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RHEOLOGY OF WHEY PROTEIN

SOLUTIONS AND GELS

Thesis submitted for the

degree of

Doctor of Philosophy

in

Food Technology

at

Massey University

New Zealand

by

Qingnong Tang

1993

RHEOLOGY OF WHEY PROTEIN SOLUTIONS AND GELS
QINGNONG TANG
TYPOGRAPHICAL AMENDMENTS

Page

- ix Line 2: for "comparison" read "comparisons"
- xxiii Line 8: for "in press" read "349-361"
- 5 Line 5: for "compositions" read "composition"
- 5 Line 4 from bottom: for "with" read "and"
- 10 Line 9: for "that whey" read "that when whey"
- 11 Line 7 from bottom: for "theory on" read "theory based on"
- 11 Last line: for "from" read "form"
- 12 Line 10 from bottom: for "Flory-Stockmayer" read "Flory and Stockmayer"
- 31 Line 5: for "surface" read "surfaces"
- 38 Line 14: for "3.2 (Data provided" read "3.2. (Data were provided".
- 40 Line 8 from bottom: for "section" read "Section"
- 45 Last line: for "thick depth" read "deep"
- 46 Line 4 from bottom: for "rigidity E_t " read "rigidity, E_t "
- 57 Last line: for "can change" read "changed"
- 58 Line 7 from bottom: for "Table 2" read "Table 4.2"
- 104 Line 12 from bottom: for "highest" read "observed maxima"
- 105 Line 13: for "staff" read "start"
- 106 Legend on x-axis of Fig. 6.14: for "pH above" read "pH well above"
- 114 Line 3: for "form 7 to 9" read "from 7 to 9."
- 120 Line 2: for "CaCl²," read "CaCl₂"
- Line 16: for "difference" read "differences"
- 124 Line 8: for "toward the end" read "for the subsequent duration"
- 125 Line 7 from bottom: for "experiment" read "experiments"
- 130 Line 2: for "widen" read "wider"
- 135 Legend on x-axis of Fig 8.10: for " $(C/C_m - 1)$, (%w/w)" read " $(C/C_m - 1)$ "
- 136 Line 8: for "was quoted" read "is given"
- Line 9: delete "has been"
- Line 12 from bottom: for "occur" read "occurs"
- Line 4 from bottom: for "be" read "lie"
- 142 Line 5: for "paper" read "work"
- Line 9 from bottom: for "comparison" read "comparisons"
- Line 6 from bottom: for "above" read "in Chapter 3"
- 144 Line 2: for "On" read "In"
- 154 Line 7: for "solution" read "solutions"
- Line 6 from bottom: delete "all"
- 155 Line 5: for "high" read "higher"
- 156 Line 11 from bottom: for "straight forward" read "straightforward"

Abstract

The use of whey protein products in foods is governed by their nutritional and functional properties. Whey protein products have increasingly been applied in a variety of food systems as functional ingredients. In order to boost applications of whey protein products and to improve, predict and control their functional attributes in food products knowledge is required about how they behave functionally under different conditions, *e.g.* when product composition, processing history, protein concentration, pH, salt concentration and temperature vary.

The flow properties of whey protein concentrate solutions were studied in a Bohlin rheometer. The effects of protein concentration, temperature, pH and salts on the gelation and gel properties of whey protein concentrates and whey protein isolate were also investigated in the same rheometer. Differences in gelation between whey protein concentrates, whey protein isolate, egg white and β -lactoglobulin were studied. Differences between dynamic shear properties determined in a Bohlin rheometer and fracture properties determined in an Instron universal testing machine were also studied.

The flow properties of whey protein concentrate solutions changed from Newtonian to pseudoplastic or even thixotropic behaviour, owing to structure formation in the solutions, *i.e.* to increases in protein intermolecular interactions. Such structure formation resulted from increases in protein concentration, temperature or CaCl_2 concentration, and from shifting the pH to extreme values.

Gelation of whey protein was dependent on protein concentration, gelation temperature, pH, salt content and lactose content. Salt content was the most important factor in determining the gelling properties of various whey protein concentrate products and whey protein isolate. Consistent gelling properties could only be achieved when salt content was carefully controlled. The degree of protein denaturation and lactose content also led to differences in gelling behaviour of whey protein concentrates.

Whey protein products, when compared with egg white, had a higher gelation

temperature, a higher minimum protein concentration for gelation, lower initial gelation rate and lower gel stiffness. The differences in initial gelation rate and gel stiffness could be compensated by adjustment of the salt content of whey protein products.

Dynamic viscoelastic measurements on whey protein isolate gels in the region of the sol-gel transition exhibited simple power law relationships between the storage (G') and loss (G'') moduli and frequency as $G' \propto \omega^{0.54 \pm 0.02}$ and $G'' \propto \omega^{0.51 \pm 0.02}$, indicating that the gel in the region of the sol-gel transition could have the geometry of a fractal. The critical exponents calculated from the protein concentration dependence of gelation time and from the site percolation model indicated that the gelation of whey protein is a realization of a percolation process.

Compression rigidity modulus (E_c), penetration rigidity (E_p), tension rigidity (E_t) and storage modulus G' all exhibited a similar pattern of variation with pH. G' , E_c , E_p and E_t , which were not closely related to the fracture properties and hardness of whey protein concentrate gels, were controlled by electrostatic interactions. The fracture forces and hardness were determined by both disulphide bonds and electrostatic interactions, while fracture strains were mainly controlled by disulphide bonds.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisors, Professor Peter A. Munro and Dr Owen J. McCarthy, for their guidance, advice and encouragement throughout this project.

I also wish to thank Professor Peter A. Munro and Dr Geoffrey Page for giving me the opportunity to work on this very interesting project.

This work was supported by a research grant from the New Zealand Dairy Research Institute (NZDRI). The Bohlin Rheometer was jointly funded by the NZDRI, the University Grants Committee and Massey University. These grants and the help provided by NZDRI during the course of this work are gratefully acknowledged. Particularly thanks must go to Dr Sheelagh A. Hewitt, Dr John J. Higgins, Dr Rex Humphrey and Dr Mark Pritchard of the NZDRI for their help and valuable advice throughout this project.

I would like to thank Mr Campbell Shearer of Bay Milk Products Ltd for samples of whey protein concentrates.

I would also like to thank all the staff of the Department of Food Technology and the Food Technology Research Centre at Massey University, especially the following:

Dr Osvaldo H. Campanella, Dr Harjinder Singh, Mr Rod Bennett, Dr Derek Haisman, Professor Ray Winger and Professor M. D. Earle for their interest, encouragement and help.

Mr Hank Van Til, Mr Garry Radford, Mr Byron McKillop, Mr Teo Cheng Tet, Mr Alistair Young, Mr Andrew Saunders and Mr Steve Glasgow for their technical assistance.

Ms June Latham, Mrs Margaret Bewley, Mrs Lesley James and Miss Rebecca Baxter for their encouragement and help during the course of this work.

All my fellow graduate students, particularly Miss Pinthita Mungkarndee, Miss Daisy Tañafrañca, Mr Hong Chen, Ms Shen Jian, Miss Netra Manvikar, Miss Rani Govindasamy, Mr David Oldfield, Mr Ranjan Sharma, Mr Salah Joudh, Mrs Gayatri Susarla, Ms Carolyn Russell, Ms Tania Ngapo and Miss Kirsten Maushake, are thanked for their encouragement,

assistance and friendship.

Finally, I am very grateful to my wife, Jing An, and to my parents for their understanding and moral support throughout these years.

Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	v
List of Figures	x
List of Tables	xxi
List of Publications	xxiii
Abbreviations	xxiv
1. Introduction	1
1.1 Manufacture of Whey Protein Products	1
1.2 Functionality and Applications of Whey Protein Products	2
1.3 Selection of Areas for Experimental Study	3
1.4 Whey Proteins and Egg White Proteins	6
1.5 Gelation	8
1.5.1 Physical and chemical gelation	9
1.5.2 Fractals and the critical gel	10
1.5.3 Percolation and gelation	11
1.6 Factors Determining Gel Properties	13
1.7 References	14
2. Rheology of Polymer Solutions and Gels	18
2.1 Rheology	18
2.2 Rheology of Protein Solutions	18
2.2.1 Concentration dependence	18
2.2.2 Shear rate dependence	20
2.2.3 Shear time dependence	20
2.3 Small Deformation Rheological Tests	21
2.3.1 Stress relaxation tests	21
2.3.2 Creep compliance tests	23
2.3.3 Dynamic shear tests	24

	vi
2.4 Large Deformation and Failure Tests	28
2.4.1 Tension tests	28
2.4.2 Compression tests	30
2.4.3 Penetration tests	31
2.4.4 Torsion tests	31
2.5 Rheological Properties in Relation to Gel Structure	32
2.6 References	32
3. Materials and Methods	37
3.1 Materials	37
3.2 Preparation of WPC Solutions	39
3.3 Steady Shear Rheological Measurements	39
3.4 Oscillatory Rheological Measurements	41
3.5 Large Deformation and Failure Tests	42
3.5.1 Compression tests	42
3.5.2 Tension tests	45
3.5.3 Penetration tests	47
3.6 Statistical Planning of Experiments and Analysis of Data	48
3.7 References	48
4. Rheology of Whey Protein Concentrate Solutions	50
4.1 Introduction	50
4.2 Experimental Procedure	50
4.3 Results	51
4.3.1 Effects of WPC(B) concentration	51
4.3.2 Effects of shear rate	53
4.3.3 Time-dependence of structure breakdown and recovery	55
4.3.4 Determination of yield stress	56
4.3.5 Effects of temperature	57
4.3.6 Determination of activation energy of flow	58
4.3.7 Effects of pH	62
4.3.8 Effects of salt type and concentration	64

	vii
4.4 Discussion	66
4.5 References	70
5. Oscillatory Rheological Exploration of WPC Gelation	73
5.1 Introduction	73
5.2 Experimental Procedure	75
5.3 Results	75
5.3.1 Effect of shear strain amplitude on gel formation and gel structure	75
5.3.2 Frequency dependence of storage modulus during gelation	77
5.3.3 Estimation of the sol-gel transition point (gelation time)	78
5.3.4 Concentration dependence of gelation time and estimation of critical concentration	79
5.3.5 Temperature dependence of gelation time	80
5.3.6 Concentration dependence of gel structure development	81
5.3.7 Temperature dependence of gel structure development	83
5.4 Discussion	84
5.5 References	87
6. Effects of pH and Salts on the Formation and Properties of WPC gels	90
6.1 Introduction	90
6.2 Experimental Procedure	90
6.3 Results	90
6.3.1 pH dependence of gelation time	90
6.3.2 pH dependence of gel development	91
6.3.3 pH dependence of gel properties	92
6.3.4 Salt concentration dependence of gelation time	95
6.3.5 Salt dependence of gel development	96
6.3.6 Salt dependence of gel properties	98
6.3.7 Brine tolerant WPCs	100
6.4 Discussion	103
6.5 References	107

7. A Comparative Study of the Gelation Characteristics of Egg White, WPCs, WPI and β-lactoglobulin	110
7.1. Introduction	110
7.2 Experimental Procedure	110
7.3 Results	110
7.3.1 Temperature sweep experiments	110
7.3.2 Minimum protein concentration for gelation	111
7.3.3 Formation and cold stiffness of egg white and whey protein gels	112
7.3.4 Effects of pH	114
7.3.5 Effects of salts	115
7.3.6 Effect of lactose	117
7.4 Discussion	119
7.5 References	121
8. Gelation of Whey Proteins: Critical Behaviour of Rheological Properties at the Sol-Gel Transition	123
8.1 Introduction	123
8.2 Experimental Procedure	123
8.3 Results	124
8.3.1 Time dependence of dynamic rheological properties	124
8.3.2 The capture of the G' and G'' crossover point	125
8.3.3 Frequency sweep and strain sweep measurements	128
8.3.4 Gelation time versus protein concentration	130
8.3.5 Storage modulus versus protein concentration	133
8.3.6 Percolation theory applied to gelation of whey proteins	133
8.4 Discussion	136
8.5 References	138
9. Comparisons between Small Deformation, Large Deformation and Failure Properties of WPC gels	141
9.1 Introduction	141
9.2 Experimental Procedure	142

	ix
9.3 Results	142
9.3.1 Comparison of G' with rigidity moduli of WPC gels	142
9.3.2 Texture profile analysis of WPC gels	145
9.3.3 Failure testing of WPC gels	145
9.4 Discussion	148
9.5 References	151
10. General Conclusions and Recommendations for Further Work	154
10.1 General Conclusions	154
10.2 Recommendations for Further Work	155
10.2.1 Further applied work	155
10.2.2 Further fundamental study	157
10.3 References	157
11. List of Symbols	158
12. Appendix 1	161

List of Figures

Figure	Page
2.1 Time profile of a simple shear stress relaxation experiment following sudden strain for a perfectly elastic solid, a viscoelastic solid and a viscoelastic liquid. From Ferry (1980) and Mitchell (1980).	22
2.2 Time profile of shear creep and creep recovery for a perfectly elastic solid, a viscoelastic solid and a viscoelastic liquid. From Ferry (1980) and Mitchell (1980).	23
2.3 Time profile of sinusoidal variation of strain and stress for a viscoelastic material.	24
3.1 Sample temperature as a function of time in the Bohlin rheometer for WPC(A) solutions containing 16% protein at water jacket temperatures of 70°C (●), 80°C (▲) and 90°C (■). Rheological measurement was usually started after 2 minutes of temperature equilibration, but for these experiments with a thermocouple inserted there was no rheological measurement.	43
3.2 Temperature histories during the preparation of WPC gels for small deformation, large deformation and failure tests.	44
3.3 Typical first and second bite compression curves (50% compression) for Instron Texture Profile Analysis (TPA) of a WPC(B) gel formed at 80°C and pH7.	44
3.4 Force versus displacement curves in tension for 12% WPC(B) gels.	47
3.5 Traces of Instron curves showing penetration tests on WPC gels.	48

Figure	Page
4.1 Apparent viscosity of WPC(B) solutions as a function of concentration at 22°C, pH7 and a shear rate of 291 s ⁻¹ .	52
4.2 Apparent viscosity of WPC(B) solutions as a function of shear rate at 22°C and pH7 determined by shear rate sweeps. Concentrations were: ○, 10; ●, 15; △, 20; ▲, 30; □, 35 and ■, 40%(TS).	54
4.3 Flow curves of WPC (B) solutions at 22°C, pH7 and concentrations (TS) of: ●, 10; ▲, 20 and ■, 30%(TS). Flow curves were determined from the data in Fig 4.2.	55
4.4 Time-dependence of structure breakdown and recovery in a 40%(TS) WPC (B) solution at 22°C and pH6.75. The sample was sheared successively at ○, 92.5 s ⁻¹ ; ●, 585 s ⁻¹ and ○, 92.5 s ⁻¹ .	56
4.5 Determination of the yield stress ($\tau_y = 0.029$ Pa) of a 40%(TS) WPC(B) solution at 22°C and pH6.75. The shear sweep was conducted from high to low shear rate.	57
4.6 Apparent viscosity of WPC(B) solutions as a function of temperature during temperature sweep tests at a shear rate of 291 s ⁻¹ , pH7 and concentrations (TS) of: ○, 15; ●, 20; △, 25 and ▲, 30%(TS).	58
4.7 Apparent viscosity of WPC(B) solutions at 22°C, pH7, a shear rate of 146 s ⁻¹ and concentrations of ○, 10; ●, 15; △, 20 and ▲, 25%(TS) after holding for 10 minutes at various holding temperatures. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.	59

Figure	Page
4.8 Apparent viscosity of 15%(TS) WPC(B) solutions versus shearing time at 22°C, pH7 and a shear rate of 146 s ⁻¹ . The solutions were held at ○, 22°C and ●, 70°C for 10 minutes before shearing.	60
4.9 Apparent viscosity of WPC(B) solutions as a function of 1/T (T = absolute temperature) at a shear rate of 291 s ⁻¹ , pH7 and concentrations of ○, 2; ●, 4; △, 8; ▲, 12 and □, 15%(TS).	60
4.10 Activation energy of flow of WPC(B) solutions as a function of concentration at 22°C, pH7 and a shear rate of 291 s ⁻¹ .	61
4.11 Effect of pH on apparent viscosity of 10%(TS) WPC(B) solutions at 22°C and a shear rate of 146 s ⁻¹ . Apparent viscosity was measured ○, 2 h; ●, 48 h; △, 98 h and ▲, 168.5 h after preparation. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.	62
4.12 Effect of pH on apparent viscosity of 20%(TS) WPC(B) solutions at 22°C and a shear rate of 146 s ⁻¹ . Apparent viscosity was measured ○, 0.5 h; ●, 2.5 h and △, 4.5 h after preparation. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.	63
4.13 Apparent viscosity of 20%(TS) WPC(B) solutions as a function of shear rate at 22°C and at pHs of ○, 7 and ●, 3. Measurement was carried out 4.5 h after preparation of the solutions.	64
4.14 Effects of sodium chloride concentration on apparent viscosity of ○, 10% and ●, 20%(TS) WPC(B) solutions at pH7, 22°C and a shear rate of 146 s ⁻¹ .	65

Figure

- 4.15 Effects of calcium chloride concentration on the apparent viscosity of 20%(TS) WPC(B) solutions at pH7, 22°C and a shear rate of 146 s⁻¹. Apparent viscosity was measured ○, 1 day; ●, 5 days; △, 8 days and ▲, 11 days after preparation of the solutions. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded after 10, 80 and 180 s. 65
- 5.1 G' versus time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, pH7, 1Hz and shear strains ○, 0.001; ●, 0.02; △, 0.04; ▲, 0.05; □, 0.1; ■, 0.14. 75
- 5.2 Shear strain sweep results at 80°C and 1Hz for WPC(A) gels containing 11.89% protein made by heating at 80°C and pH7 for 45 minutes. Shear strains used during heat-induced gel formation were ○, 0.001; ●, 0.02; △, 0.04; ▲, 0.05; □, 0.1; ■, 0.14. 76
- 5.3 Effect of shear strain amplitude on storage modulus (G') during thermal gelation of WPC(B) solutions containing 10% protein at 80°C, pH7 and 1Hz. Shear strains ○, 0.002; ●, 0.01; △, 0.02; ▲, 0.03 and □, 0.05. 77
- 5.4 Effect of frequency on storage modulus (G') during thermal gelation of WPC(B) solutions containing 10% protein at 80°C, pH7 and 0.01 shear strain. Frequencies ○, 0.1; ●, 1; and △, 10. 78
- 5.5 Viscosity (○) versus time during steady shear alone at a shear rate of 0.0231 s⁻¹, and storage modulus G' (●), loss modulus G'' (▲) and phase angle (■) versus time during oscillatory measurement alone at 1Hz and 0.002 shear strain. Both experiments used WPC(B) solutions containing 7.9% protein at pH7 and 80°C. t_c was the heating time required for sol-gel transition, or the gelation time. 79

Figure	Page
5.6 Estimation of the critical whey protein concentration of WPC(B) for gelation at 80°C, pH7, 0.1Hz and 0.002 shear strain. C_o was the critical whey protein concentration. t_c was the gelation time as defined in the text.	80
5.7 Effect of temperature on gelation time of WPC(B) solutions containing 7.9% protein at pH7, 0.1Hz and 0.002 shear strain.	81
5.8 Storage modulus (G') and loss modulus (G'') at 1Hz and 0.01 shear strain as a function of time during thermal gelation of WPC(B) solutions at 80°C, pH7 and protein concentrations of 15% (\circ), 20% (\bullet), 25% (Δ), 30% (\blacktriangle) and 35% (\square).	82
5.9 Storage modulus (G') as a function of time during thermal gelation of WPC(B) solutions containing 10% protein at 1Hz, pH7, 0.01 shear strain and \circ , 75°C; \bullet , 80°C; Δ , 85°C and \blacktriangle , 90°C.	83
5.10 Storage modulus (G') as a function of time during thermal gelation of WPC(B) solutions containing 25% protein at 1Hz, pH7, 0.01 shear strain and \circ , 68°C; \bullet , 70°C; Δ , 78°C; \blacktriangle , 85°C and \square , 90°C.	84
6.1 Effect of pH on gelation time of WPC(B) solutions containing 7.9% protein at 80°C, 0.1Hz and 0.002 shear strain.	91
6.2 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, 1Hz, 0.01 shear strain and pH values of \circ , 3.5; \bullet , 4; Δ , 5.5; \blacktriangle , 6 and \square , 7.	93

Figure

Page

- 6.3 Effect of pH on (○) storage modulus G' and (●) phase angle of WPC(A) gels at 1Hz, 0.01 shear strain and 25°C, and WPC(B) gels at 1Hz, 0.01 shear strain and 20°C. WPC(A) gels were made by heating WPC solutions containing 11.89% protein at 80°C for 44 minutes. WPC(B) gels were made by heating WPC solutions containing 12% protein at 80°C for 44 minutes. 94
- 6.4 Effect of pH on storage modulus G' of WPC(B) gels containing 11.89% protein at 1Hz, 0.01 shear strain, 80°C (○) and 20°C (●). 95
- 6.5 Effects of sodium chloride (●) and calcium chloride (○) concentration (expressed as added ionic strength) on gelation time of WPC(B) solutions containing 7.9% protein at 80°C, pH7, 0.1Hz and 0.002 shear strain. 96
- 6.6 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, 1Hz, 0.01 shear strain, pH7 and sodium chloride concentrations of ○, 0M; ●, 0.04M; Δ, 0.2M and ▲, 0.5M. 97
- 6.7 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, 1 Hz, 0.01 shear strain, pH7 and calcium chloride concentrations of ○, 0mM; ●, 5mM; Δ, 10mM and ▲, 20mM. 97
- 6.8 Effects of NaCl concentration (expressed as added ionic strength) on storage modulus G' of WPC(A) gels at 1Hz, 0.01 shear strain and 80°C after heating WPC(A) solutions containing 11.89% protein for 44 minutes at 80°C and pH values of (○) 4, (●) 7 and (▲) 8. 98

Figure	Page
6.9 Effects of calcium chloride concentration (expressed as added ionic strength) on storage modulus G' of WPC(A) gels at 1Hz, 0.01 shear strain and 80°C after heating WPC(A) solutions containing 11.89% protein at 80°C and pH values of (○) 4, (●) 7 and (▲) 8.	100
6.10 Effects of heating time on G' of (○) WPC(D), (●) WPC(E), (Δ) WPC(F) and (▲) WPC(G) solutions containing 12% protein at 75°C, pH6.5, 1Hz and 0.02 shear strain.	101
6.11 Effect of pH on G' of WPC(E) gels at 1Hz, 0.02 shear strain and 20°C after heating solutions containing 12% protein at 75°C for 44 minutes.	101
6.12 Effects of sodium chloride concentration (expressed as added ionic strength) on G' of (○) WPC(D), (●) WPC(E), (Δ) WPC(F) and (▲) WPC(G) gels at 1Hz, 0.01 shear strain and 20°C after heating WPC solutions containing 12% protein for 44 minutes at 75°C and pH6.5.	102
6.13 Effects of sodium chloride concentration (expressed as added ionic strength) on G' of WPC(E) gels at 1Hz, 0.01 shear strain and pH6.5, at (○) 75°C and (●) 20°C after heating WPC(E) solutions containing 12% protein for 44 minutes at 1Hz, pH6.5 and 75°C.	102
6.14 Diagrammatic depiction of change in gel stiffness of WPC gels with change in pH or ionic strength.	106
7.1 Storage modulus (G') versus temperature for solutions of WPI(○), WPC(B) (●), WPC(C) (Δ) and egg white (▲) containing 12% protein during a temperature sweep experiment with heating at 1°C/min and subsequent cooling at 2°C/min. Gelation was conducted at pH7, 1Hz and 0.01 maximum shear strain.	111

Figure	Page
7.2 Storage modulus (G') after 11.11 h at 80°C versus protein concentration for WPI containing 0.01M added NaCl (\circ), WPC(B) (\bullet), WPC(C) (Δ) and egg white (\blacktriangle). Experiments were performed at pH7, 0.1Hz and 0.002 maximum shear strain.	112
7.3 Storage modulus (G') versus time for solutions of WPI (\circ), WPC(B) (\bullet), WPC(C)(Δ), egg white (\blacktriangle) and β -lactoglobulin (\square) containing 12% protein during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain.	113
7.4 Storage modulus (G') of an egg white solution containing 12% protein versus time during heating and cooling cycles at pH7, 1Hz and 0.01 maximum shear strain.	114
7.5 Effects of protein concentration on $G'(80^\circ\text{C}) / G'(20^\circ\text{C})$ for WPC(B) (\bullet), WPC(C) gels (Δ), and egg white gels (\blacktriangle) after heating at 80°C and pH7 for 45 minutes, with subsequent cooling to 20°C. Measurements were performed at 1Hz and 0.01 maximum shear strain.	115
7.6 Storage modulus (G') versus time for solutions of WPC(C) containing 10% protein during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain. WPC(C) (\bullet), WPC(C) after 12 h dialysis against distilled water (\circ) and WPC(C) after 12 h dialysis against distilled water followed by 0.1M NaCl addition (Δ).	118
7.7 Storage modulus (G') versus time for 10% protein solutions during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain. WPI (\circ), WPC(C) (\bullet), egg white (Δ), WPC(C) with 0.1M KCl addition (\blacktriangle), and WPI with 0.1M KCl addition (\square).	118

Figure**Page**

- 8.1 Loss modulus G'' (\circ), storage modulus G' (\bullet) and phase angle (Δ) as a function of heating time during thermal gelation of a WPI solution containing 13% protein and 0.01M NaCl at 80°C, pH7, 1Hz and 0.002 shear strain. 124
- 8.2 Effects of pH on the increases in G'' (\circ) and G' (\bullet), and the fall in phase angle (\square) with heating time for a WPI solution containing 12% protein at 80°C, 1Hz and 0.002 shear strain. 126
- 8.3 Effects of heating at 80°C, and subsequent cooling from 80 to 20°C, on G'' (\circ) and G' (\bullet) as a function of time for a WPI solution containing 12% protein at 1Hz, 0.002 shear strain and pH7. 127
- 8.4 Effects of cooling time on G'' (\circ) and G' (\bullet) of a WPI solution containing 12% protein at 0.1Hz and 0.002 shear strain when temperature was decreased from 80°C to 30°C and held at 30°C until G' and G'' reached constant values. Before cooling, the sample was heated at 80°C, pH7, 0.1Hz and 0.002 shear strain for 45 minutes. 128
- 8.5 Effects of frequency on G' and G'' of WPI gels at 30°C, 0.002 shear strain and with different protein concentrations. G'' (\circ) & G' (\bullet), 12% protein; G' (Δ), 12.5% protein; G' (\square), 13% protein; and G' (∇), 20% protein. The WPI gels were formed by cooling after heating at 80°C for 45 minutes. 129
- 8.6 Effects of frequency on G' and G'' of WPI gels containing 12% protein at 30°C, 0.002 shear strain and with different NaCl concentrations. G'' (\circ) & G' (\bullet), 0M NaCl; G' (Δ) & G'' (\blacktriangle) 0.05M NaCl; and G' (\square), 0.01M NaCl. The WPI gels were formed by cooling after heating at 80°C for 45 minutes. 130

Figure	Page
8.7 $1/t_c$ versus $[(C/C_0)^2 - 1]$ for WPC(C) (○) and WPI (●) at 80°C, pH7, 0.1Hz and 0.002 shear strain.	132
8.8 G' versus protein concentration for whey protein gels of WPC(B) (●), WPC(C) (▲) and WPI with 0.1M NaCl (■) after heating sample solutions at 80°C, 0.1Hz, pH7 and 0.002 shear strain for 667 minutes. G' was measured at 80°C, 0.1Hz and 0.002 shear strain.	133
8.9 Data from Fig 8.8 for WPC(B) (○) and WPI (●) plotted in the form G' against $(C-C_m)$, where C_m is the minimum protein concentration for gelation, determined as indicated in Fig 8.8.	134
8.10 G' versus (C/C_m-1) after heating WPC(B) solutions at 80°C, pH7, 0.1Hz and 0.002 shear strain for 667 minutes. G' was measured at 80°C, 0.1Hz and 0.002 shear strain.	135
9.1 Effects of pH on storage modulus, G' (○) and compression modulus, E_c (●) for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each E_c data point is the average of at least four replications and the bars show one standard deviation either side of the mean.	143
9.2 Effects of pH on tension rigidity, E_t (○) and the penetration rigidity, E_p (●) for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.	144

Figure		Page
9.3	Effects of pH on hardness, cohesiveness and springiness determined by texture profile analysis for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.	146
9.4	Effects of pH on failure forces (○) and displacements (●) during tensile testing of WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.	147
9.5	Effects of pH on failure forces (○) and displacements (●) during penetration testing of WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.	147
9.6	Effects of protein concentration on failure forces (○) and displacements (●) during tensile testing of WPC gels at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of four replications and the bars show one standard deviation either side of the mean.	148

List of Tables

Table	Page	
1.1	Examples of the use of whey protein products in a variety of foods.	3
1.2	Physicochemical characteristics of whey proteins.	7
1.3	Proteins in egg white and their physicochemical properties.	8
1.4	The critical exponents of classical theory and the percolation model.	13
2.1	Rheological classification of polymer solutions and gels according to their behaviour in simple shear deformation.	19
3.1	Compositions of protein samples (% w/w).	37
3.2	Components of ash for whey protein samples (% w/w).	38
4.1	Flow behaviour index (n') and fluid consistency index (k_p) values for 10-30%(TS) WPC(B) solutions at 22°C and pH7 (Equation 4.4).	53
4.2	Values of activation energy of flow (E') and of the constant B in equation 4.5 for WPC(B) solutions at pH7 and a shear rate of 291 s ⁻¹ .	61
7.1	Effects of KCl on the storage modulus (G') of WPC(C) gels.	116
7.2	Effect of salt on storage modulus of WPI gels.	117
8.1	n values of frequency sweep tests in the vicinity of sol-gel transition for WPI solutions containing 12% protein at 0.002 shear strain.	131

Table**Page**

8.2	Results of strain sweep tests in the vicinity of sol-gel transition for WPI solutions containing 12% protein at 1 Hz.	131
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List of Publications

The results of this work have been or will be published in part in the following papers.

1. TANG, Q., McCARTHY, O. J. and MUNRO, P. A. 1993. Oscillatory rheological study of the gelation mechanism of whey protein concentrate solutions: effects of physicochemical variables on gel formation. *Journal of Dairy Research* **60**, 543-555.
2. TANG, Q., MUNRO, P. A. and McCARTHY, O. J. 1993. Rheology of whey protein concentrate solutions as a function of concentration, temperature, pH and salt concentration. *Journal of Dairy Research* **60**, in press.
3. TANG, Q., McCARTHY, O. J. and MUNRO, P. A. 1993. Oscillatory rheological characterization of the effects of pH and salts on gel formation in whey protein concentrate solutions. *Journal of Dairy Research*, draft manuscript submitted to supervisors for correction.
4. TANG, Q., McCARTHY, O. J. and MUNRO, P. A. 1993. Gelation of whey proteins: sol-gel transition and critical behaviour of the rheological properties. *Food Hydrocolloids*, draft manuscript submitted to supervisors for correction.
5. TANG, Q., McCARTHY, O. J. and MUNRO, P. A. 1993. Rheological study of gelation of egg white, whey protein concentrates, whey protein isolate and β -Lactoglobulin. *Journal of Agricultural and Food Chemistry*, draft manuscript submitted to supervisors for correction.
6. TANG, Q., McCARTHY, O. J. and MUNRO, P. A. 1993. pH dependence of whey protein concentrate gel properties: comparison of small deformation (dynamic) and large deformation (failure) testing. *Journal of Texture Studies*, draft manuscript submitted to supervisors for correction.

Abbreviations

EWP	Egg White Powder
TPA	Texture Profile Analysis
TS	total solids
WPC	whey protein concentrate
WPI	whey protein isolate
NZDRI	New Zealand Dairy Research Institute
NZDB	New Zealand Dairy Board
β -Lg	β -lactoglobulin powder containing 92% β -lactoglobulin

1. Introduction

1.1 Manufacture of Whey Protein Products

Whey protein products are mainly manufactured from two major types of whey: acid whey and sweet whey. Acid whey ($\text{pH} \leq 5.1$) is produced by direct acidification of skim milk for casein manufacture or results from Cottage cheese manufacture. Sweet whey ($\text{pH} \geq 5.6$) is the by-product of rennet-coagulated cheese manufacture from whole milk or rennet casein manufacture from skim milk (Morr, 1989). Acid whey contains a higher concentration of minerals than rennet whey due to the dissolution of the colloidal calcium phosphate component of casein micelles during the acidification process (Morr, 1989).

Whey protein concentrate (WPC), whey protein isolate (WPI), whey powder and lactalbumin are the main whey protein products produced commercially. Lactalbumin is typically made by heating whey liquor at $\text{pH} 5-6$ to $85^\circ\text{C} - 100^\circ\text{C}$ by steam injection, holding for 15-20 min and then acidifying to $\text{pH} 4.5$. The precipitated protein is recovered by centrifugation, and either spray- or drum-dried to give a final product (Kinsella and Whitehead, 1989). Lactalbumin is thus a product rich in denatured protein, which retains its nutritive value but lacks the functional properties of the native proteins (Marshall, 1982; Kinsella and Whitehead, 1989).

Whey powders are produced from whey by reverse osmosis or electrodialysis to concentrate the whey or remove minerals respectively, followed by spray-drying or roller-drying (Marshall, 1982; Kinsella and Whitehead, 1989). Whey powder contains a low protein content of up to 25% and is not suitable for most uses as a functional protein ingredient (Kinsella and Whitehead, 1989).

WPC is produced commercially on a large scale and can be considered as the most important whey protein product. WPC is made commercially from whey by pretreatment (involving pH and temperature adjustment, addition of calcium and centrifugation to remove suspended particles), ultrafiltration (and perhaps diafiltration) to concentrate protein and remove ash and lactose, vacuum evaporation to 15-20% protein and finally spray drying (Marshall 1982; Morr, 1989). The protein concentration of WPC products ranges from 30 to 90%. Heating during processing must be carefully controlled in order to reduce the denaturation of whey proteins to a minimum (Morr, 1989; Kinsella and Whitehead, 1989).

Like most globular proteins, whey proteins are amphoteric. The proteins carry a net

negative or a net positive electric charge at pH values higher or lower respectively than their isoelectric points. These ionic properties of whey proteins make it possible to adsorb them by either cation or anion exchangers. In these cases weak ionic bonds are formed between the whey protein and the opposite charge of the ion-exchanger. Lactose and other non-protein whey components which are not adsorbed by the ion exchanger are eluted with water. The adsorbed proteins are then eluted from the ion exchanger with water either by altering the pH or by increasing the ionic strength, or by a combination of both. The eluate is finally concentrated by spray drying to yield WPI containing over 90% protein (Kanekanian and Lewis, 1986). In comparison with other commercial whey protein products WPI contains high purity protein, and low ash, fat and lactose contents.

1.2 Functionality and Applications of Whey Protein Products

The functional properties and applications of whey protein products have been reviewed and discussed by Kinsella and Whitehead (1989), and by De Wit (1989a, b). The functional behaviour of proteins in food systems is influenced by both intrinsic factors (*i.e.* amino acid composition and disposition of amino acid residues, conformation, molecular size, shape, flexibility, net charge, molecular hydrophobicity, sulphhydryl groups, *etc.*) and extrinsic or environmental factors (*i.e.* temperature, pH, ion concentration, fat and lactose content, *etc.*) (Kinsella and Whitehead, 1989; De Wit, 1989a). However, how these factors influence the functional properties of whey proteins is still not well understood. Generally, whey proteins are regarded as having excellent solubility, and good gelling, emulsification and foaming or whipping properties, but these are dependent on the content of undenatured proteins, salt, fat and lactose, and on pH (Kinsella and Whitehead, 1989). High temperature during processing damages the functional properties of whey proteins or whey protein products (de Wit *et al.* 1988) by causing protein denaturation.

WPI, WPC, whey powder and lactalbumin vary greatly in functional performance mainly owing to variability in their compositions and their contents of denatured proteins. WPI and WPC have excellent nutritional value and functional properties due to their high protein content and low contents of denatured proteins, salt, lactose and fat. Whey powder and lactalbumin have excellent nutritional value but lack some key functional properties due to low protein content in the case of whey powder and a high content of denatured proteins in the case of lactalbumin. Thus, they are mainly used as nutritional rather than functional

ingredients. The use of WPI, WPC, whey powder and lactalbumin in food applications as shown in Table 1.1 is thus greatly influenced by their functional performance in food systems.

Table 1.1 Examples of the use of whey protein products in a variety of foods.

Whey protein products	Typical applications
WPI	Brine-injected meat products, sausages, comminuted chicken or turkey, surimi. Angel cake, marshmallow fillings, mousses, meringues and souffles. Mayonnaise, ice cream, margarine, low-calorie spreads. Confectionary, yoghurt, pates, frozen desserts.
WPC	Restructured meat, fish and poultry. Ham, processed meats, pasta, surimi. Egg white replacer. Dairy products such as yoghurt. Bakery products Soft drinks Infant foods Confectionery
Sweet whey powders	Bakery products, confectionery, milk replacers, batters, soups. Dairy products (yoghurt, ice cream <i>etc.</i>)
Lactalbumin	Bakery products, nutritional bars, pasta, breakfast cereals, infant foods, diet foods, protein supplements, pet food.

Source: mainly from Kinsella and Whitehead (1989), De Wit (1989a, b), Howell *et al.* (1990), and N.Z. Dairy Board (1991).

1.3 Selection of Areas for Experimental Study

As described in Section 1.1, WPCs are produced from whey in the dairy industry by four major processing steps: pretreatment, ultrafiltration, evaporation and drying. The protein concentration in whey is in the range 0.4-0.8% (Even, 1980; Marshall, 1982; Morr, 1989). This is increased to about 15-20% protein in the ultrafiltration and evaporation stages, and is further increased to about 80% protein in the WPC product after the final drying stage

(Marshall, 1982). The rheological properties of whey or whey protein solutions are expected to change during the concentration processes of ultrafiltration, evaporation and drying. The changes in rheological properties can cause handling problems and can increase energy consumption during pumping in the evaporation and drying processes. Therefore, changes in rheological properties with changes in protein concentration, temperature, pH and salt concentration could usefully be studied to with the aim of finding optimum processing conditions for the manufacture of WPCs.

With the increasing use of whey protein concentrates in food applications the importance of assessing their functional properties is evident. Heat-induced gel formation (gelation) is one of the most important functional properties of WPCs in food applications. If WPC is to sell as a gelling and nutritional ingredient for food applications, then the interactions of WPC with other food components in food systems during gel formation need to be investigated. However, such interactions are very complicated. A useful first step would be a better understanding of the effects of pH, ionic strength, temperature, protein concentration, lactose and fat on gel formation and gel properties of WPCs.

Currently, WPCs with a range of functional properties are produced commercially. It is a future goal of manufacturers to produce WPCs with consistent properties. It is known that functional properties of WPCs are affected by processing treatments and composition (Kinsella and Whitehead, 1989). For the same manufacturer, the processing treatment can be standardized, but WPCs with the same compositions are difficult to achieve since their compositions are dependent on the compositions of the original milks, which vary with season and also with other factors. It may be that some components of WPC have negligible effects on functional properties while others have major effects. It is thus important to test which components are the most important and how each component affects functional properties. With this knowledge it will then be possible for WPC manufacturers to control the critical components within narrow ranges to achieve consistent functional properties in WPC products.

In order to boost the use of WPCs as gelling ingredients in food products it is important to promote the use of WPCs as replacements of egg white. Thus, differences in the gelation properties of WPCs and of egg white need to be studied. WPC and egg white are both multicomponent protein systems. Their gelling performances should be different since they contain different protein species. It is thus important to test how large differences

are and whether is possible to compensate for differences by altering the environmental conditions to modify the gelling performance of WPC so that it matches that of egg white.

WPC and WPI are different whey products made by different processes from similar whey protein sources as described in Section 1.1. They have almost the same protein compositions but differ in other components because of different processing treatments. The functional properties of commercial WPC and WPI products are usually quite different. In the manufacture of WPCs the denaturation of proteins can be reduced to a minimum if heat treatments are carefully controlled. The denaturation of proteins during the manufacture of WPI is normally very small. Thus, the differences in functional properties between commercial WPC and WPI products are probably due to their different compositions of fat, lactose, salts and other minor components, since the denaturation of proteins in processing can be reduced to a minimal level for both types of product. Study of such factors will provide information on how to improve the qualities of WPC, and how to produce WPCs for special purposes.

A more fundamental study of gelation was also pursued. Gelation of materials is a active field of research in condensed matter physics. The revolutionary concept of fractal geometry introduced by Mandelbrot (1977) has formed a good basis for the understanding of aggregation and gelation phenomena. It is believed that at the gel point a self-similar fractal structure called a critical gel appears, which occupies the total volume of the container. The critical gel itself is said to be composed of flocs or aggregates of a fractal nature (Stinchcombe, 1985; Bremer *et al.* 1985; Hess *et al.* 1988). Acid casein gels are one of the examples which have been studied and which have a fractal dimensionality of 2.3 (Bremer *et al.* 1985). Just as at the critical point of liquid-gas transition the system behaves neither as a liquid nor as a gas, so a critical gel behaves neither as a liquid nor as a solid on any time or length scale. In fact the large fluctuations in structure or in density in such systems exist everywhere. Scaling theory (de Gennes, 1976) is widely used to study this complicated sol-gel transition. The percolation model (Stauffer *et al.* 1982) is the most popular model for the sol-gel transition. In the critical region of sol-gel transition a power law relationship between dynamic rheological properties (*e.g.* G' , G'' , dynamic viscosity, relaxation modulus) with frequency is found experimentally to exist for chemical gels (Chambon and Winter, 1986; Winter and Chambon, 1987; Hess *et al.* 1988) and some biopolymer gels (Te Nijenhuis, 1990; Cuvelier and Launay, 1990). Such power law relationships should also exist for the gelation

of whey proteins in the sol-gel transition region if the critical gel of whey proteins also has a fractal geometry.

Gelation and formed gel properties are usually studied by, respectively, small deformation dynamic shear tests and large deformation and failure tests. Dynamic rheological methods are becoming popular since they can be used to follow the gelling process without breaking the gel structure. So far, dynamic mechanical experiments provide the only direct method to determine precisely the gel point (Hess *et al.* 1988). However, it is the results of large scale deformation and failure tests that correlate better with the sensory evaluation of protein gel texture (Mohsenin and Mittal 1977; Wood 1979; Bourne, 1982). It is therefore important to compare the results of dynamic tests with those of large deformation and failure tests. WPC gels with high G' will not necessary also exhibit high rigidity moduli and high failure stresses. Such study can help us to build a bridge between the results of dynamic tests and those of large deformation and failure tests.

1.4 Whey Proteins and Egg White Proteins

The whey proteins are defined as those milk proteins remaining in the serum or whey after precipitation of the casein during cheese or casein manufacture (Walstra and Jenness, 1984; Whitney, 1988). Most of the whey proteins are globular proteins (Walstra and Jenness, 1984). Some important physicochemical characteristics of the major whey proteins are listed in Table 1.2 (Kinsella and Whitehead, 1989). Whey protein mixtures can be separated into individual protein components by the fractionation methods of gel filtration (based on molecular size) and ion exchange (based on charge). β -lactoglobulin exists as a noncovalent dimer at the pH of milk while α -lactalbumin does not polymerize at the same condition; this makes them easily separable by gel filtration chromatography (Walstra and Jenness, 1984). α -lactalbumin has the lowest denaturation temperature as shown in Table 1.2, but it is traditionally considered the most heat stable serum protein. This is because α -lactalbumin is the only major whey protein whose heat denaturation is reversible; it is stable against heat-induced aggregation since it renatures easily when cooled (Brown, 1988).

Egg white (albumen) proteins are composed of globular proteins and ovomucin fibres (Powrie and Nakai, 1985). Egg white proteins can be fractionated by the classical ammonium sulphate fractionation process, and by the more recent methodologies of polyacrylamide gel electrophoresis, sodium dodecyl sulphate polyacrylamide gel electrophoresis and ion exchange

chromatographic techniques (Froning, 1988). The major proteins of egg white, and their physicochemical characteristics, are listed in Table 1.3.

Table 1.2 Physicochemical characteristics of whey proteins.

Protein component	Molecular mass	Approximate weight of protein in skim milk (g/litre)	Isoelectric pH	T _d	Cystine groups
β-lactoglobulin	18,600	7-12	5.3	78	2 (1 -SH)
α-lactalbumin	14,200	2-5	4.8	62	4
Serum albumin	66,000	0.7-1.3	5.1	64	17 (1 -SH)
Immunoglobulin	15.0-96.0 x 10 ⁴	1.9-3.3	5.5-6.8	72	32
Proteose-peptones	-	2-6	-	-	-
Lysozyme	18,000	0.13-0.32	9.5	-	3
Lactoferrin	76,500	0.02-0.35	N/A	-	19

Source: Data from Kinsella and Whitehead (1989).

T_d is the initial denaturation temperature.

In their native state globular whey proteins and egg white proteins fold intramolecularly, burying their thiol groups (-SH), disulphide bonds (-SS-) and most of their hydrophobic residues so that extensive self-association or interaction with other proteins does not occur. Their three-dimensional globular conformation resulting from secondary structure, which is described by certain proportions of α-helix, β-sheet and unordered structure, and tertiary structure are stabilized by intramolecular disulphide bonds, hydrogen bonds, hydrophobic interactions and ionic bonds. These intramolecular bonds can be disrupted by heat, by chemical agents or by shifting to extreme pH values, leading to unfolding and hence denaturation of the protein molecules (Cheftel *et al.* 1985).

It is anticipated that the gelling performance of whey proteins should be different from that of egg white since these two protein systems vary greatly in denaturation temperature,

molecular weight, isoelectric pH and molecular structure.

Table 1.3 Proteins in egg white and their physicochemical properties.

Protein component	Egg white (% dry basis)	Isoelectric pH	Molecular weight	T _d (°C)
Ovalbumin	54	4.5	44,500	84.0
Ovotransferrin	12	6.1	76,000	61.0
Ovomucoid	11	4.1	28,000	70.0
Ovomucin	3.5	4.5-5.0	5.5-8.3 x 10 ⁶	-
Lysozyme	3.4	10.7	14,300	75.0
G ₂ Globulin	4.0	5.5	3.0-4.5 x 10 ⁴	92.5
G ₃ Globulin	4.0	4.8	-	-
Ovoinhibitor	1.5	5.1	49,000	-
Ficin inhibitor	0.05	5.1	12,700	-
Ovoglycoprotein	1.0	3.9	24,400	-
Ovoflavoprotein	0.8	4.0	32,000	-
Ovomacroglobulin	0.5	4.5	7.6-9.0 x 10 ⁵	-
Avidin	0.05	10	68,300	-

Source: Data from Powrie and Nakai (1985).

1.5 Gelation

Gelation is a subject which is both scientifically interesting and commercially important. It plays an important role in the preparation of many commercial products in the chemical industry, pharmaceutical industry, food industry and so on. Protein gelation is involved in the manufacture of many food products including yogurt, cheese, caseinate, coagulated egg white, soybean protein gels and many others. Protein gelation is also utilized for improving water absorption, thickening and particle binding, and for emulsifying or foam-stabilizing effects. Gelation is thus considered as a very important functional property of

proteins (Cheftel *et al.* 1985).

1.5.1 Physical and chemical gelation

Gelation is a transition process from liquid to solid with molecules or particles in the liquid uniting into a three dimensional network. Gelation can be divided into physical and chemical gelation. Physical gelation is usually the result of the formation of physical network junctions taking the form of helical structure or crystalline or glass domains (de Gennes, 1976; Winter, 1991). The gelation of gelatin and of certain polysaccharides are examples of physical gelation. Physical gelation can be reversible since ionic and hydrogen bonding are often the main phenomena involved in gel formation, although many physical gels show some irreversibility (de Gennes, 1976). In contrast with physical gelation, gels formed by chemical gelation involve covalent bonds (Winter, 1989) and are irreversible. Examples of chemical gels are polyethylene and polystyrene gels.

The chemical gelation process is usually characterized by a conversion fraction, p , which is defined as the ratio of the actual number of bonds formed at a given moment to the maximum possible number of such bonds. p is equal to zero and one at the beginning and at the end of gelation respectively. For small p no gel is present whereas for p close to one a gel network exists. Therefore, there is a sharp phase transition at some intermediate critical point $p = p_c$ (where $0 < p_c < 1$) where a gel network first appears and spans the whole container. For $p < p_c$, there is a liquid polymer solution called a sol which is a collection of finite aggregates formed from polymer molecules. For $p > p_c$, there is a sol embedded in a gel which has the size of the container. The point $p = p_c$ is called the gel point, and the gel network at $p = p_c$ is called the critical gel.

In chemical gelation the sol-gel transition is very well defined. However, in some physical gelation processes the gel point is not apparent since the crosslinks are not strong, and these crosslinks can break under weak stress. Two types of gelation, strong gelation and weak gelation, are distinguished by de Gennes (1976). In a strong gelation process, there is a sharp sol-gel transition threshold and the crosslinks are completely stable. In a weak gelation process, there is not a strict gel point. The crosslinks are not completely stable because there is a reversible reaction (bonding-unbonding) proceeding in both directions. Most chemical gelation processes are examples of strong gelation, while physical crosslinking leads to either strong or weak gelation (de Gennes, 1976).

When solutions of globular proteins are heated above their denaturation temperatures, the protein molecules are usually partially unfolded, and gel formation occurs at or above critical protein concentrations. The gelation of whey proteins seems to involve either physical gelation or both chemical and physical gelation. Covalent bonds such as disulphide bonds, together with physical interactions such as hydrogen bonds, ionic bonds and hydrophobic interactions, are involved in gel formation by whey proteins at pH values of 6.8 or above (Dunnill and Green, 1966; Mangino *et al.* 1987; Mangino, 1992). Thus, at this pH, gelation involves both chemical and physical gelation. Gel formation is irreversible. The present work has shown that whey proteins are heated at a high temperature, but at a concentration less than the critical concentration for the conditions used, a gel is not formed, but gelation occurs when the temperature is decreased. In this case physical gelation is occurring since only physical forces such as hydrogen bonds are involved in gel formation, and the gelation process is reversible.

1.5.2 Fractals and the critical gel

The critical gel at the gel point is neither a liquid nor a solid. It has many interesting properties. For example, the molecular weight of the largest cluster in the system diverges to infinity and the molecular weight distribution is infinitely broad. There exist clusters or aggregates ranging in size from the smallest unreacted monomer to the infinite cluster which has the size of the container (Winter, 1989). The critical gel's steady zero-shear viscosity is infinite but its shear modulus at zero frequency is zero. (Its other dynamic rheological properties are listed in Section 2.3).

Only with the introduction of the concept of the fractal by Mandelbrot (1977) can the geometric structure of the critical gel be properly understood. A fractal is scale invariant, *i.e.* it has a similar structure on all length scales. Many unordered objects in nature have this property, for example clouds, coastlines, snowflakes, clusters of stars and many others (Mandelbrot, 1977). For these irregular objects there is an order from disorder called self-similarity, which is symmetry across scale. A simple power law equation can describe such symmetry for a fractal. A fractal dimension can then be calculated from the power law equation (Mandelbrot, 1977).

The critical gel is said to consist of a self-similar distribution of self-similar clusters of all sizes, including an infinite cluster that is fractal on all length scales (Daoud and Martin,

1989). The critical behaviour in chemical gelation has been studied and discussed using the fractal concept or theory by Vilgis and Winter (1988) and Hess *et al.* (1988). Before the gel point only finite fractal clusters are present and the correlation length is the size (diameter) of a typical fractal cluster. As gelation proceeds the size of the self-similar regions or the correlation length increases and reaches infinity at the gel point. Above the gel point, with more and more crosslinks being added to the gel structure, the correlation length decreases as the size of self-similar regions decreases. The self-similar structure of the critical gel in chemical gelation has dynamic rheological properties which are related by power laws to independent variables such as frequency.

The gelation of casein has been studied by Bremer (Bremer *et al.* 1989; Bremer, 1992) using fractal concepts. In his study, aggregation of casein micelles in the acid-induced gelation process leads to clusters of a fractal nature, which form an acid casein gel that can be described as a collection of fractal clusters with a fractal dimensionality of 2.3. Vreeker *et al.* (1992) found that in the pH range 4-6 whey protein agglomerated in fractal flocs and their fractal dimensions were dependent on pH, electrolyte concentration and temperature of denaturation.

For the pure mathematical fractal the self-similarity or the scaling behaviour exists at all length scales (Mandelbrot, 1977), but in real systems the self-similarity usually does not apply to all length scales. In fact there exist lower and upper cut-off lengths between which the self-similarity exists, but outside this range the self-similarity disappears (Bremer, 1992).

1.5.3 Percolation and gelation

Gelation is a process of percolation (Stauffer and Aharony, 1992), and percolation theory is very useful in describing the sol-gel transition. The most widely used gelation models are based on the classical Flory-Stockmayer theory (Flory, 1953) (which is in fact a form of percolation theory on the Bethe lattice or Cayley tree) and the percolation model (de Gennes, 1979; Stauffer *et al.* 1982; Stauffer and Aharony, 1992). Detailed descriptions of these are given by Flory (1953), Essam (1980), de Gennes (1979), Stauffer *et al.* (1982), and Stauffer and Aharony (1992). The weakness of these gelation models in describing real gelation systems has been discussed by de Gennes (1979). For example, in the Flory-Stockmayer theory it is assumed that there is no cyclization and no steric hindrance during the polymerization process, *i.e.* small molecules branching to form larger and larger

macromolecules. Thus, excluded volume effects and intramolecular loops are not included, whereas these exist in many real gelation processes. In the percolation model the monomers are fixed on a lattice and gelation is assumed to occur without a solvent. This is a very crude representation of any real gelation process since in real systems the monomers are mixed with solvent; they are not on a lattice but are disordered (de Gennes, 1979).

Towards the gel point ($p \rightarrow p_c$) the weight average molecular weight, correlation length, gel fraction, zero-shear viscosity and elastic modulus diverge as follows (de Gennes, 1979; Stauffer *et al.* 1982; Stauffer and Aharony, 1992).

The weight average polymerization index N_w (which corresponds to the weight average molecular weight M_w) diverges to infinity slightly below the gel point as:

$$N_w \propto (p - p_c)^{-a} \quad p < p_c \quad (1.1)$$

The correlation length ξ or the linear size of the cluster diverges to infinity as

$$\xi \propto |p - p_c|^{-v} \quad p \rightarrow p_c \quad (1.2)$$

The zero-shear viscosity diverges to infinity slightly below the gel point as:

$$\eta_o \propto (p - p_c)^{-k'} \quad p < p_c \quad (1.3)$$

The gel fraction F_g (the fraction of monomers belonging to the infinite cluster) diverges from zero slightly above the gel point as:

$$F_g \propto (p - p_c)^\beta \quad p > p_c \quad (1.4)$$

The static elastic modulus at zero frequency G_e diverges from zero slightly above the gel point as:

$$G_e \propto (p - p_c)^\Delta \quad p > p_c \quad (1.5)$$

The a , v , k' , β and Δ are called critical exponents and are universal quantities. However, the classical theory of Flory-Stockmayer and the percolation model predict different critical exponents as listed in Table 1.4. The fact that $\Delta > \beta$ is due to the formation of dangling chains, which contribute to the gel fraction but do not contribute to the elastic modulus (de Gennes, 1979). There is no general agreement as to which model best describes the real gelation process, owing to experimental difficulties. Some experimental results agree with the percolation model, but others agree better with the classical theory (Stauffer *et al.* 1982).

The critical behaviour of the shear modulus of casein gels has been studied by Tokita *et al.* (1985). They found that the shear modulus of such gels scaled with a critical exponent of 2.06 and that gelation of a casein micelle solution was a realization of the percolation

process. Steventon *et al.* (1991) applied percolation theory to the gelation of a WPC and found that the gelation could be described satisfactorily by a second order kinetic model.

1.6 Factors Determining Gel Properties

The properties of polymer gels formed by chemical or physical gelation are determined by the molecular architecture, *e.g.* the monomer building blocks, molecular size, branching, chain stiffness, cross-link functionality, and solvent content (Winter, 1991). For gelation or coagulation of casein particles, the mechanical properties of a gel network are defined by three factors: the spatial distribution of the particles, the strength of the interaction forces between the particles and the structures of the particles themselves (Bremer, 1992). Bremer (1992) found that gels built with the same material (either casein or polystyrene latex) but with different geometric structures differed substantially in stiffness. Similarly, properties of globular protein gels may be governed by molecular structure, molecular size, the strengths of protein-protein interactions and the three-dimensional nature of the whole geometric structure. Many factors affect the gelation of globular proteins - such as pH, salt content, gelation temperature and protein concentration (Mulvihill and Kinsella, 1987). For the same protein sample, gels formed can range from transparent fine gels to collections of precipitated particles (Kitabatake *et al.* 1989). Thus, the geometric structure of formed globular protein gels can be altered by changes in environmental conditions such as pH and salt content.

Table 1.4 The critical exponents of classical theory and the percolation model.

Critical Exponents	Percolation Model	
	Classical Theory	(in three dimensional space)
a	1	1.8
ν	0.5	0.88
k'	?	0.8
β	1	0.39
Δ	3	1.7 - 1.9

Sources: data from de Gennes (1979) and Stauffer *et al.* (1982).

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2. Rheology of Polymer Solutions and Gels

2.1 Rheology

Rheology is the study of deformation and flow of materials, and deals with force, deformation and time. A simple way to study the rheology of a materials is to apply a shear deformation to the material and examine the relationship between shear deformation, shear force and time. This is the design principle of most modern rheometers. Using such simple shear deformation, materials can be classified rheologically. For example, polymer solutions and gels can be classified as shown in Table 2.1 (Cheng, 1986; Campanella, 1987; Winter and Chambon, 1976; Winter, 1989).

2.2 Rheology of Protein Solutions

There is a large amount of literature on the rheology of polymer solutions. Theoretical studies of the rheology of polymer solutions have been reviewed by de Gennes (1979), Dickinson and Stainsby (1982), and Ross-Murphy (1992). The rheological behaviour of protein solutions has been reviewed by Tung (1977). Here, only literature which relates to the rheology of whey protein solutions is reviewed and discussed.

Biopolymers with extended coils such as polysaccharides are much more effective in changing the flow behaviour of solutions than globular proteins. However, globular proteins can be denatured by thermal treatments to produce structure formation in the solution at low protein concentration (Ross-Murphy, 1992); this dramatically increases the viscosity and changes the flow behaviour of the solutions. This treatment could be used to make whey proteins behave as thickeners in dairy products and thus obviate the need to use additives such as polysaccharides.

2.2.1 Concentration dependence

The viscosity of whey protein solutions has been found to rise exponentially with increase in concentration (Herbert, 1972; McDonough *et al.* 1974; Hermansson, 1975). Pradipasena and Rha (1977a) studied the viscosity of β -lactoglobulin solutions using a cone and plate viscometer and found that the apparent viscosity increased linearly with increasing concentration up to 10 percent by weight and then more rapidly as concentration increased above this value.

Table 2.1 Rheological classification of polymer solutions and gels according to their behaviour in simple shear deformation.

Linear viscous liquid (Newtonian liquid)	
$\tau = \eta \dot{\gamma}$ ($\eta = \text{constant}$)	(2.1)
Non-linear viscous liquid (Pseudoplasticity, plasticity or dilatancy)	
$\tau = \eta(\dot{\gamma}) \dot{\gamma}$	(2.2)
Non-linear and time-dependent viscous liquid (thixotropy or rheopexy)	
$\tau = \eta(\dot{\gamma}, t) \dot{\gamma}$	(2.3)
Viscoelastic liquid (entanglement effect)	
$\tau = G(\gamma, t) \gamma$	(2.4)
Critical gel (neither liquid nor solid)	
$\tau = S \int_{-\infty}^t (t - t')^{-n} \gamma(t') dt'$ $0 < n < 1$	(2.5)
Non-linear viscoelastic solid	
$\tau = G(\gamma, t) \gamma$	(2.6)
Linear viscoelastic solid	
$\tau = G(t) \gamma$	(2.7)
Non-linear elastic solid	
$\tau = G(\gamma) \gamma$	(2.8)
Linear elastic solid (Hookean)	
$\tau = G \gamma$ ($G = \text{constant}$)	(2.9)

τ is the shear stress, γ and $\dot{\gamma}$ are the shear strain and the shear rate respectively. G is the shear modulus, η is the shear viscosity and t is time ($-\infty \leq t' \leq t$). S and n are two material parameters.

Source: from Ferry (1980), Cheng (1987), Campanella (1987), Winter and Chambon (1986), Winter (1989).

The effect of protein concentration on the flow properties of WPC dispersions at pH7 was investigated by Hermansson (1975). She concluded that WPC is characterized by low viscosity over a broad concentration range. The flow was found to be almost Newtonian in the range 4-12% and pseudoplastic in the range 14-16%, while at higher concentrations yield

values could be measured and the flow was therefore plastic.

2.2.2 Shear rate dependence

Whey protein solutions exhibit shear-thinning in some concentration ranges (Herbert, 1972; Hermansson, 1975; Pradipasena and Rha, 1977b). The effect of shear rate on the apparent viscosity of β -lactoglobulin solutions was studied by Pradipasena and Rha (1977b). They found that the apparent viscosity of 3% and 5% solutions was independent of the shear rate, but at higher concentrations the apparent viscosity decreased as the shear rate increased. Herbert (1972) observed that the apparent viscosity of whey protein solutions decreased as the shear rate increased and that this effect was more pronounced at higher protein concentrations.

2.2.3 Shear time dependence

Dispersions of whey proteins in some concentration ranges exhibit time-dependent flow phenomena: shear stress or apparent viscosity may decrease or increase with time at a constant shear rate (Pradipasena and Rha, 1977b). In some cases, this change is reversible, and the dispersion of protein will recover its original condition with time at rest (Hermansson, 1975). In others, the change brought about by shearing may be irreversible (Pradipasena and Rha, 1977b).

Hermansson (1975) found that a dispersion of WPC at a concentration of 20% was thixotropic, and the structure broken down during shearing at a high shear rate for 15 minutes was reversibly reformed after resting for 15 minutes. Zeng and Munro (1988) observed that WPC solutions exhibited apparent thixotropy at concentrations above 30% at the measuring temperature of 25°C: the shear stress decreased with shearing time at first and then reached a constant value after shearing for a short time. The major difference between pseudoplastic and thixotropic systems is that in pseudoplastic fluids the structural breakdown and recovery is instantaneous, whereas in thixotropic fluids a relatively long time is required (Tung, 1978).

Pradipasena and Rha (1977b) conducted an investigation into the effect of shearing time upon the apparent viscosities of β -lactoglobulin solutions. Protein solutions (3-40%) were subjected to constant rates of shear for up to 30 minutes at 25°C. These workers found that for 3 and 5% solutions the apparent viscosity remained constant, showing no shear time effect over the shear rate range of 6,850-17,000 s⁻¹. For 10-30% solutions, the apparent

viscosity increased with shearing time at a constant shear rate. However, the 40% β -lactoglobulin solution showed a time-dependent shear-thinning behaviour with its apparent viscosity decreasing with shearing time at a constant shear rate, which was distinct from the apparent rheopectic properties determined for 10-30% solutions. They assumed that the time-dependent shear thinning property of the 40% solution could be caused by the breaking of aggregates since the concentration was high enough at 40% for the protein to exist in aggregate form. The rheopectic properties of 10-30% solutions could be explained by the fact that, in shearing at high shear rates, protein molecules could become unfolded, thus changing or increasing the shape, size and effective volume of the solute in solution. Pradipasena and Rha (1977b) found also that changes in rheological properties were irreversible both for apparent rheopecty and for time-dependent shear thinning.

2.3 Small Deformation Rheological Tests

Small deformation measurements including stress relaxation, creep compliance and dynamic shear tests are used to study rheological properties of polymers within the linear viscoelastic region, *i.e.* where the ratio of stress to strain is a function of time or frequency alone and not of the stress or strain magnitude (Ferry, 1980). These are the only rheological methods available so far which can accurately and precisely define the gel point during the sol-gel transition of polymers (Chambon *et al.* 1986; Winter, 1989).

2.3.1 Stress relaxation tests

In stress relaxation tests, a strain (normally a shear strain) is suddenly imposed and kept constant while the relaxation of stress with time is measured as shown in Fig 2.1 (Tung, 1978; Mitchell, 1980; Ferry, 1980).

The relation between shear stress, τ , and shear strain, γ , is defined as

$$\tau = G(t) \gamma \quad (2.10)$$

where $G(t)$ is the relaxation modulus.

For a perfectly elastic solid the stress will be independent of relaxation time (Fig 2.1); $G(t)$ is a constant independent of time and can be written as $G = \tau / \gamma$. For a viscoelastic solid $G(t)$ will decrease initially and reach a constant non-zero value, whereas for a

viscoelastic liquid it will decay to zero.

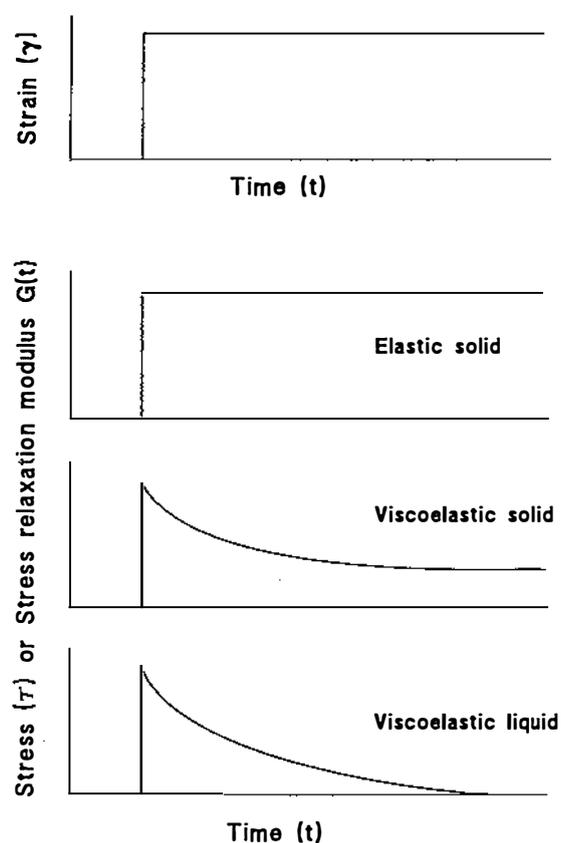


Fig 2.1 Time profile of a simple shear stress relaxation experiment following sudden strain for a perfectly elastic solid, a viscoelastic solid and a viscoelastic liquid. From Ferry (1980) and Mitchell (1980).

For a critical gel at the gel point, which is the state intermediate between a viscoelastic liquid and a viscoelastic solid, $G(t)$ follows a simple power law (Winter, 1989):

$$G(t) = S t^{-n} \quad p = p_c \quad (2.11)$$

where S is a material parameter and n ($0 < n < 1$) is the relaxation exponent (also a material parameter).

For the critical gel $G(t)$ will neither decay to zero as for the viscoelastic liquid, nor will it eventually reach a constant non-zero value as for the viscoelastic solid. The time for stress relaxation of the critical gel will be infinitely long (Winter and Chambon, 1986). The

power law behaviour of $G(t)$ versus t is said to be an expression of the self-similar structure (fractal geometry) of the critical gel at the gel point (Winter, 1987).

2.3.2 Creep compliance tests

In creep compliance tests a small constant stress is applied and maintained constant, and the resulting strain is followed with time as shown in Fig 2.2 (Tung, 1978; Mitchell, 1980; Ferry, 1980). The relationship between stress and strain is defined as

$$\gamma(t) = J(t) \tau \quad (2.12)$$

where $J(t)$ is the creep compliance.

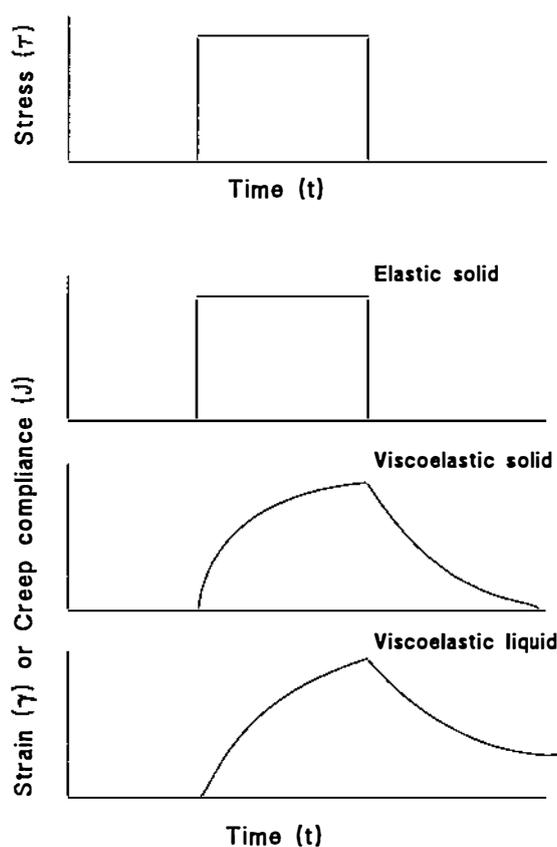


Fig 2.2 Time profile of shear creep and creep recovery for a perfectly elastic solid, a viscoelastic solid and a viscoelastic liquid. From Ferry (1980) and Mitchell (1980).

For a perfectly elastic solid the resulting strain will be independent of time and $J = 1/G$. For a viscoelastic solid the resulting strain will eventually reach a constant value, and

on removal of the stress the strain will finally return to zero. For a viscoelastic liquid a permanent deformation will remain after removal of the stress. $J(t)$ does not equal $1/G(t)$ for a viscoelastic material (Ferry, 1980).

After removal of a stress the creep strain of a critical gel will neither decay to zero at finite times as for a viscoelastic solid, nor will it reach a permanent constant deformation at finite times as for viscoelastic liquid. Complete recovery ($\gamma_{\infty} = 0$) is only attained at infinite times (Venkataraman and Winter, 1990).

2.3.3 Dynamic shear tests

In oscillation experiments a strain which is made to vary with time in a sinusoidal way is imposed on a sample. A sinusoidally varying stress will result. For an ideal elastic solid, the stress will be in phase with the strain, while for an ideal viscous fluid, the stress will be 90° out of phase with the strain. When a viscoelastic material such as a polymer gel is subjected to sinusoidally varying strain, the stress is neither exactly in phase nor 90° out of phase, but somewhere in between as shown in Fig 2.3; some of the energy input is stored and recovered in each cycle and some is dissipated as heat (Tung, 1978).

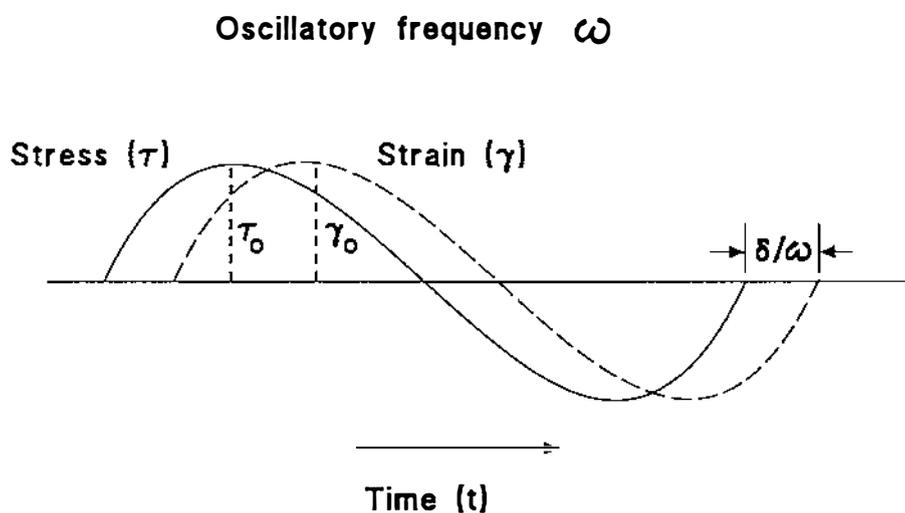


Fig 2.3 Time profile of sinusoidal variation of strain and stress for a viscoelastic material.

If a sinusoidally varying strain $\gamma(t)$ given by

$$\gamma(t) = \gamma_0 \sin(\omega t) \quad (2.13)$$

where γ_0 is the maximum strain, ω is the angular frequency and t is time is applied, the following sinusoidally varying stress $\tau(t)$ is obtained:

$$\begin{aligned} \tau(t) &= \tau_0 \sin(\omega t + \delta) \\ &= \tau_0 \{ \sin(\omega t) \cos \delta + \cos(\omega t) \sin \delta \} \end{aligned} \quad (2.14)$$

where τ_0 is the maximum stress and δ is the phase angle (loss angle) between the strain and the stress.

Within the linear region τ_0 is by definition proportional to γ_0 . Equation (2.14) can be written as:

$$\tau(t) = \gamma_0 \{ \tau_0/\gamma_0 \cos \delta \sin(\omega t) + \tau_0/\gamma_0 \sin \delta \cos(\omega t) \} \quad (2.15)$$

The elastic part of the stress, which is the part of the stress in phase with the strain, corresponds to the storage modulus G' , which is defined as:

$$G'(\omega) = (\tau_0/\gamma_0) \cos \delta \quad (2.16)$$

The viscous part of the stress, which is the part of the stress 90° out of phase with the strain, corresponds to the loss modulus G'' , which is defined as:

$$G''(\omega) = (\tau_0/\gamma_0) \sin \delta \quad (2.17)$$

Then, equation (2.15) becomes:

$$\tau(t) = \gamma_0 \{ G'(\omega) \sin(\omega t) + G''(\omega) \cos(\omega t) \} \quad (2.18)$$

The loss tangent ($\tan \delta$), the dynamic viscosity (η') and the complex modulus (G^*) are expressed as:

$$\tan \delta = G''/G' \quad (2.19)$$

$$\eta' = G''/\omega \quad (2.20)$$

$$G^* = G' + j G'' \quad (j^2 = -1) \quad (2.21)$$

$$|G^*| = \tau_s/\gamma_0 = (G'^2 + G''^2)^{1/2} \quad (2.22)$$

The storage modulus (G') is a measure of the energy stored during each test cycle whereas the loss modulus (G'') is a measure of the energy dissipated. The phase angle (δ) indicates the extent of viscous or, conversely, elastic (*i.e.* solid-like) character of a polymer gel at a particular test frequency (ω). For a perfectly elastic gel, G'' and δ would both be zero, while for an ideal fluid, G' and δ would be zero and 90° respectively.

In the linear viscoelastic region it can be said that for a viscoelastic liquid $G' = 0$ when frequency $\omega \rightarrow 0$ while for a viscoelastic solid $G' > 0$ when frequency $\omega \rightarrow 0$. Therefore, a viscoelastic liquid can be distinguished from a viscoelastic solid (Table 2.1) by whether $G' = 0$ or $G' > 0$ when frequency $\omega \rightarrow 0$. For the critical gel, which is neither liquid nor solid, G' and G'' follow a simple power law relationship with frequency as (Winter and Chambon, 1986):

$$G' \propto \omega^n, \quad G'' \propto \omega^n \quad p = p_c \quad 0 < n < 1 \quad (2.23)$$

The power law behaviour of G' and G'' versus frequency is due to the fractal nature of the critical gel. The high frequency probes the small length scale and the low frequency probes the large length scale (Vilgis and Winter, 1988). There is a self-similarity in the geometry of the critical gel between small and large length scales; thus a power law relationship exists between frequency and both G' and G'' . However, as discussed in section 1.5.2, unlike in the pure mathematical fractal, the self-similarity does not apply to all length scales of the critical gel in polymer gelation. For example, the power law behaviour will not apply if the frequency is high enough to probe length scales that are much smaller than self-similar regions of the critical gel (Vilgis and Winter, 1988).

When frequency $\omega \rightarrow 0$, G' tends to the equilibrium elastic modulus, G_e . During polymer gelation G_e is zero below the gel point and larger than zero above the gel point. The gel point can then be defined as the point when G_e starts to grow from zero. Practically, it is very difficult to measure the gel point in this way because of limitations of instruments and

fluctuation of measurements when frequency $\omega \rightarrow 0$. Tung and Dynes (1982) suggested that the gel point might occur at the point where G' equalled G'' . Winter (1987) examined this definition and found that the crossover of G' and G'' was the gel point only when the exponent value $n=1/2$ (equations 2.11 and 2.23). For $n < 1/2$ the crossover of G' and G'' occurred before the gel point and for $n > 1/2$ it occurred after the gel point. In these cases a multifrequency experiment must always be used to detect the gel point (Holly *et al.* 1987; Te Nijenhuis, 1989; Cuvelier and Launay, 1990).

A viscoelastic polymer liquid can behave like a gel, and has elastic properties, at frequencies higher than the relaxation rate of entanglements (de Gennes, 1976, 1979). The elastic entanglement network is formed not by permanent crosslinks but by points coupled by twisting or looping (Ferry, 1980). This phenomenon has been investigated, and the elastic modulus, called plateau modulus (G_N^0), has a power law relationship with polymer concentration (C) as (Daoud *et al.* 1975; de Gennes, 1976, 1979; Ferry, 1980):

$$G_N^0 \propto C^{2.00} \quad (\text{given by mean field theory}) \quad (2.24)$$

$$G_N^0 \propto C^{2.25} \quad (\text{given by scaling laws}) \quad (2.25)$$

Some experimental results agree with equation (2.24) while others favour equation (2.25), and further work is needed to resolve the difference (Ferry, 1980).

The entanglement effect has also been observed for polymer gels when the oscillatory rheological method is used to follow gel formation at high frequency. G' is not only contributed to by the elastic bonds of the gel structure, but also by the entanglement effect of polymer molecules. This entanglement effect increases with increase in frequency.

For biopolymer systems, strong gels, weak gels and entanglement networks can be characterized on the basis of their rheological behaviour, as suggested by Clark and Ross-Murphy (1987). A strong gel has a permanent network and its G' is only slightly dependent on frequency, while for a weak gel G' is more frequency-dependent. Entanglement networks do not have permanent crosslinking and show a crossover of G' and G'' in frequency sweep tests. G' and G'' of entanglement networks are much more frequency-dependent than those of weak gels. Any entanglement network behaves as a liquid at frequencies lower than the relaxation rate of the entanglements, but behaves as a gel at frequencies higher than the relaxation rate of the entanglements (de Gennes, 1976; 1979). Therefore, its G' is lower than

G'' at low frequency and is higher than G'' at high frequency (Clark and Ross-Murphy, 1987). The same arguments also apply to chemical gelation systems. The difference in rheological properties between the critical gel and any entanglement network is of great interest. For a critical gel, G' and G'' follow the simple power law relationship as shown in equation (2.23), and G' and G'' parallel each other over a wide range of frequency (Te Nijenhuis and Winter, 1989; Cuvelier and Launay, 1990; Coviello and Burchard, 1992).

The formation of a protein gel structure during the gelling process can be studied using non-destructive oscillatory rheological measurements. This oscillatory rheological technique has been used by a number of workers to monitor protein gel formation (Beveridge *et al.* 1984; Bohlin *et al.* 1984; Paulsson *et al.* 1986; 1990; Clark and Ross-Murphy, 1987; Stading and Hermansson, 1990), but few studies of heat-induced gelation of WPC using the technique have been published.

Paulsson *et al.* (1986) observed, by using the oscillatory rheological method, that bovine serum albumin had good, β -lactoglobulin intermediate and α -lactalbumin poor thermal gelation properties. Beveridge *et al.* (1984) studied gel formation in WPC as a function of time and temperature. They found that after the gel point the increasingly elastic character of WPC gels was clearly shown by the storage modulus (G') increasing with time at constant temperature, and that the rate and extent of such increases in G' were temperature-dependent.

2.4 Large Deformation and Failure Tests

Small deformation rheological tests and their application to the study of polymer gelation have been discussed above. However, large deformation and failure tests are also very important in the study of rheological properties of protein gels, particularly from the view point of commercial applications. It is failure force and failure deformation rather than shear modulus measured at small deformations which correlate better with gel strength assessed in the mouth (Wood, 1979). Four major types of large deformation and failure tests are considered here: tension, compression, penetration and torsion tests.

2.4.1 Tension tests

The tensile test is the most common mechanical test. A specimen is stretched and a stress-strain graph is produced. However, for biopolymer gels, particularly weak gels, it is difficult to carry out tensile tests because sample handling before the test involves some

deformation and maybe even premature failure (Ross-Murphy, 1992).

A tensile stress (τ_t), strain (γ_t) and Young's modulus (E) can be calculated as follow:

$$\tau_t = F_t / A \quad (2.26)$$

$$\gamma_t = \Delta l / l_o \quad (2.27)$$

$$E = \tau_t / \gamma_t \quad (2.28)$$

where F_t is the applied force, A is the cross-sectional area, l_o is the original length and Δl is the increase in length.

For a purely elastic solid, the shear elastic modulus (G) is simply related to the Young's modulus (E) as follows (Ferry, 1980):

$$E = 2 G (1 + \mu) \quad (2.29)$$

where μ is the Poisson's ratio, which is defined as the ratio of lateral contraction to longitudinal extension.

Equation (2.29) applies to a viscoelastic solid only at equilibrium; the equilibrium shear modulus (G_e) is related to the equilibrium modulus in tension (E_e) as follows (Ferry, 1980):

$$E_e = 2 G_e (1 + \mu_e) \quad (2.30)$$

For a non-linear elastic solid, equation (2.8) applies. However, just as a power law equation is used to describe a non-linear viscous solution (in which viscosity is dependent on shear rate), a similar power law relationship may be used to describe the non-linear elastic solid as follows (Stading, 1993):

$$\tau_t = k \gamma^\theta \quad (2.31)$$

where k and θ are constants.

A value of $\theta < 1$ gives a curve convex to the stress axis and $\theta > 1$ gives a curve concave to the stress axis. A linear elastic or Hookean material has $\theta = 1$ and k equal to Young's

modulus (Stading, 1993).

For a non-linear viscoelastic solid, equation (2.6) applies. The relationship between tensile stress, strain, modulus and time can be quite complicated (Ferry, 1980). Fortunately, unlike other solid materials, protein gels can be deformed considerably (*e.g.* by 10%) and still show linear viscoelastic behaviour (Walstra and Jenness, 1984); thus their rheological behaviour can be described by simple constitutive equations which apply inside the linear viscoelastic region as already discussed above.

Tensile measurements with the Instron Universal Testing Machine have been used to study the large deformation and failure properties of whey protein gels by Langley *et al.* (1986) and Stading and Hermansson (1991). Langley *et al.* (1986) used a test mould to produce a dumb-bell-shaped gel which was stretched in tension to failure. They found that the tensile strength of whey protein gels had a power law relationship with either the β -lactoglobulin or the α -lactalbumin content of the sample. Stading and Hermansson (1991) developed a method to study tensile properties of β -lactoglobulin gels and found that the stiffness (Young's modulus) of β -lactoglobulin gels at large deformation had two maxima with pH: one at pH4 and the other at around pH6.

2.4.2 Compression tests

There are two main types of compression tests: uniaxial compression and bulk compression (Bourne, 1982). In uniaxial compression, the sample is compressed in one dimension and is unrestrained in the other two dimensions. In bulk compression, the sample is compressed in three dimensions, usually by means of hydraulic pressure.

Parallel plate uniaxial compression using an Instron Universal Testing Machine, to obtain force-deformation curves, is commonly used for investigation of the rheology of protein gels. The advantages and disadvantages of compression testing have been discussed by McCarthy (1987).

In compression, a cylindrical or cubical protein gel with flat surfaces is placed between two flat plates and one of the plates is then made to move at a preselected constant speed. The force developed is recorded as a function of deformation. From force-deformation curves obtained by compression, unloading and recompression, a texture profile analysis (TPA) can be conducted to calculate the hardness, springiness, cohesiveness and adhesiveness of protein gels (Bourne, 1978, 1982). The initial slope gives the rigidity of protein gels (Egelandsdal,

1980).

Equation (2.30) can be applied also to compression tests on linear viscoelastic protein gels if the friction between the contact surface of the sample and the plate during compression can be reduced to a minimum. This is usually done by applying a lubricant to the contact surface of the sample. However, the phenomenon of self-lubrication is observed in compression testing of WPC gels (Philpott, 1987).

2.4.3 Penetration tests

Penetration testing is probably the simplest method of measuring protein gel strength. Instron Universal Testing Machines have been used in penetration experiments on protein gels (Kalab *et al.* 1971; Schmidt and Llingworth, 1978; Dunkerley and Haye, 1980; Hermansson, 1982; Paulson and Tung, 1989; Lee and Chung, 1989). For testing a gel sample, a probe attached to the Instron crosshead penetrates the sample at a constant speed, and protein gel strength is expressed as the force when the gel surface yields. Penetration tests give empirical results, but are considered to give a better correlation with sensory texture than fundamental tests (Mohsenin and Mittal 1977; Wood 1979; Bourne, 1982). Paulsson and Tung (1989) examined the relationships between the results of a penetration test and those of non-destructive dynamic shear measurement. They found that penetration forces were poorly correlated with viscoelastic parameters, whereas the slopes of force-deformation curves to the point of penetration rupture were well correlated with G' and G'' .

2.4.4 Torsion tests

The most important advantage of torsion testing is that three maximum stresses - maximum shear stress, maximum tensile stress and maximum compressive stress - are equal in magnitude (Hamann, 1983). Another advantage is that failure will occur even in a highly deformable sample. A dumb-bell-shaped protein gel is used for torsion tests; the dumb-bell is made from a cylinder of gel by removing part of the gel material with a grinding wheel of the correct profile. The dumb-bell is then placed in a modified Ferranti-Shirley cone-and-plate viscometer or some other suitable torque measuring device and twisted until failure occurs (McCarthy, 1987). However, it can be time-consuming to prepare the dumb-bell for torsion testing, and, for a weak protein gel, premature deformation and failure may occur during preparation and handling of a dumb-bell before torsion testing can take place.

Nevertheless, torsion testing is increasingly popular for the study of the failure properties of biopolymer gels (McCarthy, 1987; Foegeding, 1992; Lelievre *et al.* 1992; Mirza and Lelievre, 1992)

2.5 Rheological Properties in Relation to Gel Structure

It is the internal structure of a material that determines its mechanical or rheological properties. Rheological properties of a protein gel are always related to its gel structure. Stading (1993) investigated the rheological behaviour of whey protein gels in relation to their structure. He found that the storage modulus of fine-stranded β -lactoglobulin gels was low compared to that of particulate gels. The rheological properties of fine-stranded β -lactoglobulin gels were less frequency-dependent than the particulate gels.

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3. Materials and Methods

3.1 Materials

Several commercially available WPC powders, one WPI powder, one β -lactoglobulin powder and one commercially available egg white powder were provided by the New Zealand Dairy Research Institute (NZDRI) and the New Zealand Dairy Board (NZDB). The compositions of these protein samples as given by NZDRI are shown in Table 3.1.

Table 3.1 Compositions of protein samples (% w/w).

Samples	Protein	Moisture	Fat	Ash	Lactose
WPC (A)	75.90	4.40	6.70	3.00	10.00
WPC (B)	79.80	4.40	7.10	2.80	4.60
WPC (C)	75.99	4.53	4.90	5.53	6.30
WPC (D)	79.66	4.20	-	-	-
WPC (E)	82.02	6.40	6.13	2.32	0.34
WPC (F)	84.63	3.88	6.28	2.83	0.40
WPC (G)	86.80	3.57	-	-	-
WPI	93.60	4.15	0.86	1.81	6.5
EWP	85.78	7.36	-	-	-
β -Lg	86.60	3.50	0.75	4.0	3.0

Note:

- (1) WPC - Whey Protein Concentrate Powder
- (2) WPC (A) - Alacen 475 (This WPC powder was produced in the 1986-1987 season).
- (3) WPC (B) - Alacen 475 (This WPC powder was produced in the 1990-1991 season).
- (4) WPC (C) - Alacen 132 (from Bay Milk Products).

- (5) WPC (D) - Alacen 162 (from Bay Milk Products).
- (6) WPC (E) - Run 1D (from Bay Milk Products).
- (7) WPC (F) - Run 2D (from Bay Milk Products).
- (8) WPC (G) - LL 14 (from Bay Milk Products).
- (9) WPI - Whey Protein Isolate (from Le Sueur Isolates, Le Sueur, MN, USA).
- (10) β -Lg - β -lactoglobulin Powder containing 92% β -lactoglobulin (from NZDRI). This sample of purified β -lactoglobulin was prepared from cheddar cheese whey by the thermal separation method of Pearce (1983). Separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis indicated that 92% of the protein was β -lactoglobulin.
- (11) EWP - Egg White Powder (spray dried, egg white powder type 110, from Henningsens, USA).

Some components of the ash for some of the whey protein samples are listed in Table 3.2 (Data provided by NZDRI).

Table 3.2 Components of ash for whey protein samples (% w/w).

Component	WPC (A)	WPC (B)	WPC (C)	WPC (E)	WPC (F)	WPI
Ash	3.00	2.80	5.53	2.32	2.83	2.83
Calcium	0.30	0.33	0.22	0.01	0.04	0.20
Magnesium	0.043	-	-	-	-	-
Sodium	0.19	0.16	1.57	-	-	0.51
Potassium	1.24	0.69	0.12	-	-	0.12
Phosphorus	0.31	0.26	-	-	-	-
Phosphate	-	-	0.33	-	-	<0.005
Chloride	0.13	0.033	-	-	-	-

The proportions of the major whey proteins in WPCs were identical to those found in raw whey, and only a trace of whey proteins could be removed as insoluble material by centrifuging. There were more precipitates in WPC(A) and WPC(B) than in other WPCs after centrifuging 10% or 12% protein solutions (at $\text{pH}7.00 \pm 0.01$) for 5 minutes at 550g, indicating much more whey proteins in WPC(A) and WPC(B) were denatured during the manufacturing process. WPC(E), WPC(F) and WPC(G) were so-called brine-tolerant WPCs, and they were different from the other WPC products since they had been subjected to repeated ultrafiltration to reduce the ash and lactose contents. Thus, they had lower lactose and ash contents including calcium.

3.2 Preparation of WPC Solutions

Whey protein solutions were prepared by reconstituting WPC powder either with distilled water or with salt solutions (NaCl and CaCl_2) of fixed concentration and then mixing with a magnetic stirrer until dissolution was complete. The pHs of protein solutions were adjusted from their initial values to desired values by adding either 1M-HCl or 1M-NaOH. Generally, a protein solution with a higher than desired protein concentration (*e.g.* 22% rather than 20%) was prepared at first. After pH adjustment this was then diluted to the desired protein concentration, centrifuged for 5 min at 550 g to remove foam and dispersed air bubbles, and stored at 5.5°C prior to rheological measurements. WPC solutions for steady shear rheological measurements were stored at 5.5°C for at least one day to ensure that solution structure was in the fully recovered state. WPC solutions were stable on storage at 5.5°C in the pH range 4-8, but outside this pH range the solutions age-thickened, eventually forming very viscous solutions or even soft gels.

3.3 Steady Shear Rheological Measurements

The Bohlin VOR Rheometer System (Bohlin Rheologi AB, Lund, Sweden) (Bohlin *et al.* 1984) was used in its steady shear viscometry mode. The C25 concentric cylinders measuring system, consisting of a 25mm diameter fixed bob and a 27.5mm diameter rotating cup, was used in all experiments with water and with solutions of up to 35%, and in the determination of the yield stress of a 40% solution. The CP5/30 cone and plate measuring system, consisting of a 30mm diameter 5.4° fixed cone and a 30mm rotating plate, was used in shear rate sweep experiments on, and in measuring the time-dependent behaviour of, 40%

solutions.

All measurements with the C25 measuring system except the temperature holding and temperature dependence experiments described below were conducted as follows. For each experiment with this system the WPC solution was very carefully and slowly loaded into the cup so as to minimise the breakdown of structure, particularly for solutions with concentrations above 30%. The cup and bob were then installed in the instrument. The system temperature was adjusted to the required value, for example 22°C. The bob was then lowered very slowly into the cup until the solution just reached the top surface of the bob. In order to prevent evaporation of water and consequent surface drying the sample surface was sometimes covered with a thin layer of oil: low standard viscosity mineral oil in temperature sweep and temperature holding tests with 15-30% solutions and liquid paraffin in all tests with 40% solutions. The viscosities of these oils were considerably lower than those of the sample solutions, and the oils were immiscible with the solutions. The layer of oil was found to have little effect on the measured shear viscosity of the sample. In other experiments surface drying was not found to be a serious problem so oils were not used. Finally, rheological measurement was started after allowing 5 minutes for temperature equilibration.

The effects on apparent viscosity measured at 22°C of holding solutions at various higher temperatures were measured as follows. The C25 cup and bob were installed in the rheometer, and the water jacket was brought to the desired holding temperature. The WPC sample solution was then placed in the cup. After a holding period of 12 minutes (including a nominal 2 minutes for temperature equilibration) the jacket temperature was reduced to 22°C. The bob was then lowered into position, and rheological measurement was carried out after a 2 minute temperature equilibration period. Reasons for the selection of a 2 minute equilibration period are elaborated in section 3.4.

Temperature dependence of the apparent viscosity of WPC solutions of various concentrations up to and including 30% was measured as follows. A sample of given concentration was loaded into the rheometer, and the bob was lowered into position. The system temperature was then increased stepwise from 5 to 60°C under the control of the instrument's 'constant temperature' program; each automatic step change (increase) in temperature was accomplished in 150 s, and was then followed by a 150 s temperature equilibration period and then a 5 s period during which apparent viscosity at 291 s⁻¹ was

measured and recorded.

In temperature sweep tests, temperature was increased at the rate of 1°C/min, measurements were made at one minute intervals and shear rate was held constant.

Experiments with the cone and plate measuring system (CP5/30) were conducted as follows. The cone and the plate were installed in the instrument, and the plate temperature adjusted to 22°C. The 40% sample was then very carefully and slowly placed on the plate using a spatula. The cone was then lowered very slowly into position. The exposed sample surface was covered with liquid paraffin to prevent drying by applying several drops of liquid paraffin around the periphery of the gap between cone and plate. Finally, rheological measurement was started after allowing 3 minutes for temperature equilibration.

Shear rate sweeps to illustrate shear-rate dependence or time- dependence of viscosity were carried out by first increasing and then decreasing the shear rate incrementally with continuous shearing. The interval between successive shear rate changes was 10 seconds. A shear rate sweep designed to measure the yield stress of a 40% solution was carried out by decreasing the shear rate from an initial value of 10 s⁻¹.

In the measurement of the time-dependence of structure breakdown and recovery of a 40% solution the "Jobstream" software facility of the instrument was employed. This enabled a sequential experiment involving step changes in shear rate, and shearing for set times at the different shear rates, to be set up in advance and run automatically.

The instrument torsion bars were calibrated with both distilled water and standard viscosity mineral oils (Cannon Instrument Company, U.S.A.) before rheological measurement.

3.4 Oscillatory Rheological Measurements

The Bohlin VOR Rheometer System was used in its oscillatory mode. The C25 concentric cylinders measuring system was used in all experiments. All measurements were made with 13 ml of protein solution, which was always covered with a thin layer of liquid paraffin in order to prevent evaporation of water and consequent surface drying of the sample. The oil was immiscible with the protein solution. A layer of oil had no significant effects on shear viscosity or oscillatory properties at room temperature. The results of oscillatory rheological measurements were displayed on the PC monitor of the rheometer during experiments in the form of plots of phase angle, dynamic viscosity, and storage and loss moduli versus time.

For each experiment the cup and bob were first installed in the instrument. The system temperature was raised to the required value, usually 70, 80 or 90°C. The protein solution was stirred very slowly to ensure a homogeneous sample while avoiding the inclusion of air bubbles. A sample of 13 ml was then placed in the cup. The bob was lowered into the cup until the solution just reached its top surface. The oil was then added to the surface of the solution. Finally, rheological measurement was started after allowing 2 min for temperature equilibration.

Measurement with a small thermocouple of the change of temperature with time of WPC(A) solutions containing 16% protein inside the stationary cup showed that the temperature increased towards the desired temperature of 70, 80 or 90°C quickly before 2 min, more slowly after 2 min, and had almost reached the desired temperature after 5 min (Fig 3.1). A 2 min period was therefore chosen for temperature equilibration. During oscillatory rheological measurements the protein solutions were virtually stationary so heat transfer into the solution was largely by conduction. Temperature equilibration was therefore rather slow. However, the alternative technique of heating the protein solution to the desired temperature before placing it in the rheometer risked significant reaction and gel formation occurring before rheological measurement could be commenced.

3.5 Large Deformation and Failure Tests

3.5.1 Compression tests

WPC(B) solution was poured into 25.14 mm diameter thin plastic tubing sealed at one end. The plastic tube was then tied at the other end and placed vertically in a $80\pm 0.5^\circ\text{C}$ circulating water bath for 45 min (plus 2 min for temperature equilibration). The plastic tube was immediately immersed in cool water at about 10°C and left for one hour. It was then placed in a temperature controlled room at $20\pm 2^\circ\text{C}$. The temperature history during gel preparation is shown in Fig 3.2.

Gel sections 20 mm long were cut from the plastic tube using a razor-edged cutting device (Mulvihill and Kinsella, 1988), and the external tubing was then removed. These gel sections were transferred into the temperature controlled chamber (at $20\pm 1^\circ\text{C}$) of an Instron Universal Testing Machine (model 4502) and 30 min were allowed for temperature equilibration before testing began. Compression tests were performed on the 25.14 mm diameter x 20 mm long cylindrical specimens by compressing them with flat parallel plates

at a crosshead speed of 50 mm/min to 50% of their original height. The crosshead was then returned to its original position at 50 mm/min and a second compression cycle identical to the first was then performed following the Texture Profile Analysis (TPA) method of Bourne (1978). Force and displacement were continuously recorded. Hardness, cohesiveness and springiness were calculated from the force-deformation curves according to Bourne (1978, 1982) as illustrated in Fig 3.3. Lubricated and non-lubricated parallel plates gave identical results within experimental error, indicating the WPC gels were self-lubricating during compression tests.

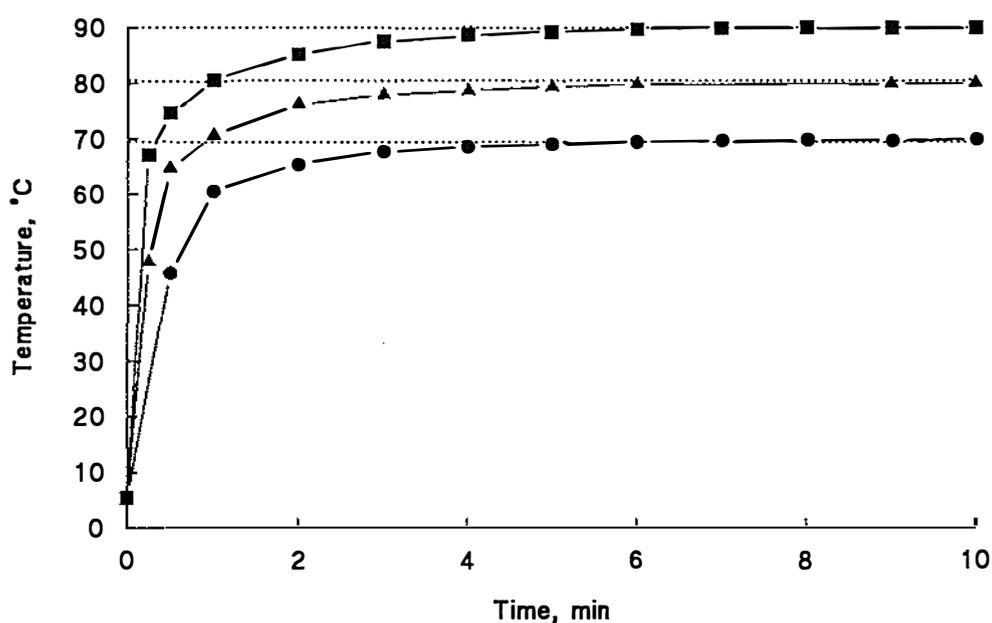


Fig 3.1 Sample temperature as a function of time in the Bohlin rheometer for WPC(A) solutions containing 16% protein at water jacket temperatures of 70°C (●), 80°C (▲) and 90°C (■). Rheological measurement was usually started after 2 minutes of temperature equilibration, but for these experiments with a thermocouple inserted there was no rheological measurement.

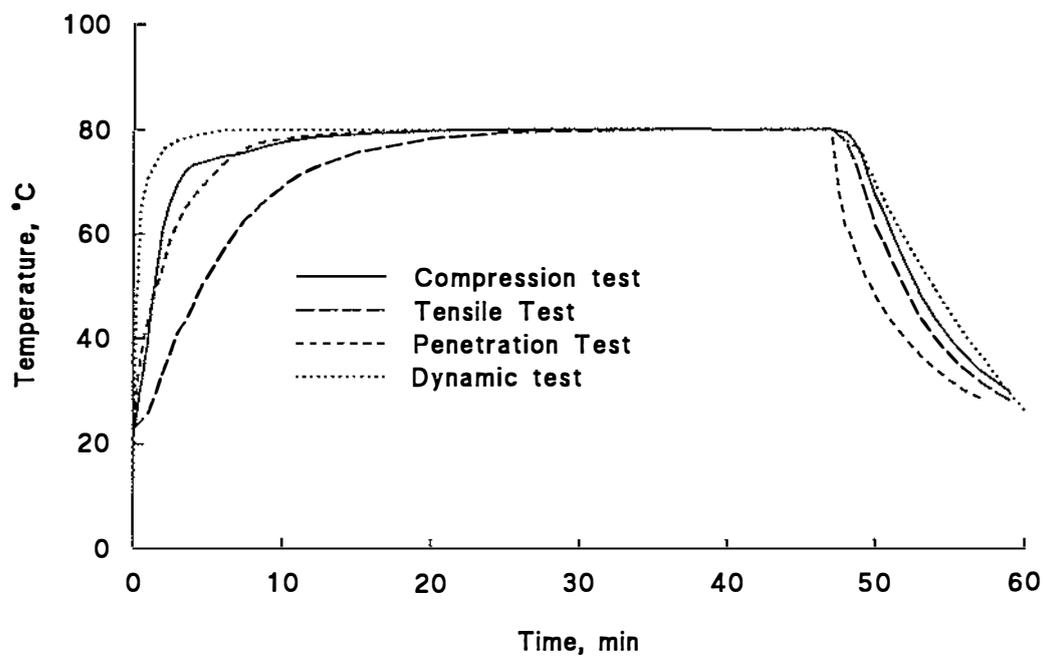


Fig 3.2 Temperature histories during the preparation of WPC gels for small deformation, large deformation and failure tests.

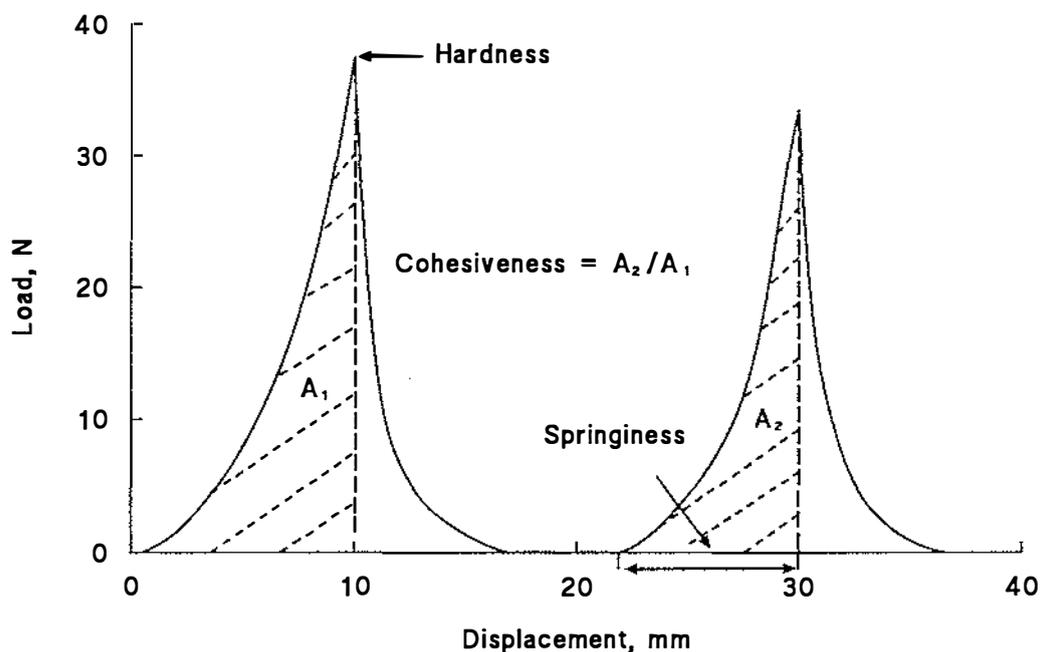


Fig 3.3 Typical first and second bite compression curves (50% compression) for Instron Texture Profile Analysis (TPA) of a WPC(B) gel formed at 80°C and pH7.

True compressive stress and strain at 10% compression during the initial compression cycle were calculated from force-deformation curves. Protein gels are normally regarded as incompressible. Poisson's ratios have been reported of 0.49 for native and modified egg white gels (Montejano *et al.* 1984), 0.50 for gelatin (van Wazer *et al.* 1963) and 0.48 for heat-induced fish muscle gels (Montejano *et al.* 1983). The Poisson's ratio of heat-induced WPC gels at 10% compression has been determined as 0.48 (Lloyd, 1986). The WPC(B) gels in this work were therefore assumed to be incompressible and the sample was assumed to retain a cylindrical shape at 10% compression. True compressive stress was calculated as follows:

$$\pi r_o^2 h_o = \pi r^2 h = \pi r^2 (h_o - \Delta h) \quad (\text{assuming volume incompressibility})$$

$$\tau_c = F_c / \pi r^2 = F_c (h_o - \Delta h) / \pi r_o^2 h_o \quad (3.1)$$

Where r_o is the original radius of the gel cylinder, r is the radius of the gel cylinder at 10% compression, h_o is the original specimen length, h is the specimen length at 10% compression, $\Delta h = h_o - h$, F_c is the force at 10% compression and τ_c is the true stress at 10% compression.

The true strain, γ_c , was calculated as

$$\begin{aligned} d\gamma_c &= -dh/h \\ \gamma_c &= \int_{h_o}^h -dh/h = -\ln(1 - \Delta h/h_o) \end{aligned} \quad (3.2)$$

The ratio of true stress to true strain gives the modulus of elasticity for compression, $E_c = \tau_c / \gamma_c$. Mohsenin and Mittal (1977) reviewed rheological terms and suggested that the term modulus of deformability should be used instead of modulus of elasticity. The term rigidity modulus is also used by some authors. For simplicity E_c will be referred to as the compression modulus.

3.5.2 Tension tests

Ring-shaped WPC(B) gels were made using a mould of 30 mm external diameter, 12 mm internal diameter and 11 mm thick depth with a hard polyethylene plastic body and an

aluminium alloy cover. The internal surface of the mould was sprayed with soy bean oil from a commercial aerosol can to prevent sticking of the samples, and tissue was then used to remove any foam from the wall of the mould. WPC(B) solution was then quickly transferred into the mould and the aluminium alloy cover was placed on top. The mould was then sealed with heat-proof tape to prevent leakage of WPC(B) solution or ingress of water. The filled mould was then heated in a $80\pm 0.5^{\circ}\text{C}$ circulating water bath for 45 min (plus 2 min for temperature equilibration). Subsequent cooling and thermal equilibration at 20°C were then performed as for the compression test samples. The temperature history during gel sample preparation is shown in Fig 3.2. Gel rings were removed from their mould in the temperature controlled room at $20\pm 2^{\circ}\text{C}$.

The ring-shaped gel samples were hung over two dowel pins, diameter 4.7 mm, one attached to the bottom plate and the other to the load cell and crosshead of the Instron Universal Testing Machine. Gel samples were loaded to failure at a cross head speed of 50 mm/min. The force-deformation curve was recorded on a computer. The gel samples tested did not fail at the point of support, which was in agreement with the tensile results of McEvoy *et al.* (1985) for ring-shaped gelatin and agarose gels. Gel failure began on the inside surface of the ring-shaped specimens as expected since stress and strain were slightly greater at this location (Smith, 1969). The failure force and the displacement at failure were measured and calculated from force-displacement curves like those shown in Fig 3.4.

The tensile test using ring samples is ideally suited for testing rubbers, and for this application Smith (1969) recommends a ratio of ring outside diameter to inside diameter of 1.10, *i.e.* a very narrow ring. McEvoy *et al.* (1985) used a diameter ratio of 1.31 for testing gelatin and agarose gels. The diameter ratio used in this work was 2.50. Narrower rings were much more fragile and easily broke before testing. Even using such wide rings no tensile results were obtained for the WPC(B) gels tested at $\text{pH}<4$. All these weak gels broke either on removal from the mould or on loading on to the tensile rig. The thick rings produced unusual Instron tensile curves (Fig 3.4). Much of the early part of the curve represents ring straightening rather than gel stretching. The final part of the curve was relatively straight, and tension rigidity E_t , was taken as the slope of the force-deformation curve (N/m) just prior to failure. The true tensile modulus could not be calculated accurately because of the geometry of the sample and because sample cross-section area was not accurately known.

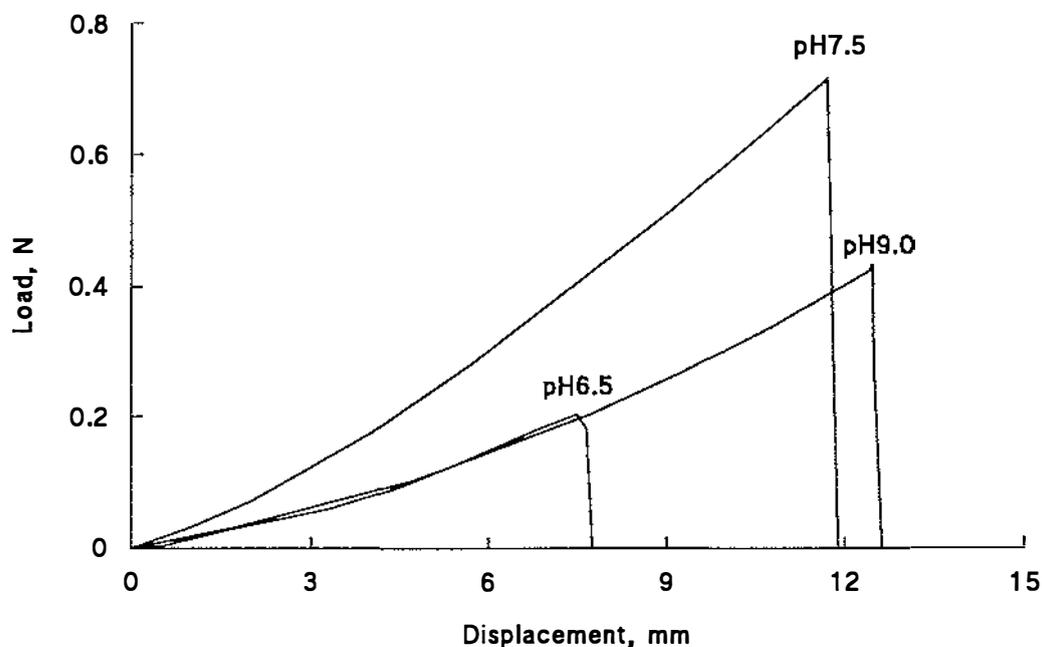


Fig 3.4 Force versus displacement curves in tension for 12% WPC(B) gels.

3.5.3 Penetration tests

WPC(B) solutions (19 ml) were placed into 22 mm diameter x 55 mm long screw top test bottles and heated in a vertical orientation in a $80 \pm 0.5^\circ\text{C}$ circulating water bath for 45 min (plus 2 min for temperature equilibration). Subsequent cooling and thermal equilibration at 20°C were then performed as for the compression test samples. The temperature history during sample preparation is shown in Fig. 3.2. Finally, gel strength was measured by penetrating the WPC(B) gels inside the bottles at 50 mm/min crosshead speed with the circular end of a 6.38 mm diameter cylindrical probe. Gel strength (failure force) was defined as the force either at the first peak on the force-deformation curve (Dunkerley and Hayes, 1980) or at the point where the slope of the curve changed suddenly as shown in Fig 3.5. Penetration rigidity E_p , was estimated as the initial slope of the force-deformation curve. The failure displacement was taken at the failure point (the point where failure force was determined) (Fig 3.5).

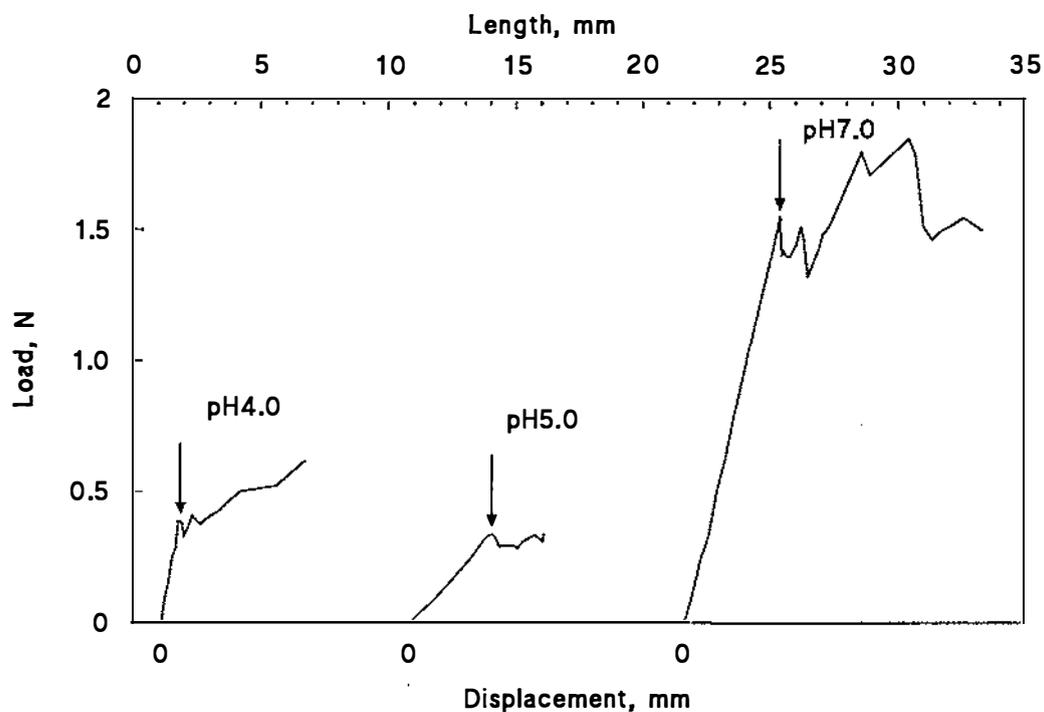


Fig 3.5 Traces of Instron curves showing penetration tests on WPC gels.

3.6 Statistical Planning of Experiments and Analysis of Data

The preparation and rheological testing of WPC solutions and gels were ordered chronologically by randomization and blocking in order to eliminate systematic error. Duplicate measurements of dynamic shear variables showed excellent repeatability. For each large deformation or failure test of WPC(B) gels four to six samples were tested independently at each experimental condition. Mean values and standard deviations were therefore calculated from at least four independent measurements.

3.7 References

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4. Rheology of Whey Protein Concentrate Solutions

4.1 Introduction

The rheological properties of WPC solutions are of practical significance in the manufacture of WPCs; for example, rheological properties of dilute solutions are important in the ultrafiltration process, and rheological properties of concentrated solutions are important in the evaporation and drying stages. The rheological properties of WPC solutions are also very important functional properties of the product and are of practical significance for applications of WPC powders in protein-containing foods.

Rheology of food colloids has been reviewed by Sherman (1970), and Dickinson and Stainsby (1982). The rheological properties of protein solutions are governed by molecular weight, size, shape, flexibility and degree of hydration, and by intermolecular interactions. These in turn are influenced by concentration, temperature, pH, ionic strength and previous processing treatments (Tung, 1978; Kinsella, 1979). Intermolecular interactions between protein molecules may be especially important with respect to rheological properties. A number of workers have reported steady shear rheological data for whey protein systems (McDonough *et al.* 1974; Hermansson, 1975, 1979; Pradipasena and Rha, 1977a, 1977b; Rha and Pradipasena, 1986; Bottomley *et al.* 1990). However, our understanding of the effects of protein concentration, temperature, pH and salts on rheological properties of WPC solutions is still limited. The aim of this work is to explore these areas in more detail.

4.2 Experimental Procedure

A commercially available WPC(B) powder was used in this series of experiments. Its composition and ash components are listed in Tables 3.2 and 3.3. The preparation of WPC(B) solutions of concentration $\leq 35\%$ (total solids), sample handling and steady shear rheological measurements are described in Chapter 3. 40%(TS) solutions were prepared by reconstituting WPC(B) powder with distilled water in a stomacher. Such solutions were stored at 5.5°C for at least one day to ensure that solution structure was in the fully-recovered state. Percentage concentrations in this chapter are all total solids concentrations. The total solids concentrations can be converted to protein concentrations using the data in Table 3.1.

4.3 Results

4.3.1 Effects of WPC(B) concentration

The effects of WPC(B) concentration on apparent viscosity are shown in Fig 4.1. Solutions with concentrations $\leq 8\%$ (TS) obeyed a form of Einstein's viscosity equation; a linear relationship existed between viscosity and concentration. This relationship at 22°C and pH7 was found to be:

$$\eta_s = \eta_w(1 + 15.62C_t) \quad (4.1)$$

where η_s is the coefficient of viscosity of WPC(B) solution (mPas), η_w is the coefficient of viscosity of distilled water (1mPas at 22°C) and C_t is the fractional weight concentration ($C_t \leq 0.08$ at pH7).

The coefficient of determination ($r^2 = 0.98$) for equation (4.1) was highly significant ($P < 0.01$). Properly, Einstein's equation relates solution viscosity to the volume fraction of the dispersed phase; here, weight fraction is assumed to be directly proportional to volume fraction.

The critical concentration, below which viscosity and fractional weight concentration were linearly related, was dependent on pH. At pH11 solutions obeyed Einstein's equation at concentrations up to only 4% (TS), after being stored 21 hours in the refrigerator; the viscosity was age-dependent at this pH.

For solutions with concentrations between 8% (TS) and 30% (TS) the relationship between apparent viscosity and concentration became non-linear (Fig 4.1). Log (apparent viscosity) versus concentration data (Fig 4.1) could be fitted well by a straight line ($r^2 > 0.99$, $P < 0.002$):

$$\log \eta_a = 5.9 C_t - 0.157 \quad (4.2)$$

where η_a is the apparent viscosity (mPas) at 22°C , pH7 and 291 s^{-1} , and $0.08 \leq C_t \leq 0.3$.

All data for solutions of $0\text{-}30\%$ (TS) could be fitted slightly less well ($r^2 > 0.989$, $P < 0.002$) by a similar equation:

$$\log \eta_a = 5.41 C_t - 0.056 \quad (4.3)$$

where $0 \leq C_t \leq 0.3$

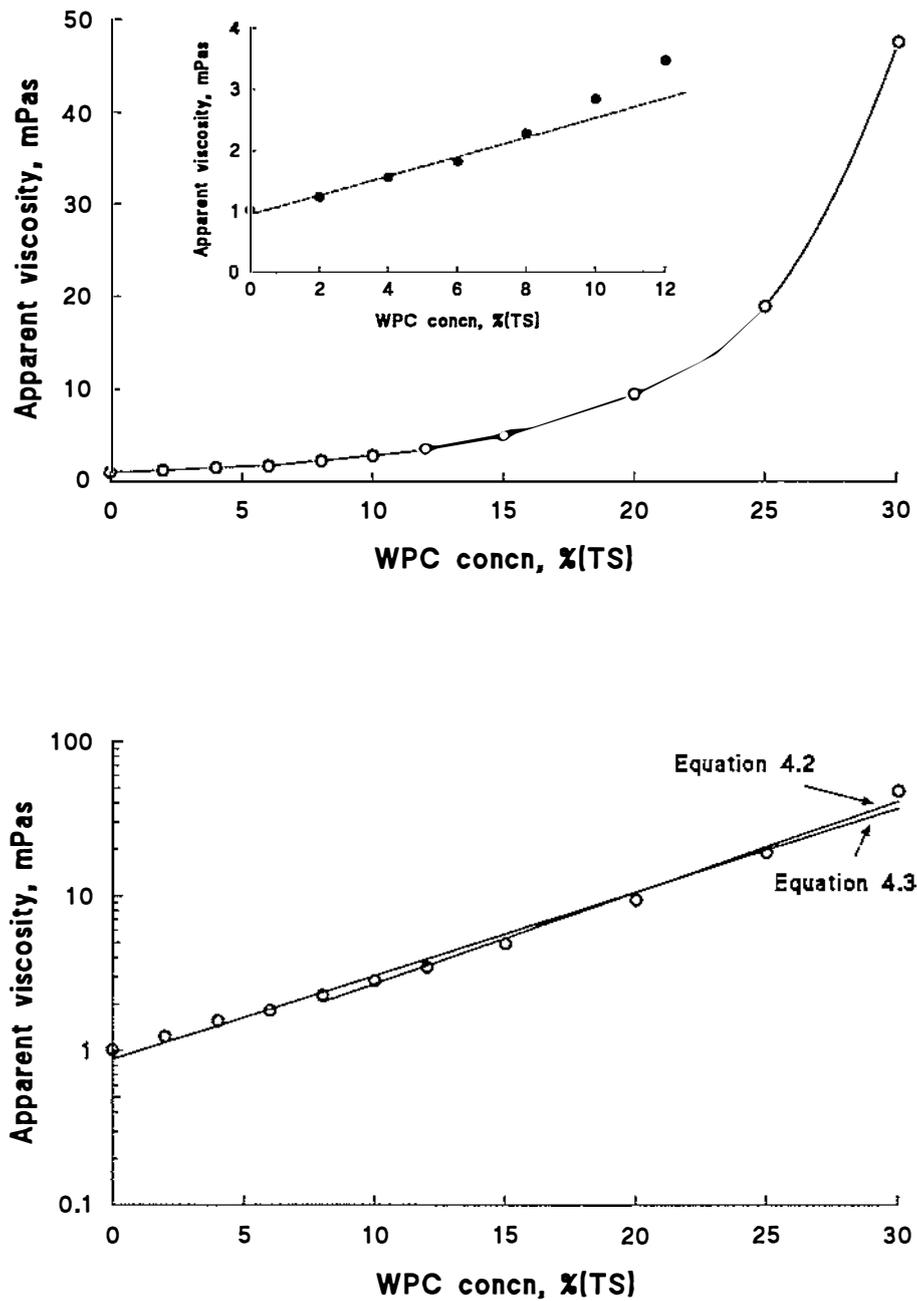


Fig 4.1 Apparent viscosity of WPC(B) solutions as a function of concentration at 22°C, pH7 and a shear rate of 291 s⁻¹.

The apparent viscosity of solutions above 30%(TS) was time-dependent (Fig 4.2), and increased very rapidly with increasing concentration.

4.3.2 Effects of shear rate

The effects of shear rate on the apparent viscosity of WPC(B) solutions are shown in Fig 4.2. At a concentration of 10%(TS) or below solutions were Newtonian: apparent viscosity was independent of shear rate. At concentrations of 15-30%(TS) apparent viscosity decreased slightly with shear rate at low shear rates, indicating slight shear thinning. At concentrations above 30%(TS) shear rate sweeps produced hysteresis loops indicating time-dependent shear-thinning. The effect of shear rate on apparent viscosity became more marked at concentrations above 30%(TS), particularly at low shear rates. Flow curves for 10%(TS), 20%(TS) and 30%(TS) solutions (Fig 4.3) could all be fitted closely by power equations of the form:

$$\tau = k_f \gamma^{n'} \quad (4.4)$$

where τ is shear stress (Pa), γ is shear rate (s^{-1}), k_f is the fluid consistency index ($\text{Pas}^{n'}$) and n' is the flow behaviour index (dimensionless).

Values of k_f and n' are given in Table 4.1. Deviation from Newtonian behaviour above 10% increased slightly (lower n') as concentration increased. k_f , which is a measure of viscosity, increased markedly with concentration. These characteristics are typical of pseudoplastic liquids.

Table 4.1 Flow behaviour index (n') and fluid consistency index (k_f) values for 10-30%(TS) WPC(B) solutions at 22°C and pH7 (Equation 4.4).

concn. (% TS)	n'	k_f ($\text{mPas}^{n'}$)
10	1.00	2.77
20	0.96	12.2
30	0.95	62.1

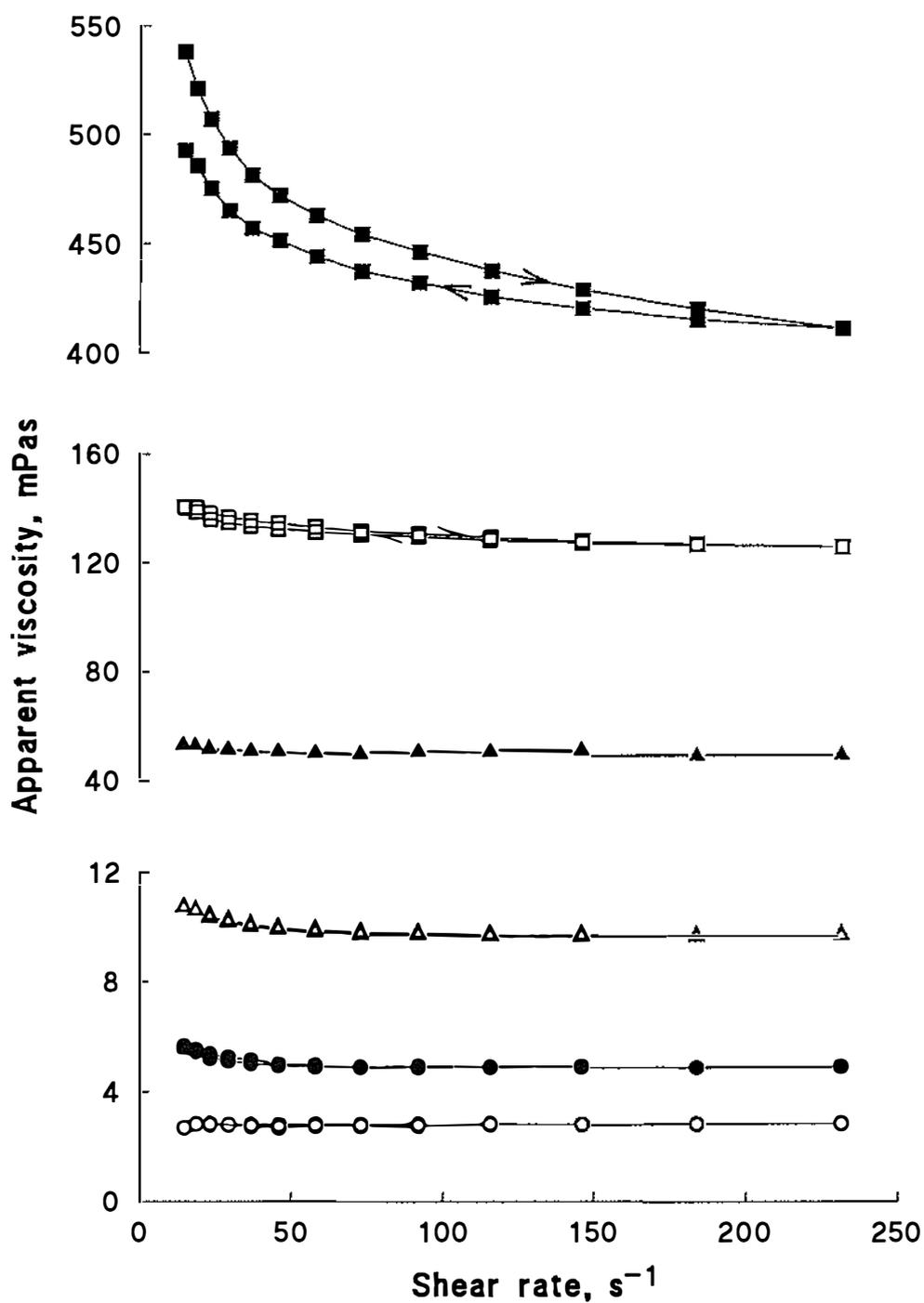


Fig 4.2 Apparent viscosity of WPC(B) solutions as a function of shear rate at 22°C and pH7 determined by shear rate sweeps. Concentrations were: ○, 10; ●, 15; △, 20; ▲, 30; □, 35 and ■, 40%(TS).

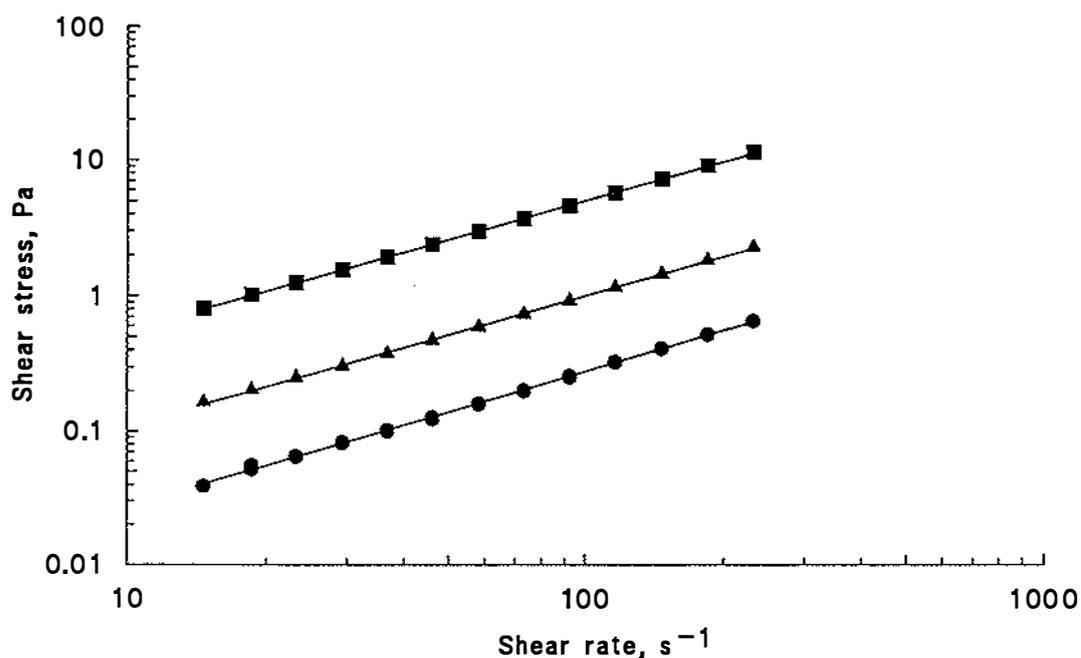


Fig 4.3 Flow curves of WPC (B) solutions at 22°C, pH7 and concentrations (TS) of: ●, 10; ▲, 20 and ■, 30%(TS). Flow curves were determined from the data in Fig 4.2.

4.3.3 Time-dependence of structure breakdown and recovery

Fig 4.4 illustrates the time-dependence of structure breakdown and recovery, as reflected in measured shear stress, in a 40%(TS) WPC(B) solution. During initial shear at 92.5 s^{-1} the shear stress decreased with shearing time and then reached a constant value after a short time. When the shear rate was increased suddenly from 92.5 s^{-1} to 585 s^{-1} , the shear stress increased immediately and then decreased with time and reached a new steady value after a short time. When the shear rate was suddenly decreased from 585 s^{-1} back to 92.5 s^{-1} the shear stress decreased immediately and then increased with time, finally reaching a constant value of 39.7 Pa, slightly less than the shear stress of 40.8 Pa existing at 92.5 s^{-1} after the first 10 minute shearing period. Solution structure initially in the fully-recovered 'at rest' state that had been broken down by shearing (at either low or high shear rates) returned to that state only after a resting period of one to two days.

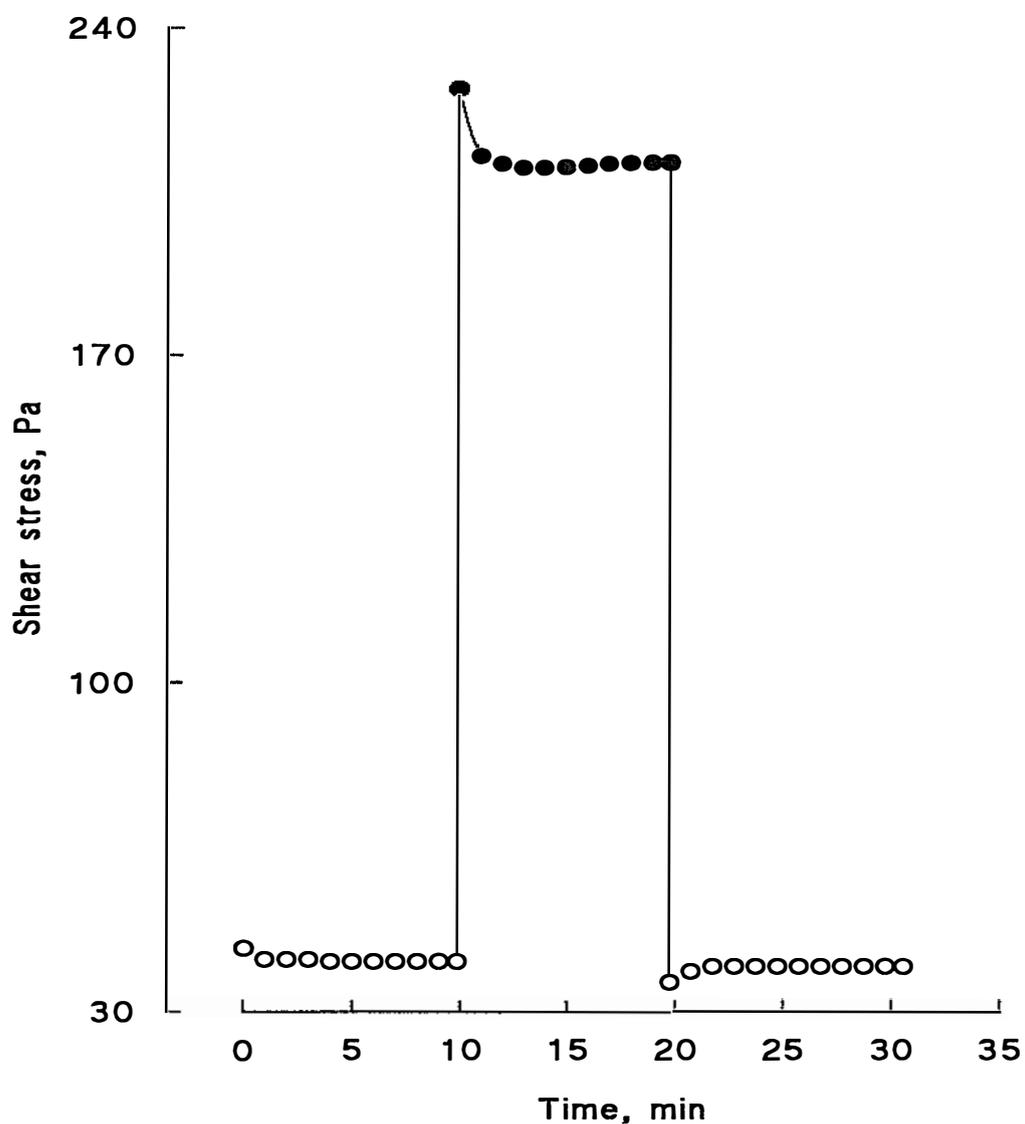


Fig 4.4 Time-dependence of structure breakdown and recovery in a 40%(TS) WPC (B) solution at 22°C and pH6.75. The sample was sheared successively at ○, 92.5 s⁻¹; ●, 585 s⁻¹ and ○, 92.5 s⁻¹.

4.3.4 Determination of yield stress

A shear sweep for a 40%(TS) WPC(B) solution over four decades is shown in Fig 4.5. A yield stress with a value of 0.029 Pa was determined from the stress axis by extrapolation of the curve - which became parallel to the shear rate axis at low shear rate. At

concentrations of 30%(TS) and below any yield stress present was too small to be measurable.

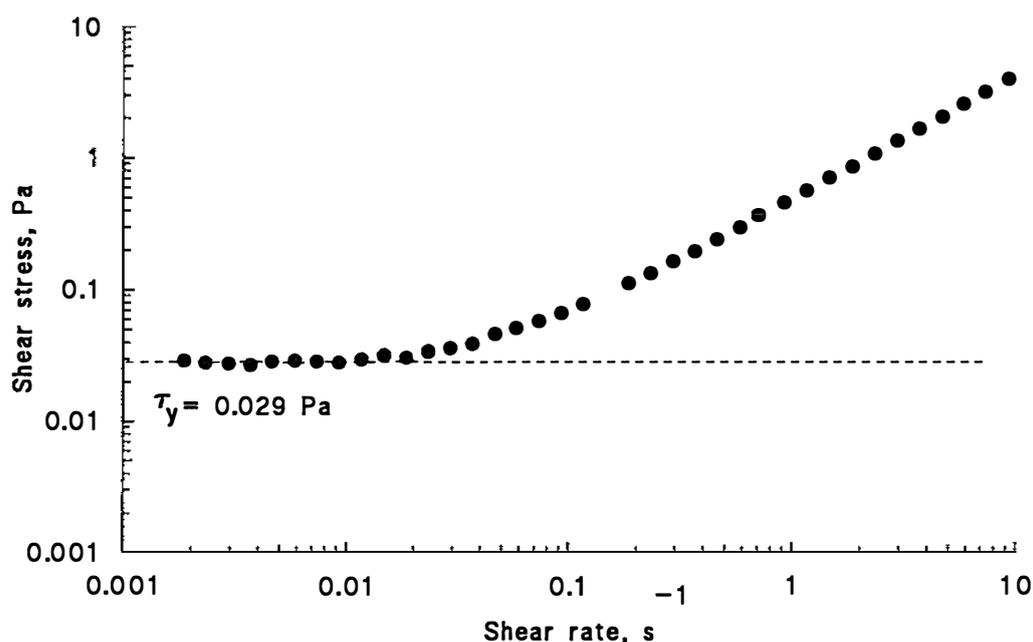


Fig 4.5 Determination of the yield stress ($\tau_y = 0.029 \text{ Pa}$) of a 40%(TS) WPC(B) solution at 22°C and pH6.75. The shear sweep was conducted from high to low shear rate.

4.3.5 Effects of temperature

Fig 4.6 shows temperature sweep results for 15-30%(TS) WPC(B) solutions. Viscosity was strongly temperature-dependent. At first, it decreased as the temperature increased and then increased rapidly with further increase in temperature.

Fig 4.7 shows apparent viscosity of 10-25%(TS) WPC(B) solutions at 22°C versus holding temperature for a holding time of 10 minutes. At and below 60°C the apparent viscosity was independent of holding temperature. Above 60°C the apparent viscosity increased with holding temperature, and this increase was more marked at higher concentrations.

Fig 4.8 shows that holding a 15%(TS) solution at 70°C for 10 minutes can change its

rheological properties at 22°C from time-independent to time-dependent shear thinning.

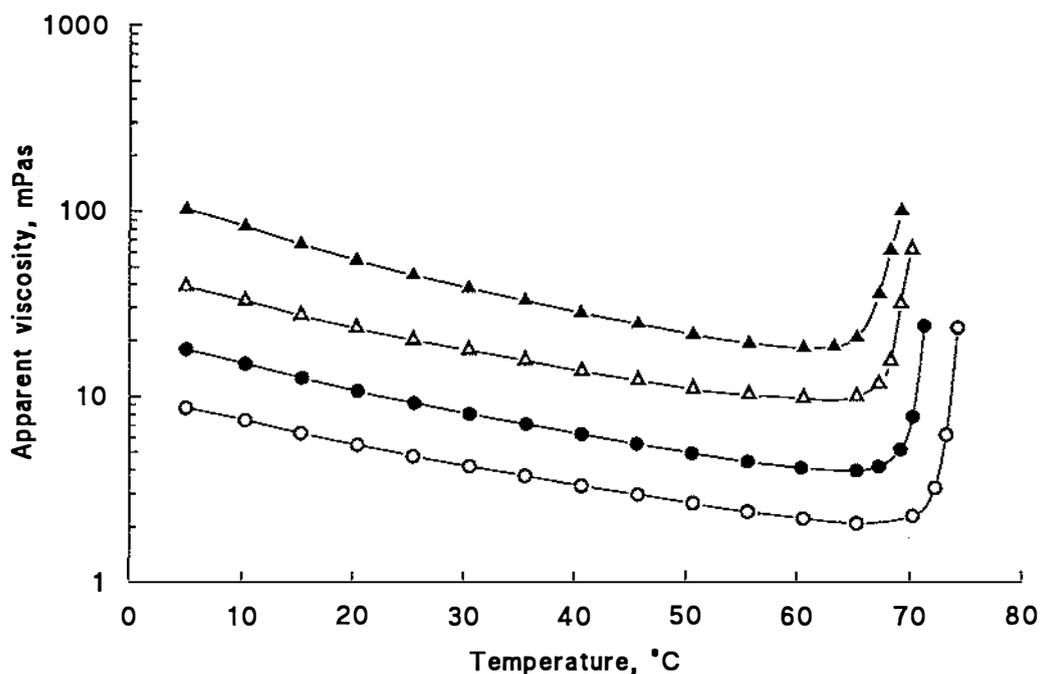


Fig 4.6 Apparent viscosity of WPC(B) solutions as a function of temperature during temperature sweep tests at a shear rate of 291 s^{-1} , pH7 and concentrations (TS) of: \circ , 15; \bullet , 20; Δ , 25 and \blacktriangle , 30%(TS).

4.3.6 Determination of activation energy of flow

Temperature-dependence data in the temperature range 5-60°C could be well fitted by straight lines (see r^2 values in Table 2) by plotting $\log(\eta_a)$ versus $1/T$ (Fig 4.9). Temperature-dependence of apparent viscosity in this temperature range for concentrations up to 30%(TS) thus follows the Arrhenius equation:

$$\eta_a = B e^{E'/RT} \quad (4.5)$$

where η_a is the apparent viscosity (at a specified shear rate) (mPas), E' is the activation energy of flow (J/mol), T is temperature (K), R is the universal gas constant (8.314 J/K mol) and B is a constant (mPas).

Values of E' and B are given in Table 4.2. $\log(E')$ was found to be linearly related ($r^2 > 0.915$, $P < 0.002$) to WPC(B) fractional weight concentration (C_t) up to 30%(TS) (Fig 4.10). The relationship was:

$$\log E' = 0.78C_t + 1.18 \quad (4.6)$$

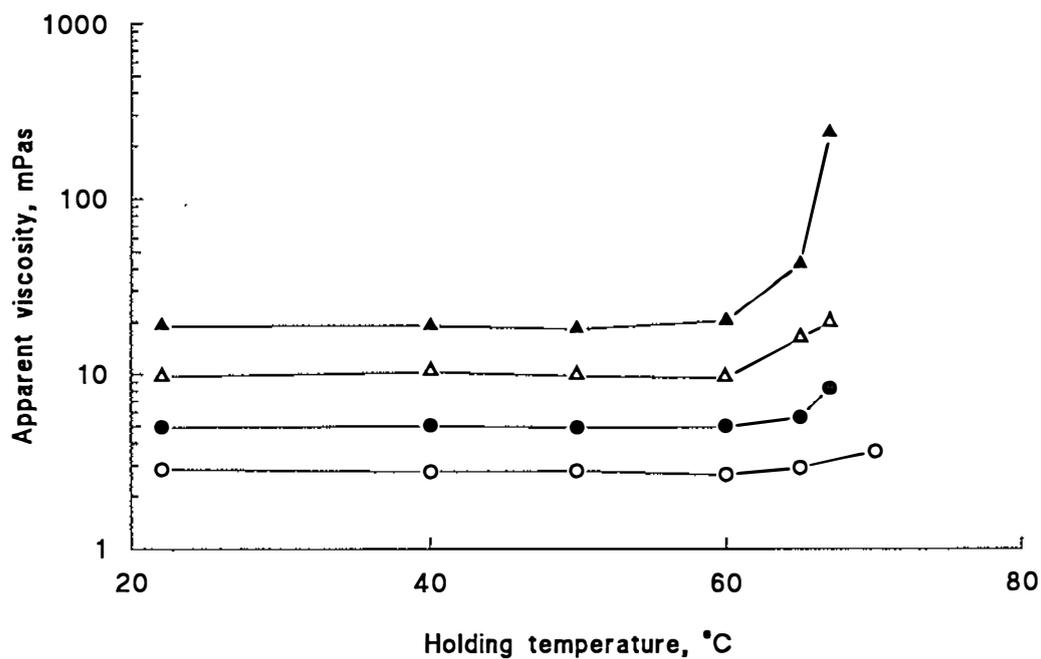


Fig 4.7 Apparent viscosity of WPC(B) solutions at 22°C, pH7, a shear rate of 146 s^{-1} and concentrations of ○, 10; ●, 15; △, 20 and ▲, 25%(TS) after holding for 10 minutes at various holding temperatures. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.

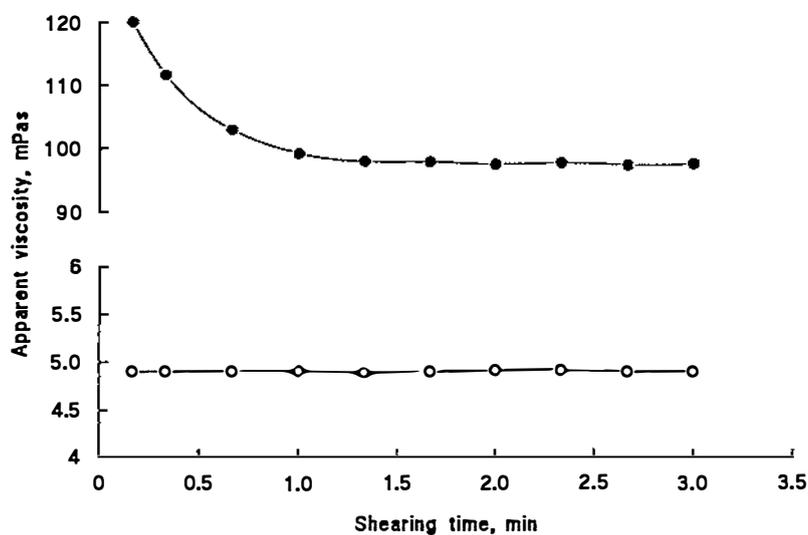


Fig 4.8 Apparent viscosity of 15%(TS) WPC(B) solutions versus shearing time at 22°C, pH7 and a shear rate of 146 s⁻¹. The solutions were held at ○, 22°C and ●, 70°C for 10 minutes before shearing.

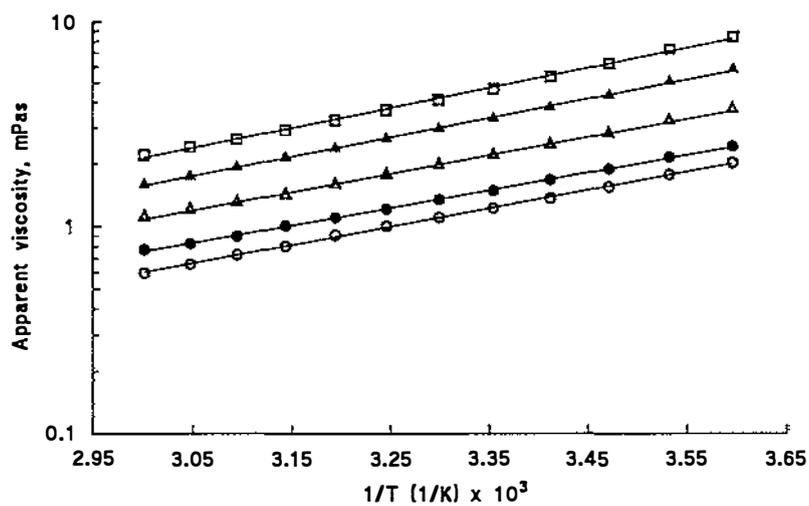


Fig 4.9 Apparent viscosity of WPC(B) solutions as a function of 1/T (T = absolute temperature) at a shear rate of 291 s⁻¹, pH7 and concentrations of ○, 2; ●, 4; △, 8; ▲, 12 and □, 15%(TS).

Table 4.2 Values of activation energy of flow (E') and of the constant B in equation 4.5 for WPC(B) solutions at pH7 and a shear rate of 291 s^{-1} .

Concn. (% TS)	E' (kJ/mol)	B (μPas)	r^2
2	16.86	1.38	0.999
4	16.19	2.20	0.999
6	16.60	2.30	1.000
8	16.97	2.37	0.999
10	17.18	2.69	0.998
12	18.11	2.28	0.999
15	18.83	2.35	0.998
20	20.13	2.71	0.996
25	25.63	0.69	0.998
30	25.96	1.36	0.997

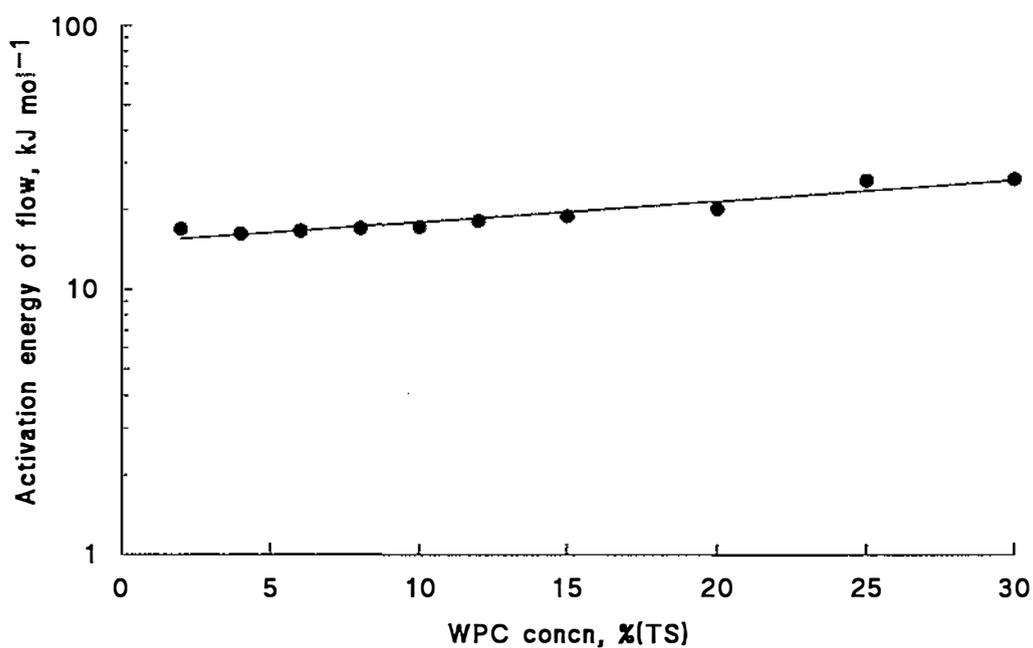


Fig 4.10 Activation energy of flow of WPC(B) solutions as a function of concentration at 22°C , pH7 and a shear rate of 291 s^{-1} .

4.3.7 Effects of pH

Figs 4.11 and 4.12 show apparent viscosity versus pH for, respectively, 10% and 20% WPC(B) solutions at 22°C. The apparent viscosity of 10%(TS) WPC(B) solutions in the pH range 2-8 was only slightly dependent on solution age up to 168.5 h after preparation. At pHs outside this range apparent viscosity increased with solution age, and this increase became more marked as pH increased above 8 or decreased below 2. The apparent viscosity of 20% solutions was independent of solution age in the pH range 4-9 up to 4.5 h after preparation. At higher and, particularly, at lower pHs apparent viscosity increased with age.

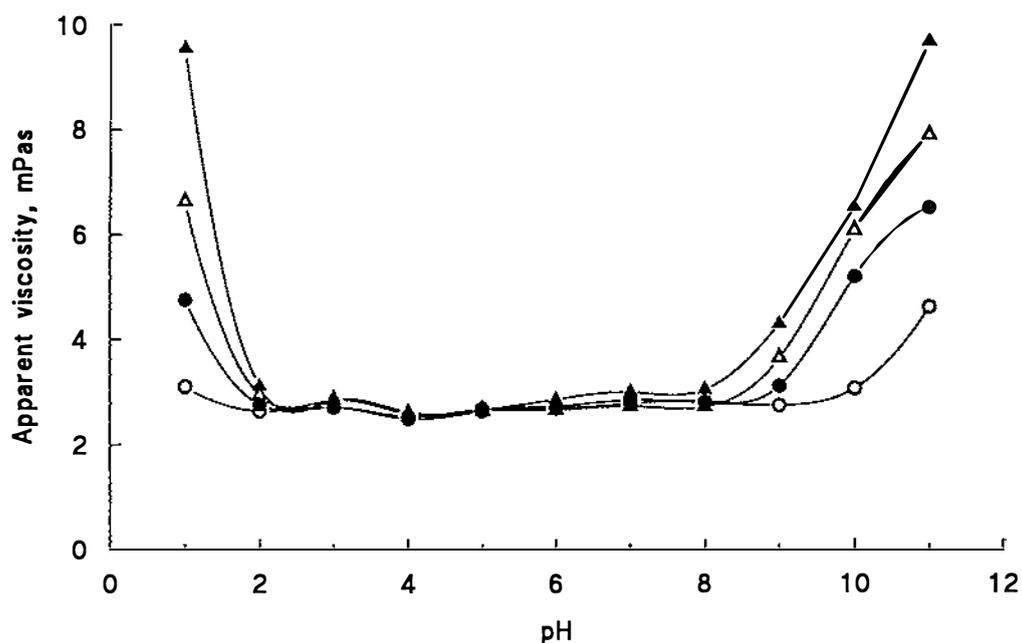


Fig 4.11 Effect of pH on apparent viscosity of 10%(TS) WPC(B) solutions at 22°C and a shear rate of 146 s^{-1} . Apparent viscosity was measured ○, 2 h; ●, 48 h; △, 98 h and ▲, 168.5 h after preparation. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.

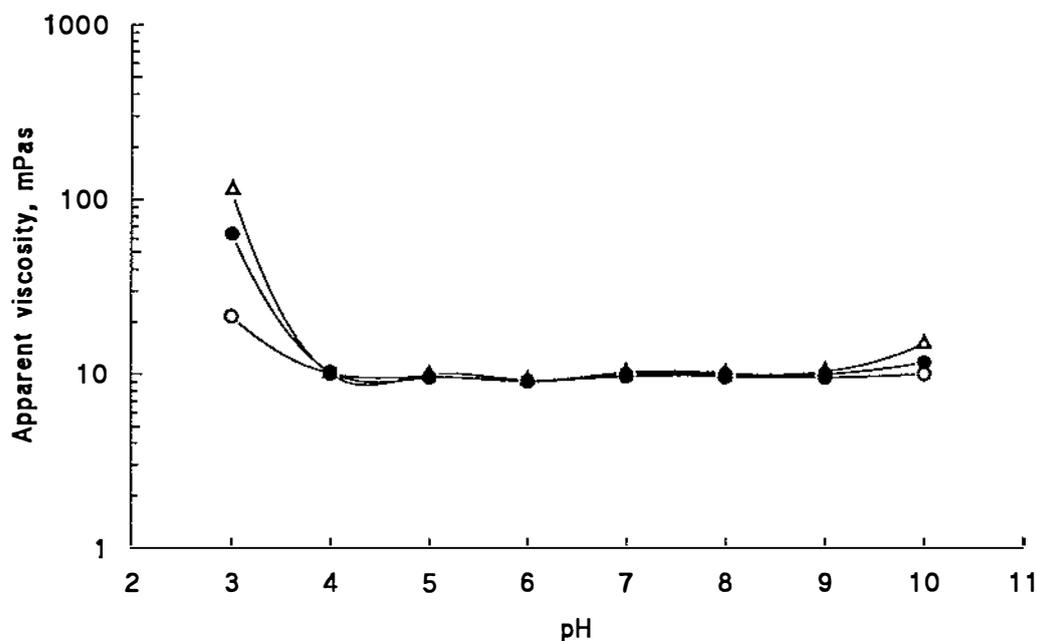


Fig 4.12 Effect of pH on apparent viscosity of 20%(TS) WPC(B) solutions at 22°C and a shear rate of 146 s^{-1} . Apparent viscosity was measured ○, 0.5 h; ●, 2.5 h and △, 4.5 h after preparation. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded at 10, 80 and 180 s.

Extreme pH values not only caused an age-dependent increase in apparent viscosity but could result also in a change in flow properties; for example, a 20%(TS) solution exhibited time-independent shear-thinning at pH7 but time-dependent shear-thinning at pH3 (Fig 4.13), while a 20% solution prepared at pH9 exhibited this same change in flow properties on being stored at 5.5°C for 24 h.

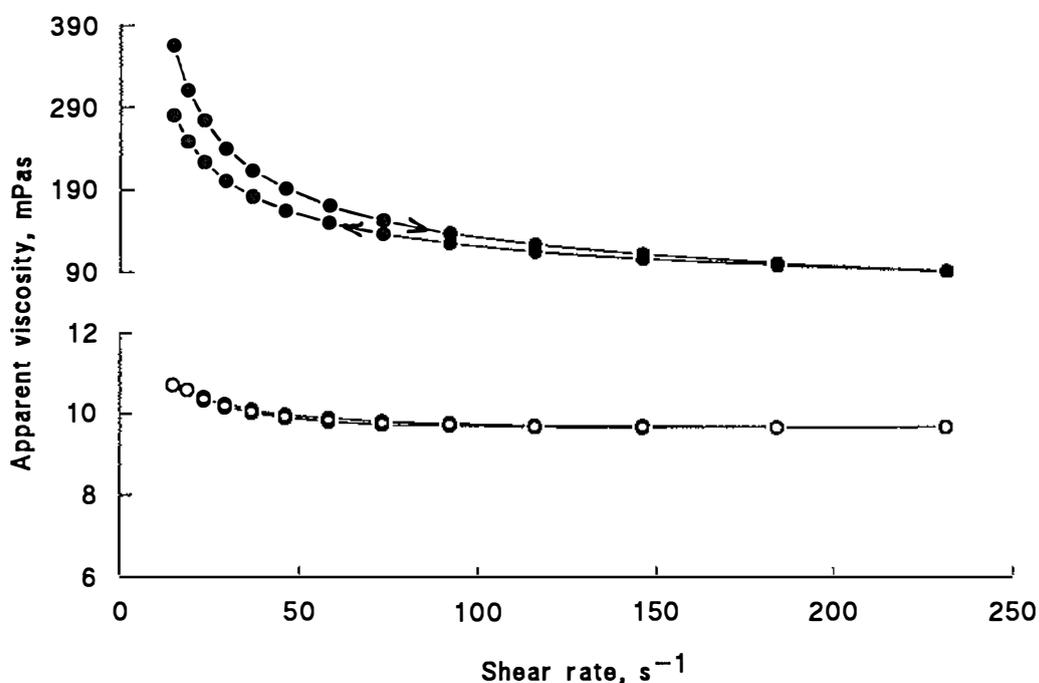


Fig 4.13 Apparent viscosity of 20%(TS) WPC(B) solutions as a function of shear rate at 22°C and at pHs of ○, 7 and ●, 3. Measurement was carried out 4.5 h after preparation of the solutions.

4.3.8 Effects of salt type and concentration

The apparent viscosity decreased slightly at first and then increased slightly as sodium chloride concentration changed from 0 to 0.8M (Fig 4.14). This phenomenon was more marked at 20%(TS) WPC(B) than at 10%(TS). The apparent viscosity of both 10%(TS) and 20%(TS) solutions was essentially independent of solution age at all salt concentrations.

At calcium chloride concentrations of 0-0.5 M apparent viscosity of 20%(TS) WPC(B) solutions increased slightly with solution age up to 11 days after preparation (Fig 4.15). At a given age viscosity first decreased very slightly and then increased as the calcium chloride concentration increased from 0 to 0.5 M. Above 0.5M apparent viscosity increased markedly with both calcium chloride concentration and solution age, the effect of age being greater at higher calcium chloride concentration. At 0.8M calcium chloride, 11 day old solutions

exhibited slight time-dependent shear-thinning. 10%(TS) WPC(B) solutions were found to follow similar trends but with smaller effects.

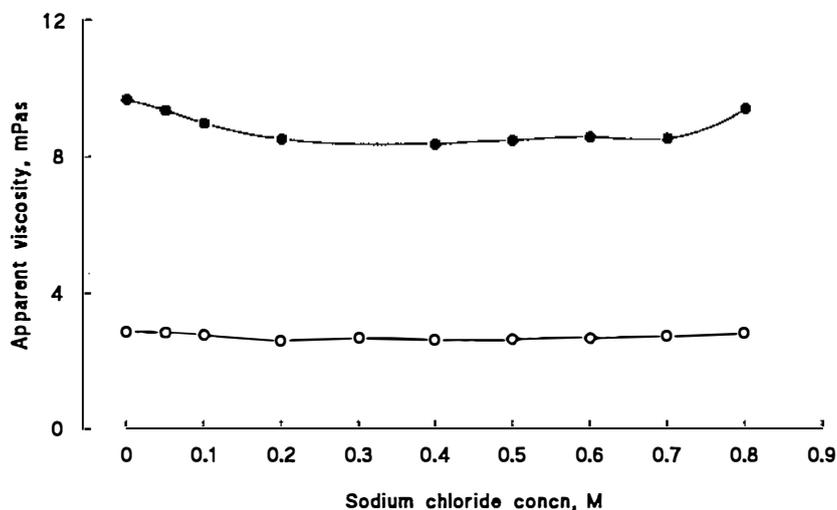


Fig 4.14 Effects of sodium chloride concentration on apparent viscosity of \circ , 10% and \bullet , 20%(TS) WPC(B) solutions at pH7, 22°C and a shear rate of 146 s⁻¹.

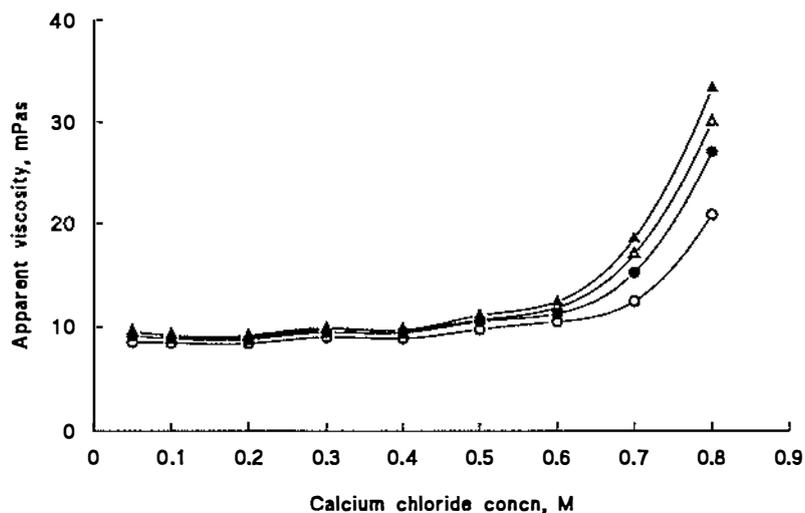


Fig 4.15 Effects of calcium chloride concentration on the apparent viscosity of 20%(TS) WPC(B) solutions at pH7, 22°C and a shear rate of 146 s⁻¹. Apparent viscosity was measured \circ , 1 day; \bullet , 5 days; Δ , 8 days and \blacktriangle , 11 days after preparation of the solutions. Shearing time was 180 s; each plotted viscosity value is the arithmetic mean of the values measured and recorded after 10, 80 and 180 s.

4.4 Discussion

The results show that the flow properties of WPC solutions depend on concentration and temperature, and may depend also on shear rate, shearing time, pH, salt type, salt concentration and solution age. Changes in such variables can affect protein molecular size, shape, flexibility and degree of hydration, and the extent of intermolecular interactions. Changes in intermolecular interactions may result in the formation of aggregates or of structure in solutions.

The effects of concentration on the rheological properties of WPC solutions (Figs 4.1-4.3) may be related to the three distinct regions of concentration identified by Dickinson and Stainsby (1982) for macromolecular solutions and by Rha and Pradipasena (1986) for globular protein solutions: 'dilute', 'semi-dilute' and 'concentrated'. Solutions with concentrations up to and including 8%(TS) were evidently 'dilute' because viscosity was linearly related to concentration by Einstein's equation, indicating that hydrodynamic interactions between protein molecules were absent. Solutions between 8%(TS) and 30%(TS) (above which time-dependent flow behaviour appears) may be considered 'semi-dilute'; the 'interactive volumes' of the dispersed protein molecules (these volumes including the effects of both hydrodynamic and molecular interactions (Rha and Pradipasena, 1986)) would have overlapped, causing a departure from linearity. The shear-thinning behaviour exhibited at concentrations above 10%(TS) is usually attributed to two phenomena for protein solutions: (a) progressive orientation of protein molecules in the direction of flow with deformation or removal of the protein hydration sphere, and (b) rupture of weak bonds such as hydrogen and ionic bonds resulting in dissociation of protein aggregates or networks (Tung, 1978; Cheftel *et al.* 1985). The second phenomenon was probably dominant for these WPC(B) solutions since shear thinning was more marked at high protein concentrations and was not observed below 10%(TS).

The power law equation (equation 4.4) was found to describe very well the shear-thinning behaviour of semi-dilute solutions (Fig 4.3). In contrast, Bottomley *et al.* (1990) reported that the Bingham equation fitted well the flow curves of 20%(TS) and 30%(TS) WPC solutions possessing small yield stresses. However, when yield stresses are small and the degree of shear-thinning slight the Bingham and power law equations are in fact very similar, and will fit flow curves about equally well.

8%(TS) may be considered a 'critical' concentration in that it represents the boundary

between 'dilute' and 'semi-dilute' behaviour - at pH7 and 22°C. Any factors causing changes in protein molecule size or shape or flexibility or hydration, or in intermolecular interactions, can shift the critical concentration to a new value; the lower critical concentration of 4%(TS) found at pH11 and 22°C was probably a reflection of protein denaturation at this high pH resulting in changes in molecular size and shape which in turn would have resulted in increased hydrodynamic interaction between molecules.

Solutions above about 30%(TS) may be considered 'concentrated'; concentrations are high enough to result in some structure formation, resulting in time-dependent flow behaviour (Figs 4.2 and 4.4) and, at 40%(TS), the presence of a measurable yield stress (Fig 4.5). Structure formation is possible at high concentrations because of the relatively high number of possible weak protein-protein bonds or linkages such as hydrogen and ionic bonds. For the major whey protein, β -lactoglobulin, Pradipasena and Rha (1977a) reported that the dilute region extended to 10% protein and that the concentrated region began at 30% protein.

The time-dependence of structure breakdown and recovery in a 40%(TS) solution at 92.5 s⁻¹ (Fig 4.4) might be attributed to two processes. Firstly, the protein-protein linkages existing at the high concentration would be broken down during shear at 92.5 s⁻¹. The rate of structure breakdown would be dependent on the number of structural linkages present and this would decrease with time. Secondly, protein-protein linkages might be rebuilt again by Brownian motion and molecular collisions, and this simultaneous rebuilding of linkages and structure would increase with time because the number of possible new structural linkages would increase. Finally, a dynamic equilibrium would be established at 92.5 s⁻¹ when rate of structural build-up equalled rate of breakdown, resulting in a steady shear stress (Fig 4.4). When the shear rate was increased quickly from 92.5 s⁻¹ to 585 s⁻¹, the shear stress increased immediately and then decreased with time to a new steady value (Fig 4.4). Presumably the new higher shear rate of 585 s⁻¹ disrupted the dynamic equilibrium set up at 92.5 s⁻¹ because it caused a greater rate of structure breakdown, and a new equilibrium then became established by the mechanism described above. When the shear rate was suddenly decreased from 585 s⁻¹ back to 92.5 s⁻¹ the shear stress decreased immediately and then increased with time to a constant value (Fig 4.4). This may indicate recovery of structure by Brownian motion at 92.5 s⁻¹ from the equilibrium structure formed at 585 s⁻¹. This kind of step-shear rate experiment, which was used here to demonstrate thixotropic behaviour qualitatively, can form part of the experimental procedure described by Cheng (1987) for characterising

quantitatively and fundamentally the rheological behaviour of thixotropic materials.

The changes of viscosity with temperature in temperature sweeps (Fig 4.6) may be the net result of three basic mechanisms. Firstly, viscosity decreases as intermolecular distances increase with increase in temperature (Holdsworth, 1971; Bakshi and Smith, 1984). Secondly, temperature-induced changes in protein structure and protein interactions occur as temperature increases, particularly at higher temperatures. Below 60°C, increase in temperature may lead to reversible physico-chemical changes such as partial unfolding of protein molecules and changes in protein hydration. Above 60°C changes in protein structure are mainly irreversible (DeWit and Klarenbeek, 1984). The extent of protein-protein hydrophobic interactions increases strongly with increasing temperature. Buried thiol (-SH) groups and disulphide (-SS-) bonds inside the native protein molecules become accessible due to unfolding of the molecules. Above 70°C the appearance of free -SH groups in whey protein increases rapidly (Lyster, 1964), and so does the consequent formation of intermolecular disulphide bonds. All these changes with increase in temperature would result in an increase in solution viscosity. Thirdly, protein aggregates formed by intermolecular interactions may be disrupted by shearing. Obviously, the first and third mechanisms, particularly the first, dominate at lower temperatures: the viscosity decreases initially with increasing temperature (Fig 4.6). As temperature increases to above 60°C the second mechanism eventually comes into balance with the first and third to give a viscosity minimum. The temperature at which this minimum occurs decreases with increasing concentration (Fig 4.6). At temperatures above this minimum the second mechanism clearly dominates; viscosity increases rapidly with increasing temperature.

Changes such as partial unfolding of protein molecules and subsequent aggregate formation caused by holding WPC(B) solutions for 10 minute periods at temperatures below 60°C are probably either reversible or negligible; apparent viscosity measured at 22°C was independent of holding temperature below 60°C (Fig 4.7). Above 60°C apparent viscosity increased with holding temperature (Fig 4.7) indicating that irreversible changes took place above 60°C. These results are consistent with those obtained by DeWit and Klarenbeek (1984) using differential scanning calorimetry.

Irreversible changes caused by high temperature can lead to a change in flow behaviour (Fig 4.8). The changes caused by holding at 70°C for 10 minutes evidently led to some structure formation in the 15%(TS) solution; flow behaviour changed from time-

independent to time-dependent shear thinning.

The Arrhenius relationship (equation 4.5) clearly describes closely the temperature-dependence of the apparent viscosity of WPC(B) solutions below 60°C (Fig 4.9). The activation energy of flow increases slightly with increasing concentration (Fig 4.10, Table 4.2 and equation 4.6) and thus with viscosity. Van Wazer *et al.* (1963) point out that the activation energy of flow of Newtonian liquids depends on the coefficient of viscosity, being greater for higher viscosities. Equation 4.6 predicts a value for the activation energy of flow of water ($C_t = 0$) of 15.4 kJ/mol (3.62 kcal/mol), which is in effect an average value for the temperature range 5-60°C. Van Wazer *et al.* (1963) tabulate values for water of 4.05 kcal/mol at 20°C and 3.30 kcal/mol at 60°C; the value predicted by equation 4.6 is thus a very reasonable one, and allows confidence to be placed in the equation.

Viscosity was independent of age in the pH range 4-8 at all WPC concentrations up to and including 20%(TS). This pH range narrowed as concentration increased (Figs 4.11 and 4.12). The age-dependent viscosity increases that occurred at low or high pHs could have been caused by denaturation of whey proteins. Electrostatic repulsion between NH_3^+ groups at very low pH and between COO^- groups at very high pH could encourage unfolding of the protein molecule (Cheftel *et al.* 1985). The conformation of the protein molecules would tend to change from globular to random coil, and intermolecular interactions such as hydrophobic interactions and/or disulphide bond formation would increase, causing a viscosity increase (Figs 4.11 and 4.12). Intermolecular interactions evidently can lead to structure formation as indicated by the appearance of time-dependent flow behaviour at very low pH (Fig 4.13).

The quantitative data presented here, visual and tactile examination of solutions, and perusal of some of the relevant literature (for example Lyster, 1964; Dunnill and Green, 1966; Hillier *et al.* 1980; Damodaran and Kinsella, 1982; Pearce, 1983; Cheftel *et al.* 1985; Morr, 1985; Mangino *et al.* 1987; Mulvihill and Kinsella, 1987; Langley and Green, 1989; Kinsella and Whitehead, 1989; Xiong and Kinsella, 1990) suggest that different types of linkage lead to the formation of time-dependent or thixotropic structure in WPC(B) solutions under different physicochemical conditions. At high WPC(B) concentration (35%TS and 40%TS), pH7 and room temperature structure is probably the result of the formation of relatively weak bonds such as hydrogen bonds and ionic bonds, and of relatively few hydrophobic interactions. In contrast, structure formation at temperatures above 65°C and pH > 6.8, and at room temperature and very high pH, is probably due to the formation of disulphide bonds,

and numerous hydrophobic interactions. At room temperature and very low pH, hydrophobic interactions and ionic bonds but not disulphide bonds might be expected to be involved in structure formation. At high calcium chloride concentration (room temperature, pH7 and 11 days) structure formation could be due to calcium bridging, and hydrophobic interactions.

Salts have two important effects on protein solubility: "salting in" and "salting out" (Cheftel *et al.* 1985; Damodaran and Kinsella, 1982). These are considered to be the net results of electrostatic and hydrophobic interactions (Damodaran and Kinsella, 1982). At low salt concentration the ions react with the charges on protein molecules, and the solvent associated with these ions serves to increase the solvation of the proteins (the "salting in" effect) (Cheftel *et al.* 1985); protein-water interactions may increase, but protein-protein interactions may decrease and so does the apparent viscosity (Figs 4.14 and 4.15). At high salt concentrations, there are not enough water molecules available for good protein solvation since salt ions bind water strongly; protein-protein interactions, particularly protein-protein hydrophobic interactions, become more powerful than protein-water interactions. This might lead to aggregation of protein molecules (the "salting out" effect) (Cheftel *et al.* 1985; Damodaran and Kinsella, 1982) and an increase in the apparent viscosity of solutions (Figs 4.14 and 4.15). Changes in apparent viscosity caused by the "salting in" and "salting out" effects of sodium chloride in the concentration range 0-0.8M are small (Fig 4.14), a result which is similar to that found by Hermansson (1975). However, the "salting out" effect of calcium chloride appears to have a much bigger effect on apparent viscosity, particularly at high WPC concentration, and this effect was age-dependent (Fig 4.15).

4.5 References

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5. Oscillatory Rheological Exploration of WPC Gelation

5.1 Introduction

The heat-induced irreversible gelation of whey protein concentrates (WPCs) in aqueous solution is a very important functional property of these products: many applications of WPCs in food (for example, meat products) depend on such gelation because it makes an essential contribution to the texture and other characteristics of the final food products.

Gelling in a WPC solution is the result of the heat denaturation and aggregation of the globular whey proteins. Such proteins exhibit a wide variety of gel types depending on such factors as protein concentration, gelation temperature, pH and ionic strength. All globular protein gels, however, can be considered to fall somewhere between two extremes: a continuous, fine, linear, stranded gel network which is usually transparent (Doi *et al.* 1987; Hermansson, 1988; Doi and Kitabatake, 1989; Kitabatake *et al.* 1989; Stading and Hermansson, 1990) and a turbid precipitate made up of discontinuous coarse particles (Robinson *et al.* 1976; Harwalkar and Kalab, 1985). The macroscopic properties of globular protein gels are governed by the microscopic and submicroscopic gel structure.

It is the macroscopic rheological properties of formed WPC gels that are important commercially; in particular, the mechanical failure properties of gels when they are subjected to large deformation are of significance because they can be correlated with gel texture (Ziegler and Foegeding, 1990). The ability to control and manipulate these properties, and the ability to change gelling behaviour in desirable ways, depend on a good understanding of the gel formation mechanism. Whey protein gelation thus has been, and is, the subject of intensive study; however, it is still not fully understood.

Clark (1991) has pointed out that the formation of a gel network in a biopolymer solution results in the transformation of a liquid-like sol (the solution) to a solid-like gel, and that, consequently, non-destructive oscillatory rheometry is an ideal way of monitoring the gelation process in real time. Measurements of dynamic viscoelastic properties made using this technique enable indirect study of the effects of physicochemical factors on the gel formation mechanism; the effects of these factors on viscoelastic properties can be used to infer their physicochemical effects at the molecular level, and thus lead to a greater understanding of the gelation mechanism.

Mulvihill and Kinsella (1987) suggested that because a WPC solution is a complex,

multicomponent system an understanding of WPC gelation might best be reached by studying separately the gelation of solutions of individual whey proteins. Paulsson *et al.* (1986, 1990) and Stading and Hermansson (1990) have done just this by using oscillatory rheometry to study the gelation of β -lactoglobulin, the principal whey protein. However, WPCs are important commercial food ingredients, and there is a practical need in the shorter term for a direct study of their gelation behaviour. Beveridge *et al.* (1984) and Steventon *et al.* (1991) used oscillatory rheometry to study the gelation of US and Irish commercial WPC preparations respectively.

The gelation of globular proteins exhibits three temporal phases: a lag phase (the gelation time), a phase of rapid structure development and a final phase during which the rate of structure development levels off (Clark, 1991). The gelation time or gel point is the heating time that elapses before the sol-gel transition results in the formation of an infinite gel network (Clark, 1991). Definitions of the gel point in terms of dynamic viscoelastic properties, and its measurement using oscillatory rheometry, have been reviewed and discussed by Stading and Hermansson (1990) and by Steventon *et al.* (1991). The gel point can be defined as the time at which the phase angle suddenly decreases from a value close to 90° (Bohlin *et al.* 1984), or the point at which the storage modulus G' first becomes detectable relative to instrument "noise" (Richardson and Ross-Murphy, 1981; Stading and Hermansson, 1990; Moritaka *et al.* 1991), or the point at which the storage modulus (G') and loss modulus (G'') versus time curves cross over (when $G'=G''$ and thus $\tan^{-1}(G''/G') = \text{phase angle} = 45^\circ$) (Tung and Dynes, 1982; Clark, 1991). Other possible definitions are the point obtained by extrapolating the steeply rising part of the G' versus time curve back to the time axis (Clark, 1991; Steventon *et al.* 1991) or the point at which G'' passes through a maximum (Stading and Hermansson, 1990). The study of gelation time can lead to a better understanding of the minimum conditions required for gelation to occur, and the kinetics of incipient gelation.

The study of gel structure formation after the gel point provides valuable information on the kinetics and extent of cross-linking inside the infinite gel network; the properties of the formed gel depend on this structure development.

The aims of this study were to use oscillatory rheometry to study the effects of protein concentration and temperature on gelation time and on gel structure formation for a commercial New Zealand WPC. The gel point was taken as the time at which the phase

angle had dropped to 45°.

5.2 Experimental Procedure

Two commercially available WPC powders (WPC A and WPC B) were used in this study. Their compositions and ash components are listed in Tables 3.2 and 3.3 respectively. WPC solution preparation, sample handling and oscillatory rheological measurements are described in Chapter 3. Protein concentration rather than total solids concentration was used in this study.

5.3 Results

5.3.1 Effect of shear strain amplitude on gel formation and gel structure

Fig 5.1 shows the effect of shear strain amplitude on the storage modulus (G') during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C and pH7. During gel structure formation G' was not affected significantly by maximum shear strain amplitudes less than 0.05 (0.005 radian). Thus, the shear strain range 0 - 0.05 can be considered to be the linear viscoelastic region for these gelation conditions.

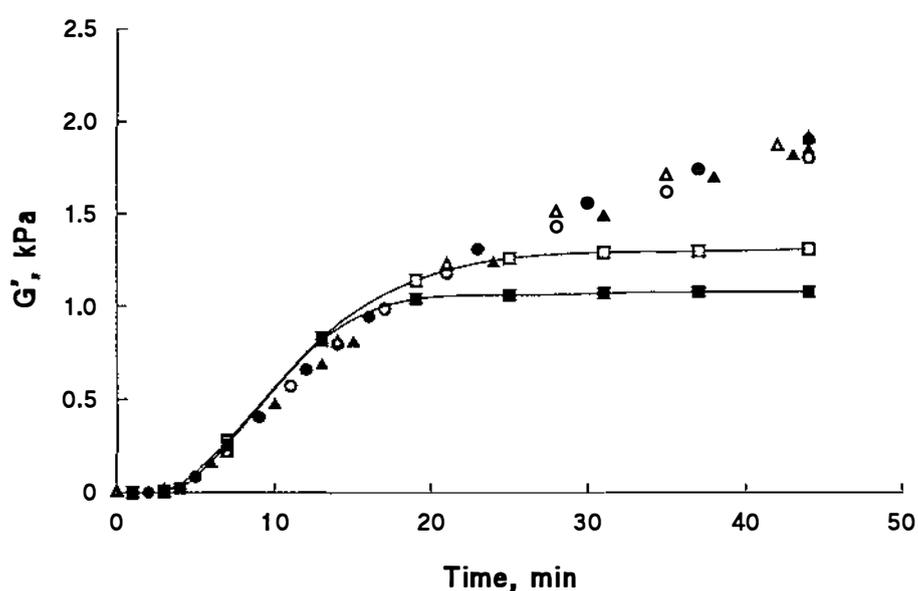


Fig 5.1 G' versus time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, pH7, 1Hz and shear strains ○, 0.001; ●, 0.02; △, 0.04; ▲, 0.05; □, 0.1; ■, 0.14.

In order to check that the results for the two higher shear strain amplitudes shown in Fig 5.1 were indeed due to these amplitudes being outside the linear viscoelastic region, rather than to their causing permanent gel structure damage during gelation, shear strain sweeps were conducted on the final gels formed at the various shear strain amplitudes at 80°C. The results for all shear strain amplitudes fell on a single curve (Fig 5.2), indicating that the gel structure, or more precisely elastic chains in the gel structure, had not been significantly damaged at the high shear strain amplitudes of 0.1 and 0.14 during gel formation. The lower G' values at shear strain amplitudes greater than 0.05 in the final stages of gel formation (Fig 5.1) were therefore due to non-linear viscoelastic behaviour rather than to gel damage. The fall in G' at shear strain amplitudes greater than 0.05 in the shear strain sweeps (Fig 5.2) is of course also due to non-linear viscoelastic behaviour.

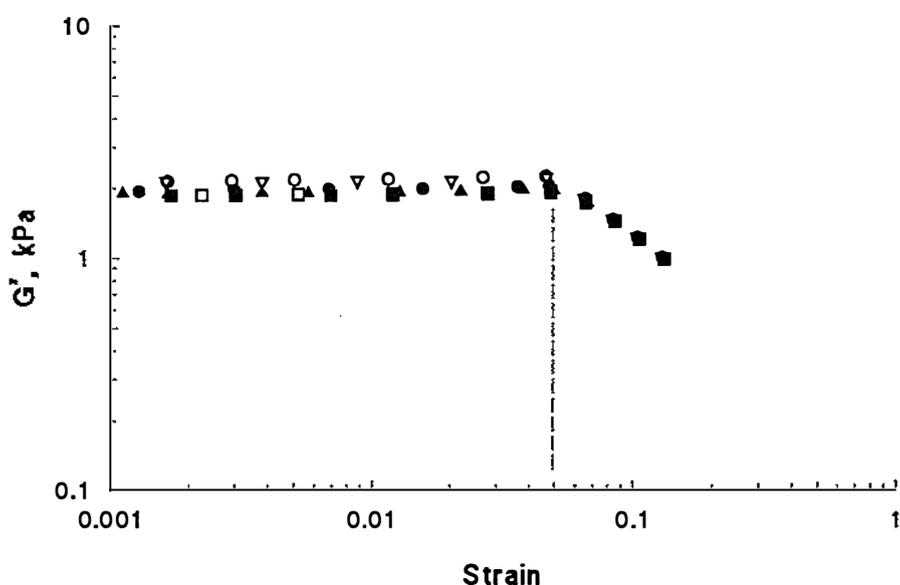


Fig 5.2 Shear strain sweep results at 80°C and 1Hz for WPC(A) gels containing 11.89% protein made by heating at 80°C and pH7 for 45 minutes. Shear strains used during heat-induced gel formation were ○, 0.001; ●, 0.02; △, 0.04; ▲, 0.05; □, 0.1; ■, 0.14.

Fig. 5.3 shows the effect of shear strain amplitude on G' during thermal gelation of WPC(B) solutions containing 10% protein at 80°C and pH7. G' during gel structure

formation was not affected significantly by shear strain amplitudes less than 0.05 (0.005 radian). Therefore, the shear strain range 0-0.05 can be considered to be within the linear viscoelastic region.

Shear strain amplitudes in the range 0.002 to 0.01 (0.0002 to 0.001 radian) were used in all later experiments. It was assumed that these were always within the linear viscoelastic region, even though this region would have been dependent on gel properties which, in turn, were governed by gelation conditions. Shear strain varies across the gap between cup and bob, and all strains quoted in this thesis are maximum values. Both WPC(A) and WPC(B) gave very similar results in the following experiments; only the results for WPC(B) are considered.

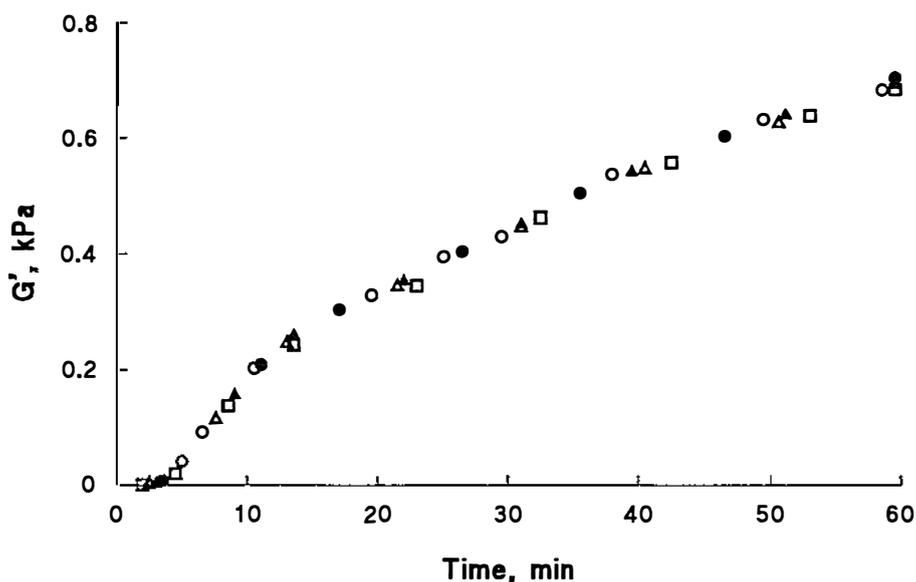


Fig 5.3 Effect of shear strain amplitude on storage modulus (G') during thermal gelation of WPC(B) solutions containing 10% protein at 80°C, pH7 and 1Hz. Shear strains \circ , 0.002; \bullet , 0.01; Δ , 0.02; \blacktriangle , 0.03 and \square , 0.05.

5.3.2 Frequency dependence of storage modulus during gelation

Fig 5.4 shows that the storage modulus (G') increased with frequency in the range 0.1Hz to 10Hz during thermal gelation of WPC solutions containing 10% protein at 80°C and pH7. At frequencies below 0.1 Hz it was difficult to obtain accurate results owing to severe

instrument "noise". At high frequencies the resistance to shear of molecular entanglements may result in erroneously high values of G' (Clark and Ross-Murphy, 1987). Thus, in subsequent experiments, either 0.1Hz or 1Hz was used.

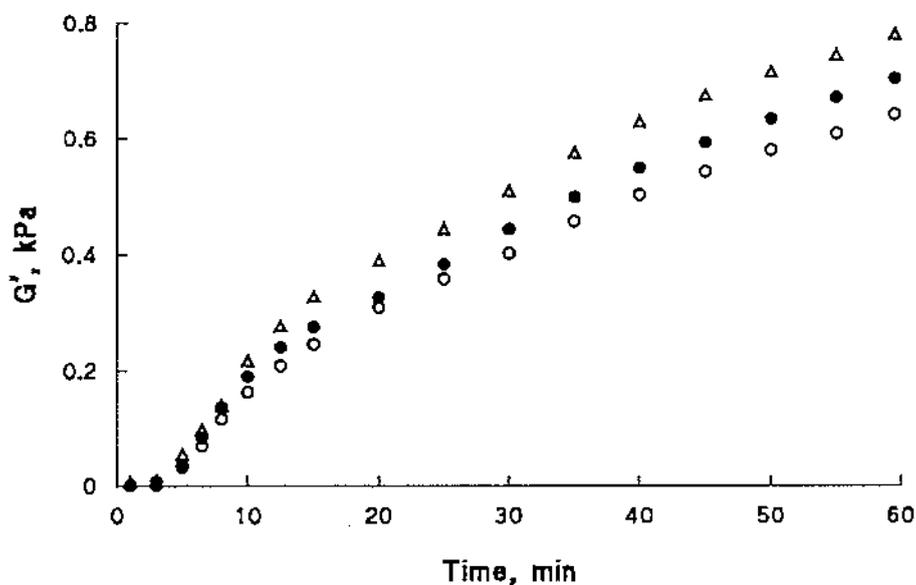


Fig 5.4 Effect of frequency on storage modulus (G') during thermal gelation of WPC(B) solutions containing 10% protein at 80°C, pH7 and 0.01 shear strain. Frequencies ○, 0.1; ●, 1; and △, 10.

5.3.3 Estimation of the sol-gel transition point (gelation time)

The changes in shear viscosity, G' , G'' and phase angle with heating time during thermal gelation of WPC solutions containing 7.9% protein at 80°C are presented in Fig 5.5. During heating the shear viscosity of the WPC solutions began to rise, slowly at first and then more rapidly. G' and G'' remained at very low values and phase angle remained at or close to 90° even when the shear viscosity had already increased dramatically. After a certain heating time G' and G'' started to rise, later crossing over, and phase angle began to fall, indicating the formation of an infinite gel network with structural continuity. Gelation time was taken as the time of heating (after the 2 min temperature equilibration period) required

for the phase angle to drop to 45° .

Gelation time under the conditions shown in Fig 5.5 was 54 min. A similar dramatic increase in viscosity prior to the gel point was observed by Richardson and Ross-Murphy (1981) during the thermal gelation of bovine serum albumin solutions.

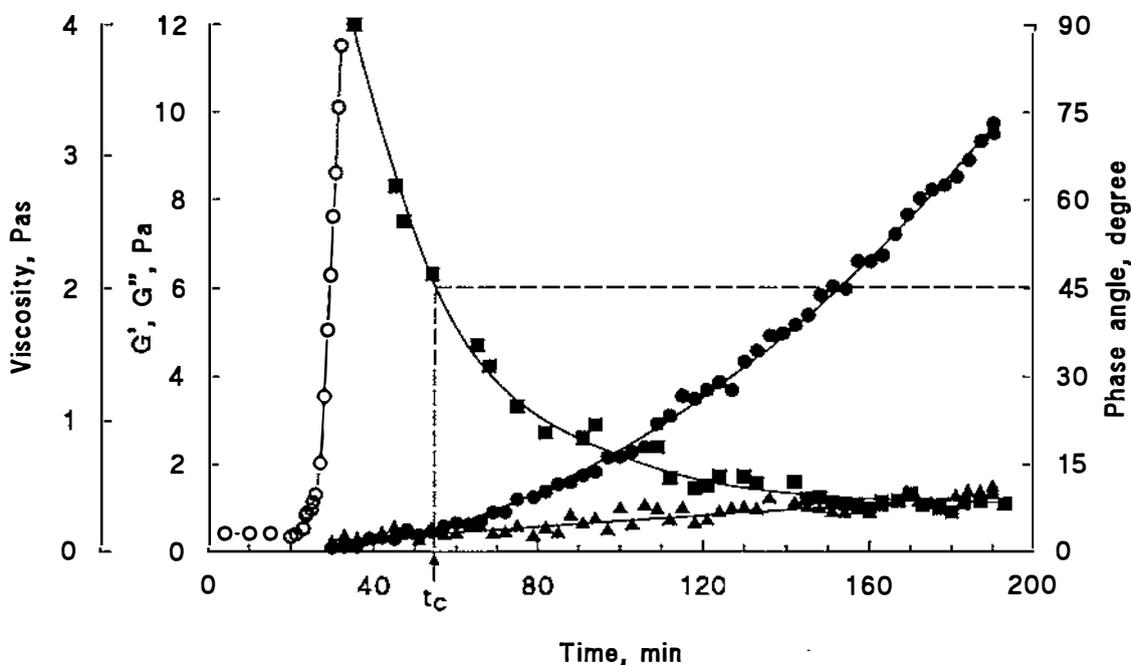


Fig 5.5 Viscosity (\circ) versus time during steady shear alone at a shear rate of 0.0231 s^{-1} , and storage modulus G' (\bullet), loss modulus G'' (\blacktriangle) and phase angle (\blacksquare) versus time during oscillatory measurement alone at 1Hz and 0.002 shear strain. Both experiments used WPC(B) solutions containing 7.9% protein at pH7 and 80°C . t_c was the heating time required for sol-gel transition, or the gelation time.

5.3.4 Concentration dependence of gelation time and estimation of critical concentration

Fig 5.6 shows the effect of protein concentration on gelation time, t_c , at 80°C . An increase in concentration resulted in a decrease in gelation time. Extrapolation of the data in Fig 5.6 to $1/t_c = 0$ ($t_c = \infty$) allowed estimation of the critical protein concentration, C_c , defined as the minimum concentration needed for gelation to occur at an infinite heating time.

For the gelation conditions used, $C_0 = 6.6\%$.

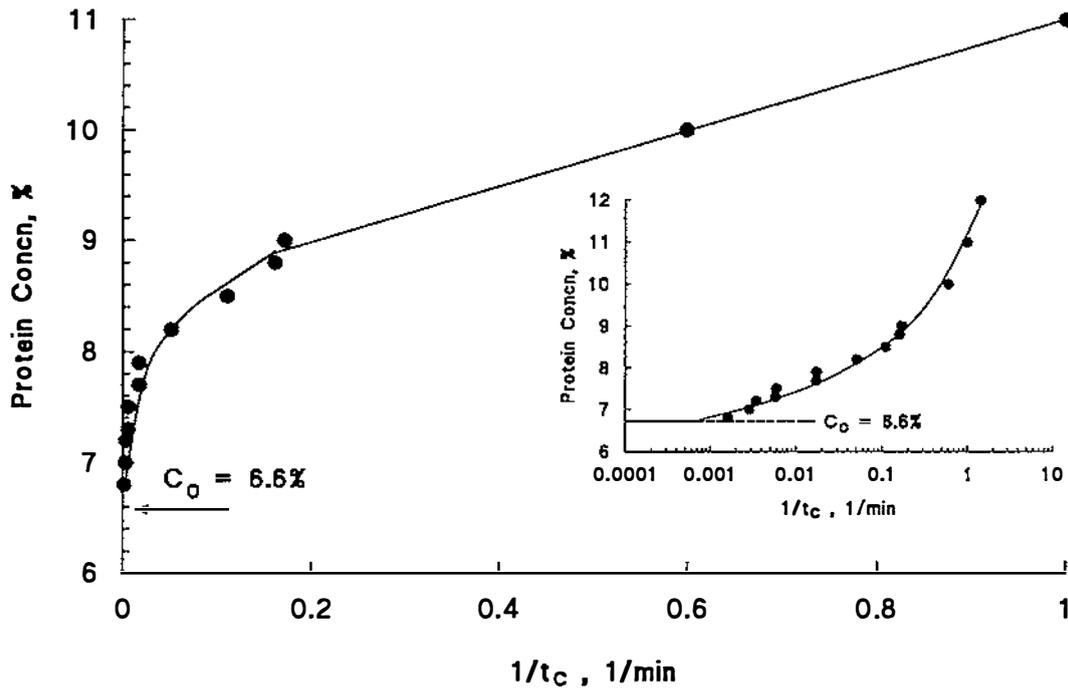


Fig 5.6 Estimation of the critical whey protein concentration of WPC(B) for gelation at 80°C , pH7, 0.1Hz and 0.002 shear strain. C_0 was the critical whey protein concentration. t_c was the gelation time as defined in the text.

5.3.5 Temperature dependence of gelation time

Fig 5.7 shows the effect of temperature on the gelation time. An increase in temperature led to a decrease in gelation time. The temperature (T) dependence of gelation time (t_c) could be described by the Arrhenius equation as shown in the insert in Fig 5.7. The equation of the straight line (coefficient of determination $r^2 > 0.988$) was:

$$\log t_c = (8.06 \times 10^3/T) - 21.04 \quad (5.1)$$

The activation energy, E' , was calculated from the slope of the line to be 154 kJ/mol.

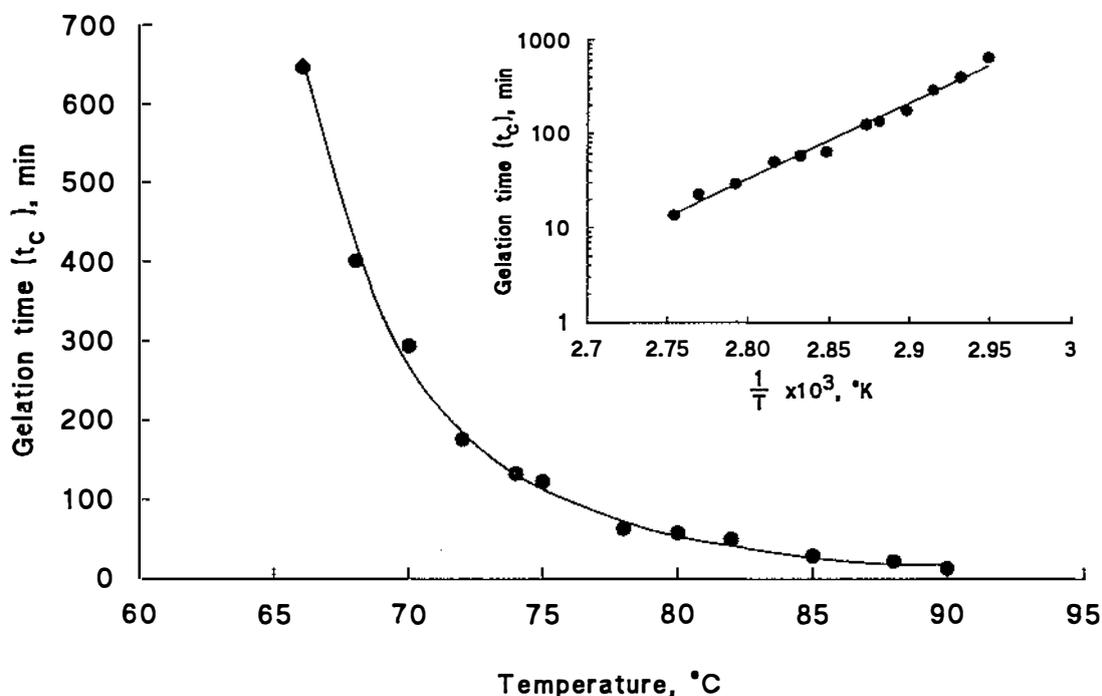


Fig 5.7 Effect of temperature on gelation time of WPC(B) solutions containing 7.9% protein at pH7, 0.1Hz and 0.002 shear strain.

5.3.6 Concentration dependence of gel structure development

The effect of protein concentration on gel structure development was investigated by following changes in G' and G'' with time for solutions of varying concentration (Fig 5.8). The storage modulus G' increased faster with time at higher protein concentrations. The final G' after 59.5 min increased with concentration. For protein concentrations above 25%, G'' increased rapidly with time after the gel point until a maximum value was reached, and then decreased again. The maximum G'' occurred earlier and had a higher value at a higher concentration. The rate of increase of G'' to its maximum and the rate of decrease from the maximum were higher at higher concentrations. For a WPC solution containing 35% protein the maximum in G'' was very sharp and occurred after only 3 min. For WPC solutions containing 15% protein G'' increased slowly and had not reached a maximum value after 59.5 min.

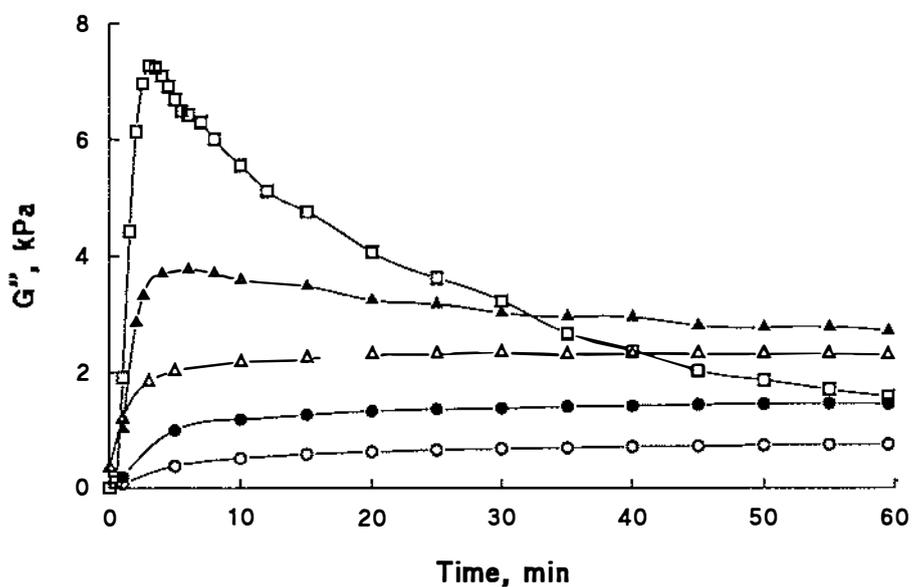
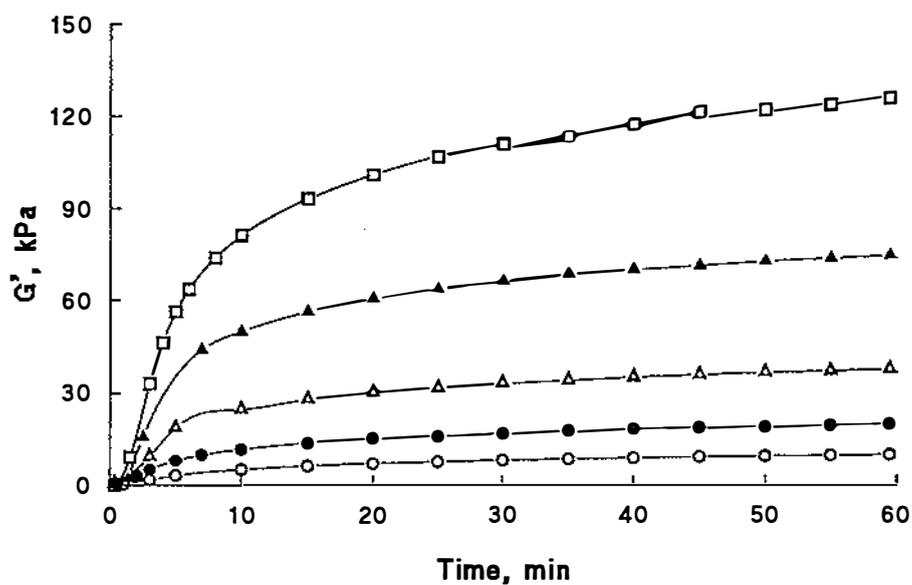


Fig 5.8 Storage modulus (G') and loss modulus (G'') at 1Hz and 0.01 shear strain as a function of time during thermal gelation of WPC(B) solutions at 80°C, pH7 and protein concentrations of 15% (○), 20% (●), 25% (△), 30% (▲) and 35% (□).

5.3.7 Temperature dependence of gel structure development

The changes in G' with time at temperatures in the range 75 to 90°C are illustrated in Fig 5.9 for WPC solutions containing 10% protein. G' was higher at higher temperature at any heating time up to 59.5 min.

Fig 5.10 shows the changes in storage modulus G' with time during thermal gelation of WPC solutions containing 25% protein at temperatures in the range 70 to 90°C. G' rose faster initially at higher temperatures. However, G' values after a heating time of 59.5 min increased with temperature up to 78°C and decreased with temperature above 78°C.

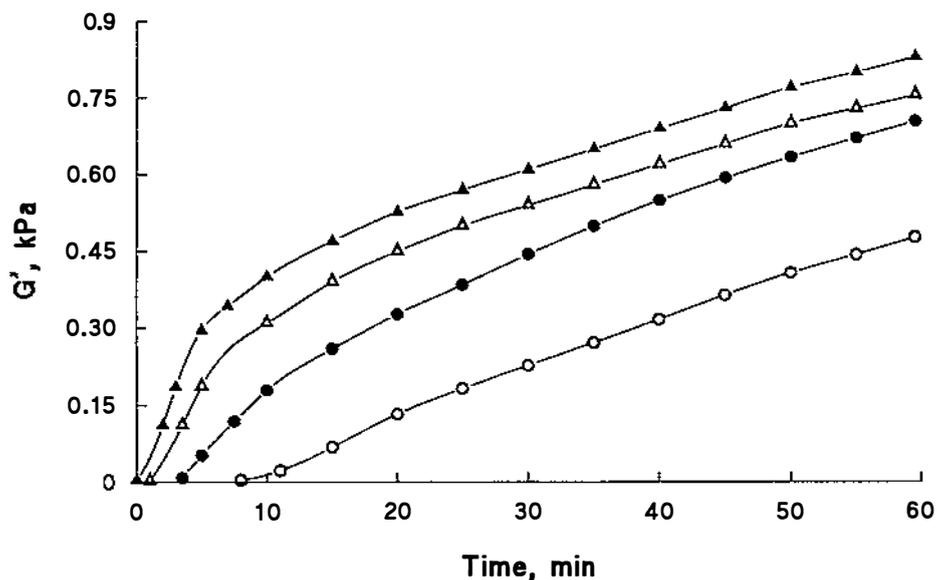


Fig 5.9 Storage modulus (G') as a function of time during thermal gelation of WPC(B) solutions containing 10% protein at 1Hz, pH7, 0.01 shear strain and ○, 75°C; ●, 80°C; △, 85°C and ▲, 90°C.

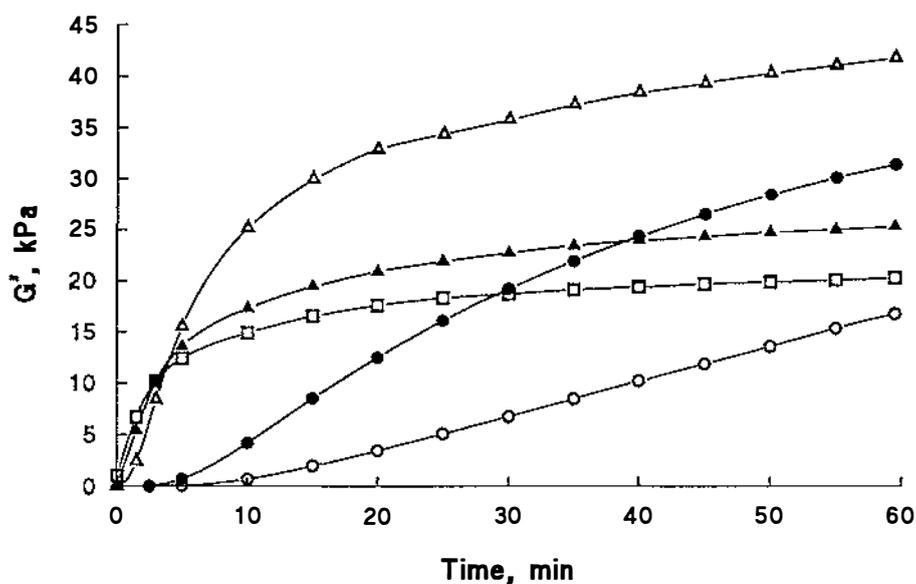


Fig 5.10 Storage modulus (G') as a function of time during thermal gelation of WPC(B) solutions containing 25% protein at 1Hz, pH7, 0.01 shear strain and ○, 68°C; ●, 70°C; △, 78°C; ▲, 85°C and □, 90°C.

5.4 Discussion

The WPC gelation process studied here was typical of biopolymer gelation including globular protein gelation. When globular protein solutions are heated above their denaturation temperature protein molecules become partly unfolded but remain in corpuscular form (Clark, 1991). These partly unfolded molecules then start to aggregate, the type of aggregate depending on net protein charge. Linear aggregates form when repulsion forces between molecules are large, "random" cluster-like aggregates when such forces are small (Clark, 1991). The formation of large aggregates by continued cross-linking would have led to the large increase in steady shear viscosity prior to the gel point. At the gel point an infinite network of aggregates is formed. Electron microscopy and other studies support this picture of globular protein gelation (Clark and Ross-Murphy, 1987).

The gelation time represents an initial lag phase in the gelation process. During this phase, both G' and G'' were very small and within the "noise level" of the rheometer (Fig 5.5). It is worth looking briefly at why, prior to the gel point, G'' (which, like steady shear viscosity, is a measure of energy dissipation) was very small even though the steady shear viscosity was very large. This apparent contradiction can be explained as follows. If the sol (the system prior to the gel point) had been a Newtonian liquid (phase angle = 90°) then its G'' would have been equal to $\omega\eta$ (where ω is the oscillatory frequency and η is the coefficient of viscosity) and G' would have been zero (Darby, 1976). The fact that G'' was actually extremely small prior to the gel point suggests that the sol was not a Newtonian liquid (which, in any case, is a rheological ideal), but a viscoelastic material possessing a very small G' and thus a phase angle very slightly less than 90° (and within the noise level of the instrument). Because of the relationship $\tan(\text{phase angle}) = G''/G'$, G'' in this case would also have been very small. For example, if phase angle = 89.99° and $G' = 0.00001$ Pa, then $G'' = 0.06$ Pa, which is considerably lower than might, without due thought, be expected on the basis of the steady shear viscosity.

The decrease in phase angle and increase in G' with time after the gel point indicated, respectively, increased solid-like (elastic) behaviour and increased rigidity (stiffness).

The dependence of gelation time on protein concentration was typical of biopolymer gelation. Very similar results were reported by Steventon *et al.* (1991) for a commercial WPC. They demonstrated by applying percolation theory that the dependence of gelation time on protein concentration was consistent with a second order kinetic process involving aggregation initiation and propagation, with the over-all aggregation process being rate limiting compared to denaturation. The results presented here indicate that a critical protein concentration (about 6.6%) existed below which a gel would not form even after an infinite heating time. The results of Steventon *et al.* (1991) suggest a similar critical concentration (slightly less than 7.0% protein) for their WPC. The effect of concentration on gelation time, including the existence of a critical concentration, agrees with the theoretical scheme for biopolymer gelation described by Clark (1991), and originally proposed by Flory and Stockmayer in the 1940's.

The ability of a single Arrhenius relationship to describe closely the dependence of gelation time on temperature over the temperature range (65 - 90°C) suggests that, in this range, the gelation process was controlled by a single rate-limiting step such as the rate-

limiting aggregation propagation process described by Steventon *et al.* (1991).

The rapid rise of G' immediately after the gel point, the tendency of G' to approach a constant plateau value and the effect of protein concentration on G' , are all predicted qualitatively by the theoretical scheme described by Clark (1991). Gel network formation by continued aggregation after the gel point would be expected to proceed faster and the "final" stiffness or rigidity of the gel would be expected to be greater at higher protein concentration. The magnitude of G' for a given protein concentration at a given time might well have been influenced by the fact that solutions of higher protein concentration contained higher concentrations of minerals and other constituents.

In biopolymer gelation, G' continues to increase slowly even after long heating times (Clark, 1991). The heating time employed here was relatively short, and it is clear that G' was still increasing at the end of this time, the rate of increase being higher at higher protein concentration.

The maximum in G'' with time that occurred at 30 and 35% protein is more common with synthetic polymers than with biopolymers, but is observed in gelatin (Clark and Ross-Murphy, 1987). Similar maxima in G'' , but in temperature ramp experiments, have been observed by Egelandsdal *et al.* (1986) in an approximately 1% (w/w) myosin suspension (pH 6, 0.2M NaCl, 1°C/min) at about 53°C and by Stading and Hermansson (1990) in a 10% β -lactoglobulin solution (pH 5.5, 0.1°C/min) at about 75°C. For β -lactoglobulin, the maximum in G'' coincided with the inflection point of the G' versus time curve and with a second step decrease in phase angle. According to Stading and Hermansson (1990) L. Bohlin has suggested that a phase transition is responsible for the maximum in G'' .

Bibbo and Valles (1982, 1984) found that for a model silicone network G'' passed through a maximum value and reached a final definite value when cross-linking was complete. They demonstrated that the maximum in G'' was associated very closely with the maximum weight fraction of pendant molecular chains that occurred during the reaction. Pendant chains were defined as those joined by only one end to the gel network, itself made up of elastic chains (joined by both ends). Chains not yet attached in any way to the network were defined as 'soluble' or 'extractable' chains. Bibbo and Valles (1984) explained that pendant chains possessed a much greater capacity for energy dissipation than either elastic or soluble chains, and were thus the main 'contributor' to G'' . It is possible that WPC gels have "energy dissipating structures" which parallel the mechanical behaviour of the pendant

chains in the system of Bibbo and Valles (1984). However, there is no direct evidence for the existence of such structures.

The effect of temperature on the rate of growth of G' after the gel point was almost certainly a kinetic effect at 10% protein. Beveridge *et al.* (1984) obtained curves of G' versus time (at 85 and 90°C), similar to those shown here, for a WPC solution containing 6.9% protein: both the initial rate of modulus growth, and the value of the modulus at the end of the heating time, increased with temperature. Beveridge *et al.* (1984) characterized their curves kinetically using a simple first order equation. Their small number of results suggested that the value of G' after an infinite heating time would be independent of temperature.

At 25% protein another factor, probably an effect of temperature on gel structure, is required to explain the results. Below 78°C, the kinetic effect was apparently dominant whereas above 78°C the effect of temperature on gel structure was dominant. A fall in G' with increase in temperature is common for biopolymer gels, and is attributed to a loss of crosslinking on heating, or a change in network strand character, or both (Clark, 1991). A similar explanation is suggested for WPC gels.

It is well recognised that protein gels of maximum strength are formed only when a critical balance of protein-protein interactions and protein-solvent interactions is attained. If protein-protein interactions are far stronger than protein-solvent interactions a precipitate rather than a gel tends to form. Presumably high temperatures increased protein-protein interactions relative to protein-solvent interactions and this may have resulted in the formation of a significant proportion of particle-like aggregates. The resulting coarse and fragile gel network would have been expected to have a lower rigidity (lower G'). Electron microscopy is needed to confirm this explanation of the effect of temperature on whey protein gel formation.

5.5 References

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6. Effects of pH and Salts on the Formation and Properties of WPC gels

6.1 Introduction

It is well established that pH, and salts such as sodium chloride and calcium chloride, have very important effects on the gelling behaviour of globular proteins such as whey proteins. A large number of studies have considered the effects of pH and salts on whey protein gel formation or gel properties. Most of these studies have involved large deformation and failure tests (Schmidt *et al.* 1979; Johns and Ennis, 1981; Dunkerley and Zadow, 1984; Kohnhorst and Mangino, 1985; Mangino *et al.* 1987; Mulvihill and Kinsella, 1988; Mulvihill *et al.* 1990; Kuhn and Foegeding, 1991; Stading and Hermansson, 1991). However, several studies have used small deformation oscillatory rheological measurements (Paulsson *et al.* 1986; Paulsson *et al.* 1990; Stading and Hermansson, 1990).

It is very important to understand the effects of pH and salts on WPC gelation since WPC powder when used as a gelling agent is often applied commercially in particular pH and salt environments, *e. g.* in meat products. With a knowledge of how salts affect WPC gelation and gel properties the dairy industry will be able to improve the quality of WPCs and produce new WPCs for specific applications such as high-salt food products. The present study was aimed at exploring the effects of pH and salts on gelation time, gel development and final gel properties using oscillatory rheological measurements. It was also aimed at gaining a greater understanding of the gelation mechanism, which is still considered to be poorly understood (Mulvihill and Kinsella, 1987; Oakenfull, 1987; Hermansson, 1988).

6.2 Experimental Procedure

Commercially available WPC powders (WPCs A, B, D, E, F and G) were used in this study. Their compositions and ash components are listed in Tables 3.1 and 3.2 respectively. WPC solution preparation, sample handling and oscillatory rheological measurements are described in Chapter 3. Concentration is expressed as protein concentration rather than as total solids concentration in this study.

6.3 Results

6.3.1 pH dependence of gelation time

The effect of pH on the gelation time of WPC(B) solutions containing 7.9% protein

at 80°C is shown in Fig 6.1. Gelation time was lowest between pH4 and pH6.5. In this pH range visual observations indicated the formation of coagula. On either side of this region gelation time increased as pH moved away. However, below pH3 gelation time started to decrease again. Between pH4 and pH4.5 solutions began to gel within the 2 min period allowed for temperature equilibration in the rheometer prior to rheological measurement.

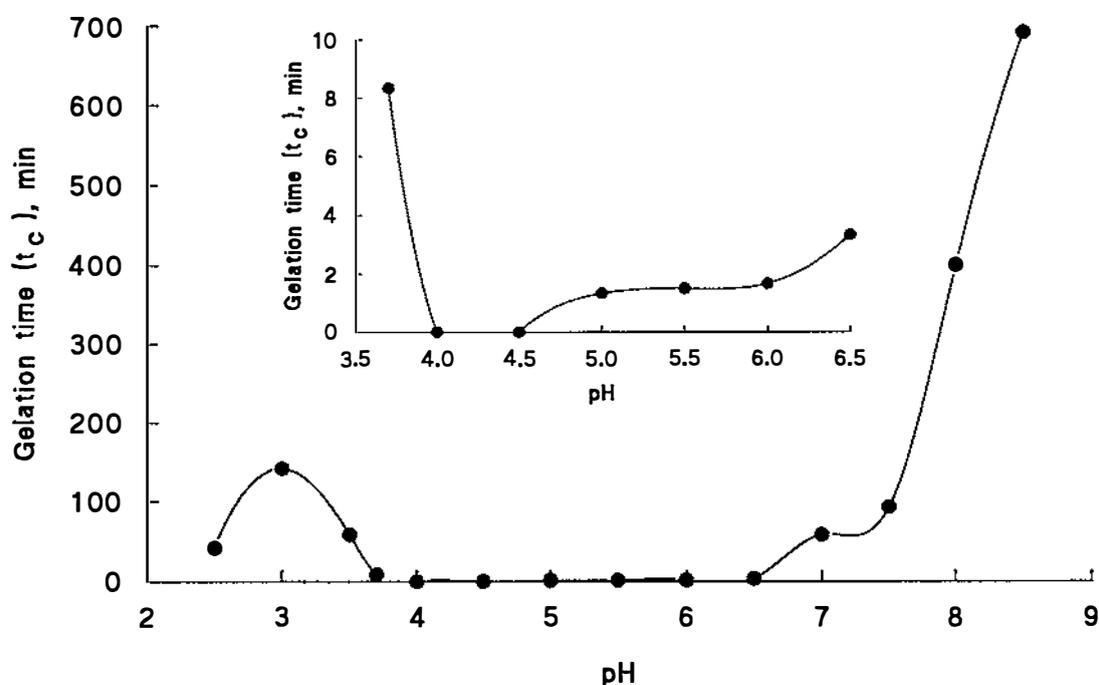


Fig 6.1 Effect of pH on gelation time of WPC(B) solutions containing 7.9% protein at 80°C, 0.1Hz and 0.002 shear strain.

6.3.2 pH dependence of gel development

Fig 6.2 shows the pH dependence of WPC(A) gelation. G' was higher at higher pH in the pH range 5.5-7 during the greater part of the 44 minute heating time (Fig 6.2). However, initial gel development (during the first five minutes) was slower at pH7 than at lower pH values above the isoelectric point region.

In the pH range 3.5-5.5 G' had the highest values at pH4 throughout the 44 minute heating time (Fig 6.2). G' was lower at pH3.5 than at pH5.5 for the initial part of the heating

time but became higher by the end of this time.

6.3.3 pH dependence of gel properties

Fig 6.3 shows pH dependence of the storage modulus (G') and phase angle of WPC(A) and WPC(B) gels. Both WPC(A) and WPC(B) gels had a similar pattern of G' versus pH. G' of both WPC(A) and WPC(B) gels exhibited maxima at somewhere near pH7 and pH4. Minima were exhibited at pH5.5 and at a pH in the range 3-3.5, while similarly low values were exhibited at high pH (8 - 9). The phase angle exhibited minima at pH7 and pH4 (corresponding to the maxima in G' at about these pH values) and maxima at pH5.5 (corresponding to the minimum in G' at this pH). Qualitatively similar curves were obtained when G' and phase angle were measured at 80°C as shown in Fig 6.4 for WPC(B) (only G' is shown), or when heating times longer than 44 minutes were used. G' always had higher values at lower temperatures than at higher temperatures because hydrogen bonding is enhanced by decrease in temperature (Cheftel *et al.* 1985).

G'' exhibited a similar pattern of pH dependence as G' , but G'' was always very much smaller than G' throughout gel development. The results for G'' are not shown here.

The formed gels were tactilely and visually observed after rheological measurements. The gels formed at pH7-8.5 were fine, uniform and semi-transparent. White coagula were produced between pH4.5 and pH6. The gels made at and near pH4 were opaque and particulate, were sticky and also appeared to be very weak in fracture in spite of their high G' . The gels made below pH4 were opaque particulate gels with some degree of stickiness.

From all these results it is considered that the most useful gels with respect to food applications of WPCs were those formed at $\text{pH} \geq 7$. This pH resulted in the formation of semi-transparent, uniform, fine gels possessing high elasticity ($\text{pH} \geq 7$) and high gel stiffness (at pH7).

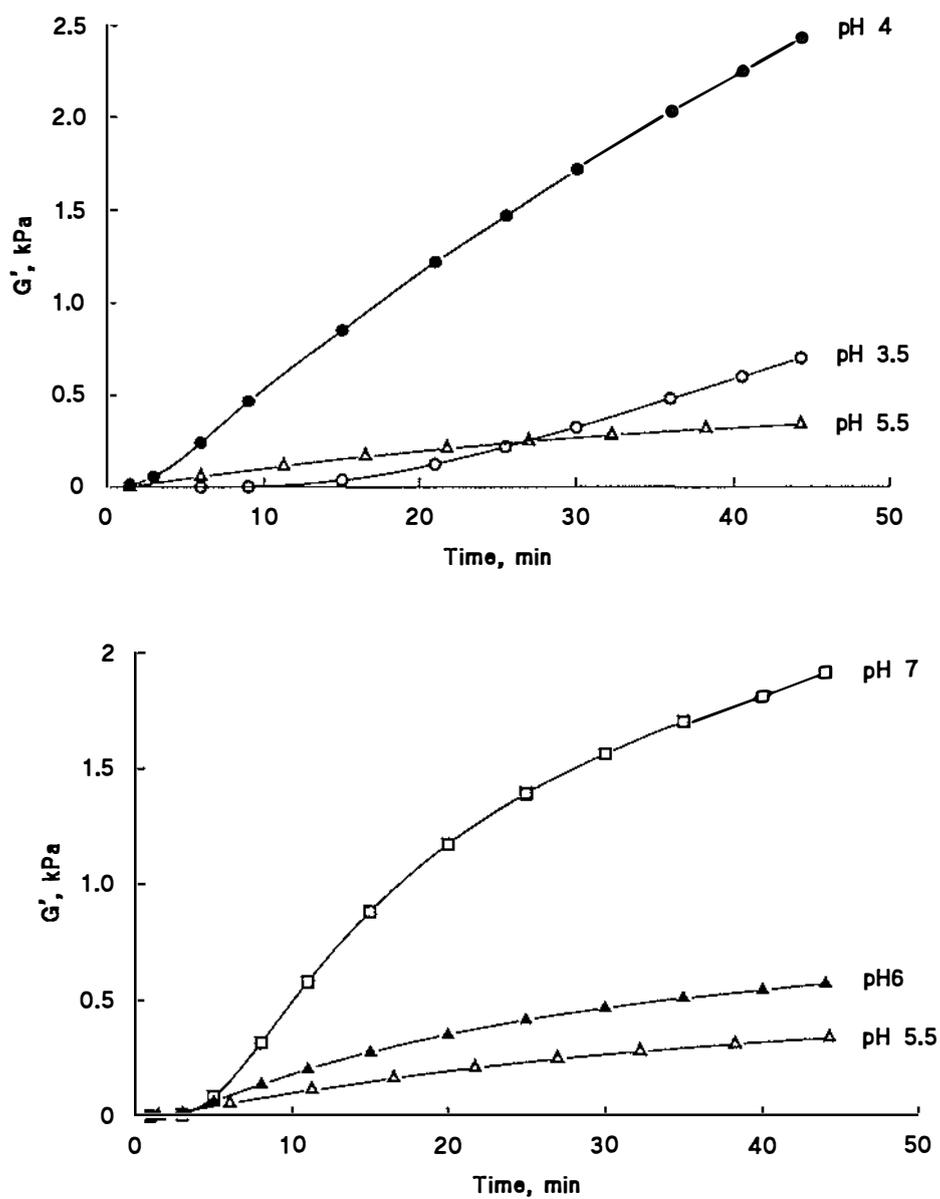


Fig 6.2 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C, 1Hz, 0.01 shear strain and pH values of ○, 3.5; ●, 4; △, 5.5; ▲, 6 and □, 7.

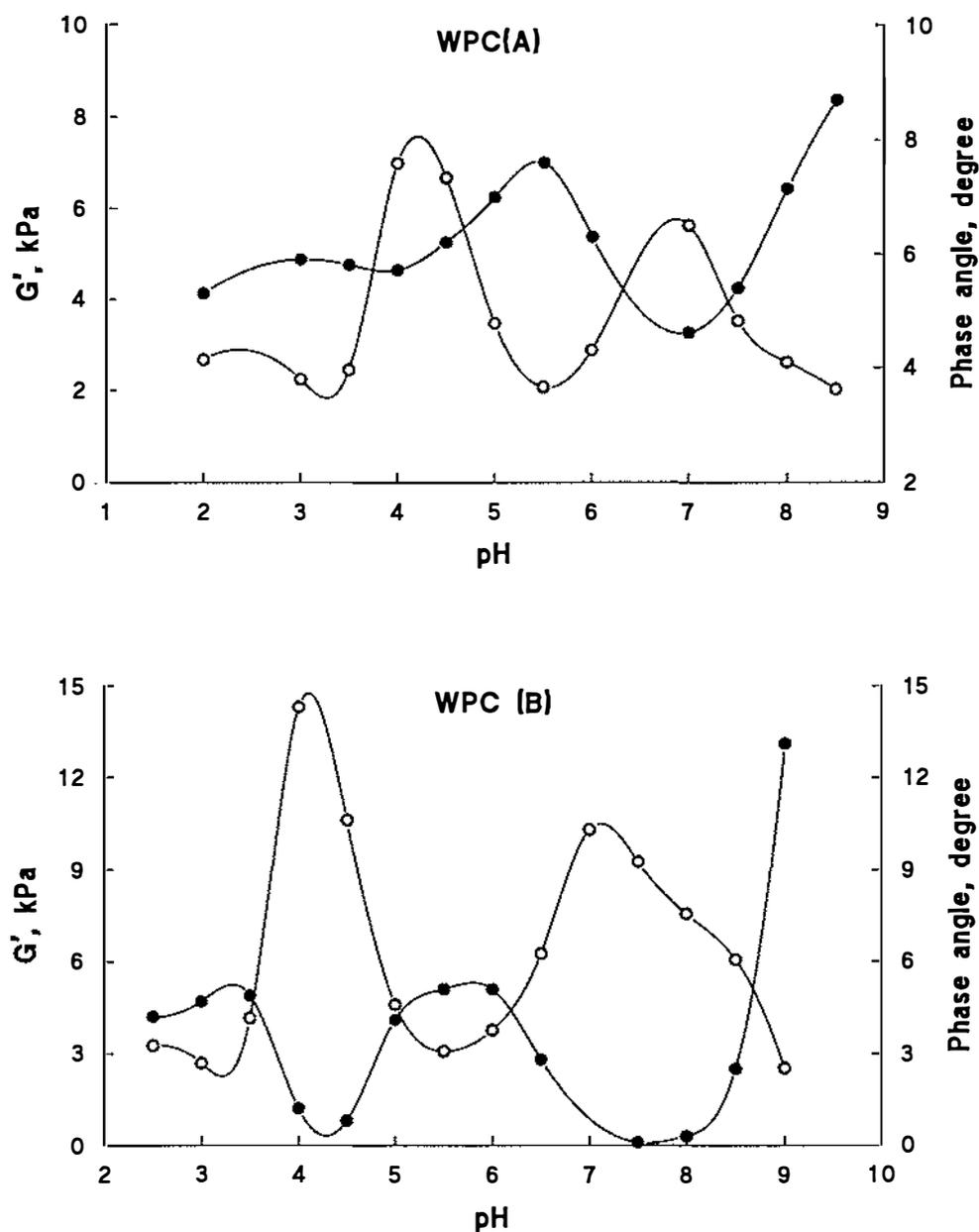


Fig 6.3 Effect of pH on (○) storage modulus G' and (●) phase angle of WPC(A) gels at 1Hz, 0.01 shear strain and 25°C, and WPC(B) gels at 1Hz, 0.01 shear strain and 20°C. WPC(A) gels were made by heating WPC solutions containing 11.89% protein at 80°C for 44 minutes. WPC(B) gels were made by heating WPC solutions containing 12% protein at 80°C for 44 minutes.

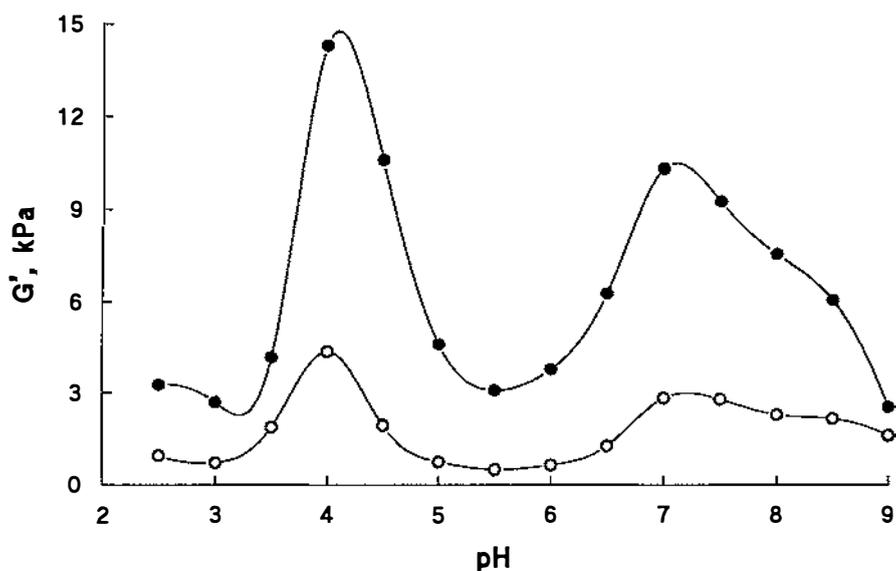


Fig 6.4 Effect of pH on storage modulus G' of WPC(B) gels containing 11.89% protein at 1Hz, 0.01 shear strain, 80°C (○) and 20°C (●).

6.3.4 Salt concentration dependence of gelation time

Fig 6.5 shows the effects of salt concentration, expressed as added ionic strength, on the gelation time of WPC(B) solutions. The addition of only small amounts of either sodium chloride (0.0005M to 0.7M) or calcium chloride (0.0002M to 0.07M) dramatically reduced gelation time, which reached a constant minimum value with increase in concentration of either salt. In the case of calcium chloride, the minimum value was reached at an added ionic strength about four times lower than in the case of sodium chloride. Above the salt concentrations at which gelation times reached constant values, coagula or precipitates tended to form with increase in salt concentration.

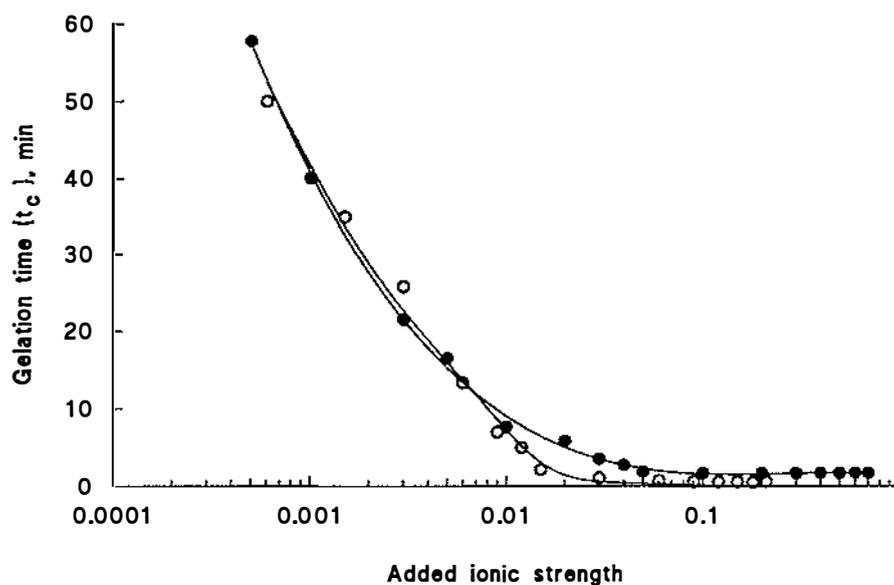


Fig 6.5 Effects of sodium chloride (●) and calcium chloride (○) concentration (expressed as added ionic strength) on gelation time of WPC(B) solutions containing 7.9% protein at 80°C, pH7, 0.1Hz and 0.002 shear strain.

6.3.5 Salt dependence of gel development

Fig 6.6 shows the effects of sodium chloride concentration in the range 0-0.5M on G' during the development of WPC(A) gels. G' increased more rapidly and maintained higher values at higher salt concentrations throughout the 44 minute heating period up to a sodium chloride concentration of 0.04M. At 0.2M salt, G' was lower than at 0.04M, and it was lower still at 0.5M.

Fig 6.7 shows the effects of calcium chloride concentration in the range 0-20mM on G' during the development WPC(A) gels. G' had higher values at 5mM than at 0mM during the whole of the 44 minutes heating period. At 10mM, G' increased rapidly at first but became smaller than at 5mM after about 8.5 minutes, and lower than at 0mM after about 20 minutes. At 20mM, G' again increased rapidly at first but became smaller than at 0mM after about 8.5 minutes.

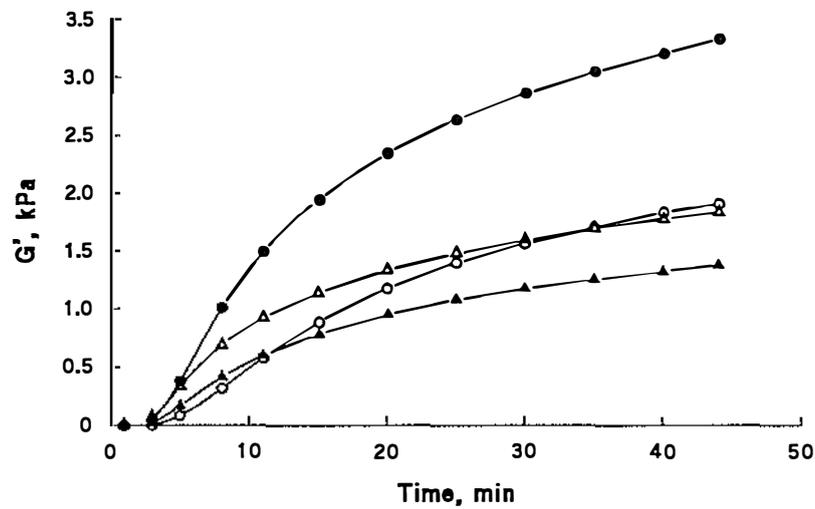


Fig 6.6 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C , 1Hz, 0.01 shear strain, pH7 and sodium chloride concentrations of \circ , 0M; \bullet , 0.04M; \triangle , 0.2M and \blacktriangle , 0.5M.

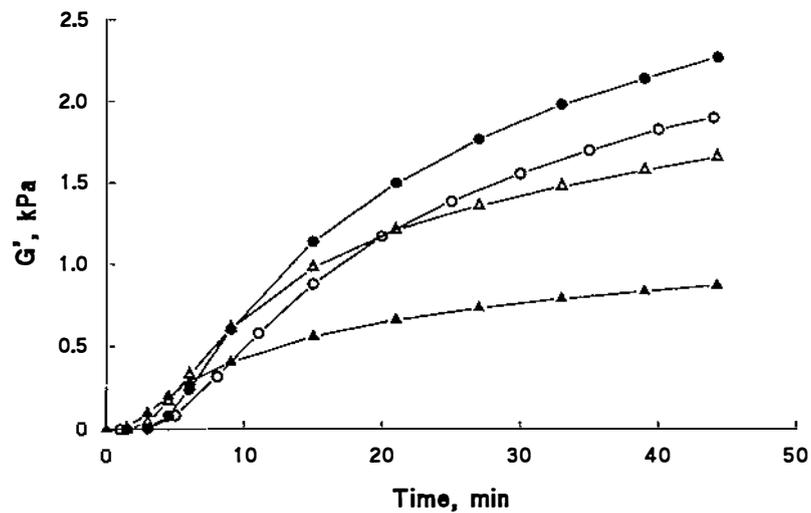


Fig 6.7 Storage modulus G' as a function of time during thermal gelation of WPC(A) solutions containing 11.89% protein at 80°C , 1 Hz, 0.01 shear strain, pH7 and calcium chloride concentrations of \circ , 0mM; \bullet , 5mM; \triangle , 10mM and \blacktriangle , 20mM.

6.3.6 Salt dependence of gel properties

Fig 6.8 shows the effects of sodium chloride concentration, expressed as added ionic strength, on G' of formed WPC(A) gels at pHs 4, 7 and 8. At pHs 7 and 8, G' increased with added ionic strength at first, then reached maxima at relatively low added ionic strength, and finally decreased. At pH8 the maximum in G' occurred at a higher added ionic strength and had a higher value than at pH7. At pH4, G' generally decreased gradually with increasing added ionic strength. G'' exhibited a similar pattern with increase in added ionic strength at pHs 4, 7 and 8. At all three pHs (4, 7 and 8) the phase angle always increased with increase in added ionic strength. The G'' and phase angle results are not shown here.

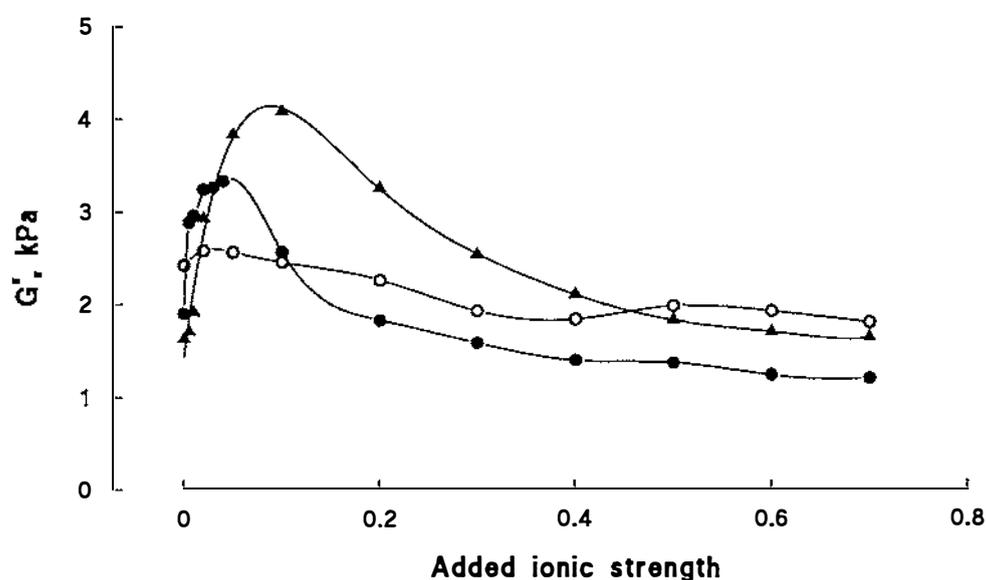


Fig 6.8 Effects of NaCl concentration (expressed as added ionic strength) on storage modulus G' of WPC(A) gels at 1Hz, 0.01 shear strain and 80°C after heating WPC(A) solutions containing 11.89% protein for 44 minutes at 80°C and pH values of (○) 4, (●) 7 and (▲) 8.

Visual and tactile observations indicated that the gel properties changed gradually with

increase in sodium chloride concentration expressed as added ionic strength. The gels formed at pH7 moved from semi-transparent, uniform, fine gels (0-0.04 added ionic strength) to opaque gels (0.05-0.1 added ionic strength), to white gels and white coagula (0.1-0.7 added ionic strength). At sufficiently high added ionic strength (*e.g.* above 1.0 added ionic strength) whey protein precipitates with no continuous gel structure were formed. The gels formed at pH8 moved from semi-transparent, uniform, fine gels (0-0.05 added ionic strength) to opaque gels (0.05-0.15 added ionic strength), to white homogeneous gels and white coagula containing visible particles (0.2-0.7 added ionic strength) and, again, even to precipitates at sufficiently high added ionic strength (*e.g.* above 1.0 added ionic strength). The gels formed at pH4 moved from opaque particulate gels (0-0.1 added ionic strength) to white particulate gels (0.1-0.7 added ionic strength).

Fig 6.9 shows the effects of calcium chloride concentration, expressed as added ionic strength, on G' of formed WPC(A) gels at pHs 4, 7 and 8. The effects of added ionic strength on G' , G'' and phase angle (results of G'' and phase angle are not shown here) were almost the same as those of sodium chloride; the only difference was that lower added ionic strength was required for calcium chloride than for sodium chloride to have significant effects on G' , G'' and phase angle.

Visual observations indicated that gel properties changed gradually with increase in calcium chloride concentration expressed as added ionic strength. The gels formed at pH7 moved from semi-transparent gels (0-0.015 added ionic strength) to opaque gels (0.015-0.03 added ionic strength), to white homogeneous gels (0.03-0.045 added ionic strength), to white coagula (0.045-0.09 added ionic strength) and even to precipitates with no continuous gel structure at sufficiently high added ionic strength. The gels formed at pH8 moved from semi-transparent gels (0-0.021 added ionic strength) to opaque gels (0.021-0.045 added ionic strength), to white gels (0.045-0.06 added ionic strength), to white coagula containing visible particles (0.06-0.09 added ionic strength) and, again, even to precipitates at sufficiently high calcium chloride concentration. The gels formed at pH4 were opaque particulate gels at added ionic strengths of 0-0.09. It is evident (Figs 6.8 and 6.9) that calcium chloride caused effects more or less identical to those of sodium chloride, but at a lower added ionic strength.

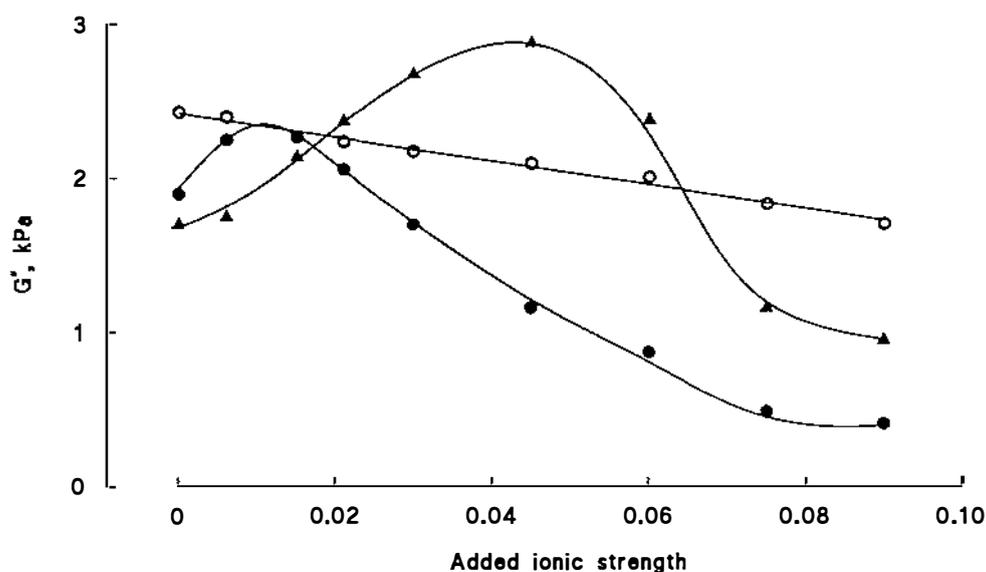


Fig 6.9 Effects of calcium chloride concentration (expressed as added ionic strength) on storage modulus G' of WPC(A) gels at 1Hz, 0.01 shear strain and 80°C after heating WPC(A) solutions containing 11.89% protein at 80°C and pH values of (○) 4, (●) 7 and (▲) 8.

6.3.7 Brine tolerant WPCs

Fig 6.10 shows G' versus heating time for WPC(D) and for the brine tolerant WPCs(E), (F) and (G). WPC(D) had higher G' values than any of the brine tolerant WPCs during 45 minutes heating at 75°C.

Fig 6.11 shows G' versus pH for brine tolerant WPC(E) gels. The maximum in G' occurred at pH5.5 whereas with normal WPC gels G' was a maximum at pH7 (Fig 6.3). The maximum in G' was shifted to lower pH by removing salts from the WPC.

Fig 6.12 shows G' versus sodium chloride concentration expressed as added ionic strength for WPC(D) gels and for brine tolerant WPC(E), WPC(F) and WPC(G) gels. The maxima in G' for brine tolerant WPCs occurred at much higher added ionic strengths and had much higher values than that of the normal WPC(D). It is evident that G' values of brine

tolerant WPC gels were higher than those of normal WPC gels at added ionic strengths larger than about 0.05M. Therefore, brine tolerant WPC gels had much higher gel stiffness at high added ionic strength than did normal WPC gels.

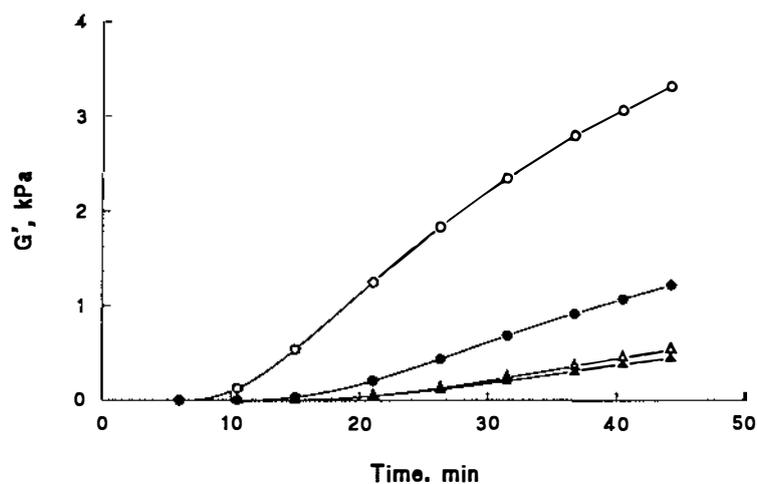


Fig 6.10 Effects of heating time on G' of (\circ) WPC(D), (\bullet) WPC(E), (Δ) WPC(F) and (\blacktriangle) WPC(G) solutions containing 12% protein at 75°C, pH6.5, 1Hz and 0.02 shear strain.

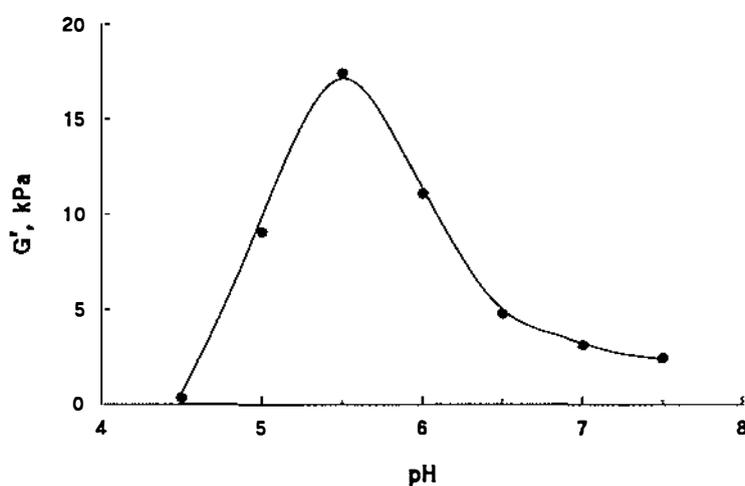


Fig 6.11 Effect of pH on G' of WPC(E) gels at 1Hz, 0.02 shear strain and 20°C after heating solutions containing 12% protein at 75°C for 44 minutes.

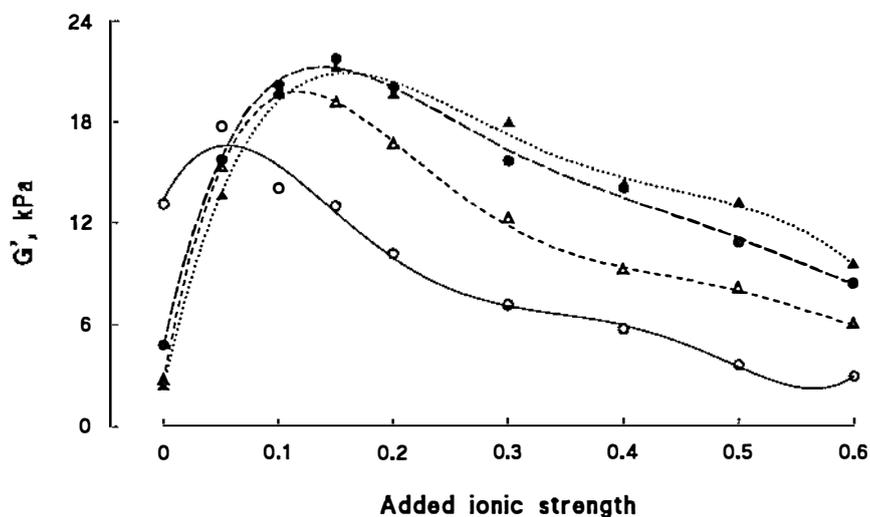


Fig 6.12 Effects of sodium chloride concentration (expressed as added ionic strength) on G' of (○) WPC(D), (●) WPC(E), (△) WPC(F) and (▲) WPC(G) gels at 1Hz, 0.01 shear strain and 20°C after heating WPC solutions containing 12% protein for 44 minutes at 75°C and pH6.5.

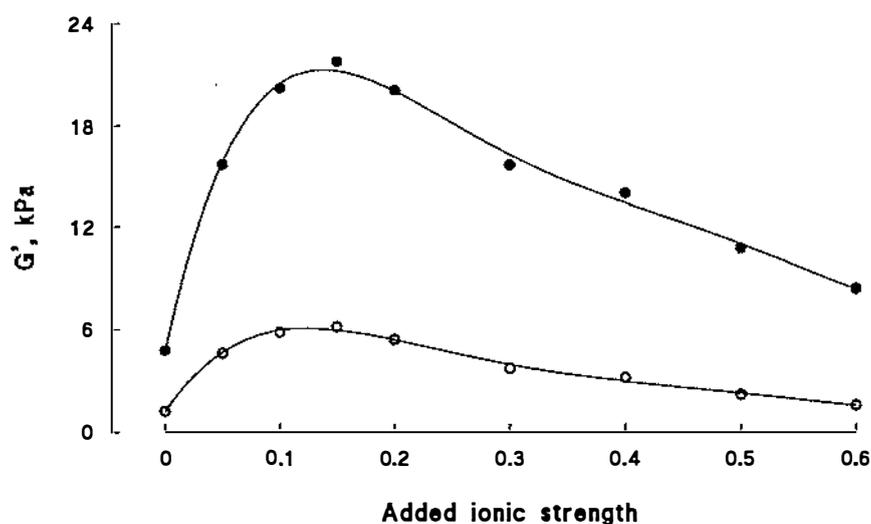


Fig 6.13 Effects of sodium chloride concentration (expressed as added ionic strength) on G' of WPC(E) gels at 1Hz, 0.01 shear strain and pH6.5, at (○) 75°C and (●) 20°C after heating WPC(E) solutions containing 12% protein for 44 minutes at 1Hz, pH6.5 and 75°C.

A decrease in measurement temperature resulted in an increase in G' owing to enhancement of hydrogen bonding, but did not change the pattern of G' versus sodium chloride concentration (expressed as added ionic strength) (Fig 6.13).

6.4 Discussion

The isoelectric pHs of the major whey proteins are 5.3 (β -lactoglobulin), 4.8 (α -lactalbumin), 5.1 (bovine serum albumin) and 5.5-6.8 (immunoglobulins) (Kinsella & Whitehead, 1989). The very short gelation times in the pH range 4-6 were probably the net result of two factors, the first being minimum repulsion between protein molecules of like species, and the second being electrostatic attraction between molecules of unlike species possessing significantly different isoelectric points (and consequently opposite net charges).

The dramatic reductions in gelation times caused by the presence of small amounts of either sodium chloride or calcium chloride were presumably caused by the screening effects of Na^+ or Ca^{2+} on the negative charges of the protein molecules at pH7 which would have reduced the electrostatic repulsion between protein molecules and aggregates. While such effects were mainly a function of added ionic strength, the greater influence of calcium chloride on gelation time at higher ionic strength is in line with the relative positions of Ca^{2+} and Na^+ in the lyotropic (Hofmeister) series. Foegeding *et al.* (1992) found that NaCl and CaCl_2 , at concentration levels very similar to those used here, has similar relative effects on the gelation time of solutions (at pH7) of β -lactoglobulin, the principal gelling whey protein.

The mechanical properties of a gel network are determined by its geometric structure, the strength of the interactive forces between the building blocks and the structures of the building blocks themselves. It has been found that gels built with the same material but with different geometric structures had substantial differences in stiffness (Bremer, 1992). Similarly, the mechanical properties or rheological properties of a WPC gel network are governed by its geometric structure, the strength of protein-protein interactions (hydrophobic interactions, and disulphide, hydrogen and ionic bonding) and the molecular structure of protein molecules prior to their incorporation into the gel network. Changes in pH or changes in the salt contents of WPC solutions alter respectively the net charges of protein molecules and, above the isoelectric point region, the screening effect of Na^+ , Ca^{2+} or other cations on the negative charges of protein molecules. This leads to changes in the strength of protein-

protein interactions which, in turn, result in different gelation rates and different geometric gel network structures. Changes in network structure are reflected in qualitative changes in the character of gels - which range from semi-transparent gels to coagula or precipitates. Changes in pH leading to significant changes in geometric structure have been reported in detail by Langton and Hermansson (1992) and by Stading *et al.* (1992). Dynamic rheological properties (G' , G'' and phase angle) are altered significantly by changes in pH and salt concentration, and this is a consequence of changes in gel structure.

The curves of G' versus pH obtained in this study are similar to that for rigidity modulus of ovalbumin gels versus pH obtained by Egelanddal (1980), and that for Young's modulus of β -lactoglobulin gels versus pH obtained by Stading and Hermansson (1991). The occurrence of maxima in G' and minima in phase angle is governed mainly by a balance between the extent of protein-protein interactions and the gel geometric structure. Excessive protein-protein interactions lead to a geometric structure (*e.g.* in coagula or precipitates) that is weak in some mechanical or rheological properties including gel stiffness, G' . However, weak protein-protein interactions also result in low gel stiffness. The maximum in G' is thus governed by the balance of these two factors and would occur somewhere between weak and excessive protein-protein interactions. Therefore, between the two (G' maximum) pHs, excessive protein-protein interactions led to lower gel stiffness; outside this pH region, weak protein-protein interactions also led to lower gel stiffness. At the (G' maximum) pHs, optimal balances between the extent of protein-protein interactions and the gel geometric structure led to the highest in gel stiffness.

Above the isoelectric point region, an increase in ionic strength increases the extent of protein-protein interactions owing to the screening and charge neutralization effects of cations on the net negative charges of the protein molecules. This increase in the extent of protein-protein interactions, and the resulting change in the gel geometric structure, would lead to a change in gel stiffness. At pHs above the (G' maximum) pH, where the balance between the extent of protein-protein interactions and the gel geometric structure is less than optimal, the net effect of a moderate increase in ionic strength is an increase in gel stiffness. At pHs between the (G' maximum) pH and the isoelectric point region, the net effect would be expected to be a decrease in gel stiffness. Thus, increasing the ionic strength by adding salt causes the maximum in G' to shift to a higher pH.

The maximum in G' (above the isoelectric point region) occurs at a much lower pH

for brine-tolerant than for normal WPCs (Figs 6.3 and 6.11). Thus, it appears that reducing the ionic strength by removing salt has exactly the opposite effect to that of increasing it: the maximum in G' is shifted to lower pH.

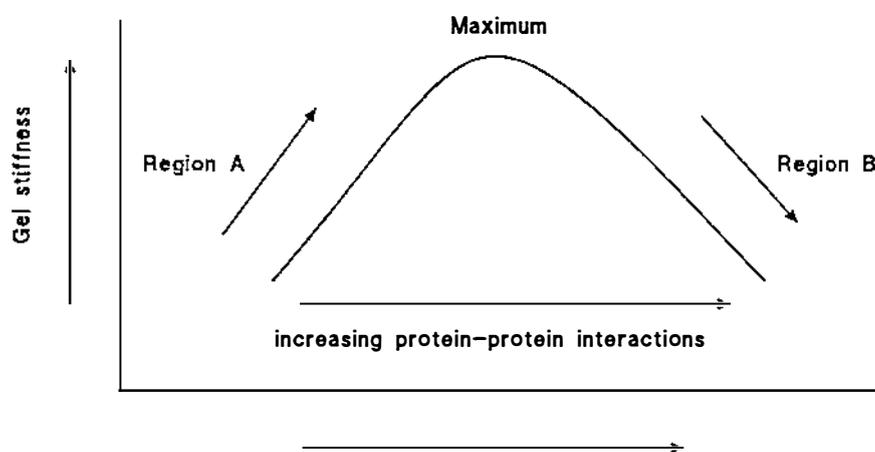
At a given pH well above the isoelectric point region, the effects of charge neutralization and screening by cations such as Na^+ and Ca^{2+} are small at low ionic strength. Electrostatic repulsion between the net negatively-charged protein molecules is high, leading to low extent of protein-protein interactions and low gel stiffness. At very high ionic strength, electrostatic repulsion is greatly reduced, resulting in high extent of protein-protein interactions and the consequent formation of coagula, again with low stiffness. Thus, at a given pH, there is a maximum in G' at an ionic strength somewhere between 'zero' and 'very high'. At a pH even further above the isoelectric point region, a higher ionic strength would be needed to achieve the maximum in G' because the protein molecules would have a higher net negative charge to staff off with. For brine-tolerant WPCs the maximum in G' at a given pH would occur at higher added ionic strength (than for normal WPCs) because of their low initial salt contents.

Different added ionic strengths are required to achieve the maximum in G' for different types of cations. For example, at pH7 and at pH8 a lower added ionic strength is required to achieve the maximum in G' of WPC(A) gels for CaCl_2 than for NaCl . This is in line with the relative positions of Ca^{2+} and Na^+ in the lyotropic (Hofmeister) series.

Most whey protein powders (WPCs or WPIs) contain some salts. The addition of salts to their solutions could thus lead to either an increase or a decrease in gel stiffness depending on their original salt contents and on the gelation pH (above the isoelectric point region). For example, below the (G' maximum) pHs (pH=6.8 for WPC(A), pH=7.2 for WPC(B) and pH=5.5 for brine tolerant WPC(E)), addition of salts would lead to decreases in gel stiffness owing to excessive protein-protein interactions. Above the (G' maximum) pHs, addition of salts would lead to increases in gel stiffness owing to increase in previously less than optimal protein-protein interactions. Therefore, at pH6.5, added ionic strength led to increase in gel stiffness for brine tolerant WPC(E), but would be expected to lead to decreases in gel stiffness for WPCs(A) and (B).

Results obtained by previous workers (*e.g.* Schmidt *et al.* 1979; Johns and Ennis, 1981; Mangino *et al.* 1987) on the effects on gel properties of adding cations to (or removing cations from) WPC preparations are often difficult to compare. As Kinsella and Whitehead

(1989) and Kuhn and Foegeding (1991) have pointed out, this is probably due to differences in composition between the different preparations studied. It is clear from the results reported here that the initial salt content of a WPC gelling system will greatly affect its response to added salt, as will the pH at which salt addition is made.



pH moving towards the isoelectric point region from extreme values, or ionic strength increasing from zero at a constant pH above the isoelectric point region.

Fig 6.14 Diagrammatic depiction of change in gel stiffness of WPC gels with change in pH or ionic strength.

In conclusion, it can be stated that the effects of pH and of salt concentration on WPC gel stiffness are the results of very similar mechanisms. Changing either the pH or the salt concentration has the principal effect of altering the surface charge on the protein molecules, this in turn altering the gelation rate and the extent of protein-protein interaction. Changes in the extent of protein-protein interactions result in changes in the geometric structure of the WPC gel network. If we start from a region of relatively low protein-protein interaction and change the pH or the ionic strength as depicted in Fig 6.14, gelation rate and protein-protein interactions increase (Region A), resulting in an increase in gel stiffness (G') to a maximum.

If the change in pH or ionic strength is continued in the same direction, protein-protein interactions become stronger and stronger (Region B). Then, coagula or precipitates possessing low stiffness tend to form.

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7. A Comparative Study of the Gelation Characteristics of Egg White, WPCs, WPI and β -lactoglobulin

7.1. Introduction

As described in Chapter 1, the heat denaturation and heat gelation of whey proteins are functional characteristics important in some confectionery products, meat products, bakery products and textured products, and in a range of new dairy analogue products formulated especially to take advantage of these characteristics (de Wit, 1989). In many of these food applications whey protein products compete with whole egg or egg white products; some of the problems of replacing egg white with whey protein have been discussed previously (Melachouris, 1984; Kinsella and Whitehead, 1989). For effective application of whey proteins, differences in the heat gelation characteristics of egg white and whey proteins must be recognised; in particular, the different gelation temperatures and the effects of physicochemical variables on gelation must be understood (Kinsella and Whitehead, 1989). Little detailed information is available in this area.

The aim of this work was to compare the gelation properties of various whey protein products with those of egg white protein, and to explore methods for eliminating some of the differences in gelation characteristics.

7.2 Experimental Procedure

Commercially available WPCs (WPCs B and C), WPI and egg white powders were used in this study. Their compositions and ash components are listed in Tables 3.1 and 3.2 respectively. A sample of purified β -lactoglobulin was prepared from cheddar cheese whey by the thermal separation method of Pearce (1983). Its composition is given in Table 3.1. Protein solution preparation, sample handling and oscillatory rheological measurements are described in Chapter 3. Concentration is expressed as protein concentration rather than as total solids concentration in this study.

7.3 Results

7.3.1 Temperature sweep experiments

Temperature sweep experiments indicated the gelation temperatures of various solutions. At 12% protein, egg white solutions began gelling at 65°C while each of the WPC

solutions began gelling at 75°C (Fig 7.1). These results are in agreement with those reported by Melachouris (1984). Egg white solution gelled faster than WPC, and egg white gels had higher G' values than WPC gels during both heating and cooling. WPI solutions at 12% protein did not form gels during heating to 90°C and formed only soft gels with low G' values on subsequent cooling.

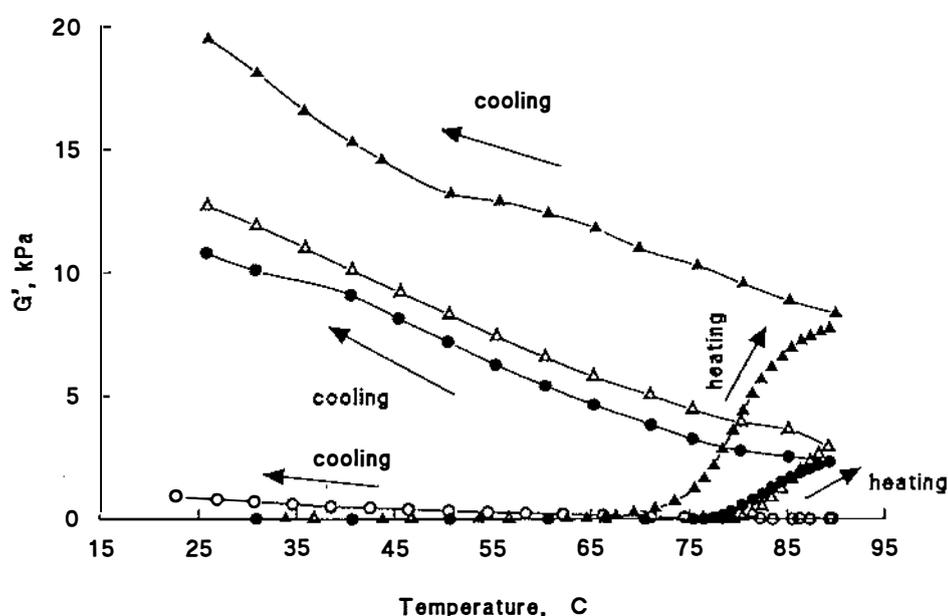


Fig 7.1 Storage modulus (G') versus temperature for solutions of WPI(\circ), WPC(B) (\bullet), WPC(C) (Δ) and egg white (\blacktriangle) containing 12% protein during a temperature sweep experiment with heating at 1°C/min and subsequent cooling at 2°C/min. Gelation was conducted at pH7, 1Hz and 0.01 maximum shear strain.

7.3.2 Minimum protein concentration for gelation

Protein solutions with a range of concentrations up to 20% were subjected to heating at 80°C and pH7 for 11.11 h, and G' was then measured at 80°C (Fig 7.2). Egg white solution was able to form gels at much lower protein concentrations (minimum 1.8%) than WPCs solutions (minimum 6%). WPC(C) had a slightly lower minimum protein

concentration for gelation than WPC(B). Even in the presence of 0.01M-NaCl WPI solutions needed to contain at least 9.8% protein before gelation would occur. The G' of egg white gels was higher than that of WPC(C) gels below 12% protein, but the two were equal above 12% protein. The G' of WPC(C) gels was always higher than that of WPC(B) gels.

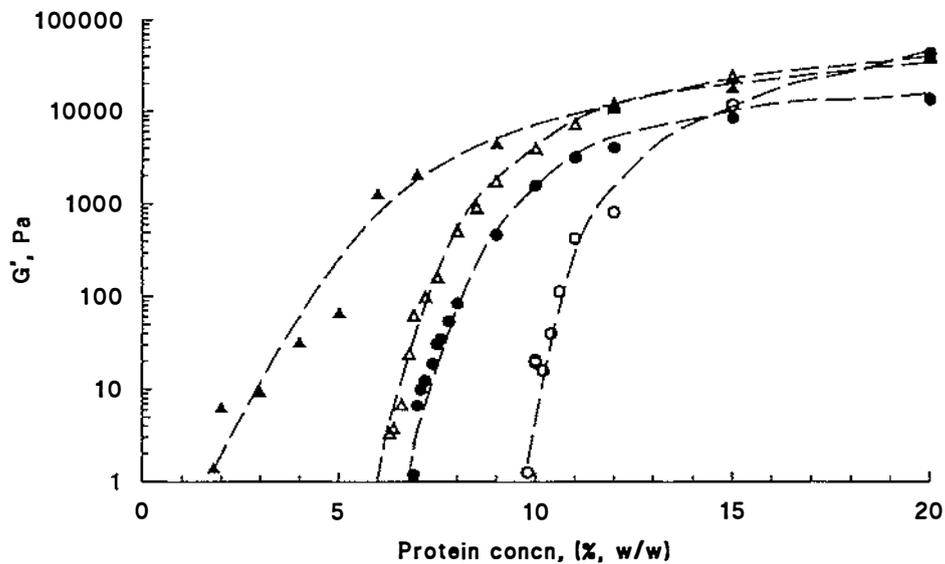


Fig 7.2 Storage modulus (G') after 11.11 h at 80°C versus protein concentration for WPI containing 0.01M added NaCl (○), WPC(B) (●), WPC(C) (Δ) and egg white (▲). Experiments were performed at pH7, 0.1Hz and 0.002 maximum shear strain.

7.3.3 Formation and cold stiffness of egg white and whey protein gels

Fig 7.3 shows the formation of gels from 12% protein solutions during heating at 80°C. WPI formed a very soft gel with very low G' values. The magnitude of G' at the end of heating increased in the order WPI < WPC(B) < WPC(C) < egg white < β -lactoglobulin. However, the initial rate of increase in G' was higher for egg white than for any of the whey proteins.

On cooling to 20°C all of the protein gels showed a substantial increase in G' (Fig

7.3). In order to determine whether the increase in G' owing to cooling was reversible or irreversible several cooling and heating cycles were conducted on the formed gels. The increase in G' on cooling egg white, WPC(B), WPC(C) and WPI gels was found to almost be completely reversible; egg white results are shown in Fig 7.4. This reversibility is similar to that observed by Beveridge *et al.* (1984); the phenomenon is most likely due to the forming and breaking of hydrogen bonds on cooling and heating respectively.

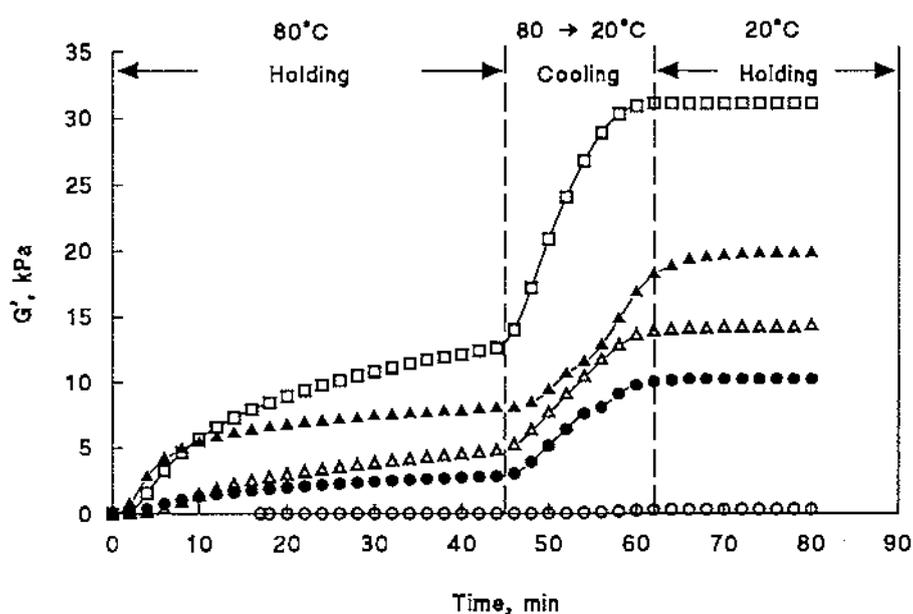


Fig 7.3 Storage modulus (G') versus time for solutions of WPI (○), WPC(B) (●), WPC(C)(△), egg white (▲) and β -lactoglobulin (□) containing 12% protein during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain.

The ratio of storage modulus at 80°C to that at 20°C is plotted as a function of protein concentration in Fig 7.5. $G'(80^\circ\text{C})/G'(20^\circ\text{C})$ was higher for egg white than for either of the WPCs below 16% protein. The ratio is probably important in the use of gelling proteins in bakery products.

7.3.4 Effects of pH

WPC(B) gels were stiff and opaque at pH7 and gradually changed to transparent, elastic soft gels as pH was increased from 7 to 9. Between pH4 and pH7 white particulate gels or coagula were formed. At and below pH4, brittle, sticky gels were formed.

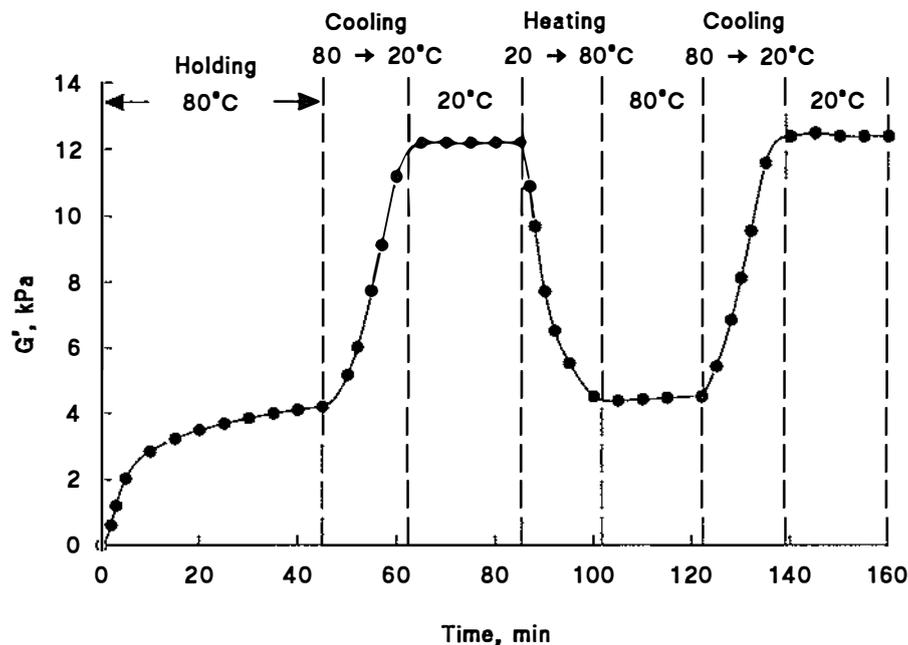


Fig 7.4 Storage modulus (G') of an egg white solution containing 12% protein versus time during heating and cooling cycles at pH7, 1Hz and 0.01 maximum shear strain.

WPI gels exhibited a maximum gel stiffness at about pH5.5. Between pHs 4 and 6 WPI gels were white, particulate and sponge-like. These gels were very porous in appearance and lost moisture very readily on gentle squeezing without fracturing, yet they had a high stiffness (high G'). WPI gels were transparent, elastic and soft between pHs 6 and 7. No gel formed above pH7. Below pH4 transparent or semi-transparent, brittle sticky WPI gels were formed.

Egg white gels exhibited maximum G' at pH4. Below pH4, brittle, sticky gels were formed. Between pH4 and pH5 strong, particulate white gels were formed. Strong, elastic

white gels were formed at and above pH6.

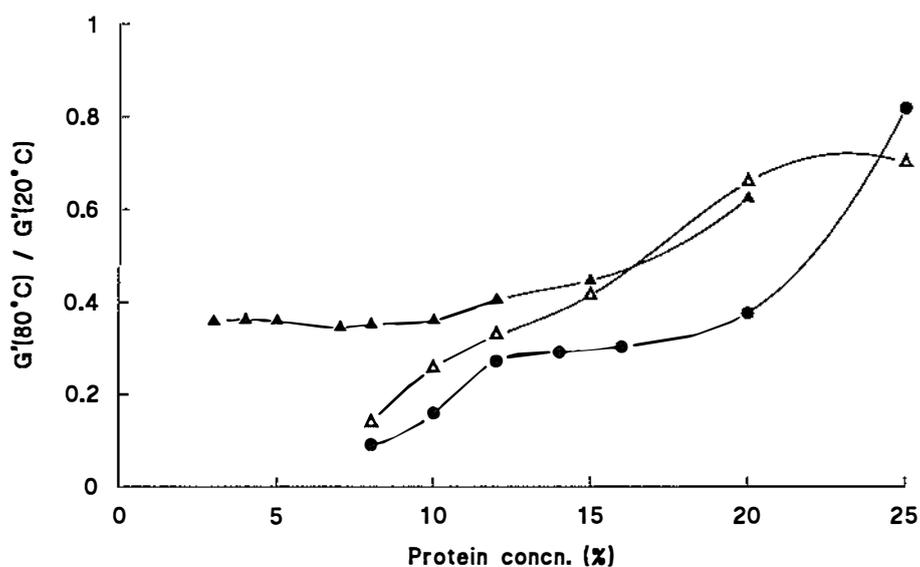


Fig 7.5 Effects of protein concentration on $G'(80^\circ\text{C}) / G'(20^\circ\text{C})$ for WPC(B) (●), WPC(C) gels (Δ,) and egg white gels (▲) after heating at 80°C and pH7 for 45 minutes, with subsequent cooling to 20°C . Measurements were performed at 1Hz and 0.01 maximum shear strain.

7.3.5 Effects of salts

The effects of KCl concentration on G' of WPC(B) gels are shown in Table 7.1. Small additions of salt caused dramatic increases in G' . However, a maximum G' was reached at about 0.1M KCl, and G' then decreased with further increase in salt concentration. $G'(80^\circ)/G'(20^\circ\text{C})$ also reached a maximum value of 0.30 at about 0.1M KCl. However, this is lower than the $G'(80^\circ\text{C})/G'(20^\circ\text{C})$ of 0.36 for egg white at 10% protein.

Table 7.2 shows the effects of different anions and cations on the G' of WPI gels. Every added salt caused a marked increase in the G' of WPI gels. Divalent (Ca^{2+} , Mg^{2+}) and trivalent (Fe^{3+}) cations had a much bigger effect on G' than monovalent cations (Na^+ , K^+).

Cations listed in increasing order of effectiveness were $\text{Na}^+ < \text{K}^+ < \text{Ca}^{2+} < \text{Mg}^{2+}$. This order follows the Hofmeister or lyotropic series except that Na^+ follows K^+ in that series. $G'(80^\circ\text{C})/G'(20^\circ\text{C})$ tended to be higher for stiff gels than for weak gels.

Table 7.1 Effects of KCl on the storage modulus (G') of WPC(C) gels.^a

KCl concn. (M)	$G'(80^\circ\text{C})^b$ (kPa)	$G'(20^\circ\text{C})^b$ (kPa)	$G'(80^\circ\text{C}) / G'(20^\circ\text{C})$
0	0.93	3.7	0.25
0.01	1.4	4.9	0.27
0.05	2.6	9.1	0.29
0.09	3.3	11.5	0.28
0.1	3.8	12.8	0.30
0.12	3.6	12.6	0.29
0.15	2.7	10.3	0.26

^a WPC(B) solutions containing 10% protein were heated at 80°C and pH7 for 45 min to form gels.

^b G' was measured at 1Hz and 0.01 maximum shear strain.

Dialysis of a WPC(C) solution to reduce its mineral content caused it to gel much more slowly, and the gel formed had a much lower G' value than was observed without dialysis (Fig 7.6). However, addition of 0.1M NaCl to the dialysed protein solution caused a large increase in the rate of gelation and of the final G' . Manipulating salt content is clearly a good method of controlling whey protein gel properties. The final G' values of WPC(C) and WPI gels could be made, respectively, equal to and greater than that of egg white by the addition of 0.1M KCl (Fig 7.7). Further, the initial gelation rate of WPI with added salt was very close to that of egg white.

Table 7.2 Effect of salt on storage modulus of WPI gels.^a

Salt	Concn (M)	G'(80°C) ^b (kPa)	G'(20°C) ^b (kPa)	G'(80°C) / G'(20°C)
-	0	0.01	0.32	0.03
NaCl	0.01	0.24	2.0	0.12
KCl	0.01	0.70	3.3	0.21
CaCl ₂	0.01	0.73	4.7	0.16
MgCl ₂	0.01	4.0	14.5	0.28
FeCl ₃	0.01	3.9	12.1	0.33
CH ₃ COONa	0.01	0.16	1.8	0.09
CH ₃ COOK	0.01	0.28	2.1	0.13
(CH ₃ COO) ₂ Mg	0.01	6.5	20.2	0.32
FeC ₆ H ₅ O ₃ ^c	0.01	1.3	4.1	0.32
Na ₃ C ₆ H ₅ O ₃ ^c	0.0033	0.04	0.76	0.05
K ₃ C ₆ H ₅ O ₃ ^c	0.0033	1.5	4.7	0.31
Na ₂ HPO ₄ ^d	0.005	0.06	0.88	0.07
K ₂ HPO ₄ ^d	0.005	1.6	4.5	0.34

^a WPI solutions containing 12% protein were heated at 80°C and pH7 for 45 min to form gels.

^b G' was measured at 1Hz and 0.01 maximum shear strain.

^c Citrate.

^d Orthophosphate.

7.3.6 Effect of lactose

Addition of lactose to WPC(C) solutions containing 12% protein at pH7, followed by heating at 80°C for 45 min, produced somewhat weaker gels than when lactose was absent.

Addition of 10% lactose produced gels with G' 25% lower at 20°C and 47% lower at 80°C than the corresponding values for gels without lactose added.

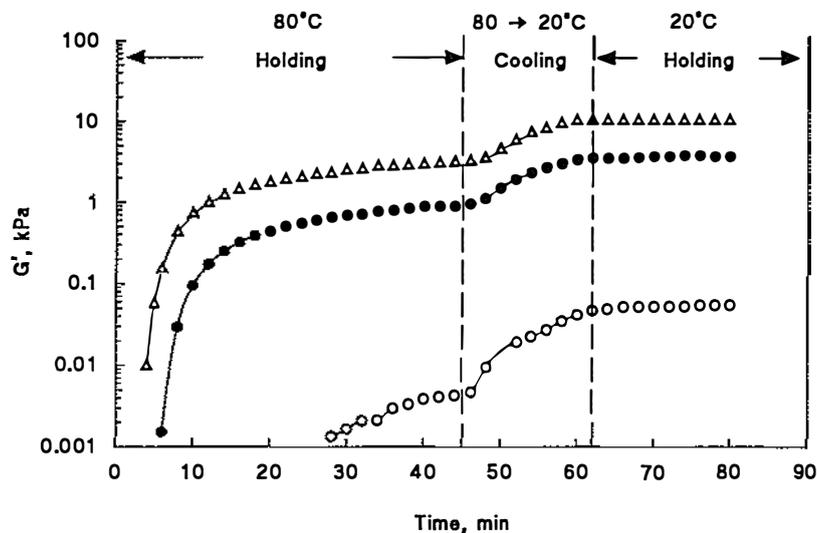


Fig 7.6 Storage modulus (G') versus time for solutions of WPC(C) containing 10% protein during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain. WPC(C) (●), WPC(C) after 12 h dialysis against distilled water (○) and WPC(C) after 12 h dialysis against distilled water followed by 0.1M NaCl addition (△).

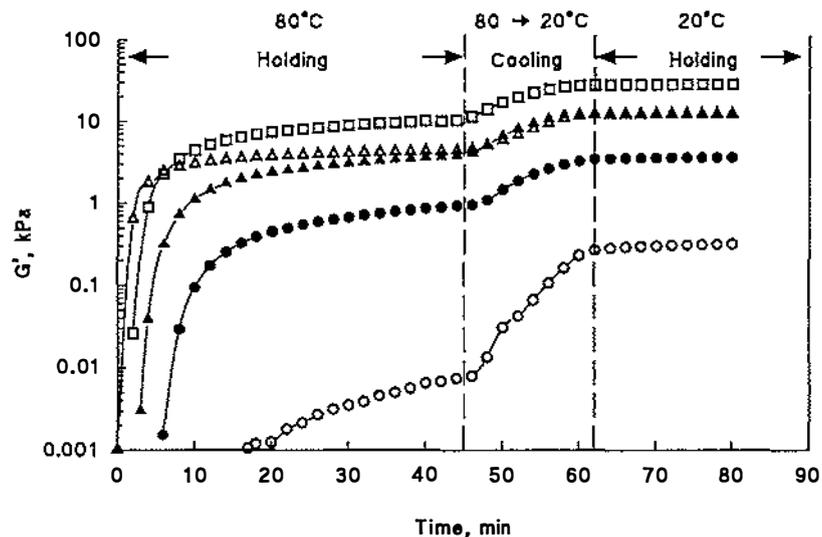


Fig 7.7 Storage modulus (G') versus time for 10% protein solutions during heating and subsequent cooling at pH7, 1Hz and 0.01 maximum shear strain. WPI (○), WPC(C) (●), egg white (△), WPC(C) with 0.1M KCl addition (▲), and WPI with 0.1M KCl addition (□).

7.4 Discussion

The least heat-stable proteins in egg white are conalbumin, ovalbumin and the G - globulins, with denaturation temperatures of 57.3°C, 71.5°C and 72°C (Froning, 1988). Of the whey proteins α -lactalbumin has the lowest denaturation temperature (62°C) but it is the whey protein most thermostable against aggregation because denaturation is highly reversible (de Wit and Klarenbeek, 1984). The other major whey proteins have denaturation temperatures of 64°C (bovine serum albumin), 72°C (immunoglobulin) and 78°C (β -lactoglobulin) (de Wit and Klarenbeek, 1984). When binary egg protein mixtures are heated denaturation occurs near the denaturation temperature of the least heat-stable protein (Froning, 1988). Since egg white, WPI and WPC are multicomponent protein mixtures their heat gelation might be expected to begin near the denaturation temperature of the least heat-stable protein in the mixture. Egg white would thus be expected to begin to gel at a lower temperature than WPC. Fig 7.1 indicates only a 10°C difference in denaturation temperature between egg white proteins and whey proteins, whereas Melachouris (1984) and Kinsella and Whitehead (1989) quote a 20°C difference in gelation temperature.

The two major egg white proteins, ovalbumin (45,000 Da) and conalbumin (76,000 Da) (together comprising 66% of egg white protein), have much higher molecular weights than the two principal whey proteins, α -lactalbumin (14,200 Da) and β -lactoglobulin (18,600 Da) (together comprising 80% of whey protein) (Froning, 1988; Kinsella and Whitehead, 1989). Linear polymers of high molecular weight form stronger gels, and gel at lower concentrations, than low molecular weight polymers. The differences in molecular weights may be one reason why egg white proteins are able to form gels at much lower concentrations than whey proteins.

The complex variation in physical appearance and rheological properties of WPC gels with pH has been discussed in detail by Stading and Hermansson (1991) and Tang *et al.* (1993b). The complex behaviour was attributed to variations in electrostatic interactions and disulphide bonding with pH. At certain pH values there are interactions resulting in maximum gel stiffness.

It is well established that salts have a major influence on the properties of whey protein gels. Increasing levels of either NaCl or CaCl₂ cause increases in gel hardness, gel shear stress and other rheological properties until maximum values of these properties are reached; values then decrease with higher salt concentrations (Schmidt *et al.* 1978; 1979;

Mulvihill and Kinsella, 1988; Kuhn and Foegeding, 1991). The same pattern has been observed for the effects of NaCl and CaCl₂ concentration (expressed as added ionic strength) on G' as shown in Chapter 6, and was observed here for the effect of KCl concentration on G' (Table 7.1). A maximum in such a gel property can be attributed to an optimum balance between protein-protein and protein-solvent interactions at a particular salt concentration as discussed in Chapter 6. Divalent cations had a much bigger effect on gel properties than monovalent cations. Kuhn and Foegeding (1991) showed in detail that a range of divalent cations (Ca, Mg, Ba) all caused a similar increase in shear stress and shear strain at failure of WPI gels, and that the increase in shear stress at failure was much larger than that caused by a range of monovalent cations (Na, Li, K, Rb, Cs).

The thermal coagulation of whey proteins can be effectively inhibited by various sugars including lactose (Garrett *et al.* 1988). This inhibition would cause a decrease in the G' values of formed WPC gels. Garrett *et al.* (1988) showed that sucrose promoted the denaturation of whey proteins but inhibited their subsequent coagulation.

Whey proteins are often suggested as a replacement for egg white in foods where heat gelation is required, for example bakery products. Five major differences have been demonstrated here between the gelation properties of egg white and those of whey proteins. At a given protein concentration egg white has a higher initial gelation rate, a higher gel stiffness, a lower gelation temperature, and a higher value of the ratio G' (80°C)/G' (20°C) (at < 16% protein). Further, egg white has a much lower minimum protein concentration for gelation. Whey protein solutions can be made roughly equivalent to egg white in terms of initial gelation rate and gel stiffness by increasing their salt contents. However, further development work is needed to produce whey protein preparations which match egg white in terms of the other three properties listed. With respect to these three properties, the use of whey protein causes particular difficulties in the manufacture of some types of cake, *e.g.* angel food cake (Melachouris, 1984; Kinsella and Whitehead, 1989). At the lower temperatures needed for this type of cake the whey protein gel network is slow to form. Also, the network suffers from low strength at high temperatures allowing loss of gas from the cake structure during the later stages of baking and during cooling. The result is a low cake volume and/or collapse of the cake (especially at its centre) on cooling.

It is commercially important to produce WPCs and WPIs with consistent functionality. However, commercial WPCs and WPIs have been reported to be highly variable in

functionality (Kinsella and Whitehead, 1989; Morr and Foegeding, 1990). Morr (1992) suggested various approaches that could be taken to reduce the wide variability in the properties of commercial WPCs. From this present work and other studies it is clear that careful control of WPC mineral content, particularly divalent and trivalent cation content, is crucial if consistent gelling properties are to be obtained.

7.5 References

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8. Gelation of Whey Proteins: Critical Behaviour of Rheological Properties at the Sol-Gel Transition

8.1 Introduction

Gelation, which involves a phase transition from a liquid state to a solid state, is understood as a critical phenomenon. The sol-gel transition of a polymer during the gelation process can be described by the classical theory of Flory and Stockmayer and by the percolation model as discussed in Chapter 1. Dynamic rheological properties of the critical gel at the gel point have a power law relationship with frequency and this behaviour is attributed to the fractal nature of the critical gel (Vilgis and Winter, 1988).

A power law dependence of the elastic shear modulus on concentration, as $G \propto C^{s'}$, has been proposed and observed for both polymer solutions and gels as discussed in Chapter 2. For polymer gels, the exponent is $s' = 2$ for gelatin (Ferry, 1948, 1980), $s' = 3\sim 4$ for polyvinyl chloride (Ferry, 1980), $s' = 5$ for soybean globulins (Bikbow *et al.* 1979), $s' = 3.4$ for soybean 11S protein (Kohyama, *et al.* 1992), $s' = 4$ for casein (Tokita *et al.* 1985), $s' = 4.1$ for whey protein isolate (Vreeker *et al.* 1992), and $s' = 2\sim 7$ for other biopolymer gels (Clark, 1989).

The major components of whey proteins are β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulin and proteose-peptone as shown in Table 1.2 (Kinsella and Whitehead, 1989). Whey protein solutions can form a three dimensional gel structure above a certain protein concentration when heated above their denaturation temperatures. The aim of this study was to investigate the dynamic rheological properties near the sol-gel transition during whey protein gelation. A power law relationship between dynamic rheological properties and frequency is expected at the sol-gel transition if the critical whey protein gel has a fractal geometry. The applicability of the percolation theory to gelation of whey proteins, and the relationship between storage modulus G' and protein concentration, are also investigated.

8.2 Experimental Procedure

Commercially available WPC(B), WPC(C) and WPI powders were used in this study; their compositions are shown in Tables 3.1 and 3.2. The preparation of whey protein solutions, instrument configuration, sample handling and data collection were as described in

Chapter 3. Concentration is expressed as protein concentration rather than as total solids concentration in this study.

8.3 Results

8.3.1 Time dependence of dynamic rheological properties

A typical gelation curve for WPI is shown in Fig 8.1. This is similar to that of a synthetic polymer (Tung and Dynes, 1982). Both storage modulus G' and loss modulus G'' increased with heating time, but G' rose more sharply than G'' . Initially, $G'' \gg G'$, and viscous behaviour predominated; after about 5 minutes heating, and toward the end of the experiment, $G' \gg G''$ and elastic behaviour predominated. Correspondingly, the phase angle fell continuously with heating time, indicating that the elastic character increased while the viscous character decreased during the gelation process. The crossover of G' and G'' was clear-cut; this crossover marks, for some gelling systems, the transition from a sol to a gel (Winter, 1987b).

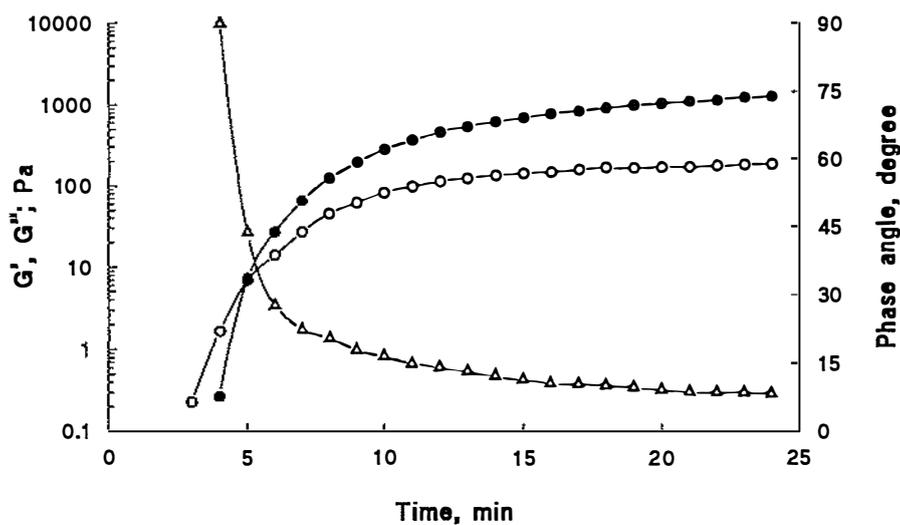


Fig 8.1 Loss modulus G'' (\circ), storage modulus G' (\bullet) and phase angle (Δ) as a function of heating time during thermal gelation of a WPI solution containing 13% protein and 0.01M NaCl at 80°C, pH7, 1Hz and 0.002 shear strain.

The crossover of G' and G'' is not always so clear-cut. When the gelation rate was

increased by decreasing pH towards the isoelectric point, the crossover of G' and G'' became less obvious, especially at lower pH (Fig 8.2). At pH6 the crossover point had almost disappeared and an expanded time scale was required to judge the crossover point. The same effect on crossover was observed when gelation rate was increased by increasing either protein concentration or salt content or both.

If the crossover point happens inside the "noise region" of the instrument it cannot be observed because of the fluctuation of experimental readings, as observed by Stading and Hermanson (1990) and mentioned in Chapter 5. This is usually the case when dynamic rheological measurement is applied to the study of whey protein gelation at low protein concentration, or high salt content, or when measurements were performed at low frequency and low shear strain. High salt content leads to a high gelation rate, while low protein concentration leads to a low density of structure both before and after the gel point. In these cases the crossover is masked by noise. In order to observe the crossover point of G' and G'' , a sample with both high protein concentration and low salt content is required; WPI seems to be a good material for such an experiment.

In gelation experiments the crossover of G' and G'' was unmistakable for WPI, as shown in Fig 8.1, but was not so evident for WPC(C), and could not even be recognized for WPC(B). The main reasons are probably that both WPC(B) and WPC(C) have high salt contents and also contain other non-protein components. They therefore gelled much faster than WPI. The WPC(B) had the highest percentage denatured protein, its gel structure was weak and dilute in the neighbourhood of the sol-gel transition, and the crossover of G' and G'' was not clear-cut.

8.3.2 The capture of the G' and G'' crossover point

It was thought that it would be interesting to conduct dynamic rheological measurements such as frequency sweeps and stress relaxation experiment on the gelling sample at the point when G' and G'' crossover *i.e.* when $G'=G''$. In the gelation of some polymers this can be done by interrupting the gelation experiment when G' and G'' start to crossover and then conducting the frequency sweeps or stress relaxation experiments. The change in the sample during the frequency sweep is considered to be negligible if the sol-gel transition is very slow since the duration of a frequency sweep is very short compared to the total gelation time (Chambon *et al.* 1986). However, in the case of thermal protein gelation

in the Bohlin rheometer the change in the sample during a frequency sweep would not normally be negligible.

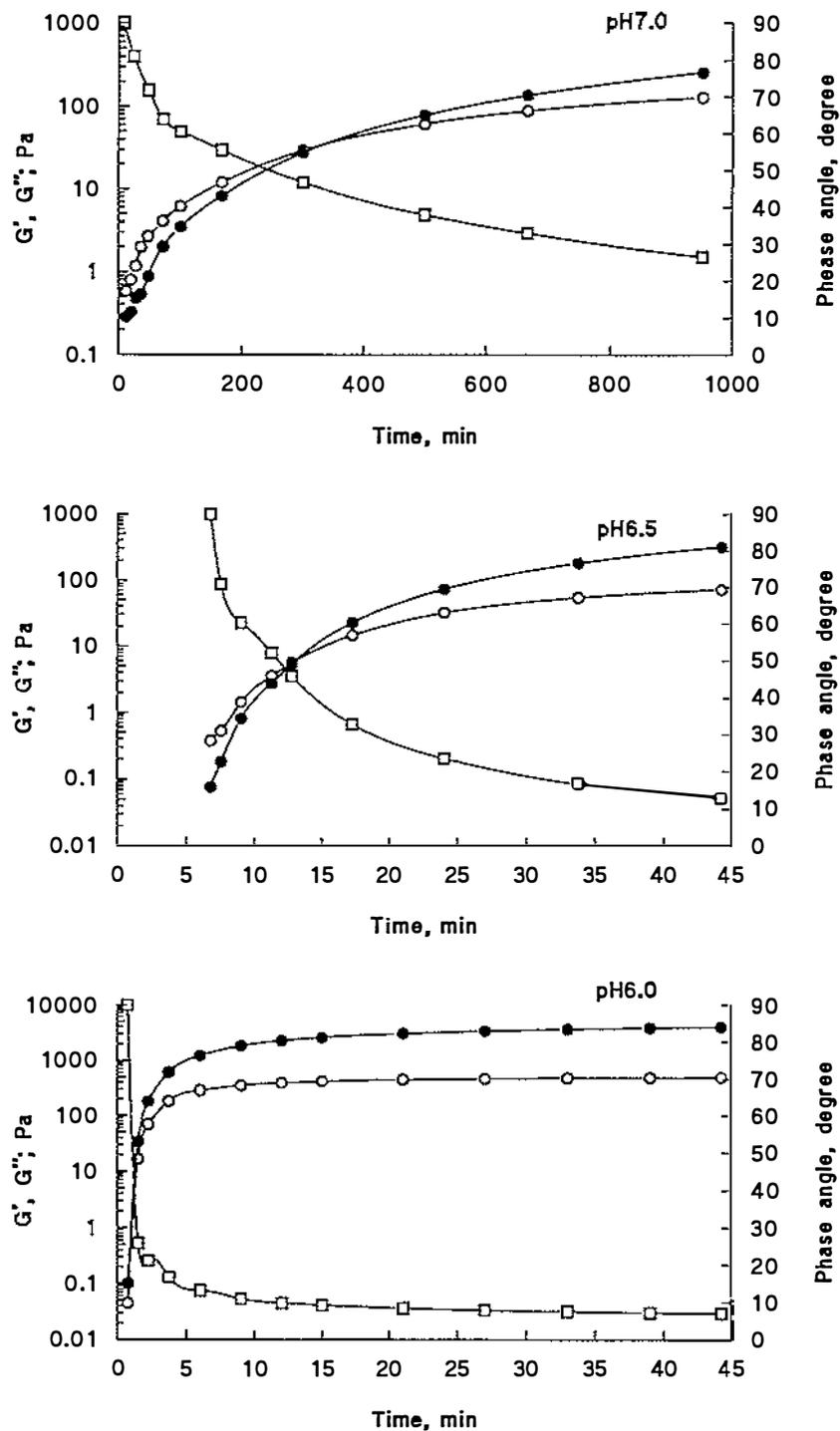


Fig 8.2 Effects of pH on the increases in G'' (\circ) and G' (\bullet), and the fall in phase angle (\square) with heating time for a WPI solution containing 12% protein at 80°C, 1Hz and 0.002 shear strain.

After about 45 minutes heating of a 12% WPI solution at 80°C the viscous behaviour still predominated ($G'' > G'$) as shown in Fig 8.3. Then, the sample was cooled in the rheometer from 80°C to 20°C by adjusting the rheometer waterbath temperature to 20°C. Both G' and G'' increased, crossed over and finally levelled off. The elastic behaviour predominated at the end of the experiment ($G' > G''$). The increases in G' and G'' is mainly the result of the build-up of hydrogen bonds which in turn is due to the decrease in temperature; the effect is completely reversible as described in Chapter 7. The final values of G' and G'' are dependent on the cooling temperature. If a suitable cooling temperature was chosen, then G' and G'' could be made to cross over and then level off at the same value, as shown in Fig 8.4. The gelling state where G' equalled G'' was therefore finally achieved in this way.

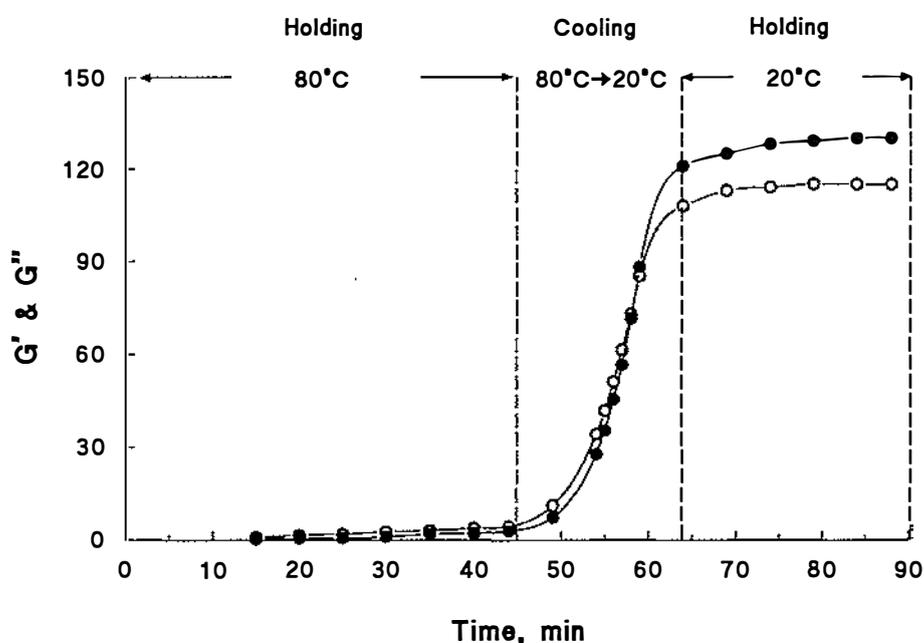


Fig 8.3 Effects of heating at 80°C, and subsequent cooling from 80 to 20°C, on G'' (○) and G' (●) as a function of time for a WPI solution containing 12% protein at 1Hz, 0.002 shear strain and pH7.

This method was suitable only for WPI in this study. For WPC(B) or WPC(C), a solution with a much lower protein concentration was required for heating at 80°C for a period of time without gelation. The sol or gel near the gel point produced by cooling the heated solution was then so dilute that the instrument could not give accurate measurements of G' and G'' in the frequency sweep experiment described in Section 8.3.3; very noisy data were produced. This method should thus be applied only to whey protein samples with very low salt contents and high protein concentrations.

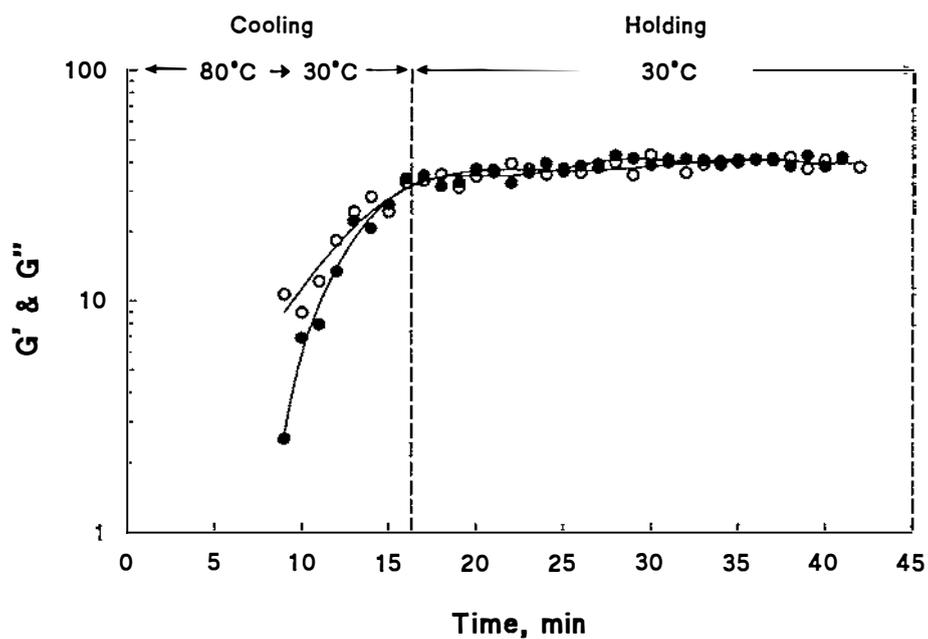


Fig 8.4 Effects of cooling time on G'' (○) and G' (●) of a WPI solution containing 12% protein at 0.1Hz and 0.002 shear strain when temperature was decreased from 80°C to 30°C and held at 30°C until G' and G'' reached constant values. Before cooling, the sample was heated at 80°C, pH7, 0.1Hz and 0.002 shear strain for 45 minutes.

8.3.3 Frequency sweep and strain sweep measurements

Frequency sweeps were performed on WPI samples after heating at 80°C for 45

minutes and then cooling to the final temperature of 30°C. Results are shown in Figs 8.5 and 8.6. For samples with 12% protein concentration and 0M salt addition (Figs 8.5 and 8.6), the frequency sweep indicated that $G' = G''$ over almost four decades of frequency and that each had a power law relationship with frequency of the form G' or $G'' \propto \omega^n$; $n=0.55$ for G' ($R^2=1$) and $n=0.51$ for G'' ($R^2=0.99$). For protein concentrations above 12% or NaCl addition above 0M the G' curve lay above the G'' curve, and both properties tended to become less frequency dependent. For clarity, only G' has been plotted for some experiments. Several such frequency sweeps were performed on samples at the gelling state $G' = G''$. The results are given in Table 8.1.

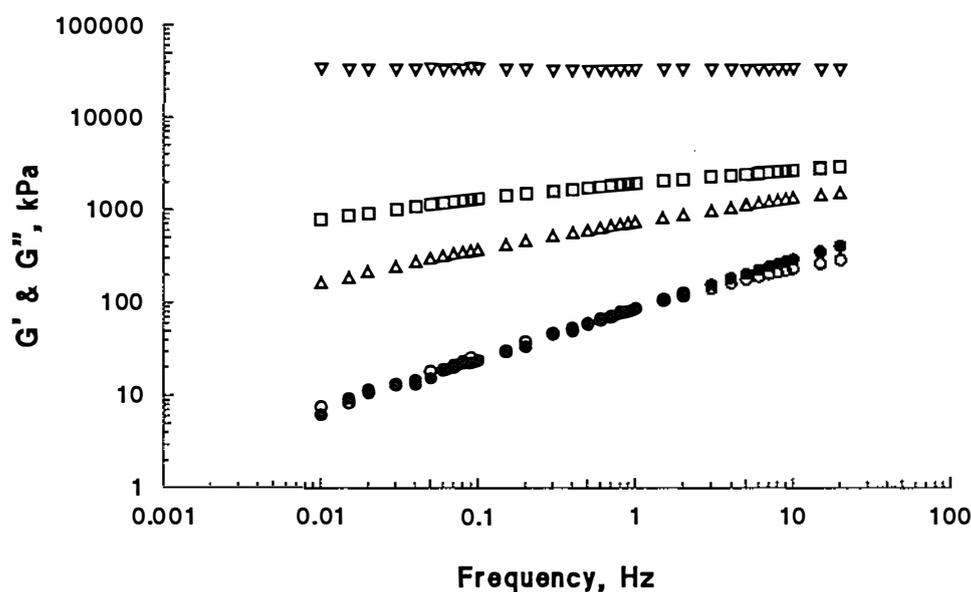


Fig 8.5 Effects of frequency on G' and G'' of WPI gels at 30°C, 0.002 shear strain and with different protein concentrations. G'' (\circ) & G' (\bullet), 12% protein; G' (Δ), 12.5% protein; G' (\square), 13% protein; and G' (∇), 20% protein. The WPI gels were formed by cooling after heating at 80°C for 45 minutes.

Strain sweep experiments, the method as illustrated in Fig 5.2 of Chapter 5, were also performed on the critical gels, and the results (Table 8.2) indicated that the strain used (0.002)

was inside the linear viscoelastic region. The critical gels at the gel point had the narrowest linear viscoelastic region. As gelation progressed the linear viscoelastic region became wider. However, from previous experiments as shown in Chapter 5 it seems that the results of gelation experiments (G' versus t) were not affected significantly provided strains used in measuring the properties of final gels were inside the linear viscoelastic region. When gelling is fast, the linear viscoelastic region quickly becomes wider, and the curve of G' versus t is not affected significantly even if the strain during the sol-gel transition is above the linear region of the critical gel.

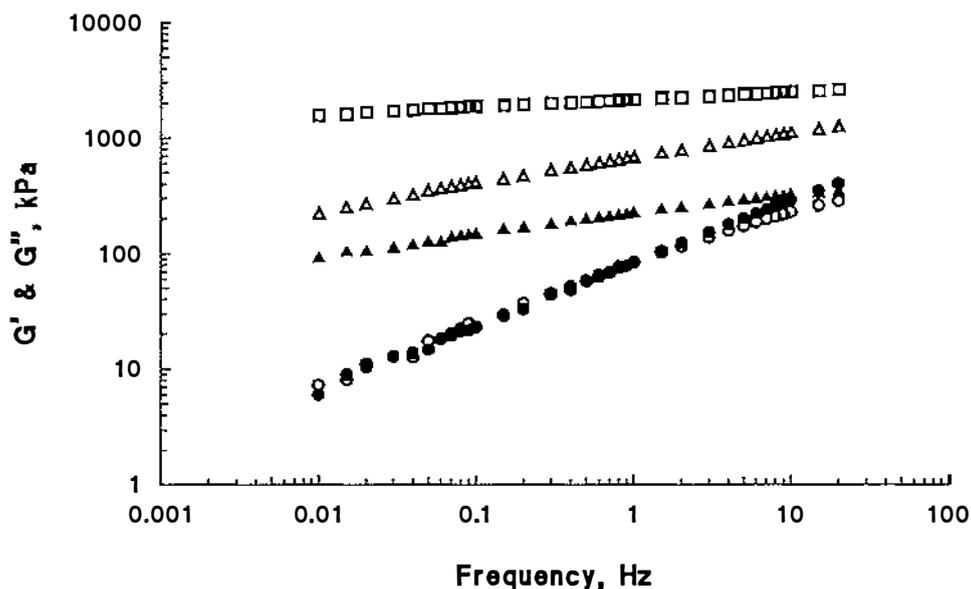


Fig 8.6 Effects of frequency on G' and G'' of WPI gels containing 12% protein at 30°C, 0.002 shear strain and with different NaCl concentrations. G'' (\circ) & G' (\bullet), 0M NaCl; G' (Δ) & G'' (\blacktriangle) 0.05M NaCl; and G' (\square), 0.01M NaCl. The WPI gels were formed by cooling after heating at 80°C for 45 minutes.

8.3.4 Gelation time versus protein concentration

By assuming the equilibrium shear modulus (G_e) was proportional to the reciprocal of the gelation time (t_g), Ross-Murphy (1991a, b, c, d) proposed the following model of

biopolymer gelation, which relates t_c with the polymer concentration (C):

$$t_c \approx K / \{[(C/C_0)^\phi - 1]^\Delta\} \quad (8.1)$$

where K is a proportionality constant, C_0 is the critical polymer concentration for gel formation, ϕ is the number of chains involved in a junction zone or the kinetic order of gelation, and Δ is a critical exponent.

Table 8.1 n values of frequency sweep tests in the vicinity of sol-gel transition for WPI solutions containing 12% protein at 0.002 shear strain.

No	Temp. (°C)	Salt concn. (mM)	n for G'	n for G''	R ² for G'	R ² for G''
1	30	0	0.55	0.50	1	1
2	30	0	0.56	0.53	1	0.99
3	30	0	0.54	0.52	1	0.99
4	50	0.625	0.52	0.49	1	1
5	50	0.625	0.53	0.49	1	1

Table 8.2 Results of strain sweep tests in the vicinity of sol-gel transition for WPI solutions containing 12% protein at 1 Hz.

NO	Temp. (°C)	Salt concn. (mM)	linear viscoelastic region (strain)
1	30	0	0 - 0.01517
2	30	0	0 - 0.01307
3	30	0	0 - 0.01547
4	50	0	0 - 0.00939

Critical protein concentrations, C_o , were measured in the way described in Chapter 5. The critical protein concentrations obtained were $C_o=6.6\%$ for WPC(B), $C_o=9.5\%$ for WPI and $C_o=6.1\%$ for WPC(C). Gelation time (t_c) was taken as the time of heating (after the 2 min temperature equilibration period) required for the crossover of G' and G'' (*i.e.* for the phase angle to drop to 45°). Steventon *et al.* (1991) applied the percolation model to the gelation of WPC and found that the gelation data could be satisfactorily described by a second order kinetic model. Thus, it can be assumed $\phi=2$. Then, equation 8.1 can be written as:

$$1/t_c = K' [(C/C_o)^2 - 1]^\Delta \quad (8.2)$$

where $K' = 1/K$.

Power law relationships of this form were obtained, with the critical exponent $\Delta=1.97$ ($R^2=0.95$) for WPI, $\Delta=2.11$ ($R^2=0.94$) for WPC(B) and $\Delta=1.96$ ($R^2=0.97$) for WPC(C). Data for WPI and WPC(C) only are shown in Fig 8.7. The results indicated that the gelation equation developed by Ross-Murphy (1991a, b, c, d) could satisfactorily be applied to whey protein gelation.

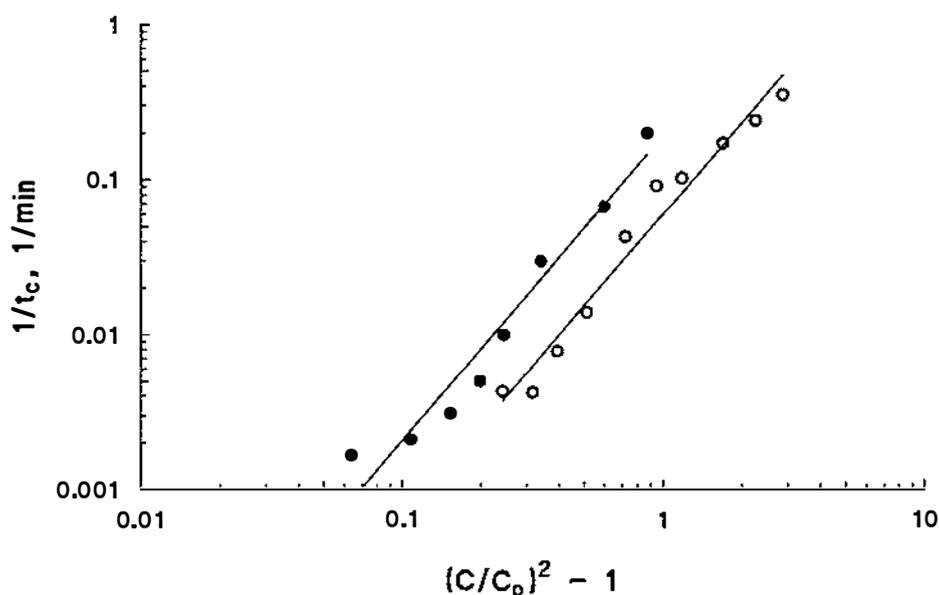


Fig 8.7 $1/t_c$ versus $[(C/C_o)^2 - 1]$ for WPC(C) (○) and WPI (●) at 80°C , pH7, 0.1Hz and 0.002 shear strain.

8.3.5 Storage modulus versus protein concentration

Gelation data for whey proteins did not show a power law relationship between storage modulus G' and protein concentration C . Rather, a power law relationship between G' and $(C-C_m)$ was obtained for WPC(B), WPC(C) and WPI. Data for WPC(B) and WPI only are shown in Fig 8.9 (for the reasons of clarity). The exponent was 2.24 for WPC(B) ($R^2=0.98$), 2.26 for WPI ($R^2=0.98$) and 2.54 for WPC(C) ($R^2=0.98$). C_m was the minimum protein concentration for gel formation in 667 minutes at 80°C , and was measured as shown in Fig 8.8. Exponents in the range 1.7-3 were obtained for WPC(B), WPC(C) and WPI depending on the heating time.

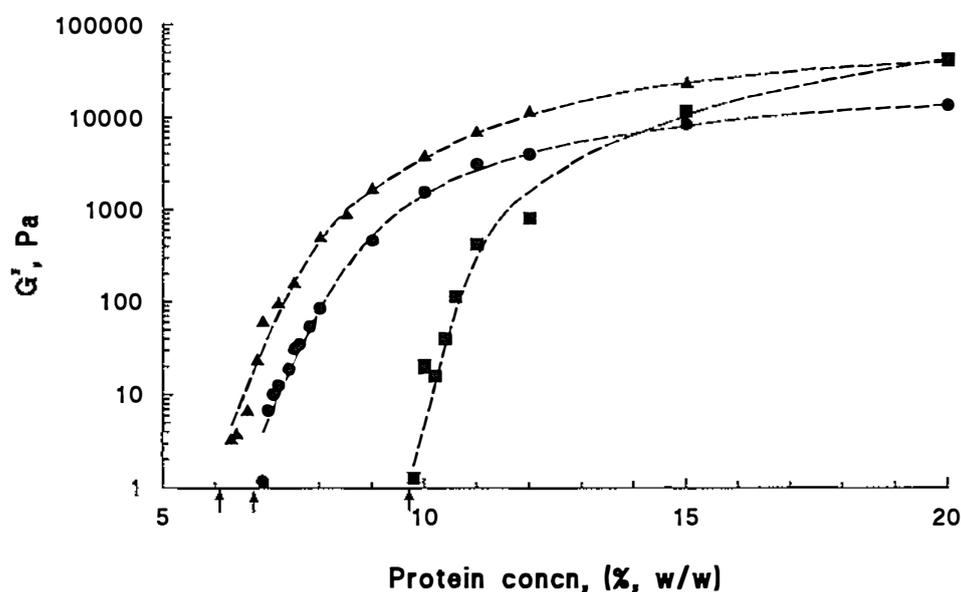


Fig 8.8 G' versus protein concentration for whey protein gels of WPC(B) (●), WPC(C) (▲) and WPI with 0.1M NaCl (■) after heating sample solutions at 80°C , 0.1Hz, pH7 and 0.002 shear strain for 667 minutes. G' was measured at 80°C , 0.1Hz and 0.002 shear strain.

8.3.6 Percolation theory applied to gelation of whey proteins

According to bond percolation theory the power law relationship $G_e \sim (p/p_c - 1)^\Delta$ should

be obtained just above the gel point. However, the fraction of bonds p (p_c is the value of p at the gel point) is difficult, if not impossible, to measure. In some cases a reduced variable $(t/t_c - 1)$ can be used to replace $(p/p_c - 1)$, where t is the elapsed time after the beginning of the gelation experiment and t_c is the gelation time. It is assumed that p is proportional to the elapsed time t (Adam *et al.* 1985).

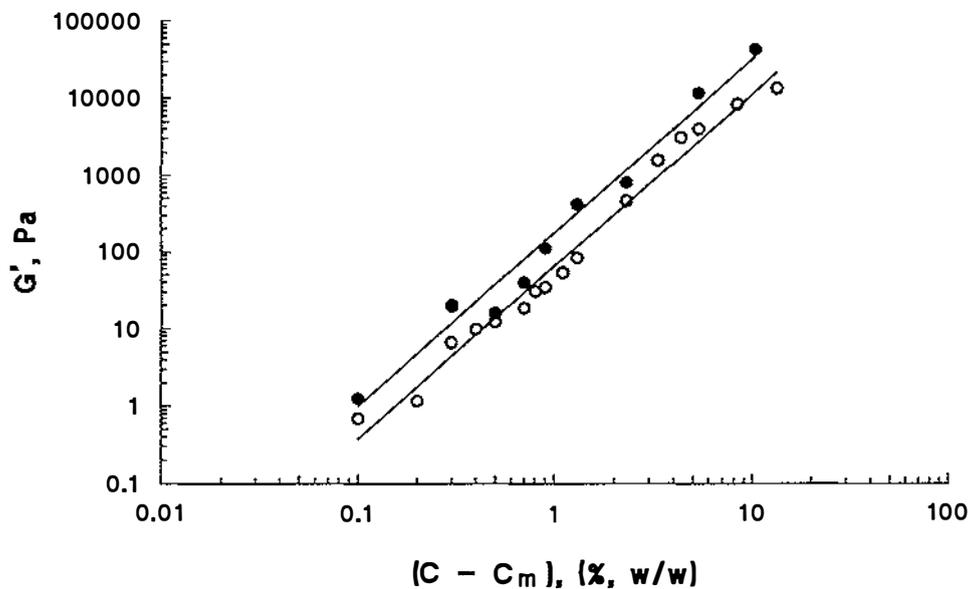


Fig 8.9 Data from Fig 8.8 for WPC(B) (○) and WPI (●) plotted in the form G' against $(C - C_m)$, where C_m is the minimum protein concentration for gelation, determined as indicated in Fig 8.8.

This analysis was applied to data for the gelation of WPC(B) and WPC(C), and a power law relationship was indeed obtained. However, the critical exponents found were 0.47 for a WPC(B) solution containing 7.9% protein at 80°C, and 0.68 for a WPC(C) containing 7.9% protein at 80°C. These values are far below those predicted by both percolation model and the classic theory. These results seemed to indicate that this analysis was not applicable to the gelation of whey protein concentrates. As pointed out by Tokita *et al.* (1985), p is not always proportional to the elapsed time t , the exact relationship depending on the mechanism

of gelation.

Site percolation theory can also be used to calculate the critical exponent. In this case it will be $G_e \propto (p'/p'_c - 1)^A$ where p' is the volume fraction of occupied sites and p'_c is the value of p' at the gel point. The protein concentration (% w/w) of whey protein solutions is proportional to p' as $C = (p'/d_s\mu_h) \times 100$ where d_s is the density of the whey protein solution (g/ml) and μ_h is the voluminosity (hydrated protein volume/dry protein mass) (Tokita *et al.* 1985). The derivation of this equation is given in Appendix 1. In the case of whey proteins, protein molecules are partially unfolded before the gel structure forms. The density of the whey protein solution (d_s) and the voluminosity of the hydrated whey protein (μ_h) change with changing protein concentration, but such changes may be considered negligible just above and near the gel point. Thus, $G_e \propto (C/C_o - 1)^A$. However, the gelation process has to be complete (heating time has to be infinite) in order for the pure site percolation theory to be applicable (Tokita *et al.* 1985).

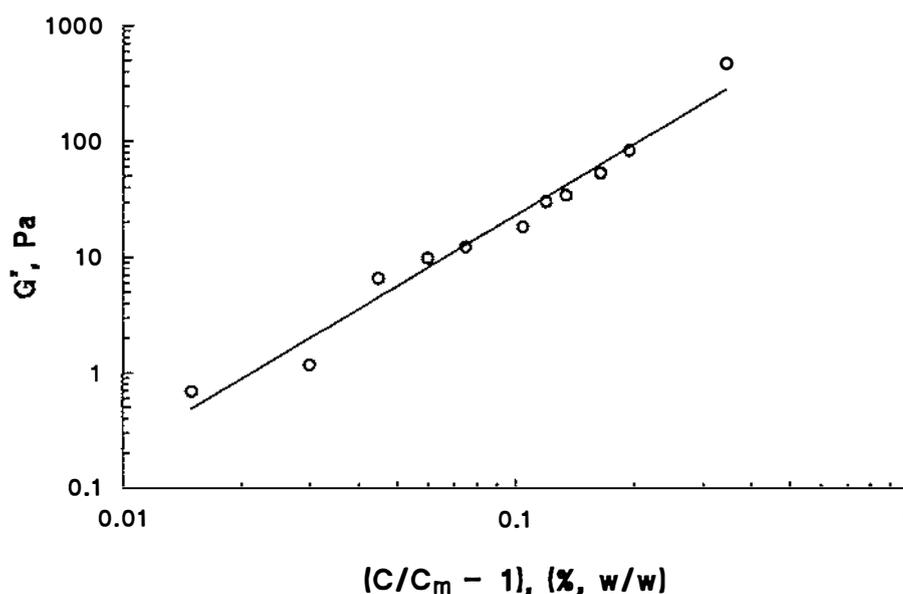


Fig 8.10 G' versus $(C/C_m - 1)$ after heating WPC(B) solutions at 80°C, pH7, 0.1Hz and 0.002 shear strain for 667 minutes. G' was measured at 80°C, 0.1Hz and 0.002 shear strain.

Fig 8.10 shows a power law relationship between G' and $(C/C_m - 1)$ for WPC(B) solutions after 667 minutes heating with an exponent value of 2.02 ($R^2=0.97$). Similar power law relationships were observed for different heating times, with the exponent decreasing slightly with increasing heating time. Values for the critical exponent Δ in the range 1.8–2.0 were obtained for WPC(B) by extrapolating to infinite heating time. At this time gelation was complete and $C_m = C_o$; the necessary conditions for a pure site percolation process were thus satisfied. (The frequency should also be extrapolated to zero to get the true critical exponent, but this minor correction was not made here). A range was quoted for Δ rather than an exact value has been because of the uncertainty introduced by extrapolation.

8.4 Discussion

The power law exponent of $n=1/2$ indicates that, for WPI, the crossover of G' and G'' is the sol-gel transition point according to the definition of Chambon and Winter (Chambon and Winter, 1985; Winter and Chambon, 1986; Chambon *et al.* 1986; Winter, 1987a, b). Gel formation by cooling from a high to a low temperature is probably mainly the result of a built-up of hydrogen bonds; the sol-gel transition is therefore of a physical nature (and thermo-reversible as discussed in Chapters 1 and 7). This gelation process is different from the heat-induced gelation of whey protein solution that occurs at 80°C. The gel produced in the latter case is thermo-irreversible; disulphide bonds, ionic bonds, hydrophobic interaction and hydrogen bonds are believed to play major roles in gel formation. The sol-gel transition of whey protein isolate that occur on cooling involves a large number of hydrogen bonds; the gelation process may be similar to stoichiometrically balanced chemical gelation in which a high degree of polymerization or cross-linking takes place, and where G' and G'' scale approximately with $n=0.5$. The sol-gel transition brought about by heating does not involve such a large number of hydrogen bonds. Therefore, it cannot be decided at present, in the case of heat-induced gelation, whether or not G' and G'' scale with the same power law exponent ($n = 0.5$) at their crossover point.

Reported power law exponents determined from frequency sweeps in the vicinity of sol-gel transition be in the range 0.2-0.9 (Winter, 1989; Muthukumar, 1989; Scanlan and Winter, 1991). In stoichiometrically balanced chemical gelation with excess crosslinker an exponent of the order of $n = 0.5$ is usually obtained, while a higher exponent, for example $n=0.70$, is obtained with stoichiometrically unbalanced chemical gelation in the presence of

the minimum amount of crosslinker necessary to achieve sol-gel transition. The percolation model predicts the exponent $n=0.7$ in three dimensional space (Martin *et al.* 1988; Muthukumar, 1989; Lairez *et al.* 1992). The discrepancies among reported exponents and their deviations from that predicted by the percolation model is attributed by some authors to either inadequately choosing the location of the gel point (Lairez *et al.* 1992) or a screening effect on viscoelasticity in the vicinity of the gel point (Muller *et al.* 1991) or both (Muthukumar, 1989). It is not clear whether or not the crossover of G' and G'' obtained by cooling in the present work is a true gel point since the exponent for G' is slightly higher than that for G'' ; this difference in exponents could be due either to inadequate location of the gel point or to experimental error. Nevertheless, the crossover of G' and G'' should be very close to the gel point for WPI if not exactly at it.

At the gel point, the critical polymer gel consists of a self-similar distribution of self-similar clusters of all sizes including a so-called infinite cluster which is fractal, as pointed out by Daoud and Martin (1989) as well as other workers (Stinchcombe, 1985). The fractal nature of the critical gel would lead to simple power law relationships between dynamic rheological properties and frequency (Vilgis and Winter, 1988). The simple power law relationship between G' and frequency for whey protein gels near the sol-gel transition suggests that they may have a fractal geometry.

A power law relationship did not exist between G' and protein concentration. This finding is consistent with the theoretical scheme for biopolymer gelation described by Clark (1991). Such a finding is not surprising since for globular proteins such as whey proteins there must exist a critical protein concentration C_o , or a minimum protein concentration C_m at a given heating time, below which a gel cannot form. Since $G' = 0$ when $C \leq C_o$ or $C \leq C_m$, the relationship $G' \propto C^{s'}$ is clearly inadequate. It is more likely that G' scales as $G' \propto (C-C_o)^{s'}$ or $G' \propto (C-C_m)^{s'}$. In the gelation of a long chain linear polymer C_o is very small and it can sometimes be assumed that $C_o \approx 0$; then, G' approximately scales as $G \propto C^{s'}$. In this study C_m rather than C_o is measured at 0.1Hz, and at a small strain which is inside the linear viscoelastic region. The results indicate that G' scales as $G' \propto C_e^{s'}$ where $C_e = (C-C_m)^{s'}$ and C_e is the effective protein concentration.

Both the exponent calculated from Ross-Murphy's equation (equation 8.1) and the exponent determined from site percolation theory, seem to be in a good agreement with the predictions of the percolation model (equation 1.5 and Table 1.4). Although this model thus

seems successful in describing the sol-gel transition of whey protein solutions in this study, it remains very crude representation of any gelation process (de Gennes, 1979). Clearly, during heat-induced gelation, the whey protein molecules are not on a lattice but are disordered, and in irregular thermal movement in the solvent (water). Further, protein-protein and protein-water interactions co-exist during gel formation, while the percolation model is a lattice model without solvent (de Gennes, 1979). Competition between gelation and precipitation is involved in the sol-gel transition process (de Gennes, 1979). When pH is near the isoelectric point or when there is a high concentration of salts, the precipitation tendency may be dominant, and deviation from the percolation model may well occur.

8.5 References

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9. Comparisons between Small Deformation, Large Deformation and Failure Properties of WPC gels

9.1 Introduction

Rheological methods used for food texture measurement can be divided into three classes (Szczesniak, 1963): fundamental tests such as compression, tension, dynamic shear, stress relaxation and creep compliance, empirical tests such as penetration and extrusion, and imitative tests. Their advantages and disadvantages for the study of food texture have been discussed by Bourne (1982). In the study of WPC gelation and gel properties, imitative tests have seldom been used and only fundamental and empirical tests have been employed.

Non-destructive dynamic shear tests have been extensively applied to the study of whey protein gelation (*e.g.* Tang *et al.* 1993a, b). They use only small deformations and can provide valuable information on gelation mechanisms, molecular interactions during gel formation, and the effects of the physical and chemical environment on gel properties, gel point and the critical protein concentration for gel formation. However, dynamic shear tests are considered to correlate poorly with sensory evaluation of gel texture (Bourne, 1982).

Compression and tension tests are also fundamental tests which can be performed at small deformation, but are typically continued until failure of the sample occurs at a relatively large deformation. Whereas dynamic shear tests can be performed on very soft gels because the gel forms in the rheometer, compression and tension tests usually require relatively firm gels. Tension tests are particularly difficult to perform on soft gels because the gels tend to break before they can be inserted into the testing machine. The ring method described by Smith (1969) is one means of performing tension tests on moderately soft gel samples.

Empirical tests such as the penetration test have been widely used to measure gel firmness in the dairy industry. The penetration test has been used to study the firmness of skim milk gels (Kalab *et al.*, 1971) and WPC gels (Schmidt and Illingworth, 1978; Dunkerley and Hayes, 1980). Paulson and Tung (1989) examined the relationships between penetration test results and non-destructive dynamic shear measurements for canola protein isolate gels. They found that the puncture force was poorly correlated with viscoelastic parameters whereas the slope of the force-deformation curve to the point of rupture was well correlated with G' and G'' . The penetration test is easy to perform and quickly provides information for quality control purposes in the dairy industry. The results of empirical tests including the penetration

test are considered to have a better correlation with sensory texture than fundamental tests using small deformation (Mohsenin and Mittal 1977; Wood 1979; Bourne, 1982). However, empirical tests provide no fundamental understanding of fracture (Bourne, 1982), and cannot provide information on molecular interactions during WPC gel formation.

The aim of this paper was to compare the results of various mechanical tests on WPC gels - because each mechanical test may provide a different part of the overall picture of gel properties. Dynamic shear testing was performed as an example of a fundamental test using only small deformations. Compression and tension tests were performed as examples of fundamental tests producing large deformation and fracture. Penetration testing was performed since it is the most common empirical test used for WPC gels by the dairy industry. Gel pH was chosen as the physicochemical variable to vary because previous work had shown that G' varied in a complex manner with pH. A wide range of gel types was also observed in the pH range 3-8 in terms of appearance, physical properties and gelation mechanism (Stading and Hermansson, 1991; Tang *et al.* 1993b).

9.2 Experimental Procedure

Commercially available WPC(B) powder was used in this study. Its composition and ash components are listed in Tables 3.1 and 3.2 respectively. WPC solution and gel preparation, sample handling, and dynamic shear, compression, tension and penetration measurements are described in Chapter 3. Concentration is expressed as protein concentration rather than as total solids concentration in this study.

9.3 Results

9.3.1 Comparison of G' with rigidity moduli of WPC gels

Typical Instron curves at 12% protein and pH7.0 for each large deformation test are shown in Figs 3.3, 3.4 and 3.5. Tension curves were concave to the load axis and have been discussed above. Compression curves were also concave to the load axis, so compression modulus was calculated from force and displacement at 10% compression, *i.e.* 1 mm displacement. Penetration curves were quite linear up to the failure point.

Visual inspection indicated that WPC gel properties varied with pH as described by Tang *et al.* (1993b). Below pH4 semi-transparent, brittle gels were formed, at pH4-6 white coagula were formed and at pH \geq 7.0 semi-transparent, strong, rubbery gels were formed.

The effects of pH on storage modulus (G'), compression modulus (E_c), tension rigidity (E_t) and penetration rigidity (E_p) are presented in Figs 9.1 and 9.2. G' , E_c , E_t and E_p of WPC gels all exhibited maxima on both sides of the isoelectric point region of whey proteins and minima in the isoelectric point region. These results are in good agreement with rigidity results for ovalbumin gels obtained by Egelanddal (1980), and Young's modulus of 12% β -lactoglobulin gels reported by Stading and Hermansson (1991). The maximum moduli or rigidity of WPC gels occurred at higher pH than the maximum Young's modulus of β -lactoglobulin gels - probably because of a difference in salt content between our work and that of Stading and Hermansson (1991)

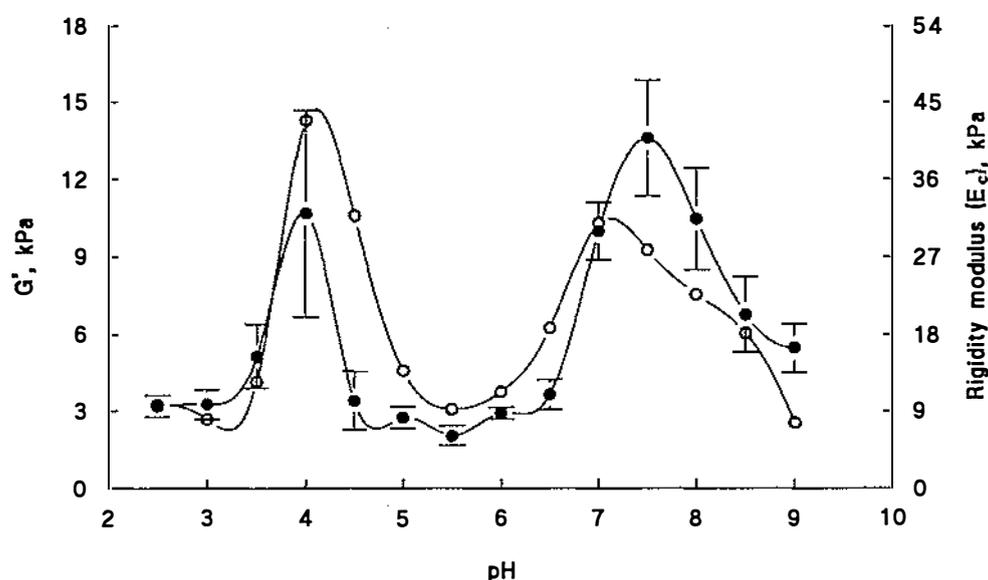


Fig 9.1 Effects of pH on storage modulus, G' (O) and compression modulus, E_c (●) for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each E_c data point is the average of at least four replications and the bars show one standard deviation either side of the mean.

For perfectly elastic materials the relationship of Young's modulus, E , and shear modulus, G , is given by $E = 2G(1+\mu)$ (where μ is the Poisson's ratio). Poisson's ratio can

be assumed to be $\mu \approx 0.5$ (*i.e.* no volume change on deformation) for protein gels (Montejano *et al.* 1984). On Fig 9.1 the E_c scale is exactly 3 times the G' scale. E_c is reasonably close to $3G'$ (as might be expected for $\mu \approx 0.5$) at most pH values. For the 14 pH values considered E_c/G' had a mean of 3.07 with a standard deviation of 1.45 and the range 0.96 (pH4.5) to 6.68 (pH9.0). WPC gels are viscoelastic rather than perfectly elastic, and E_c was measured at 10% compression while G' was measured at a maximum shear strain of 0.01, well within the linear viscoelastic region. Considering these differences the agreement between E_c and $3G'$ is reasonable.

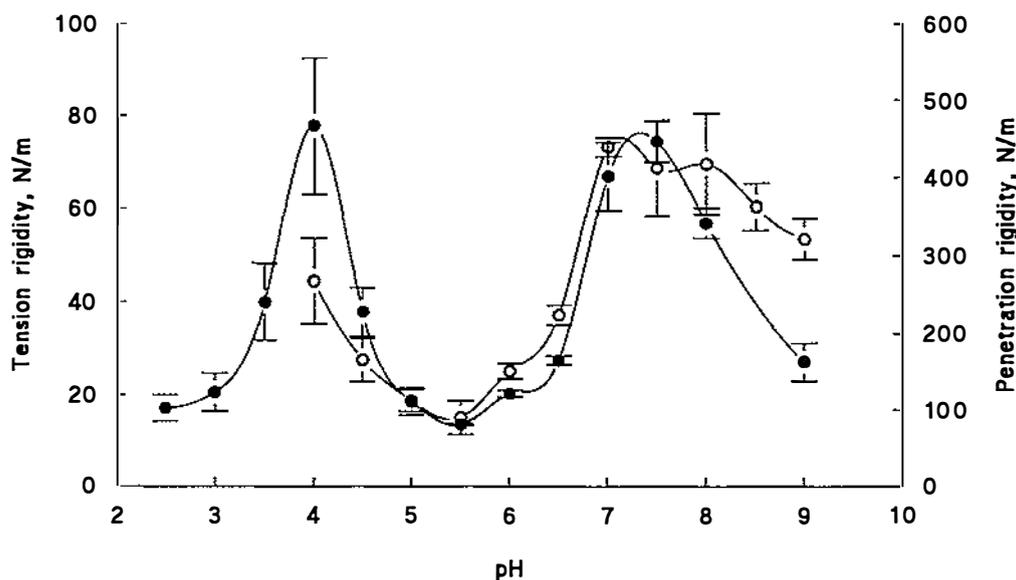


Fig 9.2 Effects of pH on tension rigidity, E_t (○) and the penetration rigidity, E_p (●) for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.

E_t and E_p (Fig 9.1) varied with pH in a very similar manner to E_c and G' . The penetration test was very simple to perform, but theoretical analysis to allow calculation of stress and strain during deformation is complicated. Shear, compression and probably some extrusion are occurring simultaneously (Bourne, 1982).

9.3.2 Texture profile analysis of WPC gels

The results of texture profile analysis are summarized in Fig 9.3. WPC gels had a very low and almost constant hardness between pH2.5 and pH5.5. Gels increased in hardness with increasing pH above pH5.5, and the hardest gels were formed at pH7.5. Above pH7.5 gels decreased in hardness with increasing pH. Cohesiveness and springiness of WPC gels both increased with increasing pH from pH4 to pH9 and both properties exhibited shallow minima at pH4. G' , E_c , E_t and E_p (Figs 9.1 and 9.2) showed completely different variation with pH compared to hardness, cohesiveness and springiness (Fig 9.3) except for hardness above pH5.5. Obviously, quite different textural parameters are being measured by the texture profile analysis.

9.3.3 Failure testing of WPC gels

In tensile testing of WPC gels the failure displacement increased fairly regularly with pH (Fig 9.4). Gels formed at pH4 and 4.5 were very brittle and failed at very low displacements. Failure forces was low over the pH range 4-6.5, rose sharply to a maximum at pH7.5 and then declined slowly from pH7.5 to 9 (Fig 9.4). This data enables more meaningful analysis of the tensile rigidity versus pH curve (Fig 9.2). The high E_t at pH4 arises because of a high force/displacement ratio at this pH, but Fig 9.4 shows that both force and deformation are very low at pH4. WPC gels at pH4 are very weak and brittle in spite of having a high E_t . The maximum in E_t at pH7 to 7.5 is caused by a maximum in failure force in this pH range.

In penetration testing of WPC gels the failure displacement remained low up to about pH7.5 and then climbed rapidly above pH8 (Fig 9.5). Failure displacement exhibited a distinct minimum at pH3.5-4. Failure force was low over the pH range 2.5-6.5, climbed rapidly to a maximum at pH7.5 and then decreased rapidly above pH8 (Fig 9.5). This data enables more meaningful analysis of the penetration rigidity versus pH curve (Fig 9.2). The maximum E_p at pH4 is caused by a very brittle gel and a very low failure displacement at pH3.5-4. WPC gels are very weak and brittle at pH4 in spite of having maxima in E_p , E_v , E_c and G' at this pH. On the other hand the maximum E_p at pH7.5 is caused by a maximum in failure force at this pH. WPC gels at pH7.5 are relatively strong and also quite tough.

The effects of pH on gel hardness (Fig 9.3), tensile failure force (Fig 9.4) and penetration failure force (Fig 9.5) were all qualitatively similar and are similar also to the

effect of pH on stress at fracture for 12% β -lactoglobulin gels (Stading and Hermansson, 1991).

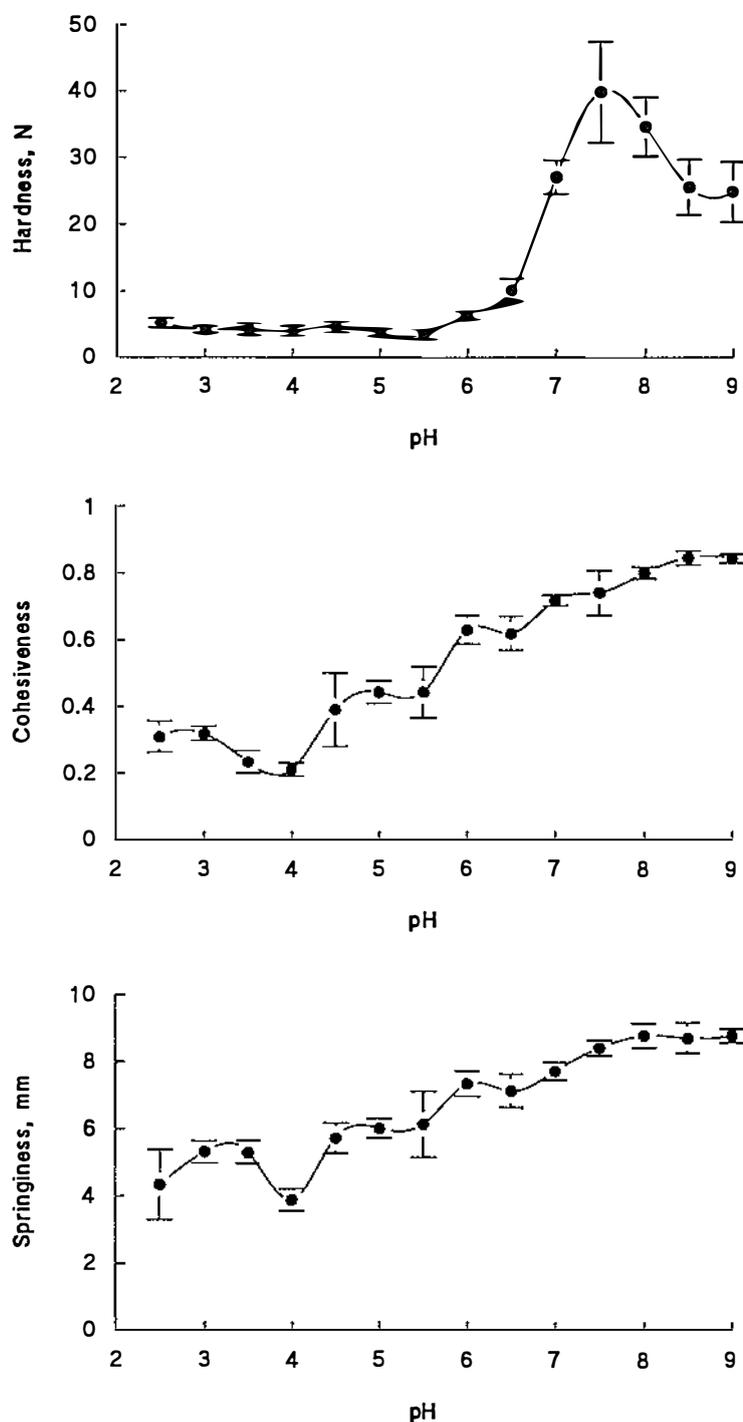


Fig 9.3 Effects of pH on hardness, cohesiveness and springiness determined by texture profile analysis for WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.

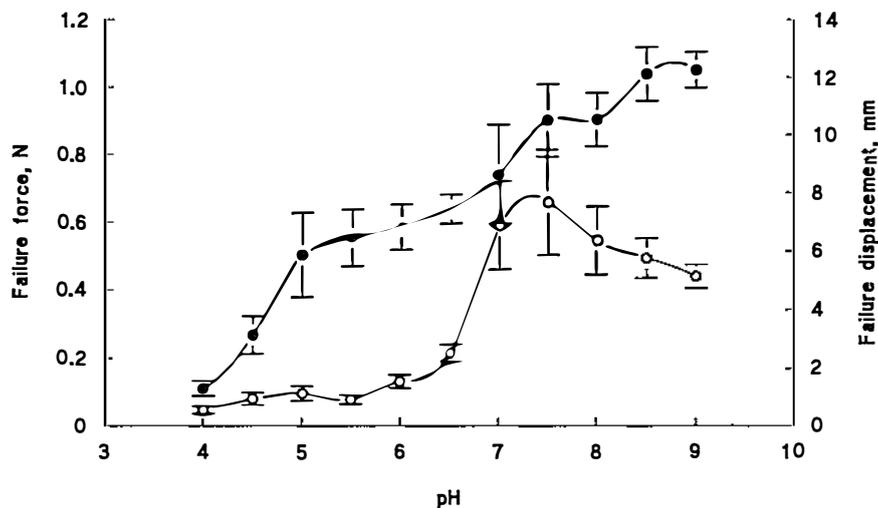


Fig 9.4 Effects of pH on failure forces (○) and displacements (●) during tensile testing of WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.

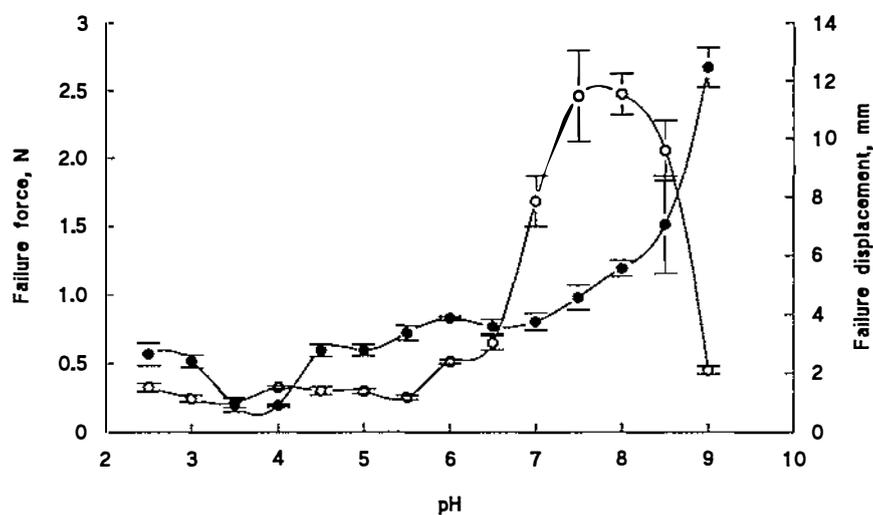


Fig 9.5 Effects of pH on failure forces (○) and displacements (●) during penetration testing of WPC gels containing 12% protein at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of at least four replications and the bars show one standard deviation either side of the mean.

Springiness (Fig 9.3), tensile failure displacement (Fig 9.4) and penetration failure displacement (Fig 9.5) all increased with increasing pH except that springiness and penetration failure displacement exhibited small minima at pH4. These results are in good agreement with the effect of pH on the strain at fracture for 12% β -lactoglobulin gels (Stading and Hermansson, 1991).

Failure force in the tensile test increased steadily with increasing protein concentration, but displacement at failure remained almost constant with increasing protein concentration (Fig 9.6).

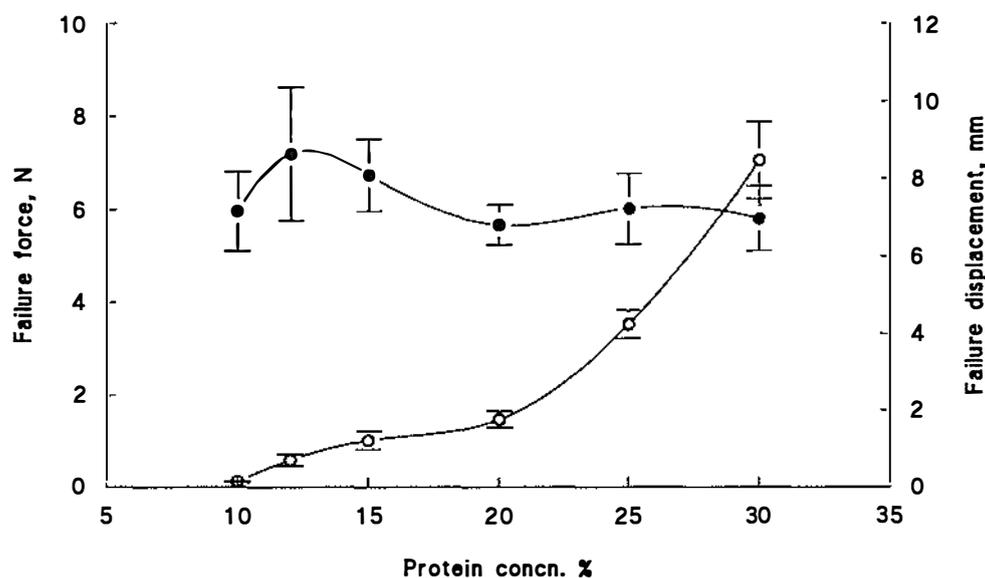


Fig 9.6 Effects of protein concentration on failure forces (○) and displacements (●) during tensile testing of WPC gels at 20°C. The WPC gels were made by heating WPC solutions at 80°C for 45 minutes. Each data point is the average of four replications and the bars show one standard deviation either side of the mean.

9.4 Discussion

There is often poor correlation between mechanical properties at small strain, and yield

or fracture properties of a food material (Mohsenin and Mittal, 1977). Such poor correlation has proved to be the case for WPC gels. At pH4 WPC gels had high values of G' , E_c , E_t and E_p but exhibited low failure forces and gel hardness, whereas at pH7-7.5 WPC gels exhibited high values for all mechanical properties. Mitchell (1980) suggested that the rupture strength of a gel was not necessarily related to its elastic modulus. Explanation of these results requires information on the effects of pH on gel microstructure and on the bonds involved in gel formation.

Langton and Hermansson (1992) used various microscopy techniques to study the effect of pH on the gel network structure of whey protein gels. They found that white particulate gels were formed at pH4-6, fine-stranded gels with stiff short strands below pH4 and fine-stranded gels with longer, more flexible strands at pH>6. This correlates well with the visual observations on the changes of WPC gel properties with pH discussed in Chapter 6.

The major chemical bonds governing the mechanical properties of WPC gels are hydrogen bonds, disulphide bonds, hydrophobic interactions and electrostatic interactions (Hillier *et al.* 1980; Zirbel and Kinsella, 1988; Mangino *et al.* 1987; Mangino, 1992). Of these, electrostatic interactions and disulphide bonds vary significantly with pH. Electrostatic interactions produce increasing protein-protein repulsion as pH moves away from the isoelectric point region (roughly pH4.8-5.3 for whey proteins), and may be shielded to some extent by salt ions. Disulphide bonds are involved in WPC gel formation only above about pH7 (Mangino *et al.* 1987; Mangino, 1992; Dunnill and Green, 1966). It has also been reported that the sulphhydryl content has little effect on WPC gel strength below about pH7 (Kohnhorst and Mangino, 1985; Mangino *et al.* 1987; Mangino, 1992). The other relevant factor is that maximum gel strength requires an optimum balance between protein-protein interactions and protein-solvent interactions, because strong protein-protein interactions result in coagulum formation, which lowers gel strength.

G' , E_c , E_t and E_p exhibited maxima at or near both pH4 and pH7. Electrostatic interactions are the dominant factor in the explanation of this behaviour, as discussed in Chapter 6. In the isoelectric point region strong protein-protein interactions lead to the formation of coagulum structures with low moduli. As pH moves away from the isoelectric point region protein-protein attractions reduce because of electrostatic repulsion until an optimum balance between protein-protein and protein-solvent interactions is achieved, leading

to maximum values in the various moduli.

TPA cohesiveness, TPA springiness, tensile failure displacement and penetration failure displacement generally increased steadily with increase in pH. The formation of disulphide bonds at pH values above 6.5 appears to be the dominant factor in explaining this behaviour. Beveridge *et al.* (1984) attributed elasticity of WPC gels to disulphide bonds, and Zirbel and Kinsella (1988) reported that elasticity, hardness and cohesiveness of whey protein isolate gels were determined by the involvement of disulphide bonds. Schmidt and Illingworth (1978) suggested that whey protein gel strength was related to the degree of intermolecular disulphide bonding in the gel network.

The remaining mechanical properties, TPA hardness, tensile failure force and penetration failure force, remained low up to pH6.5, increased rapidly to a maximum at pH7-7.5 and then decreased again. The combined effects of electrostatic interactions causing a maximum at pH7-7.5 and disulphide bonds forming strong gels at pH > 6.5 is needed to explain these results.

The penetration test is empirical, and so provides no information on fundamental mechanical properties. However, the results here show that a combination of penetration rigidity, penetration failure force and penetration failure displacement data provide as much qualitative understanding of the variation of gel properties with pH as any other single test. The penetration test is also very fast and can be performed with relatively cheap instruments. Where possible it should be performed on equipment which enables measurement of failure force, failure displacement and curve slope rather than just failure force.

Dynamic shear tests are also fast but the results here show that dynamic parameters at small deformation, such as G' , when measured alone, may not be an accurate reflection of the suitability of a WPC gel for use in foods. WPC gels at pH4 were very soft and brittle, yet G' was high. Dynamic shear tests are most useful for studying the processes occurring during gel formation, and the effects of physicochemical parameters on these processes.

In conclusion, it appears that, in the variation of WPC gel properties with pH, moduli and rigidities are governed mainly by electrostatic interactions, failure displacements are governed mainly by disulphide bond formation above pH6.5, and failure forces are governed by a combination of electrostatic interactions and disulphide bond formation.

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10. Conclusions and Recommendations for Further Work

10.1 Conclusions

1. Apparent viscosity of WPC solutions increases with increasing concentration. Rheological behaviour also depends on concentration; behaviour changes from Newtonian to pseudoplastic (time-independent shear thinning) to time-dependent shear thinning as concentration increases.
2. The change in the rheological behaviour of solution from time-independent to time-dependent shear thinning can be brought about also by extreme pHs, a high calcium chloride concentration (after aging) and heating to above 60°C. This change is considered to be caused by structure formation inside solutions.
3. The gelation time of WPC solutions is inversely dependent on protein concentration. The data obtained support the view that WPC is a percolation process.
4. Gelation time is also inversely dependent on gelation temperature in a way satisfactorily described by an Arrhenius equation.
5. Gelation time decreases if pH is moved towards the isoelectric point region, or if ionic strength is decreased (at pHs above this region). These effects are considered to be mainly electrostatic in nature.
6. The gel existing at the gel point, in the case of WPI, may have a fractal geometry.
7. The rigidities (G') of WPC and WPI gels all exhibited a similar dependence on effective protein concentration, despite differences in composition and in minimum protein concentrations required for gel formation.
8. Rigidities of well-developed WPC gels are directly related to protein content, but dependent in a complex way on pH and ionic strength. The interactive effects of pH and ionic strength can be explained largely in electrostatic terms.

9. All salt cations tested, including Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Fe^{3+} , have significant influences on the gelling behaviour of whey proteins. Divalent cations have stronger effects on gelling performance than do monovalent cations.

10. Compared with WPCs, egg white has a lower gelation temperature, a lower minimum protein concentration for gelation and a high initial gelation rate. Further, egg white gels have higher rigidities (G') than WPC gels, and the ratio (G' at 80°C / G' at 20°C) is greater for egg white than for WPC below 16% protein. The gelling performance of WPCs relative to egg white can to some extent be improved by adjusting salt content.

11. At pH4 WPC gels had high values of G' , E_c , E_t and E_p but exhibited low failure forces and gel hardness, whereas at pH7-7.5 WPC gels exhibited high values for all mechanical properties, indicating the failure strength of a WPC gel was not necessarily related to its G' , E_c , E_t and E_p .

10.2 Recommendations for Further Work

10.2.1 Further applied work

The work described in this thesis has contributed to the understanding of whey protein gelation under different environmental conditions. It forms a good basis for the future study of the interaction of whey proteins with other food components in real food systems during gel formation. Considering current applications of whey proteins in food products such future study could be divided into the following areas: interactions of whey proteins with meat proteins, interactions of whey proteins with starch, and interactions of whey proteins with egg white or other proteins.

1. Whey proteins have been used in the meat industry as functional ingredients in making cooked ham, sausage or other luncheon meat (chicken, pork and beef). It is well recognized that whey proteins increase the gel strength of meat protein, prevent fat separation, improve water binding and improve the cooked yield of meat products. However, it is not well understood how whey proteins interact with meat proteins upon cooking in an environment containing salts and fats (Jordan, 1992). It would be interesting and commercially important to explore this area.

2. Whey proteins are also used as functional ingredients in bakery products. Whey proteins are normally used as egg white replacers. It is apparent that the interactions of whey proteins with starch in structure formation during baking is very important for the quality of these products. Therefore, such interactions should be investigated, and optimum formulations and processing conditions identified.

3. This work demonstrates that egg white has some gelling properties which whey proteins cannot match - such as a low gelation temperature and a low minimum protein concentration for gelation. It would be interesting to manufacture new commercial gelling ingredients from mixtures of whey proteins and egg white. Such products may be better as egg white replacers than whey protein products. It would thus be of interest to investigate the gelling properties of mixtures of whey proteins and egg white.

4. It is known that lowering the gelation temperature of WPC could dramatically boost its application in food products as an egg white replacer. As this work has illustrated, egg white has a much lower gelation temperature than WPC. Current methods of trying to reduce the gelation temperature of WPC involve heat treatment to modify the protein molecular structure and expose -SH groups. The problems involved are both economic and technical. At low protein concentration these heat treatments of WPC are relatively straight forward, but the heating of vast amounts of WPC solutions at low protein concentration and subsequent evaporation of water would take a lot of energy. At high protein concentration, gel formation is likely during the heat treatment. It should be possible to heat treat whey protein samples with low salt contents at reasonably high protein concentrations (*e.g.* 12% protein concentration for WPI) without gel formation. However, harsh heat treatment of whey protein solutions can cause partial loss of whey protein functionality, *e.g.* by formation of insoluble precipitates.

5. The effects of salts on the dynamic rheological properties of whey protein gels have been reported in this work, but the effects of salts on failure forces, failure strain and rigidity modulus of WPC gels need to be investigated.

10.2.2 Further fundamental study

1. It will be interesting to investigate the effects of salt (*e.g.* NaCl, or CaCl₂, or MgCl₂) expressed as ionic strength on rheological properties (*e.g.* G') of pure β -lactoglobulin, α -lactalbumin and WPI gels above their isoelectric pHs. The initial salt contents of solutions of these whey protein products should be removed so that the initial ionic strength of the solutions is close to zero.

2. It will be interesting to investigate the relationships of dynamic viscoelastic properties (*e.g.* G', G'') of critical α -lactalbumin and β -lactoglobulin gels with frequency. A power law relationship should be observed if the critical gels of α -lactalbumin and β -lactoglobulin have a fractal geometry. α -lactalbumin and β -lactoglobulin samples with a very low salt content are probably needed for such study.

3. It is recommended that the protein concentration dependence of gelation time for pure α -lactalbumin and for pure β -lactoglobulin be explored. It will be interesting to see whether the results obey Ross-Murphy's equation (Ross-Murphy, 1991a, b, c, d). The site percolation model should be applied to α -lactalbumin and β -lactoglobulin gelation. The relationship between G' and protein concentration for α -lactalbumin and β -lactoglobulin should also be explored.

10.3 References

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List of Symbols

a	critical exponent	-
A	cross-sectional area of sample of specimen	m ²
B	constant	N m ⁻² s
C	protein or polymer concentration	% (w/w)
C _e	effective protein concentration (C _e = C - C _m)	% (w/w)
C _m	minimum protein concentration for gelation after a certain time	% (w/w)
C _o	critical protein or polymer concentration	% (w/w)
C _t	total solids concentration of whey protein concentrate	% (w/w)
d _s	density of whey protein solution	kg m ⁻³
E	Young's modulus	N m ⁻²
E _c	compression modulus	N m ⁻²
E _e	equilibrium modulus in tension	N m ⁻²
E _p	penetration rigidity	N m ⁻¹
E _t	tensile rigidity	N m ⁻¹
E'	activation energy	J mol ⁻¹
F _c	compression force	N
F _g	gel fraction	-
F _t	tensile force	N
G	shear modulus (τ/γ)	N m ⁻²
G(t)	relaxation shear modulus	N m ⁻²
G _e	equilibrium shear modulus (at zero frequency)	N m ⁻²
G _N ⁰	plateau shear modulus (at small but finite frequency)	N m ⁻²
G' or G'(ω)	storage modulus	N m ⁻²
G'' or G''(ω)	loss modulus	N m ⁻²
G*	complex modulus	N m ⁻²
h	specimen length at 10% compression	m
h _o	original specimen length in compression	m
J	creep compliance for a perfectly elastic solid	N ⁻¹ m ²
J(t)	creep compliance for a viscoelastic solid or liquid	N ⁻¹ m ²
k	constant	-

k_f	fluid consistency index	$N m^{-2} s^n$
k'	critical exponent	-
K	proportionality constant	s
K'	1/K	s^{-1}
l_0	original length of specimen in tension	m
Δl	increase in length in tension	m
M_w	weight average molecular weight	-
m_d	dry whey protein mass	kg
N_w	weight average polymerization index	-
n	material parameter (stress relaxation exponent, $0 < n < 1$)	-
n'	flow behaviour index	-
p	ratio of the actual number of bonds formed at a given moment to the maximum possible number of such bonds	-
p_c	p at the gel point	-
p'	volume fraction of occupied sites	-
p'_c	p' at the gel point	-
r	radius of gel cylinder at 10% compression	m
r_0	original radius of the gel cylinder in compression	m
R	universal gas constant	$J K^{-1} mol^{-1}$
s'	exponent	-
S	material parameter	-
t	time	s
t_c	gelation time	s
T	absolute temperature	K
T_d	initial denaturation temperatures of proteins	$^{\circ}C$
V_h	volume of the hydrated whey protein	m^3
V_s	volume of the whey protein solution	m^3
α	critical exponent	-
β	critical exponent	-
γ	shear strain	-
γ_c	compression strain	-
γ_0	maximum shear strain	-

γ_t	tensile strain	-
γ_∞	strain at infinite time	-
$\dot{\gamma}$	shear rate	s^{-1}
Δ	critical exponent	-
δ	phase angle	rad
η	shear viscosity	$N m^{-2} s$
η_a	apparent shear viscosity	$N m^{-2} s$
η_o	zero-shear viscosity	$N m^{-2} s$
η_s	coefficient of viscosity of WPC solutions	$N m^{-2} s$
η_w	coefficient of viscosity of distilled water	$N m^{-2} s$
η'	dynamic viscosity ($\eta' = G''/\omega$)	$N m^{-2} s$
θ	constant	-
μ	Poisson's ratio	-
μ_e	equilibrium Poisson's ratio for viscoelastic materials	-
μ_h	the voluminosity of hydrated whey protein (hydrated protein volume / dry protein mass)	$m^3 kg^{-1}$
ξ	correlation length	m
τ	shear stress	$N m^{-2}$
τ_c	compression stress	$N m^{-2}$
τ_o	maximum stress for oscillatory measurement	$N m^{-2}$
τ_t	tensile stress	$N m^{-2}$
τ_y	yield shear stress	$N m^{-2}$
ν	critical exponent	-
ϕ	kinetic order of gelation	-
ω	angular frequency	$rad s^{-1}$

Appendix 1

The relationship between the volume fraction of occupied sites (p') and the whey protein concentration (C) in Chapter 8 is given as follows:

$$p' = \frac{V_h}{V_s} \quad (a)$$

Where V_h is the volume of the hydrated whey protein and V_s is the volume of the whey protein solution.

Equation (a) can be rewritten as:

$$p' = \frac{V_h}{V_s} \times \frac{m_d}{m_d} \times \frac{d_s}{d_s} \quad (b)$$

Where m_d is the dry whey protein mass and d_s is the density of whey protein solution.

Equation (b) can be rearranged to give:

$$p' = \frac{m_d}{V_s d_s} \times \frac{V_h}{m_d} d_s = \frac{C}{100} \mu_h d_s$$

$$\therefore C = \frac{p'}{\mu_h d_s} \times 100 \quad (c)$$

Where C is the whey protein concentration (% w/w) $\{C = (m_d / V_s d_s) \times 100\}$ and μ_h is the voluminosity of hydrated whey protein ($\mu_h = V_h / m_d$).

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