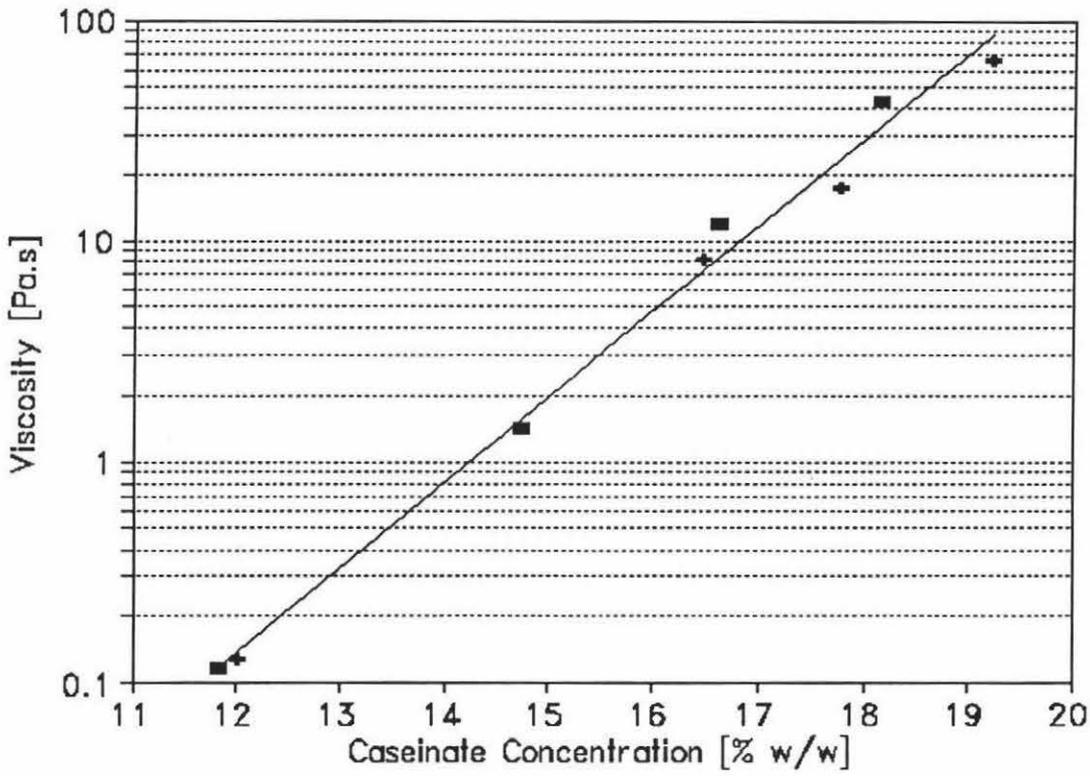


Figure 5.1 The effect of caseinate concentration on apparent viscosity of Alanate 180 dispersions in distilled water at 25°C measured with a 315 g cm (+) and 42.9 g cm (■) bar

dissolve the salt. Hence, the range of AIS investigated was limited by the amount of salt that could be dissolved. For NaCl this limit was at an AIS of 1.069. All subsequent salt studies were made over the same ionic strength range as that investigated for NaCl.

Figure 5.3 shows that the apparent viscosity of sodium caseinate increased logarithmically with added NaCl. The apparent viscosity increased by approximately 1300% over the AIS range studied.

In comparison to the increase observed with added salt, the error which could be contributed by pH variations over the entire plateau range ($\pm 26\%$) is insignificant. The control of pH of sodium caseinate solutions with added ionic strength is therefore not critically important, as long as the pH of the solution is between 6.5 and 7.45. It was found that 90 to 140 g of 14 - 15 % caseinate solutions with or without added salt fell within this pH range when 3 ml of 1 N NaOH was added. In general, pH was controlled to 6.9 ± 0.3

5.3 The phenomenon of rod climbing in solutions of Alanate 180

In the course of the experimental work it was observed that the shear sweep curves generated by some solutions showed hysteresis. The shape of the hysteresis was generally of two types, exemplified by the shear sweep curves generated by 0.4 AIS KCl replicate 1 (figure 5.4) and 1.1 AIS KCl replicate 1 (figure 5.5).

Shear sweep experiments were carried out using solutions with added CaCl_2 to investigate the nature of the hysteresis. These experiments were identical to all previous runs with the exception that the cup cover of the Bohlin was not put in place, which, while making the experiment more sensitive to changes in ambient temperature, enabled the top of

the solution to be viewed during the shear sweep.

Observations made during these experiments showed that, as the shear rate increased, a point was reached where the solution began to noticeably climb the rod of the bob. The height of the climbing solution increased with shear rate. Conversely the height of the solution decreased with decreasing shear rate. In addition to the rod climbing it was observed that as rod climbing began the solution started to change colour, becoming more milky. The milky colour did not decrease with decreasing shear rate. In solutions which produced the type of hysteresis shown in figure 5.5 it was found the solution climbed the rod to such an extent that solution was drawn up from between the walls of the cup and the bob. The point at which this phenomenon occurred corresponded with a marked decrease in apparent viscosity and a faster rate of colour change.

It is proposed that the sudden decrease in apparent viscosity, observed in figure 5.5, is due to the effective decrease in the surface area of the bob which would result in a decrease in the torque transmitted to the torsion bar. As the shear rate decreases rod climbing is decreased and the solution retakes its place between the walls of the cup and the bob. The change in colour of the rod climbing solutions is likely to be due to the entrainment of air.

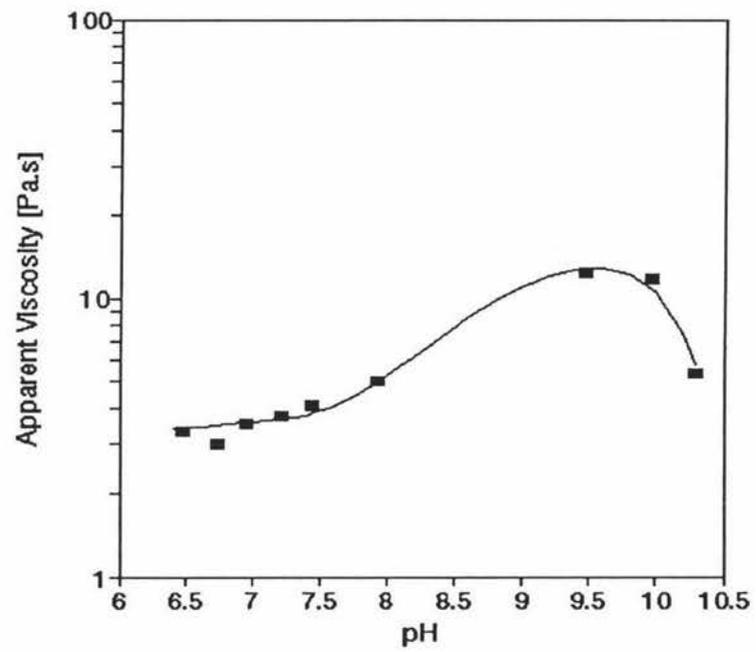


Figure 5.2 Apparent viscosity of solutions of Alanate 180 (15.6 % w/w) as a function of pH at 25°C

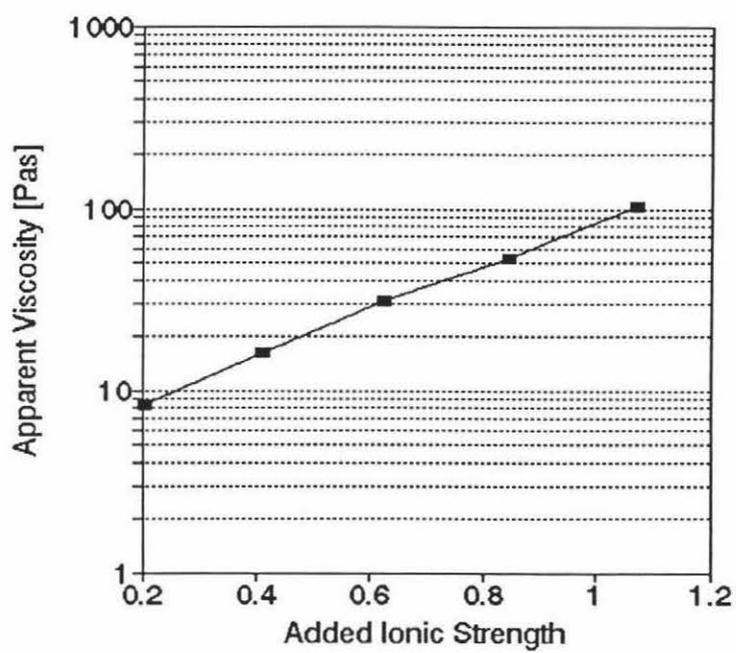


Figure 5.3 Effect of NaCl on apparent viscosity of Alanate 180 solutions (15 % w/w) at 25 °C

This proposal is supported by the results of an attempt to isolate the "particles" causing the milkiness in the solution by centrifugation, after which it was found that the solutions had reverted to their natural colour with small bubbles at the liquid surface with no traces of sediment to be found. Bubbles of air in caseinate solutions would be likely to distort apparent viscosity measurements.

For the above reasons when the viscosity/shear rate curve was noticeably affected by rod climbing, then the data collected after the commencement of rod climbing was discarded as an artifact. The closest unaffected data point from the upwards shear sweep was then used to compare the apparent viscosities of the different sodium caseinate solutions.

5.4 Reproducibility of apparent viscosity measurements for solutions of Alanate 180

The apparent viscosity data (25 °C) from the caseinate solutions with no added salt may be used as a gauge of reproducibility throughout the duration of the experimental work. The average concentration of the caseinate solutions used to generate this data was 13.74 ± 0.33 % w/w i.e. ± 2.4 % variation at a 95 % level of confidence. This concentration variation equates to a variation in apparent viscosity as calculated by equation (5.1) of ± 28 %. However, the average apparent viscosity was 1.20 ± 0.74 Pa.s i.e. ± 60 % variation at a 95 % level of confidence. A regression line was fitted to the data and the residuals were plotted against the date of solution preparation, refer figure 5.4. From this graph it appeared that solutions prepared later in the experimental stage had a higher apparent viscosity.

A regression line was fitted (equation 5.3) and the trend was confirmed at a 90 % level of confidence.

$$\Delta \eta_{app} = 2.2 \times 10^{-3} D - 2.2 \times 10^{-1} \quad (5.3)$$

where D is the date of sample preparation [days], and $\Delta \eta_{app}$ is the difference between the sample apparent viscosity and the average apparent viscosity due to time effects. The preparation date of the solution KCl replicate 1 was taken as $D = 0$.

All solutions were made from the same original bag of Alanate 180. Therefore it can be concluded that the caseinate powder was changing over time. The effect of increases in apparent viscosity due to time, as calculated by the residual regression equation (5.3), was then subtracted from the original apparent viscosity data to yield a time-modified average apparent viscosity of 1.20 ± 0.36 Pa.s (± 30 %).

The variation in the time-modified average apparent viscosity is within the limits of the variation that would be expected from protein concentration variations alone. Again when compared to the increases in apparent viscosity due to salt addition this variation is insignificant.

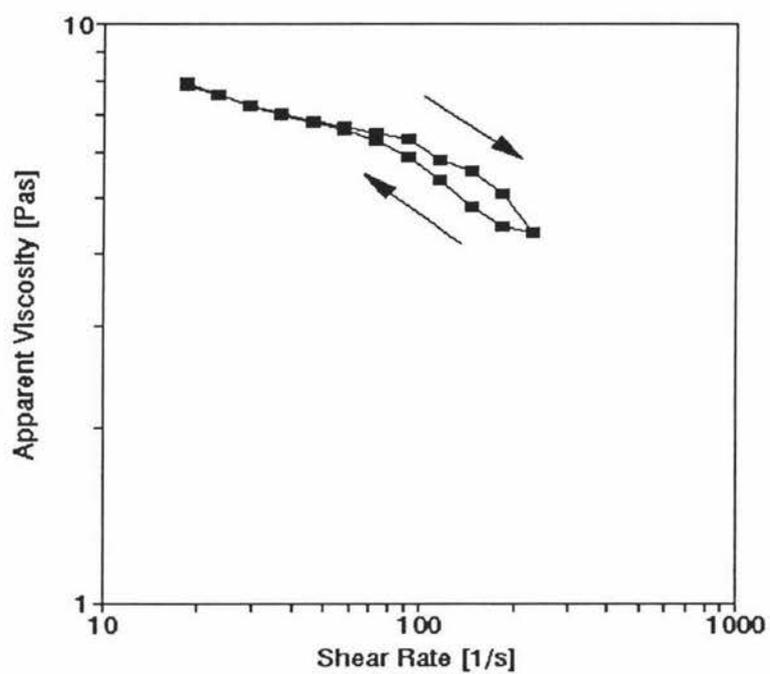


Figure 5.4 The shear sweep curve of the sodium caseinate solution with 0.4 added ionic strength KCl from replicate 1

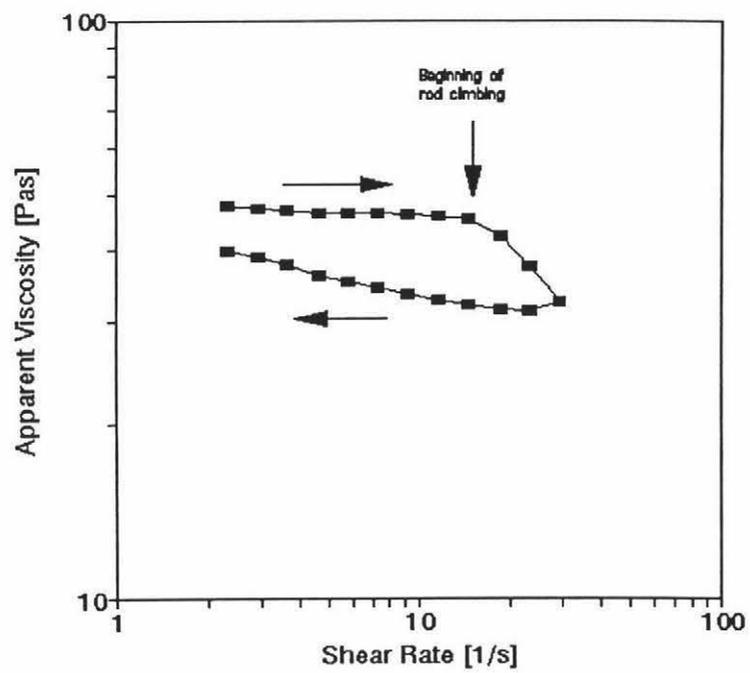


Figure 5.5 The shear sweep curve of the sodium caseinate solution with 1.1 AIS KCl from replicate 1

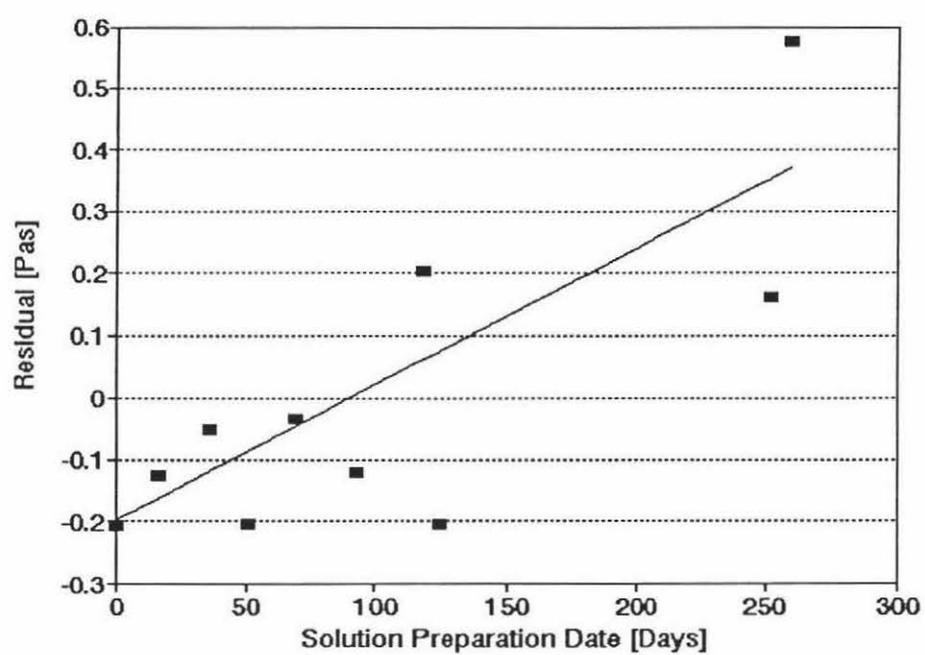


Figure 5.6 The effect of time on the apparent viscosity residual of solutions of Alanate 180 (25 °C)

6 *Effect of Monovalent Salts on the Rheology of Sodium Caseinate*

The effects of three monovalent salts; sodium chloride, potassium chloride and ammonium chloride, on the rheological properties of sodium caseinate solutions 14 % w/w were investigated. All of these salts were studied over the AIS range of 0 to 1, and at two temperatures 25 and 50 °C.

6.1 Potassium Chloride

6.1.1 The effect of potassium chloride on the shear sweep curves

There was no sign of hysteresis in the shear sweep curves from the viscometry experiments tested at 50 °C. Hysteresis was apparent in some of the curves generated from solutions tested at 25 °C.

The four solutions of replicate 1 with the highest AIS showed signs of hysteresis. The shape of the hysteresis for the solutions with 0.4, 0.6, and 0.8 AIS were similar to that shown in figure 5.4. The hysteresis exhibited by the solution 1.1 AIS KCl replicate 1 is shown in figure 5.5. Replicate 2 only showed hysteresis in the solution which has no added salt.

All of the flow curves showed a decrease in apparent viscosity with increasing shear rate. As discussed in section 4.6.2, it was not possible to test the solutions over the same shear rate range and hence a comparison of the degree of pseudoplasticity with increasing salt concentration would not be valid.

6.1.2 Effect of added potassium chloride on the apparent viscosity of sodium caseinate solutions

The apparent viscosities of all the sodium caseinate solutions were compared at a shear rate of 91 s^{-1} with the following exceptions: the data for sodium caseinate solutions ($25 \text{ }^\circ\text{C}$) with 0.8 and 1.1 AIS in replicate 1 were taken at 46 and 14.6 s^{-1} to eliminate anomalies due to rod climbing while the data for replicate 2 were recorded at 58.1 and 36.7 s^{-1} respectively due to higher shear rates being outside the range of the torsion bar; the apparent viscosity of the caseinate solution ($50 \text{ }^\circ\text{C}$) with 1.1 AIS for replicate 1 was measured at 73 s^{-1} due to torsion bar limitations.

The apparent viscosity of sodium caseinate solutions at a given shear rate as a function of AIS for KCl is shown in figure 6.1. Initial inspection of the data showed that the apparent viscosity appeared to increase in a logarithmic fashion with increases in KCl at both 50°C and 25°C . Later analysis showed that at 25°C the logarithmic increase in apparent viscosity caused by the first addition of KCl (i.e. between 0 and 0.2 AIS) was significantly larger than the increase caused by subsequent salt additions.

A linear regression of $\log[\eta_{app}]$ versus the AIS over the range 0.2 to 1.1 at 25 °C and the entire AIS range, 0 to 1.1 AIS, at 50 °C yielded the following relationships with R^2 coefficients of 0.95 and 0.94 respectively:

$$\text{at 25 } ^\circ\text{C} \quad \log[\eta_{app}] = 1.20x[A/S] + 0.27 \quad (6.1)$$

$$\text{and 50 } ^\circ\text{C} \quad \log[\eta_{app}] = 0.60x[A/S] - 0.90 \quad (6.2)$$

The increase in log viscosity for each successive addition of salt (i.e. $d[\log \eta_{app}]/d[AIS]$) was calculated. The plot of this data, figure 6.2, shows that the logarithmic change in apparent viscosity for each addition of salt appears to be constant between 0.2 and 1.1 AIS at 25 °C while the first addition of salt appears to have a greater effect on apparent viscosity. At 50 °C it appears that the logarithmic increase in apparent viscosity is constant over the entire AIS range, although the increase between 0 and 0.2 AIS is marginally higher than all the other values. Overall the logarithmic increases in apparent viscosity are lower at 50 °C than at 25 °C, however the spread of the data is reasonable leaving the possibility that $d[\log \eta_{app}]/d[AIS]$ is the same at both temperatures over the AIS range 0.2 to 1.1.

A statistical analysis of the data was performed on the basis of the following assumptions: 1) that the $d[\log \eta_{app}]/d[AIS]$ values over the AIS range of 0.2 to 1.1 at a given temperature are from the same population; 2) that the spread of data over this range is the result of random error; 3) that the variance at 25 °C is not the same as the variance at 50 °C; 4) that the variance follows a normal distribution.

The mean logarithmic increase in apparent viscosity over the AIS range of 0.2 to 1.1 was found to be 1.184 ± 0.729 at 25 °C and 0.561 ± 0.371 at 50 °C at a 95 % level of confidence. A comparison of these two

means was carried out by comparing the test-statistic, as calculated by equation (A.4) in the appendix, with a tabulated t value (Beyer, 1974) at a 95 % level with v degrees of freedom, where the degree of freedom is calculated by equation (A.5) in the appendix (Montgomery, 1985). The means were shown to be different with the mean at 50 °C being significantly lower than that at 25 °C. The logarithmic increase in apparent viscosity between 0 and 0.2 AIS compared to the mean of the constant range was found to be significantly higher for both sets of replicates at 25 °C (both replicates were approximately 8 standard deviations greater than the mean) whereas only one of the replicates at 50 °C was outside the 95% confidence limits of the mean (the replicates were 3.3 and 2.3 standard deviations greater than the mean).

Assumption 2 was tested by plotting the difference between the observed logarithmic increase in apparent viscosity and the mean increase against AIS (i.e. a plot of the residuals), figure 6.3. This plot appears to be random as most of the replicates straddle the zero line or are very close to it, and the data points are evenly scattered above and below the zero line. The replicate at 0.8 AIS tested at 25 °C seems to be an outlier. Overall assumption 2 appears to be valid.

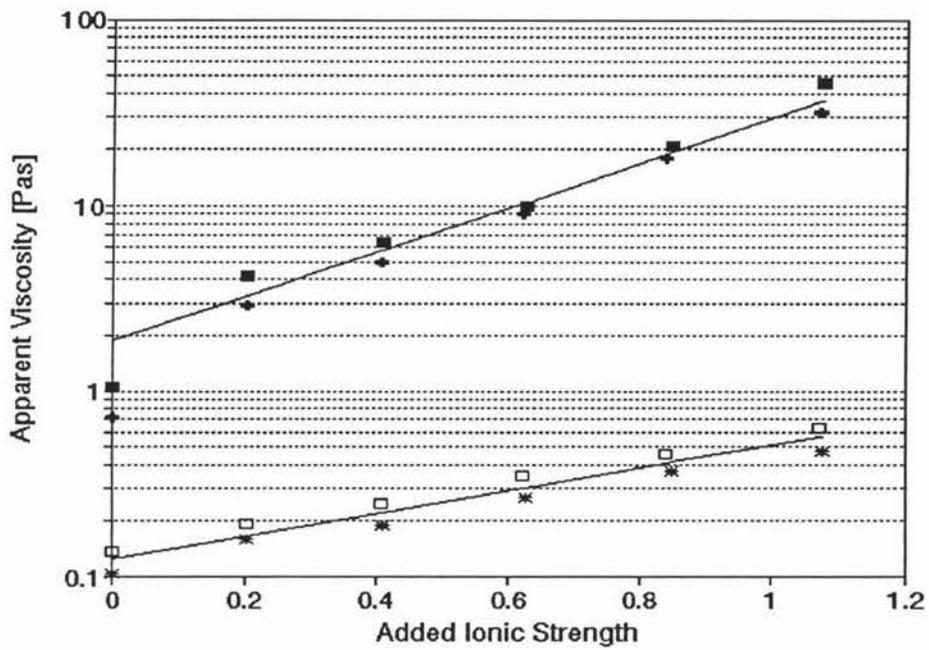


Figure 6.1 The effect of potassium chloride on sodium caseinate solutions (14 % w/w) at 25 °C replicate 1 (Δ), replicate 2 (\times), and 50 °C replicate 1 (\blacksquare), replicate 2 ($+$)

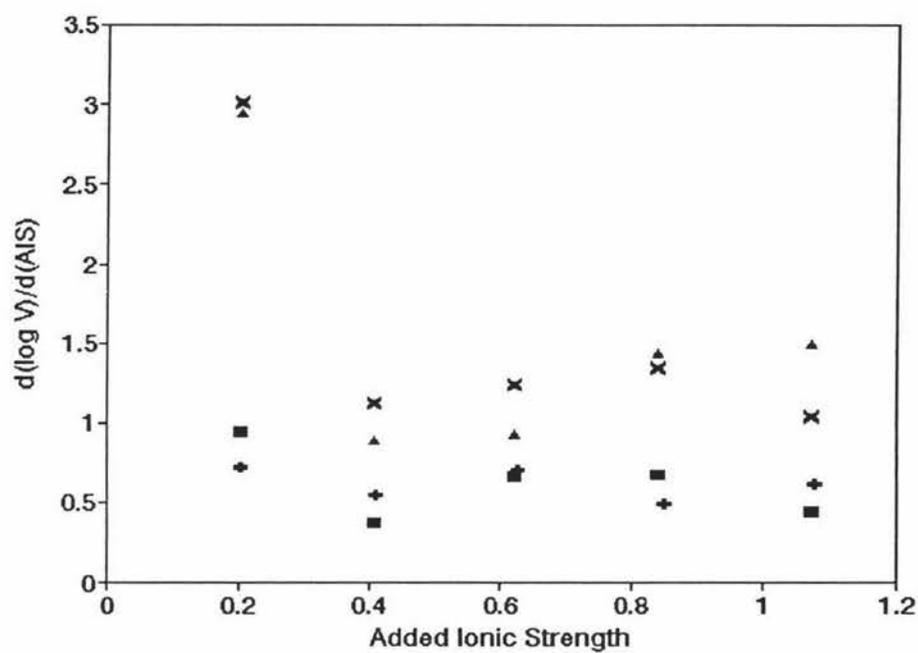


Figure 6.2 The change in apparent viscosity of sodium caseinate solutions (14 %w/w) with successive increases in the AIS KCl at 25°C replicate 1 (▲), replicate 2 (X) and 50 °C replicate 1 (■) and replicate 2 (+)

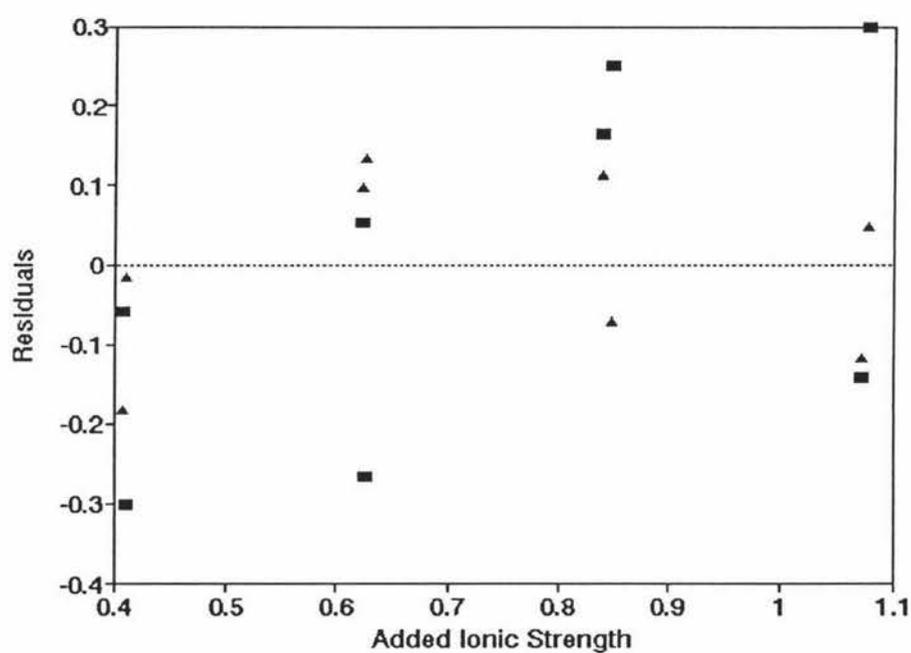


Figure 6.3 The difference between the actual and the average increase in the logarithm of apparent viscosity with AIS KCl at 25 °C (\blacktriangle) and 50 °C (\blacksquare)

6.2 Sodium Chloride

All the solutions tested had a caseinate concentration between 13.5 and 14.0 % w/w except solutions of .88 and 1.17 AIS of replicate 1 which had caseinate concentrations of 14.2 and 14.1 respectively. The range of the NaCl concentration studied was from 0 to 1.4 AIS.

6.2.1 The effect of sodium chloride on the shear sweep curves

All the solutions showed a decrease in apparent viscosity with increases in shear rate.

There was no sign of hysteresis in the solutions tested at 50 °C. Hysteresis was apparent in some of the solutions tested at 25 °C: 0.29 and 0.59 AIS of replicate 1 and at 0.29 AIS of replicate 2.

6.2.2 Effect of added sodium chloride on the apparent viscosity of sodium caseinate solutions

The apparent viscosities of all the sodium caseinate solutions were compared at a shear rate of 91 s^{-1} except for solutions of; 1.17 and 1.46 AIS of replicate 1 at 50 °C which were compared at 58.1 and 36.7 s^{-1} respectively, 0.59, 0.88, 1.17, and 1.46 AIS of replicate 1 at 25 °C which were compared at 46.0 , 18.5 , 7.31 , and 3.67 s^{-1} respectively, 0.88, 1.17, and 1.45 AIS of replicate 2 at 50 °C which were compared at 73.0 , 58.1 and 46.0 s^{-1} respectively, 0.58, 0.88, 1.17, and 1.45 AIS of replicate 2 at 25 °C which were compared at 58.1 , 18.5 , 9.21 , and 5.81 s^{-1} respectively. The main reason for the variation in the shear rate actually used for comparison of apparent viscosity was that the range of the torsion bar was exceeded.

The apparent viscosity of sodium caseinate solutions at a given shear rate as a function of AIS for NaCl is shown in figure 6.4. Initial inspection of the data showed that the apparent viscosity appeared to increase in a logarithmic fashion with increases in NaCl at both 50°C and 25°C. Later analysis showed that at 25°C the logarithmic increase in apparent viscosity caused by the first addition of NaCl (i.e. between 0 and 0.29 AIS) was significantly larger than the increase caused by subsequent salt additions.

A linear regression of $\log[\eta_{app}]$ versus the AIS over the range 0.2 to 1.5 at 25 °C and the entire AIS range, 0 to 1.5 AIS, at 50 °C yielded the following relationships with R^2 coefficients of 0.98 and 0.99 respectively:

$$\text{at } 25 \text{ } ^\circ\text{C} \quad \log[\eta_{app}] = 1.30x[A/S] + 0.57 \quad (6.3)$$

$$\text{and } 50 \text{ } ^\circ\text{C} \quad \log[\eta_{app}] = 0.64x[A/S] - 0.80 \quad (6.4)$$

The increase in log viscosity for each successive addition of salt (i.e. $d[\log \eta_{app}]/d[AIS]$) was calculated. A statistical analysis of the data was performed in the same manner as for KCl.

The mean logarithmic increase in apparent viscosity over the AIS range of 0.29 to 1.4 was found to be 1.29 ± 0.68 at 25 °C and 0.62 ± 0.34 at 50 °C with a 95 % level of confidence.

A comparison of the two means showed that the mean logarithmic increase in apparent viscosity was greater at 25 °C than at 50 °C with a 95% level of confidence. The logarithmic increase in apparent viscosity between 0 and 0.29 AIS compared to the mean of the constant range was found to be significantly higher for both sets of replicates at 25 °C

(both replicates were approximately 3.4 standard deviations greater than the mean) whereas none of the replicates at 50 °C were outside the 95% confidence limits of the mean.

A plot of the residuals, showed the data to be randomly scattered indicating that the logarithmic increase in apparent viscosity with AIS over the range 0.2 to 1.2 is constant.

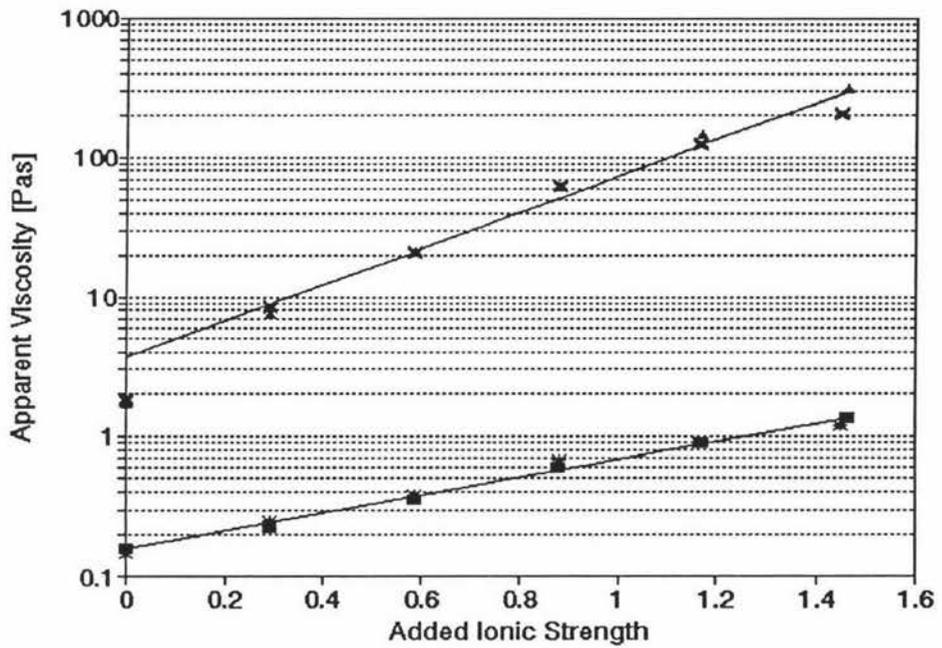


Figure 6.4 The change in apparent viscosity of sodium caseinate solutions (14 % w/w) with successive increases in the AIS by NaCl at 25°C replicate 1 (▲), replicate 2 (X), and 50°C replicate 1 (■), replicate 2 (+)

6.3 Ammonium Chloride

All the solutions tested had a caseinate concentration between 13.5 and 14.0 % w/w. The range of the NH_4Cl concentration studied was from 0 to 1.1 AIS.

6.3.1 The effect of ammonium chloride on the shear sweep curves

All the solutions showed a decrease in apparent viscosity with increases in shear rate.

There was no sign of hysteresis observed in any of the solutions tested at either 25 °C or 50 °C.

6.3.2 Effect of added ammonium chloride on the apparent viscosity of sodium caseinate solutions

The apparent viscosities of all the sodium caseinate solutions were compared at a shear rate of 91 s^{-1} .

The apparent viscosity of sodium caseinate solutions at a given shear rate as a function of AIS for NH_4Cl is shown in figure 6.6. Initial inspection of the data showed that the apparent viscosity appeared to increase in a logarithmic fashion with increases in NH_4Cl at both 50°C and 25°C. Later analysis showed that at 25°C the logarithmic increase in apparent viscosity caused by the first addition of NH_4Cl (i.e. between 0 and 0.2 AIS) was significantly larger than the increase caused by subsequent salt additions.

A linear regression of $\log[\eta_{\text{app}}]$ versus the AIS over the range 0.2 to 1.1 at 25 °C and the entire AIS range, 0 to 1.1 AIS, at 50 °C yielded the following relationships with R^2 coefficients of 0.94 and 0.92 respectively:

$$\text{at } 25 \text{ }^{\circ}\text{C} \quad \log[\eta_{app}] = 0.64x[A/S] + 0.33 \quad (6.5)$$

$$\text{and } 50 \text{ }^{\circ}\text{C} \quad \log[\eta_{app}] = 0.30x[A/S] - 0.90 \quad (6.6)$$

The increase in log viscosity for each successive addition of salt (i.e. $d[\log \eta_{app}]/d[A/S]$) was calculated. A statistical analysis of the data was performed in the same manner as for KCl.

The mean logarithmic increase in apparent viscosity over the AIS range of 0.2 to 1.1 was found to be 0.704 ± 1.429 at $25 \text{ }^{\circ}\text{C}$ and 0.251 ± 0.799 at $50 \text{ }^{\circ}\text{C}$ at a 95 % level of confidence.

A statistical analysis showed that there is no significant difference between the two means at a 95% level of confidence. The logarithmic increase in apparent viscosity between 0 and 0.2 AIS compared to the mean of the constant range was found to be significantly higher at $25 \text{ }^{\circ}\text{C}$ (approximately 2.7 standard deviations greater than the mean) whereas it was not significantly higher at $50 \text{ }^{\circ}\text{C}$ (approximately 1 standard deviation greater than the mean).

A plot of the residuals showed the data to be randomly scattered indicating that the logarithmic increase in apparent viscosity with AIS over the range 0.2 to 1.2 is constant.

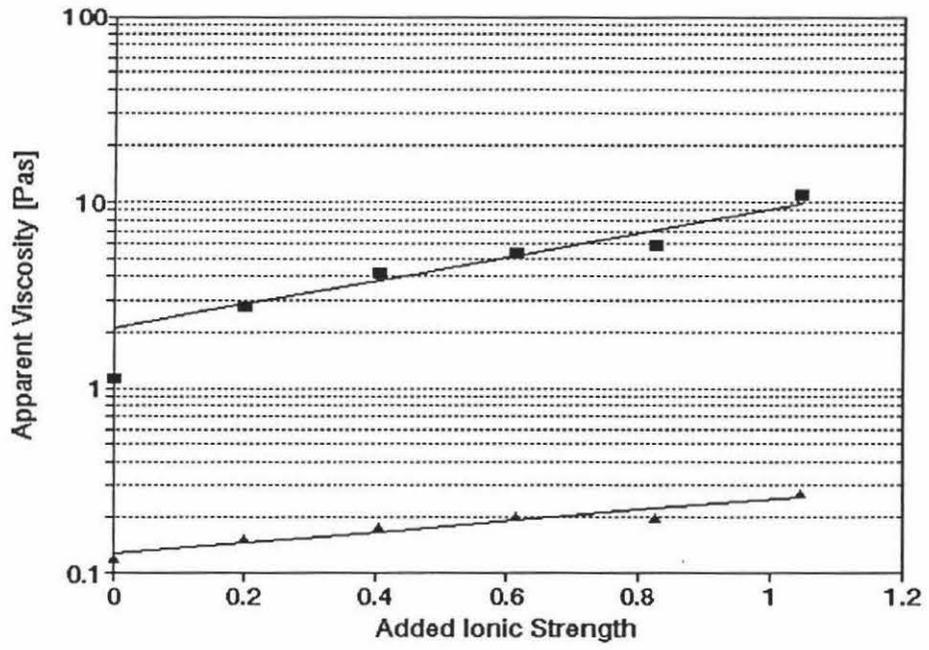


Figure 6.5 The effect of ammonium chloride concentration on the apparent viscosity of sodium caseinate solutions (14 % w/w) at 25 °C (■) and 50 °C (▲)

6.4 Discussion

6.4.1 General protein interactions

The nature of the interactions occurring in pure aqueous protein solutions is very complex. In general there are three types of interactions that are possible: protein-protein, protein-solvent, and solvent-solvent.

Table 6.1 lists specific protein-protein bond types which proteins can participate in and their characteristics. Towler et al (1981) reported that in 15 % w/w sodium caseinate solutions (pH 9.0) two of the major caseins, α_{s1} - and β - casein contribute to viscosity through hydrophobic and hydrogen bonding. κ -casein is thought to interact strongly with the other casein components through non-bonded interactions and κ -casein molecules linked together by way of disulphide bridges (Towler et al, 1981). Previously, the viscosity of casein solutions has been lowered by the use of urea (Belche and Ellis, 1957; Robinson, 1966) Urea alters the structure of the water solvent and interferes with hydrogen bonding.

In the system studied in this work the solvent is water. Proteins interact with water through their peptide bonds (dipole-dipole or hydrogen bonds) or through their amino acid side chains (interactions with ionized, polar, and even nonpolar groups).

The rheological behaviour of proteins is strongly influenced not only by the presence of water but also by the activity of the water (Cheftel et al, 1985). The water associated with proteins can assume essentially three different states: vicinal, multilayer and bulk-phase. These states have been discussed in section 2.3.1.

6.4.2 Ionic strength and electrolytes

It was observed in all the caseinate solutions that the apparent viscosity of the solution increased with increases in salt concentration and that the initial addition of salt at 25 °C caused a proportionately larger increase in apparent viscosity than subsequent salt additions.

The effect of a neutral electrolyte present in an aqueous solution of a macromolecule, such as a protein, upon the structure or conformational stability of the protein is the sum of several differing and perhaps competing interactions, such as protein-solvent (discussed in section 6.4.1), protein-electrolyte, and electrolyte-solvent interactions.

Macromolecules such as proteins are particles of colloidal size in their own right. Charge effects on colloidal particles give rise to an electric double layer which is largely responsible for the colloidal stability of the particle in the case of hydrophobic particles, but only partly so in the case of hydrophilic particles such as proteins (Schnepf, 1992).

Flocculation, in the case of the hydrophobic colloids, occurs when the electric double layer is effectively suppressed to less than 1.0 nm by the concentration of the neutral electrolyte; the thickness of the double layer is also a function of the valence of the counter ion and is independent of the charge on the colloidal particle (Eaglund, 1975). For a neutral uni-univalent electrolyte the thickness of the particle double layer is found to be of the order of 1.0 nm at an electrolyte concentration of 0.1 mol l⁻¹.

Table 6.1 Protein-Protein Linkages and Interactions

Type	Energy (kJ/mol)	Interaction distance (Å)	Functional groups involved	Disrupting solvents	Enhancing conditions
Covalent bonding	330 - 380	1 - 2	Cystine S-S	Reducing agents: mercaptoethanol cysteine, dithiothreitol, sulphites	
Hydrogen bonding	8 - 40	2 - 3	Amide; hydroxyl; phenol,	Solutions of urea, guanidine hydrochloride, detergents, heating	Cooling
Hydrophobic interactions	4 - 12	3 - 5	Amino acid residues with long aliphatic or aromatic side chains	Detergents, organic solvents	Heating
Electrostatic interactions	42 - 84	2 - 3	Carboxyl (COO ⁻), amino (NH ₃ ⁺), etc.	Salt solutions, high or low pH	
van der Waals	1 - 9		Permanent, induced, and instantaneous dipoles		

Cheftel et al, (1985) in Fennema p264

Flocculation does not occur in the case of hydrophilic colloids such as the proteins and nucleic acids, because of the marked hydration of the particle and its subsequent stabilization. Salt addition does however, mean that the intercharge repulsion between charge centres on the macromolecule will be at a minimum (Eaglund, 1975).

Concentrations of neutral electrolyte lower than 0.1 mol l^{-1} in the case of hydrophobic colloids result in a double layer of increased thickness and hence increased stability with regard to flocculation for the colloidal particle. Hydrophilic colloids, however, produce a situation which is much more complex; the macromolecule, unlike the hydrophobic particle, is capable of marked changes in conformational order. Lowering of the electrolyte concentration from 0.1 mol l^{-1} leads to increased thickness of the double layer, as in the case of hydrophobic particles, but additionally, because of the internal mobility of the macromolecular chain, to repulsion between adjacent charged groups and resulting changes in conformation. Such changes in conformation, since they may lead to the exposure of previously buried hydrophobic or hydrophilic groups, may also lead to changes in hydration of the macromolecule (Eaglund, 1975).

Changes in the hydration and conformation of the macromolecule at neutral electrolyte concentrations in excess of 0.1 mol l^{-1} do not involve changes due to variation of the thickness of the double layer, since this is effectively suppressed (Eaglund, 1975). Such changes are most likely to occur because of interactions of the ions with the solvent and the exchange between ions in the solvent and ions more firmly held by the macromolecule (Eaglund, 1975).

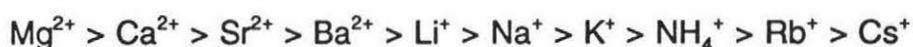
It has been observed (Eaglund 1975; Kuntz and Brassfield 1971) that at low concentrations of neutral electrolytes the amount of water bound to proteins increases with increasing salt concentration. This trend

continues until an ionic strength of 0.1 - 0.15 is reached, after which further increases in ionic strength result in marked decreases in bound water. With uni-univalent electrolytes, this maximum in hydration is associated with abrupt changes in conformational structure for a wide variety of proteins.

Such conformational changes induced by the suppression of the electric double layer at ionic strengths less than 0.15 may explain the proportionately large increase in viscosity observed between 0 and 0.2 AIS compared to viscosity increases at higher AIS.

According to Eaglund (1975 in Fennema, 1977), at high salt concentrations, electrostatic interactions are apparently of little importance with regard to the amount of water bound to proteins, whereas competition of ions and proteins for the existing water is of considerable importance.

The degree to which a protein is dehydrated by an electrolyte depends on the tendency of the electrolyte to become hydrated. Cations can be arranged in the following Hofmeister or lyotropic series of approximately decreasing hydration tendency (Singh, 1991):



Based on the AIS coefficients of equations 6.1, 6.3 and 6.5, the monovalent salts may be arranged in order of decreasing effect on apparent viscosity of sodium caseinate solutions at 25 °C over the AIS range 0.2 to 1.5: Na^+ (1.3) > K^+ (1.2) > NH_4^+ (0.64). A similar series, for the effect of monovalent salts on solutions of sodium caseinate at 50 °C, based on the AIS coefficients of equations 6.1, 6.2, and 6.3 over the AIS range 0 to 1.5 is: Na^+ (0.64) > K^+ (0.60) > NH_4^+ (0.30). It should be noted that the AIS coefficients at 50 °C are almost half of the AIS coefficient

value at 25°C. This series is further evidence to support the theory that the increases in apparent viscosity of sodium caseinate solutions with increases in salt concentration greater than 0.15 AIS are the result of dehydration of the protein by the salt rather than ion-protein interactions.

The dehydration of the caseinate molecules by the added salt would effectively increase the concentration of caseinate with respect to unbound water. As discussed in section 2.3.1, it is well known that the apparent viscosity of caseinate solutions is a logarithmic function of caseinate concentration. The constant increase in the logarithm of apparent viscosity at AIS > 0.2 also supports the concept that the primary effect of adding salt is to increase the effective caseinate concentration by binding some of the solvent water.

6.4.3 Effect of temperature

The results outlined in section 6.2, 6.3, and 6.4 showed that in all cases the apparent viscosities of caseinate solutions with or without added salt were approximately an order of magnitude lower at 50 °C than at 25 °C. It was also found that the effect of added salt on the logarithmic increase in apparent viscosity (the AIS coefficient from equations 6.1 to 6.6) at 25 °C, was approximately twice that at 50 °C for all salts. The initial addition of salt caused a significantly greater increase in apparent viscosity than subsequent additions at 25 °C whereas this phenomena was not observed at 50 °C.

Water binding by proteins generally decreases as the temperature is raised, because of decreased hydrogen bonding (Cheftel et al, 1985). Korolczuk (1982) found that for sodium caseinate solutions at neutral pH the hydration of sodium caseinate was 3.0 g water/g protein at 25 °C and decreased almost linearly to about zero at 80 °C

When salt is present in water the ions will distribute themselves relative to each other. The two factors that play roles are the thermal jostlings and the electrical interactions between charged particles. Around any positive ion there will be an increase in the concentration of negative ions and a decrease in the concentration of positive ions. These changes result in ions moving to energetically more favoured regions, i.e., those where their potential energy is low. The tendency for this movement must compete with random thermal motion (Barrow, 1979).

Therefore as temperature increases the tendency for ions to structure water and charged particles such as protein will decrease due to the increase in random thermal motion.

If the hydration of sodium caseinate and salts decreases with increasing temperature then there would also be a concomitant increase in the ratio of free bulk-phase water to the vicinal and multilayer water. This would effectively dilute the concentration of caseinate. Hermansson et al. (1975) suggested that the amount of swollen not fully solvated caseinate particles present is responsible for the enormous increase in viscosity with concentration. She proposed that at high concentrations, the swollen particles will come closer together causing friction and weak interaction forces between particles.

Presumably, assuming a homogeneous solution, because of the increase in free bulk-phase water the caseinate particles will be further apart thus decreasing the interactive forces and the degree of friction. The effect of these two mechanisms may explain the decrease in apparent viscosity with increasing temperature.

This phenomena may play a part in the observation that salt additions have a greater effect on apparent viscosity at 25 °C than at 50 °C.

In section 6.4.2 it was proposed that the proportionately large increase in apparent viscosity between 0 and 0.2 AIS compared to salt additions greater than 0.2 AIS was due to unfolding caused by suppression of the electric double layer. The reason why a similar proportionately large increase in apparent viscosity was not observed at 50 °C may be due to unfolding caused by the higher temperature. Increasing temperature progressively disorders both protein and solvent by disruption of hydrogen and ionic bonding which destabilizes protein structure and causes unfolding (Kinsella et al., 1989; Cheftel, 1985).

7 *Effect of Divalent Salts on the Rheology of Sodium Caseinate*

The effects of three divalent salts; calcium chloride, magnesium chloride and zinc chloride, on the rheological properties of sodium caseinate were investigated. The AIS range of the divalent salts studied in this section was limited from 0 to a maximum of 0.12, because precipitation occurred at higher AIS. Shear sweep experiments were conducted at two temperatures; 25 and 50 °C.

7.1 Calcium Chloride

7.1.1 The effect of calcium chloride on the shear sweep curves

Hysteresis was observed to occur in the solutions of the following AIS: 0.06, 0.08, and 0.10 of replicate 1 at 25 °C; 0.10 and 0.12 of replicate 1 at 50 °C; 0.06 of replicate 2 at 25 °C; 0.08 and 0.10 of replicate 2 at 50 °C.

The shape of the hysteresis in the curves varied between the two types observed to occur in some of the solutions with added KCl shown in figures 5.4 and 5.5. The degree of hysteresis, for either type, was generally more pronounced than those found in solutions with KCl. The shear rate at which hysteresis, occurred. in all cases, was greater than 91 s^{-1} .

All the solutions exhibited pseudoplasticity.

7.1.2 Effect of added calcium chloride on the apparent viscosity of caseinate solutions

The apparent viscosities of all the sodium caseinate solutions were compared at a shear rate of 91 s^{-1} . Figure 7.1 shows that for solutions tested at both $25 \text{ }^{\circ}\text{C}$ and $50 \text{ }^{\circ}\text{C}$ the apparent viscosity increases logarithmically with increases in the concentration of calcium chloride to reach a sharp maximum after which subsequent additions of calcium chloride result in a logarithmic decrease in apparent viscosity. The maximum at both temperatures was observed to occur at an AIS of 0.08. All the solutions with an AIS greater than 0.08 were milk coloured. The milky colour was an intrinsic property of the solutions.

A linear regression analysis was performed separately on the increasing data and the decreasing data. The AIS point 0.08 was included in both the increasing and decreasing data sets.

A linear regression of $\log[\eta_{app}]$ versus AIS at $25 \text{ }^{\circ}\text{C}$ yielded the following relationships for the increasing and decreasing data with R^2 coefficients of 0.98 and 0.97 respectively:

$$\log[\eta_{app}] = 8.80x[A/S] + 0.029 \quad (7.1)$$

increasing

$$\log[\eta_{app}] = -24.63x[A/S] + 2.80 \quad (7.2)$$

decreasing

At $50 \text{ }^{\circ}\text{C}$ the following relationships were found for the increasing and decreasing data with R^2 coefficients of 0.95 and 0.98 respectively:

$$\log[\eta_{app}] = 4.18x[A/S] - 0.95 \quad (7.3)$$

increasing

$$\log[\eta_{app}] = -18.65x[A/S] + 1.00 \quad (7.4)$$

decreasing

The rate of decrease in viscosity with increases in CaCl_2 above an AIS of 0.08 appears to be greater than the rate of increasing viscosity due to increases in CaCl_2 over the AIS range of 0 to 0.08. Solutions at 25°C are more sensitive to changes in salt concentration.

7.2 Zinc Chloride

7.2.1 The effect of zinc chloride on the shear sweep curves

Hysteresis was not observed to occur in any of the solutions. All solutions exhibited pseudoplasticity.

7.2.2 Effect of added zinc chloride on the apparent viscosity of caseinate solutions

The apparent viscosities of all the sodium caseinate solutions were compared at a shear rate of 91 s^{-1} . Figure 7.2 shows that, for solutions tested at both 25 °C and 50 °C, the apparent viscosity increases with successive additions of ZnCl_2 to reach a broad maximum over the AIS range of 0.02 to 0.06 after which further increases in ZnCl_2 concentration result in a decrease in apparent viscosity. No milkiness was observed in any of the solutions.

7.3 Magnesium Chloride

An attempt was made to establish the effect of MgCl_2 on the rheological characteristics of sodium caseinate solutions over the AIS range of 0 to 0.12. From the results of the viscometry experiments, it appeared that

the addition of $MgCl_2$ had no effect on the apparent viscosity of solutions of sodium caseinate at either 25 or 50 °C. The moisture analysis of each solution showed that the concentration of the solutions decreased from 13.8 at 0 AIS to 11.2 at 0.12 AIS. A moisture analysis of each centrifuge tube demonstrated that a concentration gradient existed in all the samples, except the solution with 0 AIS. Due to the presence of the moisture gradient in all the solutions the data is not considered reliable and has not been included in this work. Since all the solutions tested exhibited constant apparent viscosity despite the significant concentration gradient, it would seem that $MgCl_2$ must increase the apparent viscosity of sodium caseinate solutions. The most likely cause of the concentration gradients would be the formation of aggregates on the addition of $MgCl_2$ which subsequently are spun out during the centrifugation step of the method (section 4.4). It is known that substantial amounts of magnesium are found in the micelles of casein (Swaisgood, 1985)

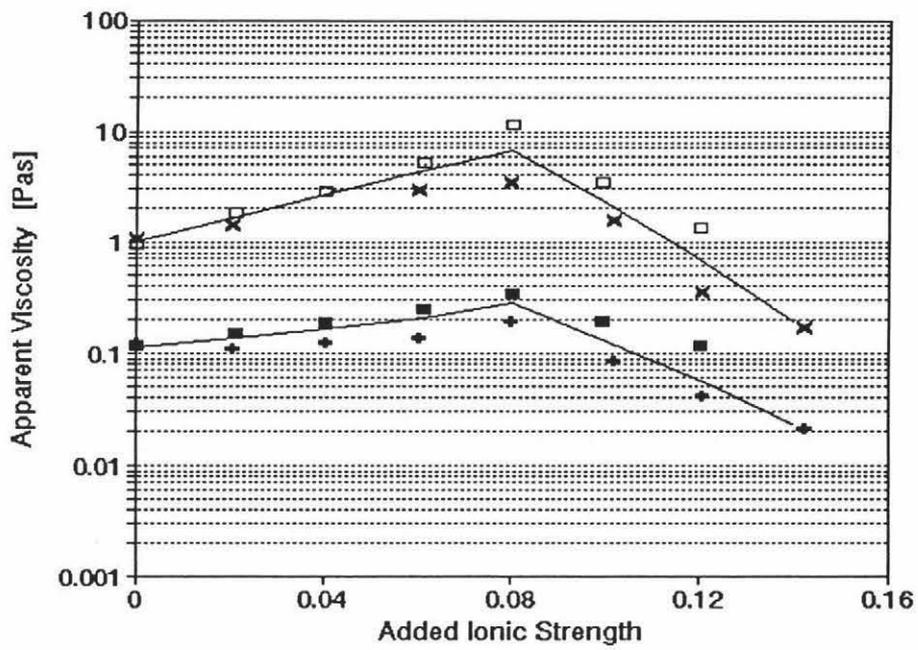


Figure 7.1 The effect of calcium chloride concentration on apparent viscosity of sodium caseinate solutions (14% w/w) at 25 °C (□ replicate 1, x replicate 2), and 50 °C (■ replicate 1, + replicate 2)

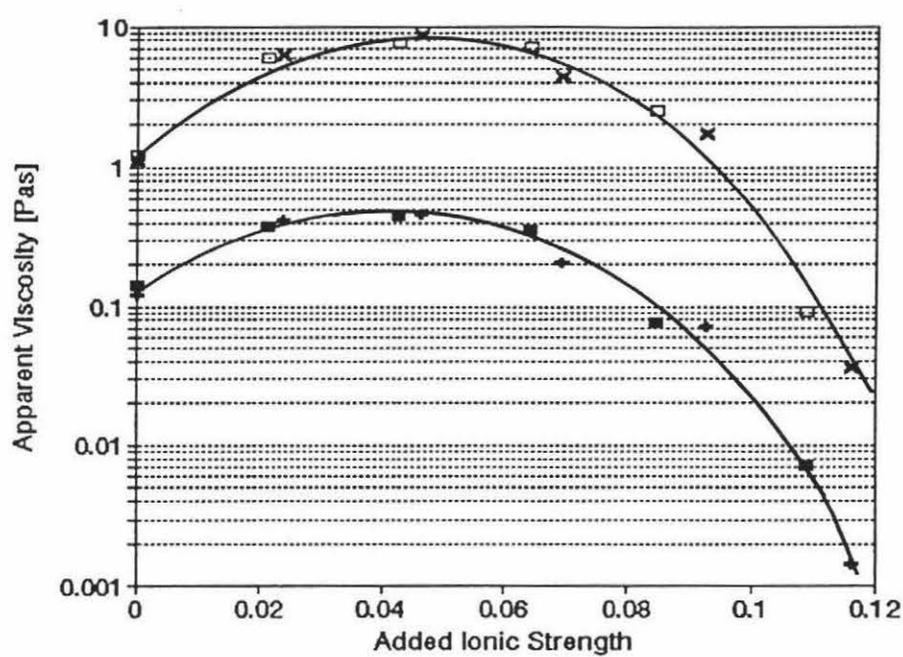


Figure 7.2 The effect of zinc chloride concentration on apparent viscosity of sodium caseinate solutions (14% w/w) at 25 °C (□ replicate 1, x replicate 2), and 50 °C (■ replicate 1, + replicate 2)

7.5 Discussion

The main effects of divalent salts on the rheological properties of sodium caseinate solutions are: 1. Low salt concentrations increase apparent viscosity until a maximum is reached after which increases in salt dramatically decrease apparent viscosity; 2. The maximum of the ZnCl_2 graph appears to be more curved than the sharp maximum for CaCl_2 ; 3. The effect of divalent salt on apparent viscosity is greater at 25 °C than at 50 °C; 4. The concentration of divalent salt that can be added to caseinate solutions is limited due to precipitation which occurs at approximately > 0.12 AIS.

The shape of the apparent viscosity - AIS graphs indicates that there are two mechanisms affecting apparent viscosity: one which acts to increase viscosity and the second which acts to decrease viscosity.

The increases in apparent viscosity with divalent salts at low concentration and the decreased viscosity at high temperatures are probably due to the mechanisms that were described in sections 6.4.2 and 6.4.3 respectively for monovalent salts.

The maximum of the ZnCl_2 graph appears to be more curved than the sharp maximum for CaCl_2 . This would indicate that for ZnCl_2 both of the viscosity changing mechanisms occur at the same time, with a gradual change in the dominance of the mechanisms compared to the sharp, abrupt maximum (coupled with a dramatic colour change) for CaCl_2 which suggests the effective loss of the mechanism which causes an increase in apparent viscosity and the gain of one which causes a decrease in the apparent viscosity and a colour change in the sodium caseinate solutions.

The mechanism responsible for decreases in apparent viscosity was not

observed with the addition of monovalent salts. The decrease in apparent viscosity is probably due to the formation of micelles. The process of micellization involves a massive change in conformation and association of the caseinate molecule (Mulvihill and Fox, 1989). Micelle formation decreases apparent viscosity because protein is bound into little tight balls. This explanation for the decrease in apparent viscosity at AIS > 0.08 for CaCl_2 and 0.06 for ZnCl_2 is supported by a number of literature references.

From the data collected in this study the first CaCl_2 solution to show a change in colour was the solution with an AIS of 0.1. This is equivalent to 7.3 mg calcium per gram of protein. This value is in agreement with Mulvihill and Fox (1989) who reported that addition of calcium to sodium caseinate increased viscosity, especially at >pH 7.0 but at <pH 7 addition of calcium >8mg/g caused a decrease in viscosity due to micelle formation.

The formation of aggregates or micelles in the presence of zinc or calcium ions is contrary to what one would expect from the place of these ions in the Hofmeister series (section 6.4.2). The reason for this discrepancy is that in addition to the hydration and electric double layer affect of ions (which follows the Hofmeister series) (section 6.4.2), ions may also affect the protein by preferentially binding to the protein molecule itself (Schnepf, 1992).

In a general review on the interactions of ions with proteins Barrow (1983) reported that alkaline metals (such as sodium and potassium ions) react to only a limited extent with proteins, whereas alkaline earths, such as calcium and magnesium, are somewhat more reactive. Barrow also noted that Ca^{2+} ions (also Fe^{2+} , Cu^{2+} , and Mg^{2+}) may be an integral part of certain molecules or molecular associations .

The interactions reported by Barrow appear to hold true for caseinate. Clarke (1989) reported that sodium ions have approximately 1/100 th of the affinity of calcium ions for binding to casein. Organic phosphate groups are the predominant substrates in ion binding to casein (Clark et al, 1989). Clarke et al, (1989) in an electrochemical study of calcium ion binding to sodium caseinate (15 % w/w) found that prior to the addition of calcium chloride mainly sodium ions are bound. A model of initial ion exchange (two sodium ions for one calcium ion) followed by more extensive calcium binding past the stage of complete desorption of sodium ions was proposed. It is well known that caseinates such as sodium and potassium lack micellar structure whereas calcium caseinate may have some.

The reason for the strong interactions of caseinates with polyvalent ions has been attributed to the cross-linking of carboxyl and ester phosphate groups as well as through intermolecular hydrophobic bonding that is favoured at appropriate conditions of temperature, pH, and $[Ca^{2+}]$ (Morr CV, 1982). The caseins, α_{s1} , α_{s2} , and β -casein have a high concentration of phosphoserine residues and a high net negative charge at neutral pH. Ca^{2+} is preferentially bound and Zn^{2+} can also be bound. The binding of these ions leads to charge neutralization, aggregation and eventually precipitation (Coker, 1991).

8 Gelation of Sodium Caseinate Solutions with added Salt

8.1 Oscillation experiments

The reason for the existence of this chapter was the observation that some of the high apparent viscosity caseinate solutions with added NaCl, KCl, NH_4Cl and ZnCl_2 appeared to form gels when refrigerated. Reference to the phenomenon of cold gelation of caseinate solutions has not been published. The most well documented case of cold gelation is that of gelatin, but a number of polysaccharides also form gels on cooling. These include agarose, pectin, and carrageenan (Evans and Wennerstrom, 1994).

Like a solid, a gel has elastic properties; when it is deformed by stress, it recovers its original shape after removal of the stress, as the deformation has left the bonds between the particles intact. But a gel also has viscous properties, because part of the deformation is not recovered at removal of the stress; in other words the gel flows when stressed as bonds are broken and new bonds formed, as in a liquid. If the stress is applied for a very short time, the elastic (i.e., recoverable) deformation is always predominant; if the stress lasts long, the viscous deformation (i.e., flow) is most prevalent (Walstra and Jenness, 1984).

A gel thus shows viscoelastic behaviour, which can be characterized by two rheological parameters, the elastic or storage modulus (G') and the viscous or loss modulus (G''). If G' is much greater than G'' the elastic properties prevail, if G' is much less than G'' the viscous properties prevail. The overall resistance to deformation is expressed in the combined modulus G^* . The moduli give the ratio of the stress to the

relative deformation and thus have the dimension of stress (e.g., Pa); they depend, often strongly, on the time scale of the deformation (Walstra and Jenness, 1984). The dependence of the moduli on the time scale of the deformation was beyond the scope of this study. The relationship between G^* and G' and G'' is shown in the vector diagram below, figure 8.1.

In a review of gels and gelling, Clarke AH (1992) reports that in a typical gelling system G'' is initially greater than G' , indicating the starting solution to be a viscoelastic fluid of low G^* , and one which is relatively more viscous than it is elastic. At a certain time the G' and G'' curves cross-over, and then G' rises rapidly as the system develops a much higher modulus, and shows much greater relative elasticity. After the cross-over, G'' rises too, and in many cases passes through a maximum before falling again as the network becomes more completely established, and still more elastic. In the work presented here the temperature at which the crossover point occurs is taken to be the gel-sol transition temperature. The cross-over corresponds to a phase angle of 45° . An ideal elastic body has a phase angle of zero and an ideal viscous body has a phase angle of 90° .

8.2 Results

The cold gelation characteristics were studied for each series of solutions in which it was observed to occur using the solution with highest apparent viscosity. For the monovalent salts this was the solution with an AIS of about 1.1 whereas for zinc chloride this was 0.04 AIS. The effect of AIS on the gelation properties of sodium caseinate was examined with NaCl. The method used for observing gelation of these solutions is detailed in section 4.7.

The rheological parameters measured by the Bohlin during a typical

gelation experiment are shown graphically in figure 8.2. The data of interest from these experiments are the gel-sol transition temperature, the elastic modulus (G'), the viscous modulus (G'') and the phase angle (δ). These values are shown for: a) the effect of different salts on gelation characteristics and b) the effect of concentration of NaCl on gelation characteristics, in tables 8.1 and 8.2 respectively. The rheological parameters, i.e G' , G'' and δ , tabulated are the average of the values recorded at 5 °C.

Figure 8.3 shows the effect of NaCl concentration on the gelling characteristics of sodium caseinate. G' increases in a linear fashion with AIS. G'' by comparison appears to increase more slowly with AIS. The gelation temperature reported in table 8.2 is the average of the gel-sol transition temperature measured by the Bohlin on the cooling of the sol and the heating of the gel. The raw data showed the gelation temperature (as measured by the Bohlin waterbath) to be consistently 4.6 °C colder than the melting temperature. An experiment to gauge the accuracy of the Bohlin's measuring system revealed a lag, during heating and cooling, of approximately 3 °C between the Bohlin measured temperature of the sample and the actual temperature of the sample as measured by a thermocouple.

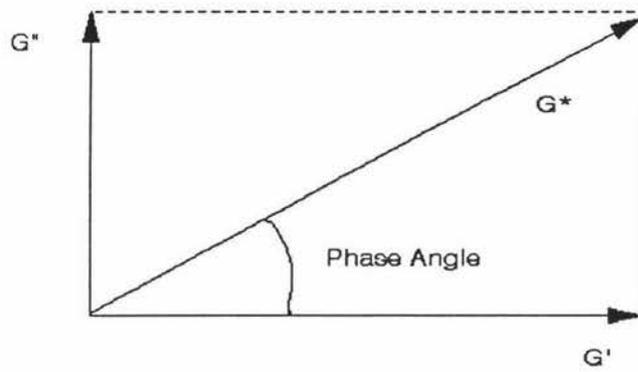


Figure 8.1 Vector diagram showing the relationship between G' , G'' , G^* and the phase angle (Tang 1990)

Hence the apparent difference in the gel-sol transition temperature on cooling and heating (4.6 °C) is believed to be an artifact of the Bohlin with the real gel-sol transition temperature lying at the mid-point between the two measured values. The gel-sol transition temperature of the caseinate solutions increased approximately linearly with AIS.

Figure 8.4 shows that the phase angle appears to decrease linearly with increased NaCl concentration over the AIS range of 0.3 to 1.4, whereas between 0 and 0.3 the decrease in phase angle is greater. The solution with no added salt was the only solution not to form a gel.

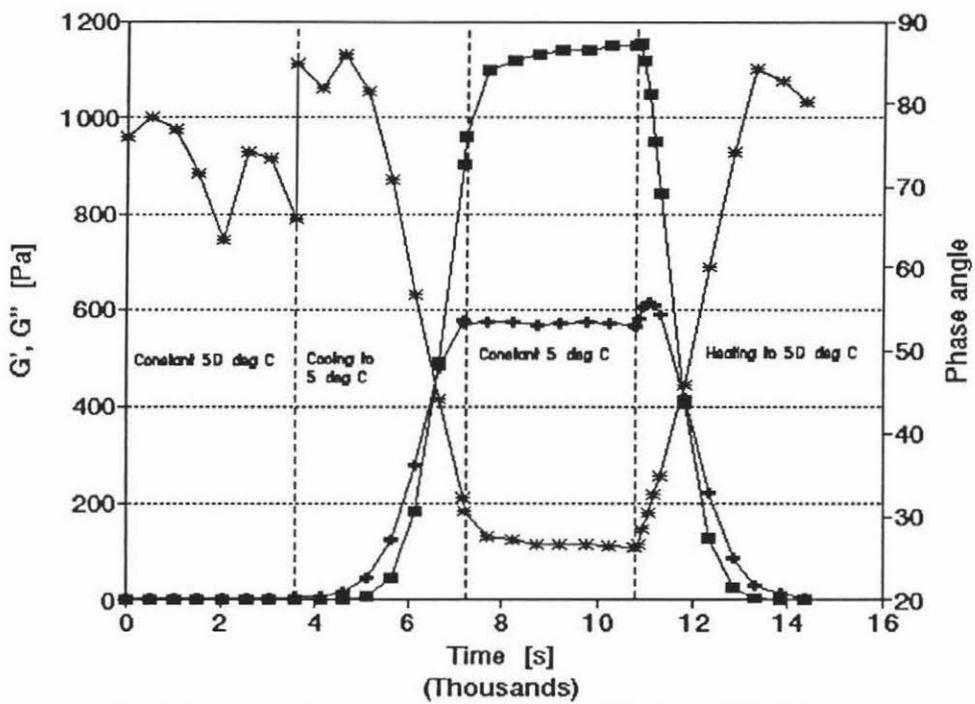


Figure 8.2 The rheological parameters: G' (■), G'' (+), and phase angle (*), as a function of time and temperature for the gelation of a 1.06 AIS NaCl sodium caseinate solution

Table 8.1 Gelling characteristics of sodium caseinate solutions with different ions

Ion	AIS	Caseinate concentration [% w/w]	Gel-sol transition temperature [°C]	G' at 5°C [Pa]	G'' at 5°C [Pa]	Phase angle at 5°C [Pa]
Na	1.06	14.0	14.8	1112	571	26
K	1.08	14.0	14.9	1254	613	26
NH ₄	1.04	13.5	8.0	353	295	39
Zn	0.04	13.4	—————	497	613	51

Table 8.2 The effect of NaCl concentration on the gelling characteristics of sodium caseinate solutions

AIS	Caseinate concentration [% w/w]	Gel-sol transition temperature [°C]	G' at 5°C [Pa]	G'' at 5°C [Pa]	Phase angle at 5°C [°]
0	13.8	————— —	16.2	33.3	64.1
0.3	13.8	9.6	322	238	36.5
0.6	13.9	13.2	658	381	30.1
0.9	14.2	17.0	1255	516	22.4
1.2	14.1	19.7	1861	590	17.6
1.4	13.9	22.4	2284	537	13.3

8.3 Discussion

In general, gelation of proteins is thought to proceed by a two step mechanism. The first step involves the denaturation of the protein. The protein begins to change its native conformation and unfold. The second step, which often proceeds more slowly, involves the reorientation of the denatured proteins into a three-dimensional network (Schnepf, 1992).

In the traditional formation of jelly, a polymer solution is made up, by dissolving gelatin in hot water. At the elevated temperature, the thermal vibrations will carry the molecules past one another, but, on cooling, the thermal vibrations cease to be strong enough to overcome the attractive forces at entanglements. The complete movement of whole chains away from one another is then prevented. The structure solidifies as a gel, which will be reversible: it will 'melt' on heating (Hearle, 1982).

In the results it was found that although the melting temperature was higher than the gelation temperature the difference was not larger than the temperature lag between the temperature of the actual fluid and the temperature measured by the Bohlin. Hence the difference was deemed to be an artifact of the Bohlin. However, Evans and Wennerstrom (1994) in a discussion on the formation of physical gels stated that the process is not thermodynamically reversible, i.e. the heating and cooling curves are slightly displaced relative to one another. It may be that this was the case with the solutions tested here, however in this study the noise generated by the temperature lag is such that this is not possible to determine.

The plot of phase angle at 5 °C versus salt concentration, figure 8.4, shows that the initial addition of salt causes a much greater increase in the elastic nature of a caseinate solution than subsequent increases in

salt concentration.

The shape of the phase angle-AIS plot can be explained by the mechanisms described in section 6.4.2; i.e. that the initial addition of salt > 0.15 AIS suppresses the electric double layer resulting in a change in conformation and that subsequent salt additions have a dehydrating effect on the protein. There is evidence in the literature that supports this explanation.

In a review on gelation, Cheftel et al, (1985) reported that electrostatic repulsions (caused by the electric double layer) especially at pH values far from the isoelectric point and protein-water interactions tend to keep polypeptide chains apart. Intermolecular protein attraction (and gelations) takes place more readily at high protein concentrations because of the greater probability of intermolecular contacts (Cheftel et al, 1985; Hearle, 1982).

The conclusions reached in section 6.4.2 may also explain why the solution of ZnCl_2 at 0.06 AIS did not form a gel when the solution of NaCl at 0.3 AIS did, even though the ZnCl_2 solution had a higher apparent viscosity. The zinc solution had a higher apparent viscosity due to the strong salt bridges formed between caseinate molecules as a result of the strong affinity of the protein for the ion. However the added ionic strength of the solution was < 0.15 hence the suppression of the electric double layer and therefore the changes in conformation necessary for cold gelation could not occur.

9 *Conclusions and Recommendations*

9.1 Effect of monovalent salts on the rheology of sodium caseinate solutions

The effects caused by the addition of monovalent salts on the apparent viscosity of sodium caseinate solutions at 25 °C and 50 °C are summarised by figure 9.1. The main sections of the figure are:

- A) The initial addition of salt (0.2 AIS) at 25 °C causes a significantly greater proportional increase in apparent viscosity than subsequent additions of salt. It is proposed that this increase in apparent viscosity is due to conformational changes induced by the suppression of the electric double layer. The reason why a similar proportionately large increase in apparent viscosity was not observed at 50 °C may be due to unfolding caused by the higher temperature.

- B) The logarithm of apparent viscosity increases linearly with increases in salt concentration greater than 0.2 AIS. It is proposed that this increase is the result of dehydration of the protein by the salt rather than ion-protein interactions. The slope of section B for sodium caseinate solutions with added salt increased in the order: $\text{NH}_4^+ < \text{K}^+ < \text{Na}^+$. This series is in accordance with the Hofmeister series for the hydration tendency of cations.

- C) The logarithm of apparent viscosity for sodium caseinate solutions increases linearly with salt addition over the range 0 to 1.4 AIS at 50 °C. It is proposed that increase in the logarithm of apparent viscosity is due to the dehydrating effect of the salt. The slope of

section C is approximately half that of section B. The reason for the difference in slope is probably due to the tendency for ions to structure water and charged particles (e.g. proteins) being disrupted by the increase in random thermal motion at 50 °C.

- D) The apparent viscosity of a given solution with or without added salt was an order of magnitude greater at 25 °C than at 50 °C. The reason for this difference is related to the decrease in water binding by proteins because of the disruption of hydrogen bonding at high temperatures.

9.2 Effect of divalent salts on the rheology of sodium caseinate solutions

The apparent viscosity of sodium caseinate solutions increases with divalent salt concentration until a maximum is reached. Increasing the salt concentration above that necessary for maximum apparent viscosity results in a decrease in viscosity followed by precipitation. The shape of the apparent viscosity - AIS curves were similar at 25°C and 50°C. The apparent viscosity of a given solution with or without added salt was an order of magnitude lower at 50°C than at 25°C.

The mechanisms responsible for the increase in apparent viscosity are probably the suppression of the electric double layer and dehydration effects caused by the increase in ionic strength and protein binding by the divalent ion.

The maximum and subsequent decrease in apparent viscosity and precipitation observed with further increases in AIS are probably the

result of aggregate and/or micelle formation due to ion binding to the caseinate molecule.

The difference between the apparent viscosity of a solution at 50 °C and 25 °C is due to the decrease in water binding by proteins because of the disruption of hydrogen bonding at high temperatures.

9.3 Effect of salts on the cold gelation of sodium caseinate solutions

It was found that in order for a sodium caseinate solution with added NaCl to form a gel at 5 °C, the salt concentration must exceed 0.29 AIS (the lowest AIS tested). It is proposed that the suppression of the electric double layer is necessary if a gel is to form. All the sodium caseinate solutions with 1.1 AIS monovalent salt formed gels.

Solutions containing divalent salts, even though they had a higher apparent viscosity than some of the gel forming solutions containing monovalent salts, did not form gels. It was concluded that their inability to form gels at 5 °C was that their AIS was less than that necessary to suppress the electric double layer (0.15 AIS).

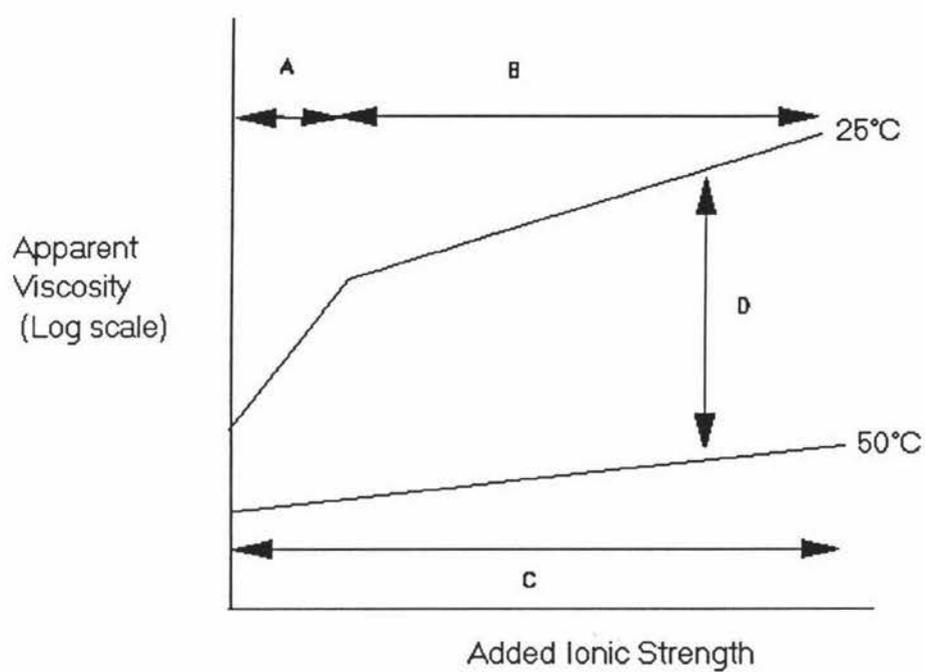


Figure 9.1 Summary of effects of monovalent salt concentration on the apparent viscosity of solutions of sodium caseinate

The gel-sol transition temperature was found to increase with increasing salt concentration. It was concluded that this was the result of dehydration of the caseinate molecules by the monovalent salts.

9.4 Application of findings to industry

In many applications of casein and caseinate significant quantities of salts of some type are present in the final formulation e.g. whipped toppings, low fat spreads, coffee whiteners, adhesives, paper coatings. The results in this study help our understanding of the interactions between caseinate and salt in such final applications.

9.5 Recommendations for future work

In future work on this subject, it is recommended that:

- a) the effect of K^+ , and NH_4^+ concentration on the rheological characteristics of cold setting sodium caseinate gels be examined, particularly over the AIS range 0 to 0.3;
- b) the effect of salt concentration on the apparent viscosity of sodium caseinate solutions over the temperature range 25 to 50 °C be studied. This would enable the temperature at which salt addition of 0.2 AIS ceases to cause a proportionately greater increase in apparent viscosity than subsequent salt additions to be determined.
- c) the effect of salts on the rheological characteristics of solutions and gels made from different caseinates i.e. calcium, ammonium, potassium and aluminium caseinates be studied.

- d) the effect of trivalent ions on the apparent viscosity and gelling characteristics of sodium caseinate solutions be investigated.
- e) the viscometry experiments reported here be repeated on a rheometer that is capable of measuring normal stress. This would enable a more complete characterisation of the rheological behaviour of caseinate solutions to be made.

This study has concentrated on the rheological effects of salts on sodium caseinate solutions. In most cases, however, texture, consistency or viscosity modification are only one of the reasons cited for the incorporation of caseinate in an application. Other reasons for caseinate usage include: adhesive qualities, ability to accept colour, emulsion stabilizer, foam formation and nutrition. A comprehensive review on the uses of caseins and caseinates is given by Southward (1989). Therefore it would be useful to investigate what effect salts have on these other functional properties.

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Appendix

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

Hypothesis

$$H_0: \mu_1 = \mu_2 \quad (\text{A.1})$$

$$H_1: \mu_1 < \mu_2 \quad (\text{A.2})$$

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

Criteria for Rejection

$$t_0 < -t_{\alpha, v} \quad (\text{A.3})$$

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

Test Statistic

$$t_0 = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \quad (\text{A.4})$$

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 for t_0

$$v = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2} \right)^2}{\frac{(S_1^2/n_1)^2}{n_1 + 1} + \frac{(S_2^2/n_2)^2}{n_2 + 1}} - 2 \quad (\text{A.5})$$