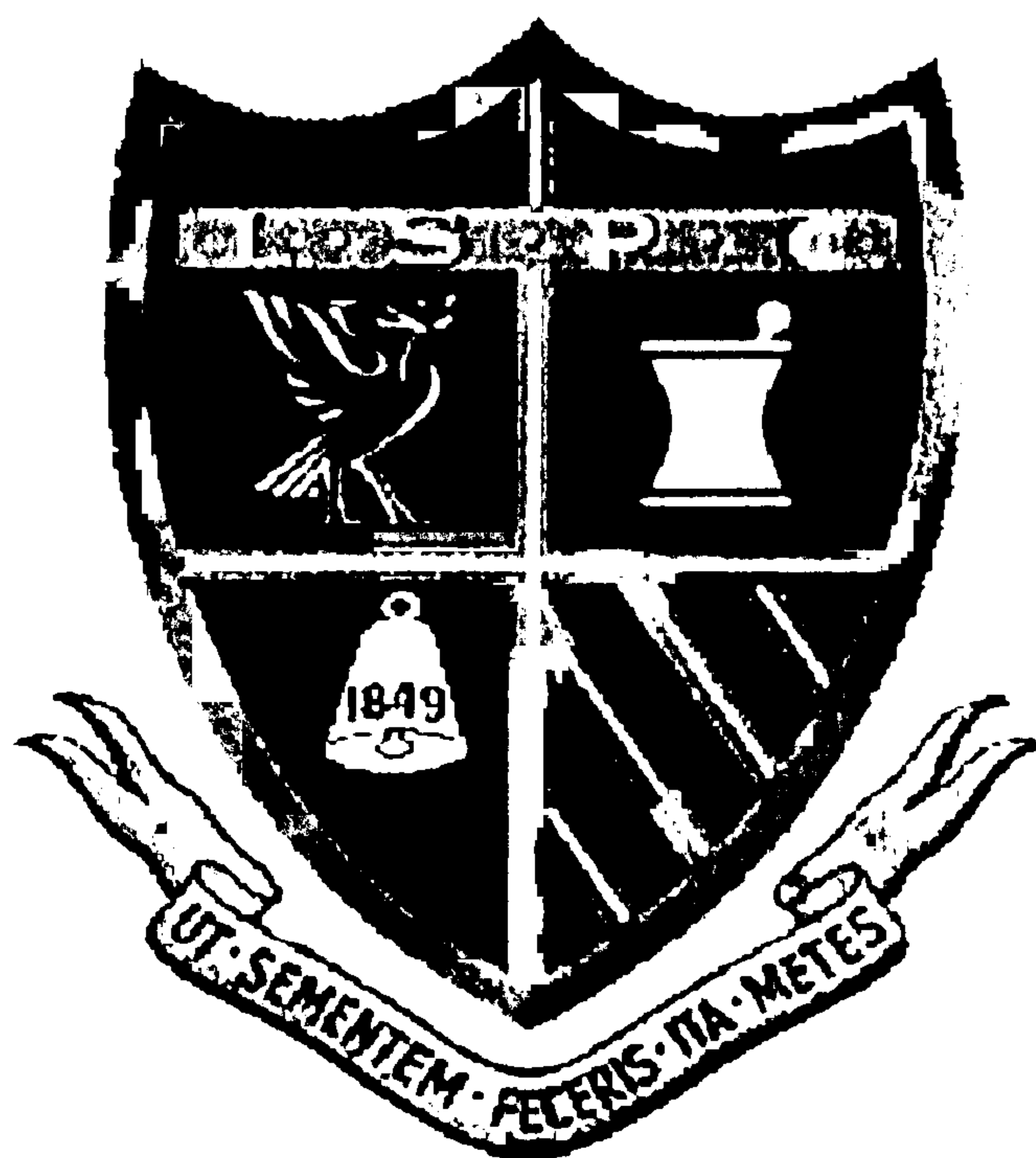


**STUDIES INTO THE DEVELOPMENT OF AN IN-SITU  
GELLING WOUND DRESSING**

**BY**

**JONATHAN WILLIAM GILKES BSc (Hons) MSc**

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**DEPARTMENT OF PHARMACY AND CHEMISTRY  
LIVERPOOL JOHN MOORES UNIVERSITY  
BYROM STREET  
LIVERPOOL  
L3 3AF**

# **TABLE OF CONTENTS**

	Page No
Table of contents	i
List of Figures	vii
List of Tables	xiii
Glossary	xvi
Acknowledgements	xvii
Abstract	xviii
<b>1 GENERAL INTRODUCTION</b>	<b>1</b>
<b>1.1 Wounds</b>	<b>1</b>
1.1.1 Classification of Wounds	1
1.1.1.1 Mechanical injury	1
1.1.1.2 Burns	1
1.1.1.3 Ulcers	1
<b>1.2 Healing</b>	<b>2</b>
1.2.1 Healing mechanisms	2
1.2.1.1 Primary intention	2
1.2.1.2 Secondary intention	2
1.2.1.3 Delayed primary closure	2
1.2.1.4 Grafting and flap formation	3
1.2.2 The healing response	3
1.2.2.1 Inflammation	3
1.2.2.2 Proliferation	4
1.2.2.3 Maturation	5
1.2.3 Factors that influence the wound healing process	6
1.2.3.1 Circulation	6
1.2.3.2 Infections of the wound	6
1.2.3.3 Malnourishment	6
1.2.3.4 Chemical agents	6
1.2.3.5 Age	7
<b>1.3 Wound management</b>	<b>7</b>
1.3.1 History	7
1.3.2 Occlusive wound management	7
1.3.3 The properties of occlusive dressings	8
1.3.3.1 Gaseous permeability	8
1.3.3.2 pH	9
1.3.3.3 Freedom from toxins and debris	9
1.3.3.4 Impermeability of a dressing to microorganisms	9
1.3.3.5 Thermal properties	9
1.3.4 Dressing groups	10
1.3.5 Occlusive wound healing	10
1.3.6 Wound microbiology	12
<b>1.4 Alginates in wound management</b>	<b>13</b>
1.4.1 Structure and properties of alginates	14
1.4.1.1 Chemical composition.	15
1.4.1.2 Molecular weight distribution.	15
1.4.1.3 Solubility	17
1.4.1.4 Stability	17



1.4.2	Gel formation in sodium alginate	17
1.4.2.1	Molecular basis of gel formation	17
1.4.2.2	Methods of gel preparation	18
1.4.2.2.a	The diffusion method of gel formation	18
1.4.2.2.b	The internal method of gel formation	20
1.4.2.2.c	Gels by other methods	20
1.4.2.3	Gel strength	20
<b>1.5</b>	<b>Hyaluronic acid in healing</b>	<b>21</b>
1.5.1	Structure and properties of hyaluronic acid	21
1.5.2	Role in healing	22
1.5.3	Scarless healing	23
<b>1.6</b>	<b>Aims of this thesis</b>	<b>23</b>
<b>2</b>	<b>EXPERIMENTAL</b>	<b>24</b>
<b>2.1</b>	<b>Sample preparation</b>	<b>24</b>
2.1.1	Sample preparation using cellulose based polymers	24
2.1.1.1	Materials	24
2.1.1.2	Equipment	24
2.1.1.3	Formulation methods	25
2.1.1.3.a	Formulation of sodium alginate, and sodium alginate and polymer solutions	25
2.1.1.3.b	Calcium solution preparation	25
2.1.2	Sample preparation using delta gluconolactone and calcium carbonate	25
2.1.2.1	Materials	25
2.1.2.2	Equipment	25
2.1.2.3	Formulation methods	26
2.1.2.3.a	Sample preparation for investigation of rheological and water uptake properties	26
2.1.2.3.b	Preparation of phosphate buffer	27
2.1.2.3.c	Preparation of fresh delta gluconolactone solution	27
<b>2.2</b>	<b>Rheological methods for the characterisation of formulated gels and solutions</b>	<b>28</b>
2.2.1	Brief introduction to the theory of rheology	28
2.2.1.1	Viscosity and elasticity of materials and ideal behaviour	28
2.2.1.1.a	The viscosity of material and Newtonian behaviour	28
2.2.1.1.b	The elasticity of material and hookean behaviour	29
2.2.1.2	The measurement of rheological properties	30
2.2.1.3	Non-Newtonian behaviour of materials	32
2.2.1.3.a	Plastic flow	32
2.2.1.3.b	Pseudoplastic flow	32
2.2.1.3.c	Dilatant flow	33
2.2.1.3.d	Thixotropy	33
2.2.1.4	Linear viscoelasticity	34
2.2.1.4.a	The oscillation technique	35
2.2.1.4.b	The creep technique	35
2.2.2	The methods and methodology used to characterise the rheological properties of formulated gels and solutions	36
2.2.2.1	Equipment	36

2.2.2.1.a	A description of the equipment	37
2.2.2.2	Oscillation methods used to investigate the viscoelasticity of formulations	37
2.2.2.2.a	Determination of the linear viscoelastic region	37
2.2.2.2.b	Investigating formulation structure with a frequency sweep	38
2.2.2.2.c	Investigating structure change with a time sweep	39
2.2.2.3	Investigating elasticity using creep	39
2.2.2.4	Determining viscosity using flow methods	41
2.2.2.4.a	Determining maximum stress	41
2.2.2.4.b	Determining viscosity and rate index.	42
<b>2.3</b>	<b>A method for determining the absorptive capacity of amorphous hydrogels</b>	<b>43</b>
2.3.1	Introduction	43
2.3.2	Materials	45
2.3.3	Equipment	45
2.3.3.1	Franz cell diameters	45
2.3.4	A method for determining the absorptive capacity of amorphous hydrogels using Franz cells and its validation	46
2.3.4.1	The Franz cell method	46
2.3.4.2	Franz cell method validation parameters	46
2.3.4.2.a	Determination of method precision	46
2.3.4.2.b	Linearity of response	46
2.3.4.2.c	Determination of method sensitivity	47
2.3.4.2.d	Determination of method accuracy	47
2.3.4.2.e	Determination of method robustness.	47
2.3.4.3	Validation results	47
2.3.4.3.a	The precision of the Franz cell method	47
2.3.4.3.b	Linearity of response of the Franz cell method	48
2.3.4.3.c	The sensitivity of the Franz cell method	49
2.3.4.3.d	The accuracy of the Franz cell method	49
2.3.4.3.e	The robustness of the Franz cell method	50
2.3.4.4	Discussion	50
2.3.4.5	Summary	51
<b>3</b>	<b>INTRODUCTION TO EXPERIMENTAL DESIGN IN FORMULATION DEVELOPMENT</b>	<b>52</b>
<b>3.1</b>	<b>Introduction</b>	<b>52</b>
<b>3.2</b>	<b>Experimental designs</b>	<b>53</b>
3.2.1	Full factorial experimental designs	53
3.2.1.1	An example of a full factorial experiment	54
3.2.1.1.a	Calculating the main effects of factors	54
3.2.1.1.b	Calculating interactions	57
3.2.1.1.c	Interpreting the main effects and interactions	57
3.2.2	Fractional factorial experimental design	58
3.2.2.1	Calculating the main effects and interactions	59
3.2.3	The use of experimental design to screen large numbers of factors	56
3.2.3.1	Calculating factor effects in a Plackett and Burman screening experimental design	60

<b>3.3</b>	<b>Summary of the experimental designs used in this project</b>	<b>63</b>
<b>4</b>	<b>THE FORMULATION OF SODIUM ALGINATE GELS WITH CELLULOSE BASED POLYMERS</b>	<b>64</b>
<b>4.1</b>	<b>Introduction</b>	<b>64</b>
<b>4.2</b>	<b>Commercial amorphous hydrogel dressings</b>	<b>64</b>
4.2.1	Experimental	65
4.2.2	Results	65
<b>4.3</b>	<b>Polymers of choice</b>	<b>66</b>
4.3.1	Celquat SC240C®	66
4.3.2	Natrosol 250 MPharm®	67
4.3.3	Aquasorb A500®	67
<b>4.4</b>	<b>Experimental</b>	<b>67</b>
4.4.1	Materials	67
4.4.2	Methods	68
4.4.2.1	Water uptake determination	68
4.4.2.2	Rheological evaluation	68
4.4.3	The formulation of sodium alginate and calcium chloride	69
4.4.3.1	Experimental design	69
4.4.3.2	Results	69
4.4.3.2.a	Water uptake	69
4.4.3.2.b	Rheological evaluation	71
4.4.4	The formulation of Celquat® with sodium alginate and calcium chloride	71
4.4.4.1	Experimental design	71
4.4.4.2	Results	75
4.4.4.2.a	Water uptake	75
4.4.4.2.b	Rheological evaluation	75
4.4.5	The formulation of Natrosol® with sodium alginate and calcium chloride	79
4.4.5.1	Experimental design	79
4.4.5.2	Results	81
4.4.5.2.a	Water uptake	81
4.4.5.2.b	Rheological evaluation	81
4.4.6	The formulation of Aquasorb® with sodium alginate and calcium chloride	84
4.4.6.1	Experimental design	84
4.4.6.2	Results	87
4.4.6.2.a	Water uptake	87
4.4.6.2.b	Rheological evaluation	87
<b>4.5</b>	<b>Discussion</b>	<b>90</b>
<b>4.6</b>	<b>Conclusion</b>	<b>95</b>
<b>5</b>	<b>THE APPLICATION OF DELTA GLUCONOLACTONE IN THE FORMULATION OF ALGINATE GELS AS IN-SITU GELLING WOUND DRESSINGS</b>	<b>96</b>
<b>5.1</b>	<b>Introduction</b>	<b>96</b>



<b>5.2</b>	<b>Experimental</b>	<b>96</b>
5.2.1	Materials	96
5.2.2	Equipment	96
5.2.3	Methods	97
5.2.3.1	Water uptake determination	97
5.2.3.2	Rheological evaluation	97
5.2.3.3	pH Determination	97
5.2.4	The gelling of alginate with calcium carbonate and delta gluconolactone.	97
5.2.4.1	Experimental design	97
5.2.4.2	Results	98
5.2.4.2.a	Gelling time	98
5.2.4.2.b	Gel strength	98
5.2.4.2.c	Post sterilisation pH	101
5.2.4.2.d	48h Post gelation pH	101
5.2.4.2.e	Zero rate viscosity	101
5.2.4.2.f	Rate index	101
5.2.5	The gelling of alginate in the presence of sodium chloride, calcium carbonate and delta gluconolactone	106
5.2.5.1	Experimental design	106
5.2.5.2	Results	106
5.2.5.2.a	Gelling time	106
5.2.5.2.b	Gel strength	107
5.2.5.2.c	pH Post sterilisation	107
5.2.5.2.d	pH 48h Post gelation	111
5.2.5.2.e	Zero rate viscosity	111
5.2.5.2.f	Rate index	111
5.2.5.3.g	Water uptake	115
<b>5.3</b>	<b>Discussion</b>	<b>115</b>
<b>5.4</b>	<b>Summary</b>	<b>119</b>
<b>6</b>	<b>THE USE OF A SCREENING EXPERIMENTAL DESIGN TO INVESTIGATE FACTORS IN THE DEVELOPMENT OF AN <i>IN-SITU</i> GELLING ALGINATE FORMULATION</b>	<b>120</b>
<b>6.1</b>	<b>Introduction</b>	<b>120</b>
<b>6.2</b>	<b>Experimental</b>	<b>120</b>
6.2.1	Materials	120
6.2.2	Equipment	120
6.2.3	Methods	121
6.2.3.1	Experimental design	121
6.2.3.2	Characterisation of formulations	121
<b>6.3</b>	<b>Results and discussion</b>	<b>121</b>
6.3.1	The effects profile of factors influencing gelling time	123
6.3.2	The effects profile of factors influencing water uptake	123
6.3.3	The effects profile of factors influencing viscosity	125
6.3.4	The Effects Profile Of Factors Influencing pH	127
6.3.5	The Effects Profile Of Factors Influencing Gel strength	129



6.3.6	Choice of the most active factors	130
6.4	<b>Summary</b>	<b>131</b>
7	<b>INVESTIGATION OF THE FACTORS MOST INFLUENTIAL IN THE PERFORMANCE OF AN <i>IN-SITU</i> GELLING ALGINATE SYSTEM, USING A FRACTIONAL FACTORIAL EXPERIMENTAL DESIGN.</b>	<b>132</b>
7.1	<b>Introduction</b>	<b>132</b>
7.2	<b>Experimental</b>	<b>132</b>
7.2.1	Materials	132
7.2.2	Equipment	132
7.2.3	Methods	133
7.2.3.1	Experimental design	133
7.2.3.2	Characterisation of formulations	133
7.3	<b>Results and discussion</b>	<b>134</b>
7.3.1	Water uptake	134
7.3.2	pH	138
7.3.2.1	The influence of factors on the pH of the sterile formulations in the experimental design	139
7.3.2.2	pH 48h post gelation	141
7.3.3	Rheological characterisation of the formulations in the experimental design	141
7.3.3.1	Gelling time	143
7.3.3.2	Gel strength	145
7.3.3.2.a	Max G'	145
7.3.3.2.b	The use of the linear viscoelastic range (LVR) to determine the effects of factors on gel strength	147
7.3.3.3	Viscosity	151
7.3.3.3.a	Zero rate viscosity of the sterile formulations	151
7.3.3.3.b	Rate index	151
7.4	<b>Summary</b>	<b>154</b>
8	<b>GENERAL DISCUSSION</b>	<b>155</b>
8.1	<b>Overview</b>	<b>155</b>
8.2	<b>Formulation characterisation</b>	<b>155</b>
8.3	<b>In-situ gelling wound dressing development</b>	<b>155</b>
8.4	<b>Conclusion</b>	<b>167</b>
9	<b>RECOMMENDATIONS FOR FURTHER WORK</b>	<b>169</b>
10	<b>REFERENCES</b>	<b>170</b>
	<b>APPENDICES</b>	<b>188</b>

## **LIST OF FIGURES**

Figure 1.1	A schematic diagram showing the leapfrog migration of epidermal cells to form a new basement membrane during wound healing <sup>[8]</sup> .	5
Figure 1.2	Schematic diagram comparing the benefits of occlusive over normal healing, notice the path of the migrating epidermal cells <sup>[8]</sup> .	12
Figure 1.3	The structural formulae of sodium alginate highlighting the different conformations. The G block structure is particularly important for complexing divalent ions.	16
Figure 1.4	Schematic diagram of the egg box model <sup>[83]</sup> , showing the binding of calcium ions by G block regions to form junction zones <sup>[84]</sup> .	19
Figure 1.5	Schematic diagram showing the binding of calcium by oxygen atoms <sup>[55,62,80]</sup>	19
Figure 1.6	The structure of Hyaluronic acid: N-acetyl- $\beta$ -D- glucosamin and $\beta$ -D-glucuronic acid joined at the 1,3 and 1,4 positions respectively <sup>[103]</sup> .	22
Figure 2.1	Schematic diagram of the application of shear stress <sup>[115]</sup> to a Newtonian liquid: stress $\sigma$ applied in direction U, F (Where A = area, d = distance, U= and F = Force.).	29
Figure 2.2	A schematic representation of the change of shear stress with increasing shear rate for a Newtonian liquid <sup>[118]</sup> .	29
Figure 2.3	Schematic diagram of the application of shear stress <sup>[115]</sup> to a Hookean solid: stress $\sigma$ applied to surface DC (Where A, B, C and D are the surface positions without stress applied and A', B', C' and D' are the surface positions with a stress ( $\sigma$ ) applied).	30
Figure 2.4	The truncation of a cone <sup>[118]</sup> .	31
Figure 2.5	A sample under shear between a cone and plate <sup>[118]</sup> .	31
Figure 2.6	A schematic representation of the thixotropic and anti-thixotropic hysteresis loops <sup>[118]</sup> .	34
Figure 2.7	A schematic representation the response of a viscoelastic material to the application and removal of a constant stress <sup>[118]</sup> .	36
Figure 2.8	The linear viscoelastic region of a sample solution of 4%w/w sodium alginate.	37
Figure 2.9	The structure of a solution of 4%w/w sodium alginate determined using a frequency sweep.	38
Figure 2.10	The phase transition of an 8%w/w sodium alginate solution, induced by internal release of calcium ions (section 1.4.5.2.b). The gel point is where G' and G'' cross.	40
Figure 2.11	The use of a plastic cover and silicone oil to prevent sample dehydration during extended analyses. The sudden increase of G' in plot 1, is due to dehydration and not gelation.	40
Figure 2.12	Determination of the maximum stress a 4%w/w sodium alginate solution could withstand.	41
Figure 2.13	The determination of the flow profile of a 4%w/w sodium alginate solution used a steady state method.	42



Figure 2.14	Schematic diagram of a Franz cell. Key: lower compartment A, upper compartment B, Visking tubing C, plastic tube and cover D and horse shoe clamp E.	43
Figure 2.15	The linear (red) and polynomial (blue) regression curves of the data in Table 2.3.	49
Figure 3.1	The results from Table 3.2 processed using an analysis of variance in Minitab® to calculate the main effects and interactions of factors.	56
Figure 3.2	The main effects profile of factors, a graphical representation from Minitab®.	56
Figure 3.3	The interaction profile of factors, a graphical representation from Minitab®.	58
Figure 3.4	Example effects profile for a 10 factor Plackett and Burman design. The effect can be presented with the units of the test carried out.	62
Figure 4.1	Viscosity (flow) profiles of 1) Nugel® 2) Intralene® and 3) Sterigel®.	65
Figure 4.2	Creep recovery of 1) Nugel®, 44.3% 2) Intralene®, 59.3% and 3) Sterigel®, 84.3%.	66
Figure 4.3	The structure of two anhydroglucose units in a cellulose derivative. The number of R groups per monomer unit depends on the degree of substitution. Where for Cellulose $R = H$ , or for Hydroxyethylcellulose, $R = (CH_2CH_2O)_nH$ , Sodium carboxymethyl cellulose, $R = CH_2COO^- Na^+$ Polyquaternium-10, $R = (CH_2CH_2O)_n R'N^+(CH_3)_3Cl$ .	67
Figure 4.4	The main effects of sodium alginate and calcium concentration on water uptake.	70
Figure 4.5	The interaction profile of alginate and calcium on water uptake.	70
Figure 4.6	The main effects of sodium alginate and calcium concentration on viscosity.	72
Figure 4.7	The interactions of sodium alginate and calcium concentration on viscosity.	72
Figure 4.8	The main effects of sodium alginate and calcium concentration on creep recovery.	73
Figure 4.9	The interactions of sodium alginate and calcium concentration on creep recovery.	73
Figure 4.10	The main effects of sodium alginate and calcium concentration on rate index.	74
Figure 4.11	The interactions of sodium alginate and calcium concentration on rate index.	74
Figure 4.12	The main effects of factors sodium alginate and Celquat® on water uptake.	76
Figure 4.13	The interactions profile of factors Celquat® and alginate on water uptake.	76
Figure 4.14	The main effects of factors Celquat®, alginate and calcium on viscosity.	77
Figure 4.15	The interactions profile of factors Celquat®, alginate and calcium on viscosity.	77

Figure 4.16	The main effects of factors Celquat®, alginate and calcium on % structure recovered.	78
Figure 4.17	The interactions profile of factors Celquat®, alginate and calcium on % structure recovered.	78
Figure 4.18	The main effects of factors Celquat®, alginate and calcium on rate index.	80
Figure 4.19	The interactions profile of factors Celquat®, alginate and calcium on rate index.	80
Figure 4.20	The main effects profile of factors alginate and Natrosol® on water uptake.	82
Figure 4.21	The interactions profile of factors alginate and Natrosol® on water uptake.	82
Figure 4.22	The main effects profile of factors Natrosol®, alginate and calcium on viscosity.	83
Figure 4.23	The interactions profile of factors Natrosol®, alginate and calcium on viscosity.	83
Figure 4.24	The main effects of factors Natrosol®, alginate and calcium on % structure recovered.	85
Figure 4.25	The interactions profile of factors Natrosol®, alginate and calcium on % structure recovered.	85
Figure 4.26	The main effects of factors Natrosol®, alginate and calcium on rate index.	86
Figure 4.27	Interactions profile of factors Natrosol®, alginate and calcium on rate index.	86
Figure 4.28	The main effects of factors alginate and Aquasorb® on water uptake.	88
Figure 4.29	The interaction profile of factors alginate and Aquasorb® on water uptake.	88
Figure 4.30	The main effects of factors Aquasorb®, alginate and calcium on viscosity.	89
Figure 4.31	The interactions profiles of factors Aquasorb®, alginate and calcium on viscosity.	89
Figure 4.32	The main effects of factors Aquasorb®, alginate and calcium on % structure recovered.	91
Figure 4.33	The interactions profile of factors Aquasorb®, alginate and calcium % structure recovered.	91
Figure 4.34	The main effects of factors Aquasorb®, alginate and calcium on rate index.	92
Figure 4.35	The interactions profile of factors Aquasorb®, alginate and calcium on rate index.	92
Figure 4.36	Comparing the viscosity profile of the top formulation 1) 8%w/w sodium alginate, 3%w/w Aquasorb® and 0.15%w/w calcium with 2) Nugel®, 3) Intrasil® and 4) Sterigel®.	94
Figure 4.37	Comparing the elasticity of the top formulation 1) 8%w/w sodium alginate, 3%w/w Aquasorb® and 0.15%w/w calcium with 2) Intrasil®, 3) Sterigel® and 4) Nugel® using creep.	94
Figure 4.38	Comparing the water uptake performance of the top formulation 8%w/w alginate, 3%w/w Aquasorb® and 0.15%w/w calcium with Intrasil®, Sterigel® and Nugel®.	95



Figure 5.1	The main effects of sodium alginate and calcium concentration on gelling time.	99
Figure 5.2	The interaction profile of sodium alginate and calcium concentration on gelling time.	99
Figure 5.3	The main effects of sodium alginate and calcium concentration on gel strength.	100
Figure 5.4	The interaction profile of sodium alginate and calcium concentration on gel strength.	100
Figure 5.5	The main effects of sodium alginate and calcium concentration on the post sterilisation pH.	102
Figure 5.6	The interaction profile of sodium alginate and calcium concentration on the post sterilisation pH.	102
Figure 5.7	The main effects of sodium alginate and calcium carbonate on the 48h post gelation pH.	103
Figure 5.8	The interaction profile of sodium alginate and calcium carbonate on 48h post gelation pH.	103
Figure 5.9	The main effects of sodium alginate and calcium carbonate on zero rate viscosity.	104
Figure 5.10	The interaction profile of sodium alginate and calcium carbonate on zero rate viscosity.	104
Figure 5.11	The main effects of sodium alginate and calcium carbonate on rate index.	105
Figure 5.12	The interaction profile of sodium alginate and calcium carbonate on rate index.	105
Figure 5.13	Main effects of sodium alginate, sodium chloride and calcium carbonate on gelling time.	108
Figure 5.14	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on gelling time.	108
Figure 5.15	Main effects of sodium alginate, sodium chloride and calcium carbonate on gel strength.	109
Figure 5.16	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on gel strength.	109
Figure 5.17	Main effects of sodium alginate, sodium chloride and calcium carbonate on post sterilisation pH.	110
Figure 5.18	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on post sterilisation pH.	110
Figure 5.19	Main effects of sodium alginate, sodium chloride and calcium carbonate on 48h post gelation pH.	112
Figure 5.20	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on 48h post gelation pH.	112
Figure 5.21	Main effects of sodium alginate, sodium chloride and calcium carbonate on zero rate viscosity.	113
Figure 5.22	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on zero rate viscosity.	113
Figure 5.23	Main effects of sodium alginate, sodium chloride and calcium carbonate on rate index.	114
Figure 5.24	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on rate index.	114
Figure 5.25	Main effects of sodium alginate, sodium chloride and calcium carbonate on water uptake.	116

Figure 5.26	Interaction profile of sodium alginate, sodium chloride and calcium carbonate on water uptake.	116
Figure 6.1	The effects profile of factor influence over gelling time (factors b1-b10).	124
Figure 6.2	The effects profile of factor influence over water uptake (factors b1-b10) $XE^{-y} = X \times 10^{-y}$ .	124
Figure 6.3	The effects profile of factor influence over viscosity (factors b1-b10).	125
Figure 6.4	The effects profile of factor influence over rate index (factors b1-b10).	127
Figure 6.5	The effects profile of factor influence over pH of the sterile formulation (factors b1-b10).	128
Figure 6.6	The effects profile of factor influence over pH 48h post gelation (factors b1-b10).	129
Figure 6.7	The effects profile of factor influence over G' max (gel strength) (factors b1-b10).	130
Figure 6.8	Frequency chart of the influential of factors in the alginate gelling system.	131
Figure 7.1	The profiles of the main effects and interactions of factors affecting water uptake (8h).	136
Figure 7.2	The interaction profile of factors affecting water uptake (8h).	136
Figure 7.3	The profiles of the main effects and interactions of factors affecting water uptake (24h).	137
Figure 7.4	The Interaction profile of factors affecting water uptake (24h).	137
Figure 7.5	The profiles of the main effects and interactions of factors affecting pH of the sterile formulations.	140
Figure 7.6	The interaction profiles of factors affecting pH of the sterile formulations.	140
Figure 7.7	The profiles of the main effects and interactions of factors affecting pH 48h after gelation.	142
Figure 7.8	The interaction profiles of factors affecting pH 48h after gelation.	142
Figure 7.9	The profiles of the main effects and interactions of factors affecting gelling time.	144
Figure 7.10	The interaction profiles of factors affecting gelling time.	144
Figure 7.11	The profiles of the main effects and interactions of factors affecting gel strength (G' max).	146
Figure 7.12	The interaction profiles of factors affecting gel strength (G' max).	146
Figure 7.13	The profiles of the main effects and interactions of factors affecting gel strength (G*).	148
Figure 7.14	The interaction profiles of factors affecting gel strength (G*).	148
Figure 7.15	The profiles of the main effects and interactions of factors affecting gel strength (%G* lost 0-150Pa).	150
Figure 7.16	The interaction profiles of factors affecting gel strength (%G* lost 0-150Pa).	150

Figure 7.17	The profiles of the main effects and interactions of factors affecting zero rate viscosity of the sterile formulations.	152
Figure 7.18	The interaction profiles of factors affecting zero rate viscosity of the sterile formulations.	152
Figure 7.19	The profiles of the main effects and interactions of factors affecting the rate index of the sterile formulations	153
Figure 7.20	The interaction profiles factors affecting the rate index of the sterile formulations	153



## **LIST OF TABLES**

Table 1.1	Classification of mechanical wounds.	1
Table 1.2	Classification of wounds arising as a result of burning.	1
Table 1.3	Classification of ulcerating wounds.	2
Table 1.4	A summary of the three stages of wound healing <sup>[3]</sup> .	3
Table 1.5	Desirable properties of an ideal occlusive dressing <sup>[20,22]</sup> .	7
Table 1.6	Conditions considered ideal for wound healing <sup>[1]</sup> .	8
Table 1.7	The characteristics of the major dressing types, from references <sup>[20,43]</sup> .	11
Table 1.8	Phaeophyceae species and their gelling properties. The G/M block frequency gives an idea of the effect of the G Block content.	14
Table 2.1	The diameters of Franz cells used in this study.	45
Table 2.2	Results determining the precision of the Franz cell method, 8%w/w Sodium alginate 24h time point data (n=18).	48
Table 2.3	Different concentrations of sodium alginate, 24h timepoint data (g/g/cm <sup>2</sup> ), used to calculate the Franz cell methods' linearity of response (n=33). Shown graphically in Figure 2.13.	48
Table 2.4	8%w/w Sodium alginate with 0.5, 1 and 3%w/w Aquasorb <sup>®</sup> 24h time point data (g/g/cm <sup>2</sup> /24h), used to calculate the sensitivity of the method.	49
Table 2.5	The absorptive capacity of commercial amorphous hydrogels using different glassware at the 24h time point (g/g/cm <sup>2</sup> /24h).	50
Table 3.1	The experimental design domain, giving the 4 experiments that are required to investigate the system.	55
Table 3.2	The use of coded variables to represent the levels of each factor in Table 3.1. The results from the breaking strength tests are included.	55
Table 3.3	The mean breaking strength values for determining the effects of factors: mean values of row, column and all cells.	55
Table 3.4	A 2 <sup>5</sup> experiment, by partitioning the design i.e., performing the experiments, where abcde is positive allows the main effect and first order interactions of factors to be estimated.	59
Table 3.5	Plackett and Burman experimental design for 10 factors. Coded variables + = high level, - = low level and 0 = central point.	61
Table 4.1	Water uptake experiment factors and factor levels. For the investigation of sodium alginate alone the levels of calcium were 0%w/w (-1) and 0.15%w/w (1).	68
Table 4.2	The factors and factor levels for the rheological evaluation.	68
Table 4.3	Water uptake: factorial experimental design domain for sodium alginate and calcium.	69
Table 4.4	Rheological evaluation: factorial experimental design for sodium alginate and calcium.	69
Table 4.5	Results for the sodium alginate and calcium chloride water uptake experiments ( $\bar{X}$ , n = 6 ( $\pm$ standard deviation)).	69



Table 4.6	Results for the rheological evaluation of sodium alginate and calcium (n = 1).	71
Table 4.7	Factorial experimental design for the water uptake experiments of Celquat® formulated with alginate in the presence of calcium ions.	71
Table 4.8	Factorial experimental design for the rheological evaluation of Celquat® formulated with alginate and calcium.	75
Table 4.9	Water uptake results for the Celquat®, alginate and calcium formulation ( $\bar{X}$ , n = 6 ( $\pm$ standard deviation)).	75
Table 4.10	Results for the rheological evaluation of Celquat® alginate and calcium (n = 1).	79
Table 4.11	Factorial experimental design for the water uptake experiments of Natrosol® formulated with alginate in the presence of calcium ions.	79
Table 4.12	Factorial experimental design for the rheological evaluation of Natrosol® formulated with alginate and calcium ions.	79
Table 4.13	Water uptake results for Natrosol® formulated with alginate ( $\bar{X}$ , n = 6 ( $\pm$ standard deviation)).	81
Table 4.14	Results for rheological evaluation of Natrosol® formulated with alginate and calcium (n = 1).	81
Table 4.15	Factorial experimental design for the water uptake experiments of Aquasorb® formulated with alginate in the presence of calcium.	84
Table 4.16	Factorial experimental design for the rheological evaluation of Aquasorb® formulated with alginate and calcium.	84
Table 4.17	Water uptake results for Aquasorb® formulated with alginate in the presence of calcium ( $\bar{X}$ , n = 6 ( $\pm$ standard deviation)).	87
Table 4.18	Results for rheological evaluation of Aquasorb® formulated with alginate and calcium (n = 1).	87
Table 5.1	Factors and levels for the experimental design investigating the gelation of sodium alginate and calcium carbonate.	97
Table 5.2	Two factor, two level experimental design investigating the gelation of sodium alginate and calcium carbonate.	98
Table 5.3	Results of the physical tests performed on each formulation, given in Table 5.2.	98
Table 5.4	Factors and Levels for the experimental design investigating the effects of sodium alginate, calcium carbonate and sodium chloride.	106
Table 5.5	Three factor, two level experimental design investigating the effects of sodium alginate, calcium carbonate and sodium chloride.	106
Table 5.6	Results of the physical tests performed on each formulation, given in Table 5.5.	107
Table 6.1	The factors and their levels for the Plackett and Burman screening experimental design investigating the development of an in-situ gelling alginate formulation.	121
Table 6.2	Plackett and Burman screening experimental design for 10 factors at 2 levels, with a central domain (coded 0) to estimate the precision of the experiment.	122

Table 6.3	Results of the characterisation tests carried out on each of the sixteen formulations in the Plackett and Burman experimental design. The sterile pH was obtained from the liquid formulation at 0h and the gel 48h post gelation.	122
Table 6.4	The effects of factors (b1-b10) for each characterisation test, calculated using Minitab <sup>®</sup> .	123
Table 6.5	Tally of the five most active factors for each characterisation method, to determine the most influential for further investigation.	130
Table 7.1	A $2^{5-1}$ fractional factorial experimental design, allows the estimation of main effects and first order interactions without confounding.	133
Table 7.2	Factors and their levels for the $2^{5-1}$ fractional factorial design.	134
Table 7.3	Water uptake method results for each formulation in the $2^{5-1}$ fractional factorial experimental design ( $\bar{X}$ , $n = 3$ ( $\pm$ standard deviation)).	135
Table 7.4	The main effects and interactions of factors for water uptake characterisation, in the fractional factorial experimental design.	135
Table 7.5	pH determination results for each formulation in the $2^{5-1}$ fractional factorial experimental design ( $n = 1$ ) (48h = 48h post gelation).	138
Table 7.6	The main effects and interactions of factors for pH determination, in the fractional factorial experimental design (48h = 48h post gelation).	139
Table 7.7	The results from the rheological characterisation of each formulation in the $2^{5-1}$ fractional factorial experimental design ( $n = 1$ ).	143
Table 7.8	The main effects and interactions of factors from the rheological characterisation of formulations in the fractional factorial experimental design.	143
Table 8.1	A summary of results from the characterisation of amorphous hydrogels from Chapter 4 (Section 4.2.2 and Tables 4.5, 4.6, 4.9, 4.10, 4.13, 4.14, 4.17 and 4.18).	159
Table 8.2	A formulation for an in-situ gelling wound dressing.	168



## **GLOSSARY**

Acute wound:	Wounds with no underlying healing defect that proceed to restore skin structure and function in an orderly fashion.
Chronic wound:	Long lasting wound [US national health statistics 3 months or longer].
Debride:	To excise or remove devitalised or dead tissue and foreign matter from a wound.
Decubitus ulcer:	Ulcer in the skin over a bony prominence.
Dehiscence:	A bursting open, splitting or gaping along natural or sutured lines.
Eschar:	(scab) Thick, leathery crust of dead tissue often covering an underlying necrotic processes.
Exudate:	Drainage, which comes from a wound.
Granulation tissue:	Soft pink fleshy projections of tissue that form during the healing process in a wound.
Granuloma:	Indefinite term applied to nodular inflammatory lesions, usually small or granular, firm, persistent and containing compactly grouped mononuclear phagocytes.
Hypertrophy:	General increase of a part of an organ, not due to tumor formation. Synonymous with hyperplasia.
Ischaemic:	Local anemia due to mechanical obstruction (mainly arterial narrowing) of the blood supply.
Keloid scar:	A nodular, firm, moveable non-encapsulated linear mass of hyperplastic scar tissue, tender and frequently painful.
Macerate:	Softening by the action of liquid.
Maceration:	Softening by soaking or steeping.
Necrosis:	Pathological death of cells or a portion of tissue or organ, resulting from irreversible damage.
Necrotic:	Pertaining to or affected by necrosis.
Slough:	Necrosed tissue separated from the living structure or to separate from living tissue, said of a dead or necrosed part.
Trauma:	An injury physical or mental.
Wound:	Trauma/ injury to living tissues of the body.
Wound Dehiscence:	Disruption of apposed surfaces of a wound.

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## **ABSTRACT**

The management of wound healing using occlusive dressings is a relatively recent development. Winter (1962) in a landmark investigation, proved the ability of wounds to re-epithelialise more quickly under an occlusive dressing. There are a limited number of occlusive dressings that are capable of forming *in-situ*. The benefits of a dressing that can form *in-situ*, and covering the wound area completely, could include increased patient comfort and reduced maceration from excess exudate. This thesis investigates the development of an alginate based wound dressing capable of gelling *in-situ*.

A new method for determining the absorptive capacity of wound dressings was developed and validated. The method used a Franz cell by filling the lower compartment with a gel and then used the upper compartment to hold a donor solution. This allowed the uptake of water to be monitored by weight change over time. The Franz cell method was used to characterise the physical properties of the hydrogel formulations developed in this thesis.

The use of cellulose based polymers as gelling additives was investigated. The gels produced were not thought capable of forming gels *in-situ*. The method of manufacture was not representative of the intended application. The polymers used were Natrosol® 250MPharm, Aquasorb® A500 and Celquat® SC240C. The polymers were formulated with sodium alginate and sterilised. The resultant gels could be described as amorphous hydrogels and were compared to the commercial products Intrasil® (Smith and Nephew Ltd.), Nugel® (Johnson and Johnson) and Sterigel® (SSL). The formulation containing 3%w/w Aquasorb® A500 and 8%w/w Sodium alginate was considered as better than the commercial formulations, based on the characterisation methods used.

The use of delta gluconolactone was reported in combination with sodium alginate by McDowell (1954). More recently delta gluconolactone was used in the manufacture of microbiological cell culture media (Draget *et al* 1989). The technique incorporated the use of calcium carbonate to ensure homogeneous gelation of sodium alginate with non-toxic by products. This method of alginate gelation was used to develop an *in-situ* gelling wound dressing. A formulation comprised of: Sodium alginate (5.5%w/w), Sodium alginate type LF 10/60, Sodium chloride (0.25 %w/w), Calcium carbonate (0.22%w/w), Phosphate buffer (0.25%w/w), Glycerol (5%w/w), Sodium carboxymethyl cellulose type low viscosity, Sodium carboxymethyl cellulose (0.62%w/w), Hyaluronic acid (0.05%w/w). This would form a gel *in-situ* within an hour and in combination with a secondary dressing allow the treatment of leg ulcers, cavity and burn wounds.

**CHAPTER 1 GENERAL INTRODUCTION**

**1.1 WOUNDS**

Wounds arise from cellular damage or death, usually resulting from trauma, and are classified by the nature of trauma.

**1.1.1 Classification of Wounds**

Thomas<sup>[1]</sup> defined a wound as a defect or break in the skin that results from physical, mechanical or burn damage, or one that develops as a result of the presence of an underlying medical or physiological disorder. It is possible to classify the type of wound as a result of trauma.

**1.1.1.1 MECHANICAL INJURY**

Mechanical injuries are classified by the cause of the injury. Brief summaries of different types of mechanical injury are found in Table 1.1.

Table 1.1 Classification of mechanical wounds.

Type of injury	Cause
Abrasion/laceration	friction from a rough surface
Penetrating	sharp object or bullet
Bite	human or animal
Surgical	scalpel, suture etc

**1.1.1.2 BURNS**

Thermal, chemical, electrical or radiation injuries are classified by the extent of injury caused and are briefly summarised in Table 1.2.

Table 1.2 Classification of wounds arising as a result of burning.

Type of Burn	Area affected
1 <sup>st</sup> Degree	superficial burns of the epidermis and upper layers of the dermis
2 <sup>nd</sup> Degree	deep dermal burns where most of the epidermal layer is destroyed
3 <sup>rd</sup> Degree	full thickness burns where all elements of the skin are destroyed

**1.1.1.3 ULCERS**

Ulcerative wounds are classified by the cause of the injury. A brief summary of different types of ulcer can be found in Table 1.3.



Table 1.3 Classification of ulcerating wounds.

Type of Ulcer	Origin
Decubitus	pressure to load bearing surface
Ischaemic	vasoconstriction
Other	systemic infection

## 1.2 HEALING

Immediately upon injury the wound begins to heal. It is a complex three phase process, which takes a minimum of 14 days to complete<sup>[2,3]</sup>. The three phases of healing: inflammation, proliferation and maturation are discussed in section 1.2.2, following an introduction to the methods/mechanisms of wound closure (section 1.2.1). The reader is directed to the text by Clark<sup>[4]</sup> which covers the molecular and cellular biology of wound healing in detail.

### 1.2.1 Healing Mechanisms

The four general mechanisms of wound healing are classified by the nature in which the medical professional prepares the wound for healing. For each mechanism, the healing response is essentially the same (section 1.2.2).

#### 1.2.1.1 PRIMARY INTENTION

Healing by primary intention or primary closure is the mechanism used on mechanical wounds, such as surgical incisions, which are injuries that have not become infected. The wound edges are drawn and held together *e.g.*, by suture. The resultant wound contains minimal quantities of granulation tissue and leaves only a thin scar.

#### 1.2.1.2 SECONDARY INTENTION

Healing by secondary intention (open granulation) is applied to wounds when it is not possible to draw the edges together without stretching the skin, *i.e.* the injury is long standing or infection has occurred. As re-epithelialisation occurs, the wound becomes progressively filled with granulation tissue which, when it comes to the edges of the wound, triggers the reformation of the epidermis.

#### 1.2.1.3 DELAYED PRIMARY CLOSURE

This is a procedure where the wound is prepared for suture but left 'open' to allow infection to be treated or when the blood supply is poor, to allow it to improve.



1.2.1.4 GRAFTING AND FLAP FORMATION

A graft, a piece of skin containing the dermis and epidermis, is removed from a different site on the body and placed over the wound. A successful graft reduces healing time and the chances of infection. When a skin flap is employed, the tissue is not completely detached from the body, allowing the skin to continue to receive a blood supply.

**1.2.2 The Healing Response**

Wound repair is not a simple linear process in which growth factors are released by physiological events, but rather it is an integration of dynamic processes involving soluble mediators, formed blood elements, extracellular matrix and parenchymal cells<sup>[4]</sup>. The three phases of this process: inflammation, proliferation and maturation are summarised in Table 1.4.

Table 1.4 A summary of the three stages of wound healing<sup>[3]</sup>.

<b>Phase</b>	<b>Time /days</b>	<b>Response at the Wound space</b>	<b>Tissues surrounding the wound</b>
Inflammation	0-4	Coagulation	Oedema and Inflammation
Proliferation	5-14	Granulation tissue and Collagen formation	Increased blood flow
Maturation	15-(1 year)	Regress of vessels Maturation of collagen	Normalisation

1.2.2.1 INFLAMMATION

The acute inflammatory response usually extends from the onset of injury to approximately the fourth day of healing, characterised by the symptoms of erthyema (redness), oedema (swelling), pain and clot formation. Haemostasis and the inflammation stage are intrinsically linked and will therefore be discussed as one. The process begins with vasoconstriction, reducing blood flow to the injured area. Platelets then start to aggregate, stimulated by the damaged tissue, swell and become adherent<sup>[4]</sup>. A positive feedback cycle is activated by platelet degranulation, releasing factors that attract more platelets and begin clot formation, which in turn facilitates the migration of epidermal cells into the wound stimulating fibroblast proliferation<sup>[5]</sup>.

Blood coagulation, or clotting, is initiated at the time of injury and follows two pathways: the intrinsic and extrinsic pathways. Platelet aggregation in the blood vessels initiates the intrinsic pathway. The extrinsic pathway is initiated by the release of

thrombin tissue factor by damaged endothelial cells, coagulating blood within the wound space, which results in eschar (scab) formation<sup>[6]</sup>. Clot formation is managed by fibrinolysis, ensuring the forming clot remains local to the wound area<sup>[4]</sup>.

The large surface area provided by the aggregation of platelets and subsequent coagulation of blood initiates the cascades and pathways of healing. The surface activation of Hageman factor (factor XII)<sup>[6]</sup> leads to the release of vasoactive agents such as bradykinin<sup>[4,6,7]</sup>. The subsequent increase in blood vessel permeability allows the influx of leukocytes into the wound, the start of the next stage of healing, proliferation.

#### 1.2.2.2 PROLIFERATION

The second phase of healing comprises two processes: i) re-epithelialisation and ii) granulation tissue formation, which occur simultaneously after trauma and continue throughout the inflammation and proliferation phases. Protection from the environment is ensured by re-epithelialisation. Granulation tissue formation leads to a return of dermal integrity.

During re-epithelialisation the wound is covered by the migration of cells, in a leapfrog fashion<sup>[8,9,10]</sup>, from the stratified epidermis (Figure 1.1). Phenotypic changes prevent cells from undergoing terminal differentiation allowing the wound space to be covered. The migrating cells attach themselves to the basement membrane, if this has been removed by the injury, the cells attach to the provisional matrix. The provisional matrix is formed from the fibrin clot, which provides an ideal environment for the influx cells to the wound site promoting the formation of granulation tissue<sup>[4]</sup>. Once the wound surface is covered, the epidermal cells return to their intended morphology.

Angiogenesis commences 2-3 days after injury. It is the process of new capillary formation from existing small venules in close proximity to the wound site. The wound takes on a granular appearance as the inflammation stage subsides. The capillaries are covered by an extracellular matrix, formed by the influx of material into the wound by fibroplasia. Fibroblasts are attracted to the wound to secrete collagenous and non-collagenous matrices. The extracellular matrix consists of macrophages, fibroblasts and endothelial cells embedded in a loose matrix of collagen (types I, II and III), fibrin, fibronectin and proteoglycans (hyaluronic acid)<sup>[4,6,7]</sup>.



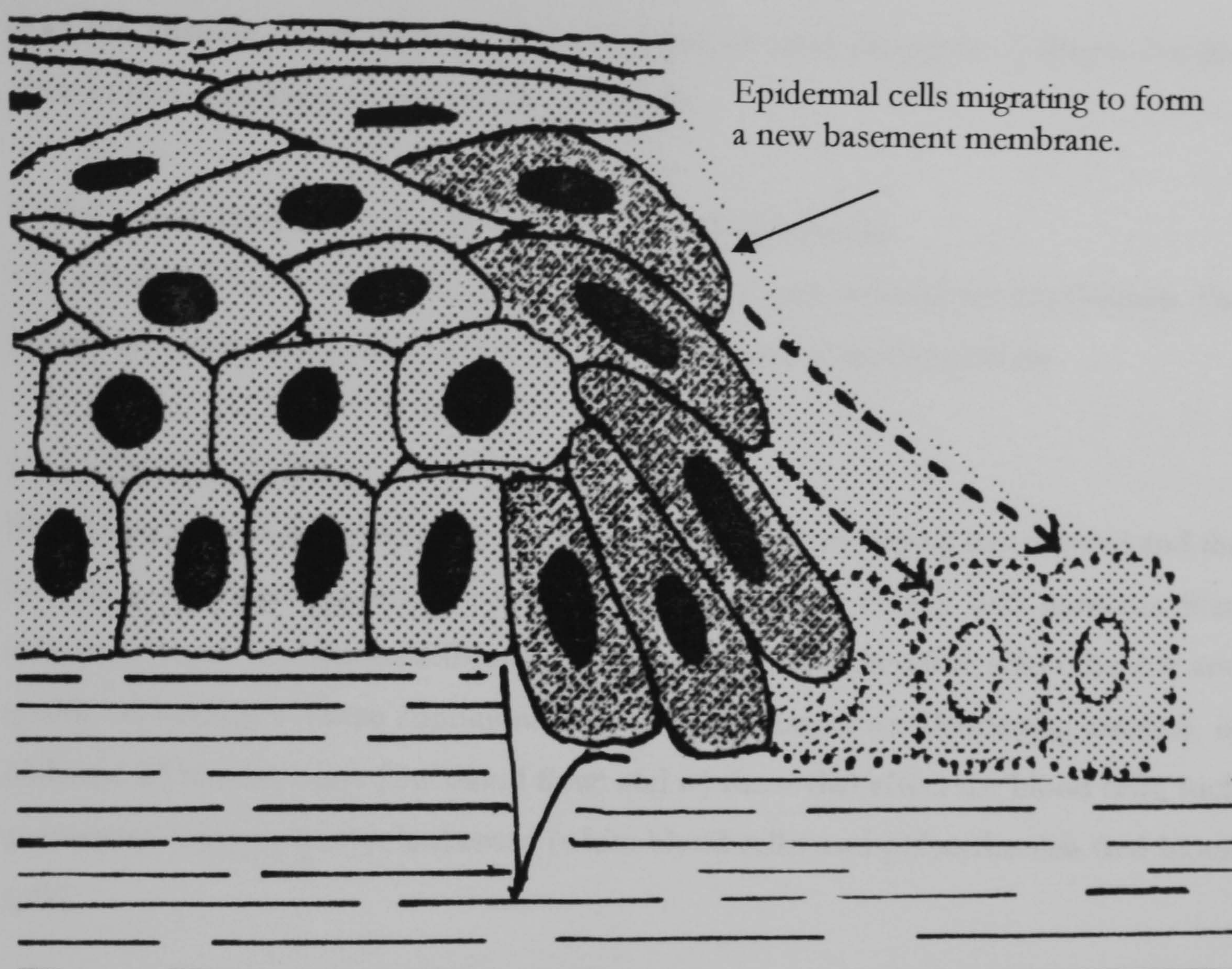


Figure 1.1 A schematic diagram showing the leapfrog migration of epidermal cells to form a new basement membrane during wound healing<sup>[8]</sup>.

#### 1.2.2.3 MATURATION

The final phase of healing sees the remodelling of the extracellular matrix, cell maturation and apoptosis of endothelial cells and myofibroblasts. Wounds gain only about 20% of their final strength by the third week after trauma. Thereafter, the rate at which wounds gain tensile strength is slow and they fail to attain the same breaking strength as uninjured skin. At its maximum strength a scar is only 70% as strong as intact skin<sup>[2]</sup>.

Extracellular matrix components serve several critical functions for effective wound repair. First, during granulation tissue formation, fibronectin provides a provisional substratum for the migration of and growth of cells, a linkage for myofibroblasts to effect wound contraction and a focus for collagen fibrillogenesis. The presence of large quantities of highly hydrated hyaluronic acid in granulation tissue provides a matrix that is easily penetrated by migrating parenchymal cells. The early formation of types I, III and V collagen fibrils, provides tensile strength for the wound. As the matrix matures



over the ensuing weeks fibronectin and hyaluronic acid disappear. Collagen bundles grow in size, increasing wound tensile strength<sup>[4]</sup>.

### **1.2.3 Factors That Influence The Wound Healing Process**

The discussion has so far assumed that healing has progressed without interference. The following section details factors that can impair the wound healing process.

#### **1.2.3.1 CIRCULATION**

Blood supply has a great influence on healing. Once haemorrhaging has stopped and the healing process has started, the wound consumes more energy and nutrients than uninjured tissue. Conditions affecting the blood supply will therefore affect the rate and quality of healing<sup>[3]</sup>. These conditions include i) arteriosclerosis (hardening arteries), ii) diabetes, iii) venous stasis (low blood flow) and iv) those that affect the blood cells, such as anaemia (oxygen levels), leukaemia (white blood cells) and polycythaemia (red blood cells).

#### **1.2.3.2 INFECTIONS OF THE WOUND**

The presence of bacteria in the wound does not necessarily lead to infection. The balance between the number and virulence of bacteria and the effectiveness of the immune response determines whether colonisation leads to infection. Wound microbiology is discussed in section 1.3.6.

#### **1.2.3.3 MALNOURISHMENT**

Vitamin, protein (and amino acid) and trace element deficiencies have the potential to impair healing. Vitamin A aids epithelisation<sup>[11]</sup> and vitamin C<sup>[12]</sup> is required for collagen synthesis. Proteins and amino acids are required for new tissue and cell formation. Trace elements such as zinc are required for protein synthesis<sup>[13,14]</sup> and calcium is very important for clot formation<sup>[4]</sup>.

#### **1.2.3.4 CHEMICAL AGENTS**

Drugs affect wound healing indirectly, *e.g.*, vasoconstrictors such as nicotine reduce blood flow<sup>[15]</sup>, topical antibiotics were found to impair healing<sup>[16,17]</sup>. Lineweaver *et al*<sup>[18]</sup> found that antiseptic solutions of, 1% povidone iodine, 0.25% acetic acid, 3% hydrogen peroxide and 0.5% sodium hypochlorite, delayed wound healing.

1.2.3.5 AGE

Physiological differences of the skin affect healing; the skin of the aged is thinner, drier, more fragile and more easily bruised<sup>[7,19]</sup>. Inappropriate use of adhesive bandages to treat ulcers for example, can remove new epithelial tissue leading to new sites of ulceration<sup>[20]</sup>.

1.3 WOUND MANAGEMENT

Clinicians require dressings that are capable of maintaining the wound in an ideal environment for successful, rapid healing. The desirable properties of an ideal dressing are listed in Table 1.5. However, it must be noted that it is not possible for a single dressing to heal all types of wound<sup>[20]</sup>. The huge variety of dressings available on the market is testament to this<sup>[20,21]</sup>.

Table 1.5 Desirable properties of an ideal occlusive dressing<sup>[20,22]</sup>.

Properties of an ideal dressing
Ability to handle excess exudate
Removal of toxic substances
Moist environment over the wound
Gaseous exchange permitted
Barrier to microorganisms
Thermal insulation provided
Freedom from particulate contaminants
Removal without trauma to new tissue

1.3.1 History

For an in depth history of wound management the reader is referred to the work of Spyratou<sup>[23]</sup>.

1.3.2 Occlusive Wound Management

Florence Nightingale, during the Crimean war (1851-1853), made the first steps towards effective wound management practice by establishing the foundations of modern nursing techniques. The mainstay of dressings, from then up to the 1950’s, were absorbent non-occlusive materials retained by various adhesive tapes or bandages. The advice to allow the wound to breathe was followed unquestioningly<sup>[24]</sup>. It was generally accepted that in order to prevent infection a wound should be kept as dry as possible<sup>[3]</sup>.

The most significant advance in the development of wound management occurred with the advent of occlusive healing. Early investigators of occlusive wound management



were Bloom<sup>[25]</sup>, Bull *et al*<sup>[26]</sup> and Schilling *et al*<sup>[27]</sup>. The first investigation to prove that wounds healed more rapidly under occlusion was carried out by Winter<sup>[28]</sup>. Using a porcine model, Winter<sup>[28]</sup> showed that an occlusive dressing (polyethylene) epithelialised the wound twice as rapidly as a wound left open to the air.

An occlusive dressing can be defined as a dressing that forms a barrier between the wound and the outside environment. Semipermeable dressings are permeable to water vapour, oxygen and other gases but are impermeable to water and bacteria. Semiocclusive dressings have a greater absorptive mechanism, and together with semipermeable films, maintain a moist wound surface<sup>[29]</sup>. A water vapour exchange, of approximately 2500g/m<sup>2</sup>/24h compared to a 5-7000g/m<sup>2</sup>/24h loss of water from an uncovered wound, prevents dehydration<sup>[29,30]</sup>.

### 1.3.3 The Properties Of Occlusive Dressings

The ideal properties of an occlusive dressing are outlined in (Table 1.6) with the conditions required for rapid cutaneous wound healing.

Table 1.6 Conditions considered ideal for wound healing<sup>[1]</sup>.

<b>Ideal conditions for healing</b>
Moist with exudate, not macerated
Free of toxins & debris from the dressing
Optimal temperature for healing
Undisturbed from frequent dressing changes
At an optimum pH value
Free of clinical infection & excessive slough

#### 1.3.3.1 GASEOUS PERMEABILITY

The gaseous permeability of an occlusive dressing exerts a certain amount of control over the wound environment. The wound dressing should prevent dehydration but allow the evaporation of excess wound fluid. Film dressings such as Opsite<sup>®</sup>, which has a vapour permability four times that of skin, can still retain exudate<sup>[30]</sup>. The oxygen levels in the wound are also important. Gradients of oxygen concentration exist and tend towards zero at the centre of the wound<sup>[31]</sup>. Consequently angiogenic migration progresses towards the centre of the wound, stimulated by the secretion of angiogenic factors by hypoxic macrophages <sup>[32,33]</sup>.



### 1.3.3.2 pH

Oxygen levels, cellular activity<sup>[32]</sup> and bacterial proliferation<sup>[30]</sup> can all be potentially affected by the pH of the wound and, by default, so can the dressing. Leveen *et al*<sup>[34]</sup> demonstrated how a 0.9 unit change in wound pH resulted in a 5-fold increase in oxygen level. In a study on wounds left for delayed primary closure Lipton *et al*<sup>[35]</sup> found that of 51 clean uncontaminated wounds 18 became infected. Wounds closed below the critical pH, pH 7.2, would undergo suppuration. However, under the occlusive dressing Granuflex<sup>®</sup> wound pH was found to be pH 5.6 24h after dressing<sup>[36]</sup>. Thomas<sup>[1]</sup> concluded that dressings that directly or indirectly reduce the pH of exudate might help to prevent infection. The microbiology of the wound is dealt with in section 1.3.6.

### 1.3.3.3 FREEDOM FROM TOXINS AND DEBRIS

It is important to ensure that dressings are free from material that will cause adverse reactions. As early as 1913 it was recognised that small cellulose particles entering a wound could cause a foreign body reaction and granuloma<sup>[37]</sup>. Wood fibres in the wound (from surgical gowns) were found to cause amongst other reactions, keloid scars and wound dehiscence<sup>[38]</sup>. Fibres from cotton dressings become stuck in wound exudate and glue the dressing to the wound<sup>[39]</sup>. Dressing removal damaging the wound retards healing. Alginates are thought to be biodegradable because they are natural polymers, although this theory has been reconsidered<sup>[19]</sup>.

### 1.3.3.4 IMPERMEABILITY OF A DRESSING TO MICROORGANISMS

The ability of a dressing to prevent microbial penetration is important, equally important is the ability of the same dressing to prevent the escape of pathogens. Two methods used to prevent the exudate reaching the dressing surface (strike through) which provides a pathway through which bacteria can enter the wound are i) cellulose layers (isolated from the wound surface) to transport exudate laterally and ii) hydrophobic layers to physically prevent strike through<sup>[1]</sup>.

### 1.3.3.5 THERMAL PROPERTIES

Enzymatic activity in the wound is probably susceptible to temperature, which will have an affect on healing. A wound does not quickly regain normal metabolic rates<sup>[39]</sup>, because phagocytic and mitotic activities are sensitive to temperatures below 28°C<sup>[40]</sup>. The role of the dressing is therefore important to ensure that the wound is kept at an

ideal temperature. In a study using pigs housed at 21°C, Lock<sup>[40]</sup> compared wound temperatures under different dressings. The temperature of an open wound was found to be 21°C, covered with cotton wool 25-27°C, with a polyurethane sheet 30-32°C and with Synthaderm (polyurethane foam) 33-35°C. The latter two dressing are occlusive and raise the wound temperature above the critical value of 28°C.

### **1.3.4 Dressing Groups**

There are 5 main groups of occlusive and semi-occlusive dressings, manufactured from a variety of products. They are: 1) hydrogel, 2) alginate, 3) hydrocolloid, 4) foam and 5) film. The characteristics and the advantages/disadvantages associated with products from the different groups are summarised in Table 1.7.

### **1.3.5 Occlusive Wound Healing**

The formation of a scab (eschar) is nature's method of protecting the wound as the processes of wound healing continue. The scab is not vapour (water) proof, but protects the underlying tissue from contamination and damage. Under the layer of dried exudate is a dehydrated and non-viable layer of the dermis. There is no obvious layer separation. Water loss by evaporation is balanced by the re-wetting of the tissue below. There is a clear disadvantage of allowing a wound to heal this way, as seen in Figure 1.2. Under the occlusive dressing the epidermal cells migrate unimpeded over the wound bed compared to the route taken by the epidermal cells under the scab, first studied by Hinman and Maibach<sup>[41]</sup>. The epidermal cells must cleave a passage under the dry dermis before the latter stages of healing can commence<sup>[8]</sup>. The scab is detached by the regeneration of the epidermis.

Wounds covered with an occlusive dressing do not form a scab and remain hydrated. The survival of epidermal cells in the hair follicles and sweat glands within the wound site improves the chances of rapid healing. They are the source of the new basement membrane. Hence, the hydrated wound surface allows the rapid movement of cells and during dressing changes, avoids secondary damage. The result of dehydration of the dermal layer is to cause further trauma. The occlusive dressing prevents maceration of the wound by absorbing excess exudate.



Table 1.7 The characteristics of the major dressing types, from references<sup>[20,43]</sup>.

Dressing Type	Composition	Indication	Advantages	Disadvantages
Hydrogel	Crosslinked polymer matrix, 96% water.	Acute/chronic partial thickness exudative, painful wounds.	Soothing, cooling, non adherence and absorptive.	Secondary dressing required, cost and frequency of changes.
Alginate	Calcium alginate fibres woven/non woven.	Full/partial thickness with high exudate and post operative wounds.	Highly absorbent, haemostat, gelling provides moist environment.	Secondary dressing, cost, not ideal for dry wounds.
Hydrocolloid	Mixture of adhesive, absorbent polymers and a gelling agent.	Acute/chronic or full thickness wounds, pressure sores, stasis ulcers.	Debrides wound, non adherence, absorptive, protects and reduced dressing frequency.	Cost, foul smell, may disrupt new epithelium.
Foam	Polyurethane foam, with hydrophilic and hydrophobic surfaces.	Acute/chronic partial thickness wounds, wounds with surrounding dermatitis.	Debrides, absorptive, offers extra protection against further damage.	May become incorporated into wound, secondary dressing required.
Film	Polyurethane or co-polyester film with adhesive backing.	Superficial burns, graft donor sites, acute partial thickness wounds with low exudate.	Fluid retention, decreases wound pain at donor site, transparency.	Cost, low absorption, may adhere to wound, does not debride.



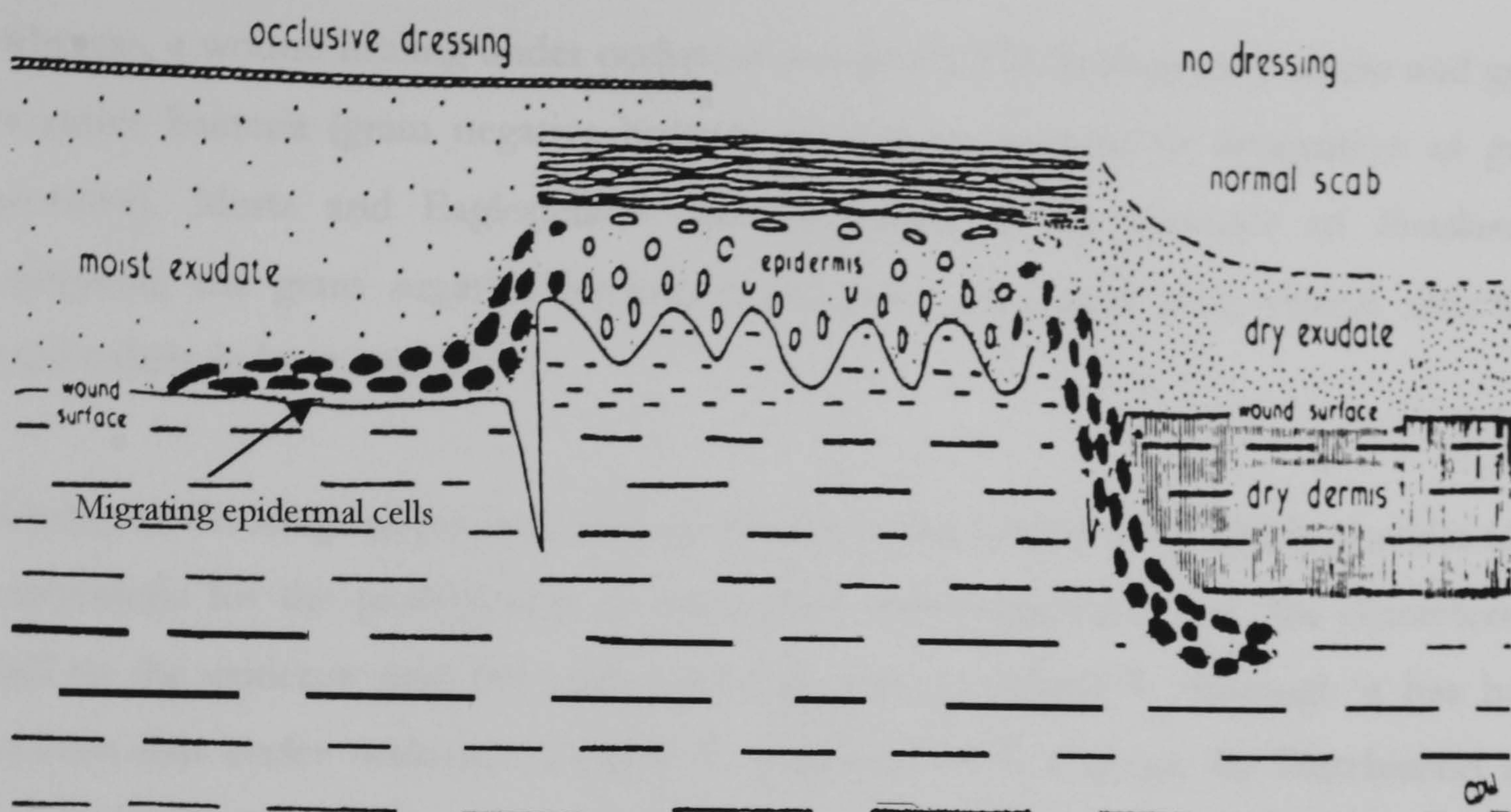


Figure 1.2 Schematic diagram comparing the benefits of occlusive over normal healing, compare the paths of the migrating epidermal cells<sup>[8]</sup>.

The effects of pH, oxygen level and temperature are complex and can have both detrimental as well as beneficial effects on the healing wound. In the case of macrophages, important mediators in the healing process, the hypoxic environment of the wound stimulates the phages to secrete the factors which encourage angiogenic migration towards the centre of the wound<sup>[33]</sup>. Consequently an occlusive dressing can promote or prolong the hypoxic environment. The effect on macrophages in this respect is particularly important since hypoxia may reduce their production of some inflammatory and pain reducing mediators such as prostaglandin and at the same time enhance others that encourage angiogenesis<sup>[42]</sup>. Wounds without a blood supply would not, however, benefit from dressings promoting hypoxia<sup>[43]</sup>.

Collagen synthesis and fibroblast proliferation under occlusion affect the healing wound. Occluded wounds are infiltrated by fibroblasts and collagenous material earlier than non-occluded wounds<sup>[28]</sup>, producing wounds with reduced scarring<sup>[44]</sup>. The biosynthesis of collagen in occluded wounds is 20-60% greater than normal wounds<sup>[43]</sup>.

### 1.3.6 Wound Microbiology

The skin harbours a large number of microbes and it is therefore very difficult to keep a wound free from bacteria. The three species of bacteria associated with clinical infection are *Staphylococcus aureus*, *Staphylococcus hyicus* and *Pseudomonas aeruginosa*<sup>[45,46,47]</sup>. Mertz and Eaglestein<sup>[48]</sup> indirectly showed that the type of bacteria found in a wound depends on the dressings used. A wound healing normally is colonised by gram positive bacteria,



whereas, a wound healing under occlusion is colonised by both gram positive and gram negative bacteria (gram negative bacteria are not as resistant to desiccation as gram positive). Mertz and Eaglestein<sup>[48]</sup> failed to mention the presence of *Pseudomonas aeruginosa*, the gram negative bacteria most often associated with clinical infection, particularly in burn wounds<sup>[46]</sup>.

Occlusive dressings characteristically produce a moist environment which provides ideal conditions for the proliferation of pathogenic microorganisms, and this characteristic led to the criticism that infection would be sure to follow<sup>[24]</sup>. Although it has been shown that under occlusion bacteria do proliferate<sup>[49,50]</sup>, a review by Hutchinson and McGukin<sup>[24]</sup> showed that infection rates of occlusive and non-occlusive dressings were, 2.6% and 7.6% respectively. It was concluded that the proliferation of bacteria under occlusive dressings is inevitable but is in general not detrimental to wound healing. In fact, the presence of bacteria are thought to promote more rapid healing<sup>[24]</sup>.

An inflammatory response is induced by both wound healing and infection. It is therefore essential to differentiate between colonisation and infection of a wound to ensure it is treated correctly. The characteristics of infection are the invasion of viable tissue by pathogens and a polymorphonuclear response. Burn wounds are the wounds most notorious for becoming infected<sup>[3]</sup>. Clinical practice dictates that occlusive dressings are not used for infected wounds. Occlusive dressings that reduce the proliferation of bacteria have been investigated<sup>[46]</sup>, but the use of such dressings on infected wounds was considered questionable until the benefits are clinically proven.

#### **1.4 ALGINATES IN WOUND MANAGEMENT**

The early history of alginates in wound management can be traced to records of the use of seaweed by coastal populations for the healing of burns<sup>[51]</sup>. Blaine<sup>[52]</sup> performed animal experiments to investigate the histological effects of alginate implanted into various tissues and the use of a calcium alginate gel on experimental wounds. Passe and Blaine<sup>[53]</sup>, and Olivier and Blaine<sup>[54]</sup> found calcium and sodium alginate were absorbed, with no evidence of adverse histological changes when used without antiseptic agents. The rate of absorption varied with location and vascularity of tissue. The use of alginates was widespread up to the 1970's and a survey showed that alginates were being

used in some 70 hospitals in one form or another<sup>[1]</sup>. The high cost of manufacture led a decline in their use<sup>[55]</sup>.

Since this decline modern manufacturing processes have enabled the widespread use of alginates in wound management<sup>[55,56]</sup>. Gilchrist and Martin<sup>[57]</sup> were the first to use the modern material in a clinical study of foot injuries and supported the findings of Blaine<sup>[52]</sup>. The biodegradability of sodium alginate has, however, come into question<sup>[58,59,60]</sup>. The fact remains that it is biocompatible<sup>[19,61]</sup> and therefore a useful basis for further studies into wound management products. Calcium alginate fibre dressings, which were successfully used and clinically proven to improve wound healing<sup>[51,58,61]</sup>, have a very high content of calcium and exhibit good haemostatic properties.

### 1.4.1 Structure And Properties Of Alginates

Alginates are recovered commercially from marine brown algae (*Phaeophyceae*), agar and carrageenans from red marine algae (*Rhodophyceae*). Some of the species of *Phaeophyceae* used for the production of alginates are summarised in Table 1.8. The frequencies ( $F_G$  or  $F_M$ ) of G and M blocks, were calculated from  $^{13}C$  NMR<sup>[62,63]</sup> and give an indication of the physical properties of the alginate types, which are discussed in section 1.4.1.1, in more detail. The more useful physical properties of alginates include water retention, enhanced gelling and viscosity (thickener)<sup>[62]</sup>. Alginates are widely used in over 300 industrial, pharmaceutical and medical applications<sup>[55,56]</sup>.

Table 1.8 *Phaeophyceae* species and their gelling properties. The G/M block frequency gives an idea of the effect of the G Block content.

<i>Phaeophyceae</i> Species	Alginate gel properties	Frequency $F_G/F_M/F_{GG}$
<i>Macrocystis pyrifera</i>	medium strength	0.39/0.61/0.16
<i>Laminaria hyperborea</i>	high strength	Stipe 0.68/0.32/0.56
<i>Laminaria digitata</i>	medium strength	0.41/0.59/0.25
<i>Laminaria japonica</i>	low strength	0.35/0.65/0.18
<i>Ascophyllum nodosum</i>	low strength	0.36/0.64/0.16
<i>Ecklonia maxima</i>	medium strength	0.45/0.55/0.22
<i>Lessonia nigrescens</i>	medium strength	0.38/0.62/0.19

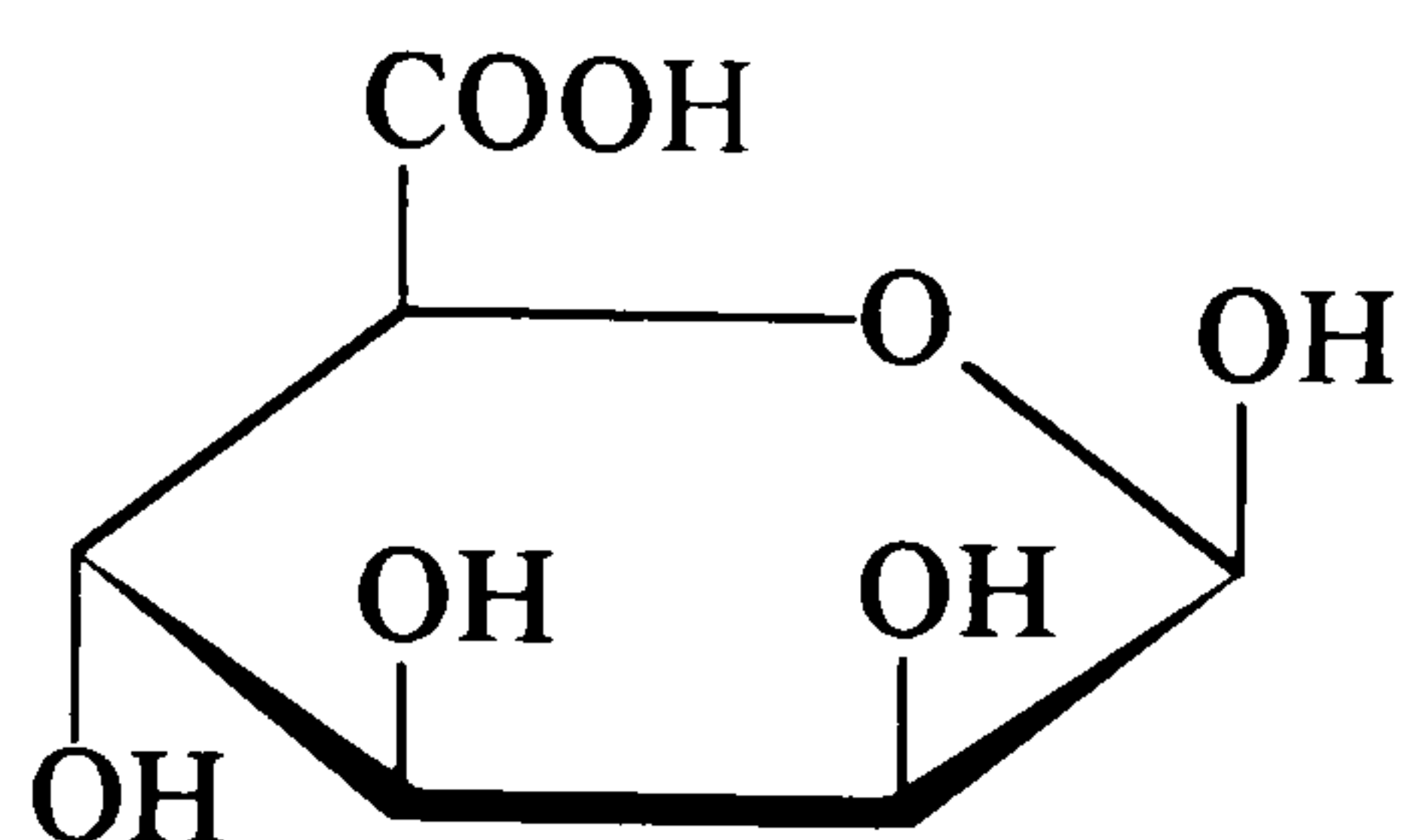


#### 1.4.1.1 CHEMICAL COMPOSITION.

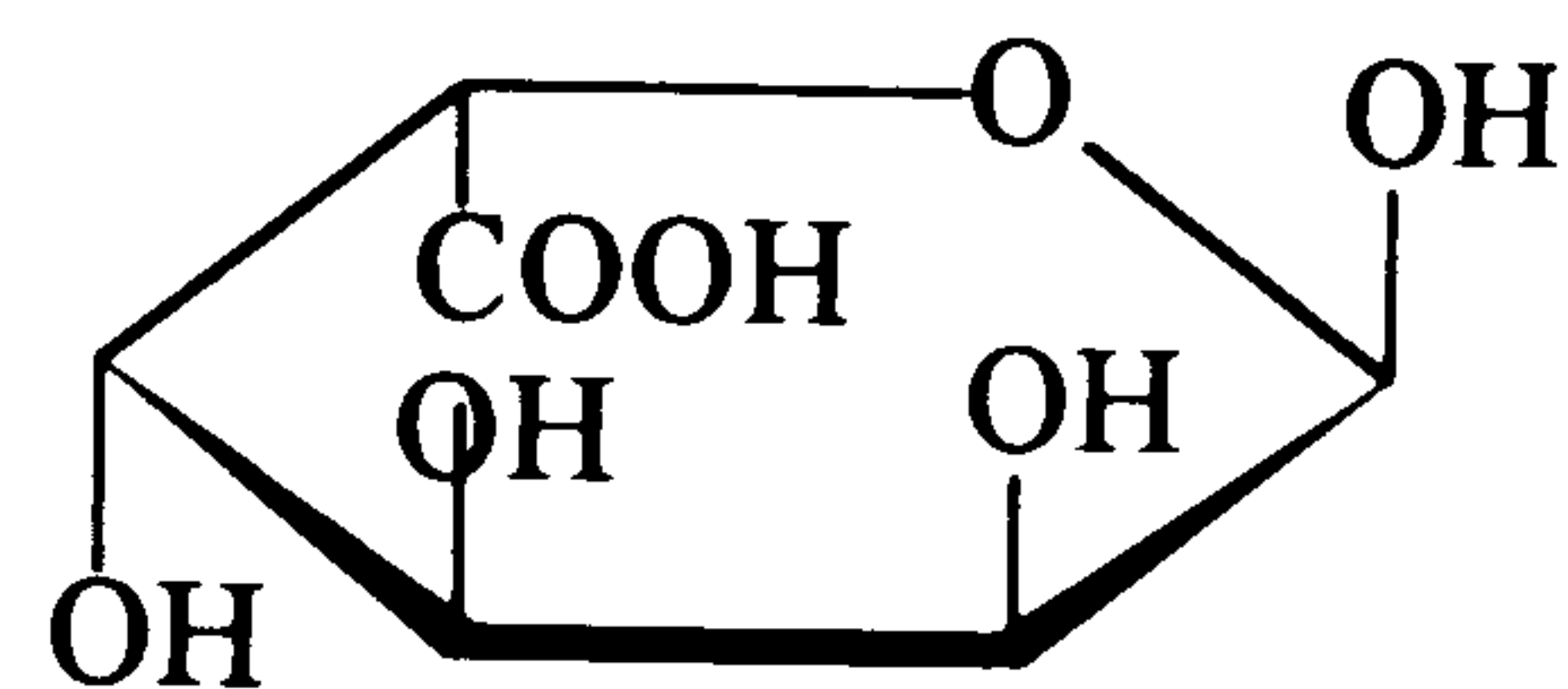
Sodium alginate is comprised of two monosaccharide units;  $\alpha$ - L- gulopyranuronic acid (abbreviated G) and  $\beta$ - D- mannopyranuronic acid (abbreviated M), linked by (1 $\rightarrow$ 4) glycoside bonds (Figure 1.3). A true block copolymer sodium alginate has alternating regions of G, M, GM, GGM and MMG blocks with no repeating unit<sup>[62]</sup>. The structure or block sequence of the polymer is dependant upon factors such as growth environment, plant age and area of extraction from the plant<sup>[64]</sup>. The prevalence of G blocks increases with age and structural importance within the plant (leaf < stem < stipe), providing a certain amount of species and regional dependence upon the structure and therefore properties of sodium alginate (Table 1.8). The randomness associated with the structure of an alginate polysaccharide chain, leads to a composition distribution<sup>[62]</sup> with regard to the true ratio of G and M. The G block content in particular affects the physical properties of alginate gels and has a direct bearing on their commercial application.

#### 1.4.1.2 MOLECULAR WEIGHT DISTRIBUTION.

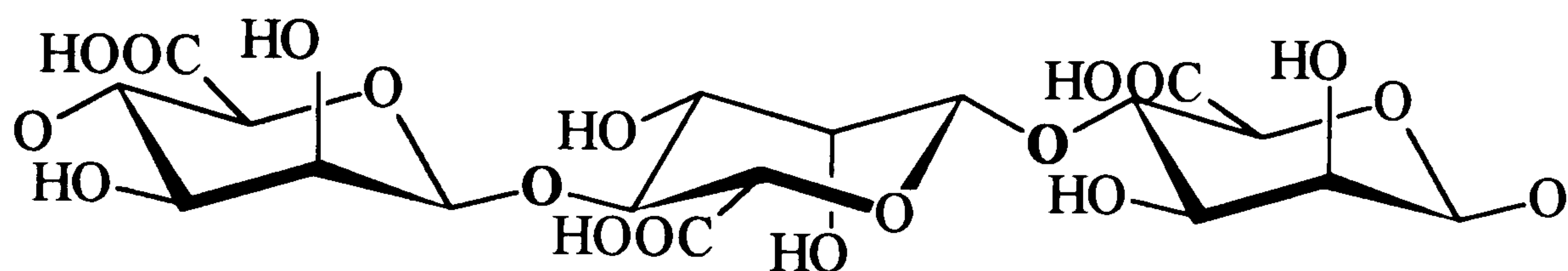
Alginates, as with all polysaccharides are polydisperse, *i.e.* they differ in small constitutive details<sup>[65]</sup>. Thus molecular weights are given as an average: the number ( $M_n$ ) or weight average ( $M_w$ ), the ratio  $M_w/M_n$  is the polydispersity index PI, where, for uniform polymers the PI value is 1 and for narrowly distributed polymers PI is about 1.04<sup>[65]</sup>. Smidsrød and Haug<sup>[66]</sup> reported a PI value of 3 for *Laminaria digitata*. In a study which involved the fractionation of alginate samples based on their molecular weight, Martinsen *et al*<sup>[63]</sup> found that separation was not based on monomer composition for *Macrocystis pyrifera* and *Laminaria hyperborea*. Differences in molecular weight of alginates were related to the different types of preparation and purification processes<sup>[55]</sup>. The role of molecular weight with regard to gel strength is discussed in section 1.4.2.3. Martinsen *et al*<sup>[63]</sup> noted that the molecular weight may be of importance in controlling the porosity and pore size distribution of the capsular membrane during microencapsulation. The importance of a narrow molecular weight distribution is, however, emphasised when considering the immobilisation of cells for implantable materials. The leakage of low molecular weight fragments potentially affects biocompatibility<sup>[67]</sup>.



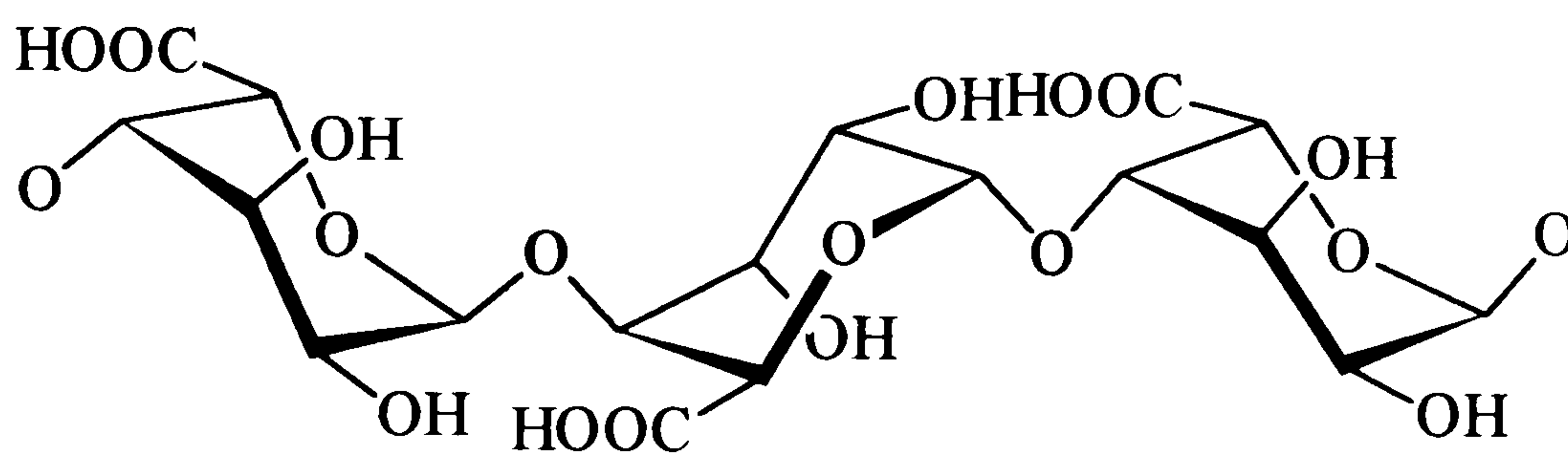
$\beta$ -D-Mannopyranuronate



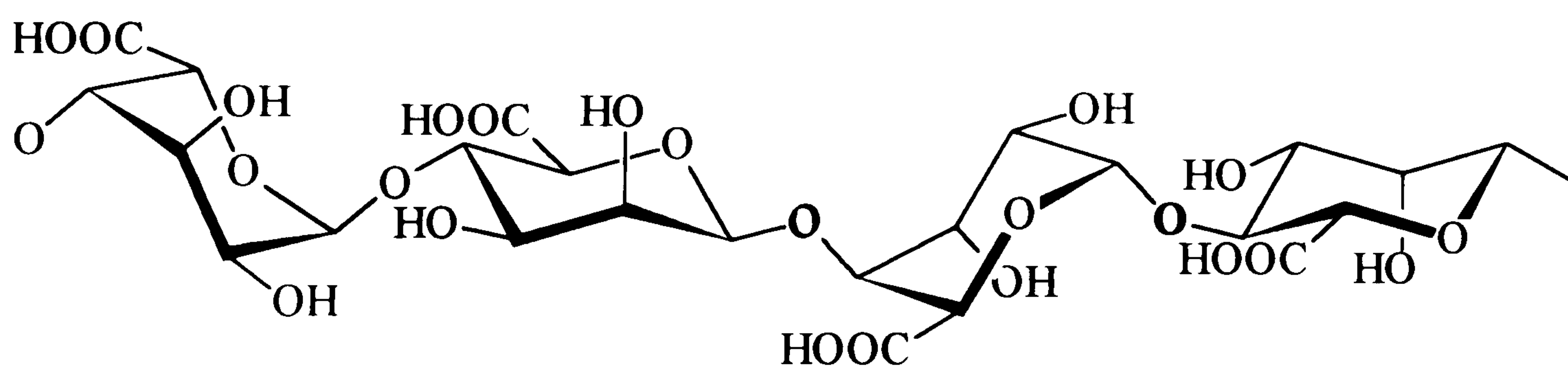
$\alpha$ -L-Gulopyranuronate



M Block



G Block



G and M Block

Figure 1.3 The structural formulae of sodium alginate highlighting the different conformations. The G block structure is particularly important for complexing divalent ions.



#### 1.4.1.3 SOLUBILITY

A major factor contributing to the wide application of alginates is their solubility in cold water<sup>[55]</sup>. As an anionic polyelectrolyte, the solubility is to a large extent governed by the fact that the driving force of swelling and dissolution is the entropy of mixing, caused by free counter ions<sup>[62,68]</sup>. Solubility is affected by pH, salt and divalent ion concentration. The pKa values of G and M monomers are 3.38 and 3.65 respectively<sup>[55]</sup> reducing pH to these and lower pH values will cause the precipitation of alginic acid from solution<sup>[69,70]</sup>. The addition of divalent ions to sodium alginate solutions leads to the formation of insoluble gels<sup>[52]</sup>.

#### 1.4.1.4 STABILITY

Alginate salts and free acids are generally stable<sup>[62]</sup> but under the correct conditions are prone to hydrolysis<sup>[62]</sup>. The glycosidic linkages of alginates are susceptible to both acid and alkaline degradation<sup>[71,72]</sup> and oxidation by free radicals<sup>[73]</sup>. Microorganisms can also degrade alginates<sup>[74]</sup>. The mechanism responsible for degradation at high pH is an alkali-catalysed  $\beta$ -alkoxy elimination reaction<sup>[71,75]</sup>. At or below pH 5, proton catalysed degradation is responsible and as the pH reaches the pKa of the uronic acids the mechanism of degradation is again different, due to intramolecular catalysis<sup>[76]</sup>.

### **1.4.2 Gel Formation In Sodium Alginate**

Alginate solutions immediately form gels on addition of divalent metal ions, for which there is an order of affinity e.g., with *Laminaria digitata* Pb > Cu > Cd > Ba > Sr > Ca > Co, Ni, Zn, Mn > Mg<sup>[55]</sup>. The discussions throughout this text will be based around calcium because its salts have been used extensively throughout the project, due mainly to its haemostatic properties<sup>[77,78]</sup> and toxicity compared to cadmium and lead<sup>[79]</sup>. The immediacy of this room temperature reaction is such that, without controlling the availability of calcium ions, gel formation is heterogeneous.

#### 1.4.2.1 MOLECULAR BASIS OF GEL FORMATION

In the solid state, M regions have a flat ribbon like twofold conformation, similar to that of other equatorial linked hexosans such as cellulose<sup>[80]</sup>. A buckled conformation is adopted in the G block regions as a consequence of the residues being linked di-axially restricting rotation about the glycosidic bond<sup>[80]</sup> (Figure 1.3). This molecular conformation is ideal for complexing divalent ions as described by the egg box

model<sup>[81,82,83]</sup> (Figure 1.4). The carboxy groups are positioned so that a 'cavity' is formed between two juxtaposed G regions allowing the divalent ions to be chelated by the alginate (Figure 1.5) forming junction zones. Morris *et al*<sup>[81]</sup> studied gelation using circular dichroism to determine the extent of junction zone formation with increasing calcium concentration. They found that, at calcium concentrations higher than the stoichiometric requirement of the alginate, aggregates were formed (Figure 1.4). The formation of dimers of different multiplicity were confirmed by Stokke *et al*<sup>[84]</sup> using small angle X-ray diffraction. They suggested that the egg box model should include the use of aggregation numbers of laterally associated chains that depend both on the calcium concentration and the type of alginate.

The binding of calcium ions in the junction zones is thought to proceed by a co-operative mechanism<sup>[80,85]</sup>. Binding of the first cation between any pair of chains causes alignment which facilitates binding of the next cation, and so on along the sequence<sup>[85]</sup>. The minimum block length required for this to occur is not known, but it is at least 2 G monomers<sup>[86]</sup>. The average length of junction zones is thought to be in the region of 20 G monomers<sup>[87]</sup>. Once formed the junction zones are considered irreversible because they are kinetically trapped<sup>[86]</sup> by the high activation energy of reopening<sup>[88]</sup>.

The gel point, as Matsumoto *et al*<sup>[89]</sup> state, occurs when the number of intermolecular interaction points per polymer molecule reaches a certain value, which is independent of molecular weight. Up to this point it is possible that a pre-gel state is formed<sup>[90]</sup>.

#### 1.4.2.2 METHODS OF GEL PREPARATION

The two main methods for preparing calcium alginate gels are diffusion and internal (*in-situ*) gelation.

##### 1.4.2.2.a THE DIFFUSION METHOD OF GEL FORMATION

The immediacy of gelation is such that a solution of sodium alginate can be dropped into a bath of free calcium ions, without the alginate dispersing any further. The gel is formed by the diffusion of the calcium ions into the alginate solution. Though the method is simple and relatively rapid, there are disadvantages. The gels produced are inherently heterogeneous<sup>[86]</sup>, factors affecting homogeneity include the concentration of calcium ions, molecular weight and G block distribution<sup>[55,62]</sup>.



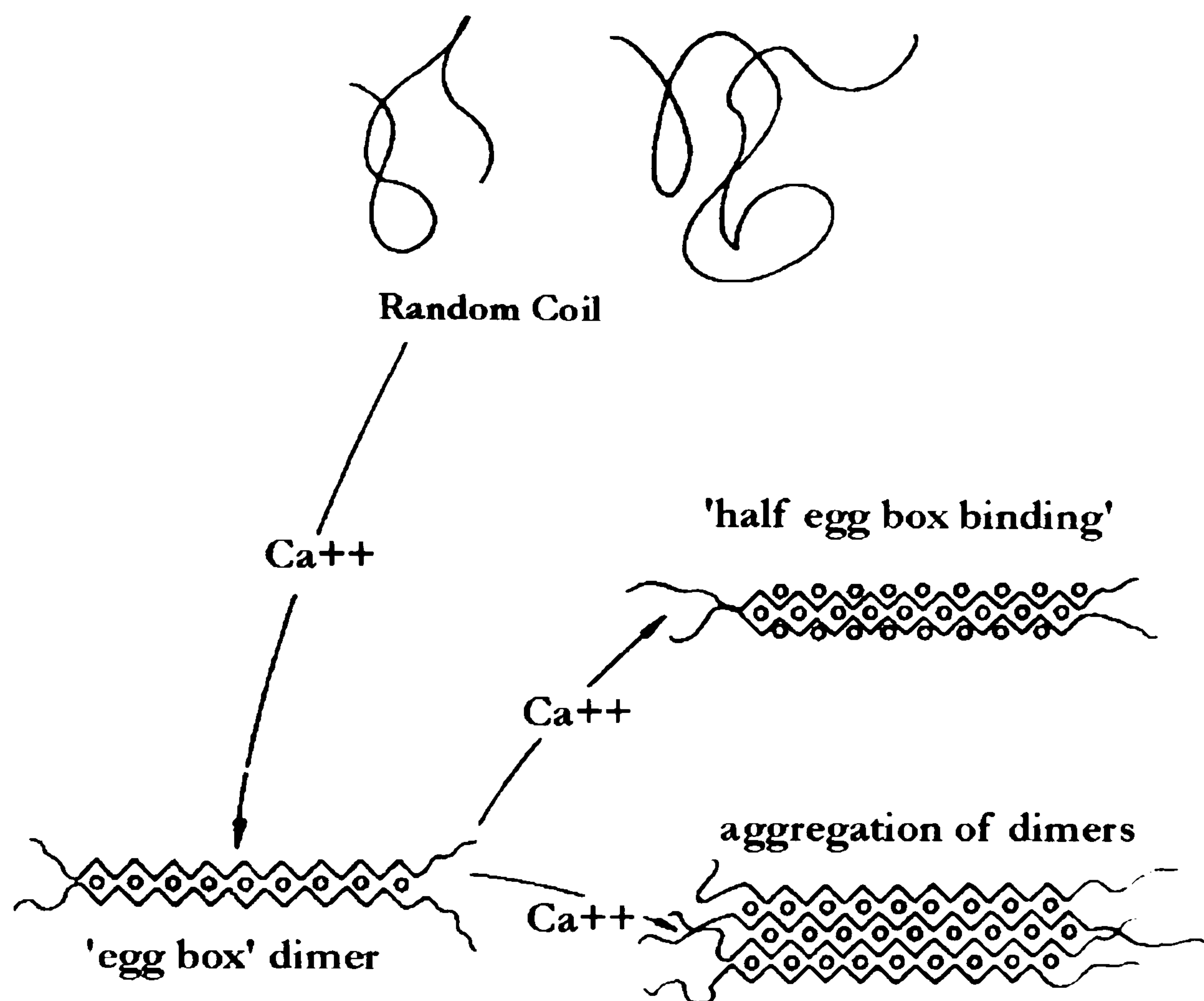


Figure 1.4 Schematic diagram of the egg box model<sup>[83]</sup>, showing the binding of calcium ions by G block regions to form junction zones<sup>[84]</sup>.

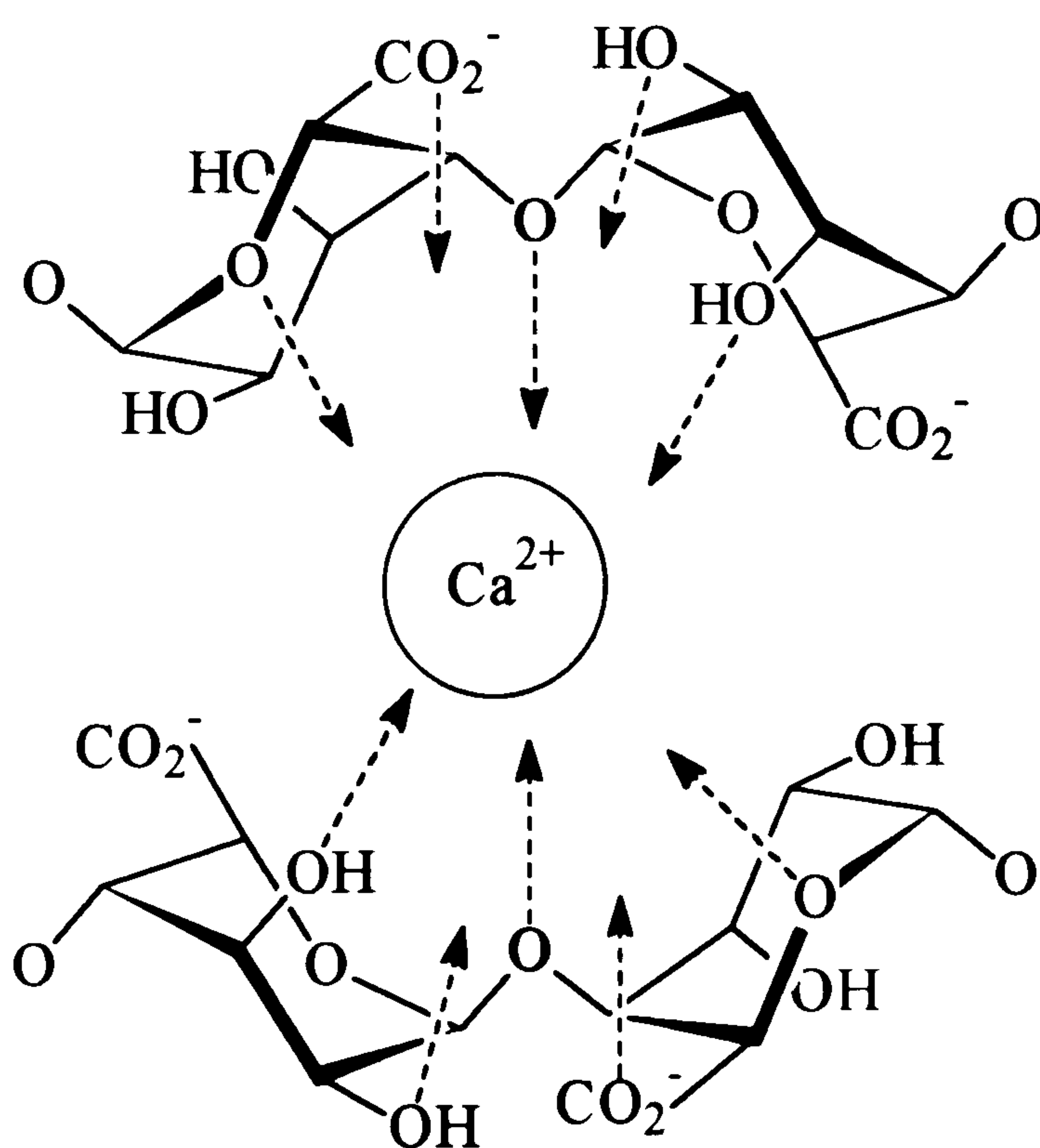


Figure 1.5 Schematic diagram showing the binding of calcium by oxygen atoms<sup>[55,62,80]</sup>

#### 1.4.2.2.b THE INTERNAL METHOD OF GEL FORMATION

The use of insoluble calcium salts allows the dispersion of calcium throughout a solution of sodium alginate. Until the salts are dissolved to release calcium ions a gel cannot be formed. The controlled (slow) release of an acid liberates calcium ions by reaction with the insoluble salt. The release of the acid must be controlled. Draget *et al*<sup>[91]</sup> found that simply adding hydrochloric acid lead to heterogeneous gels. By far the best method for producing gels is the use of the slowly hydrolysing compound delta gluconolactone. The initial chemical reaction is hydrolysis, delta gluconolactone hydrolyses in water, with ring opening, to produce a carboxyl group. Upon this slow acidification, the insoluble calcium carbonate converts to the carbonate anion ( $\text{HCO}_3^-$ ) and free calcium ions<sup>[92]</sup>. The gel produced has a homogenous structure with properties dependant upon the concentration of calcium ions, molecular weight and G block distribution of the alginate. The method was first patented in 1954 by McDowell<sup>[93]</sup> and has found many applications especially in the area of cell immobilisation <sup>[24]</sup>. However, its use for wound management products has yet to be demonstrated.

#### 1.4.2.2.c GELS BY OTHER METHODS

Delta gluconolactone was used to set gels without the presence of divalent ions. Morris and Chilvers<sup>[94]</sup> used a 1:1 mixture of alginate and pectin to form a stable low pH gel. Draget *et al*<sup>[95]</sup> achieved similar results with just alginate. The use of EDTA to complex a soluble calcium salt was reported<sup>[96]</sup>. Cole and Garbel<sup>[97,98]</sup> have patented formulations using acetic acid to dissolve a salt producing a foam. Rapid, even mixing through a double barrel syringe and mixing nozzle avoided heterogeneity.

#### 1.4.2.3 GEL STRENGTH

The strength of alginate gels depends strongly on the composition and sequence of monomers in the alginate molecule<sup>[62]</sup>. Not only is the presence of glucuronic acid important, but the quantity and distribution are vital to the properties of the gel<sup>[88,99]</sup>. Clare<sup>[55]</sup> noted that low G alginates form gels more quickly than high G alginates but are unable to increase their strength by increasing the calcium content once all the G block regions have been filled. High G alginates form strong brittle gels and low G alginates form softer more malleable gels.



The dependence of alginate gel strength on molecular weight reaches a maximum and then gel strength becomes independent of molecular weight<sup>[88,92,95]</sup>. Smidsrod and Haug found<sup>[88]</sup> gel strength (stiffness) became independent from molecular weight at a degree of polymerisation (DP) of 400. This independence was confirmed more recently by Draget *et al*<sup>[92,95]</sup> for calcium alginate gels<sup>[92]</sup> and alginic acid gels<sup>[95]</sup>, though the molecular weights vary. In the study on calcium alginate gels<sup>[92]</sup> the method of preparation had a profound effect on the magnitude of the point of independence from molecular weight. The alginate gels formed *in-situ* became independent from molecular weight at a level 3-4 times greater than gels formed by dialysis<sup>[92]</sup>.

The concentration of alginate has a bearing on the strength of gel produced. There is a linear relationship between the square of alginate concentration and gel strength<sup>[88]</sup>. As a function of calcium ion concentration, gel strength reached a maximum and then further addition of alginate resulted in a weaker gel<sup>[74]</sup>.

## **1.5 HYALURONIC ACID IN HEALING**

Hyaluronic acid was associated with more rapid<sup>[100]</sup>, even scarless healing<sup>[101]</sup>. Its properties and role in healing are discussed below.

### **1.5.1 Structure And Properties Of Hyaluronic Acid**

Hyaluronic acid (now more commonly known as hyaluronan)<sup>[102,103]</sup> is a high molecular weight polysaccharide. It has a glycosaminoglycan structure with a repeat disaccharide unit of N-acetyl- $\beta$ -D- glucosamin and  $\beta$ -D-glucuronic acid, linked together by alternate  $\beta$ 1,3 and  $\beta$ 1,4 glycosidic bonds forming a long linear unbranched polysaccharide chain<sup>[103]</sup> (Figure 1.6). Hyaluronan is found in most eukaryotic tissues and synthesis takes place on the inner surface of the plasma membrane of a cell<sup>[104]</sup>. The molecular weight of the polymer varies greatly, depending on its source<sup>[104]</sup>, from thousands to millions of Daltons<sup>[102,103,104]</sup>. Hyaluronan is found throughout the human body, soft connective tissue having the highest and the blood the lowest concentrations, with half of its total amount found in the skin<sup>[101]</sup>. Degradation occurs in the lymphatic system, at the lymph nodes<sup>[104,105]</sup>. The low molecular weight fractions that are absorbed by the blood are either metabolised in the liver or excreted by the kidney<sup>[104]</sup>. The enzymes responsible for the degradation of hyaluronan are hyaluronidase,  $\beta$ -N-acetyl-D-hexosamidase and  $\beta$ -

D-glucuronidase<sup>[104,106]</sup> with hyaluronan being broken down to its constituent monosaccharides.

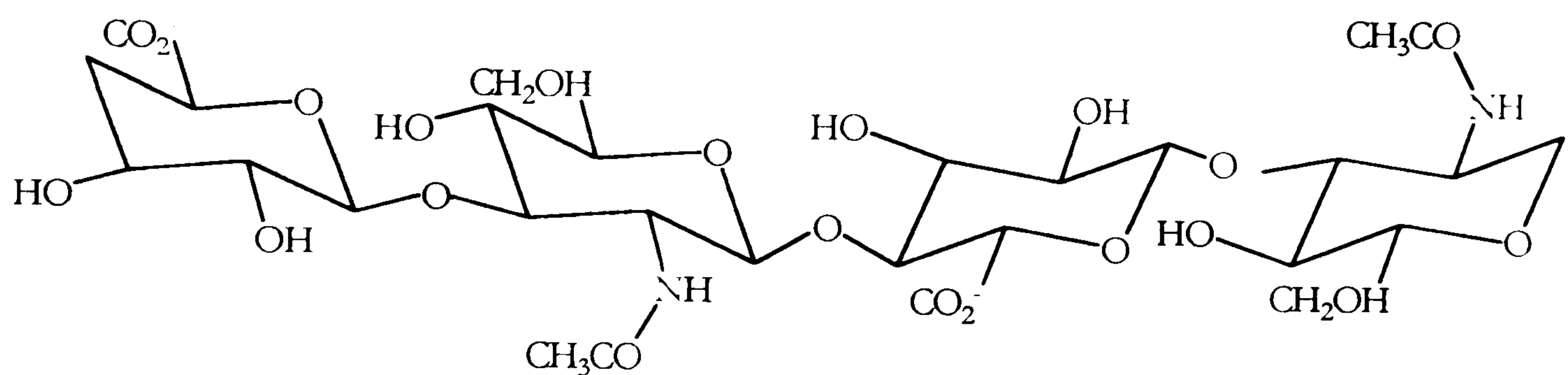


Figure 1.6 The structure of Hyaluronic acid: N-acetyl-β-D- glucosamin and β-D-glucuronic acid joined at the 1,3 and 1,4 positions respectively<sup>[103]</sup>.

### 1.5.2 Role In Healing

The beneficial effects of hyaluronan on the wound healing process are reported as being more rapid<sup>[100]</sup> and scarless healing<sup>[101]</sup>. The healing process was discussed in section 1.3. The discussion below gives an overview of the role of hyaluronan. It should be noted however, that the full extent of the role of hyaluronan is still unknown<sup>[107]</sup>.

Hyaluronan plays a dual role. During the inflammatory phase it activates and moderates this phase of healing. Activation is seen through the enhancement of cell infiltration, and an increase in the production of proinflammatory cytokines. Moderation is seen through inhibition of inflammatory proteinases, free radical scavenging, and antioxidant properties<sup>[107]</sup>. During the granulation or proliferation phase, cell proliferation is aided by facilitating cell detachment. The hyaluronan rich hydrated extracellular matrix aids cell migration. The low molecular weight fragments of hyaluronan stimulate angiogenesis<sup>[108]</sup>. In contrast, high molecular weight fragments of hyaluronan retard angiogenesis<sup>[107]</sup>.

The most exciting developments toward the healing process become apparent in the final stages of healing. The migration of basal keratinocytes is facilitated by the presence of hyaluronan, via a CD44 mediated mechanism, the remodelling phase, where a hyaluronan rich matrix may reduce collagen deposition leading to reduced scarring, as seen in foetal healing<sup>[107]</sup>.



### 1.5.3 Scarless Healing

The ability of a wound to heal without scarring was shown in a number of foetal models<sup>[109]</sup> and by Hellström and Laurent<sup>[110]</sup> in an experiment on the tympanic membrane using a hyaluronan based dressing. The ability of a foetus to heal without scarring is not a foregone conclusion. Healing with scarring will occur after a certain point during gestation, which was found to be approximately the second trimester in humans and 100-120 days for the foetal lamb<sup>[111]</sup>. The healing of foetal wounds differs from those in the postnatal period. The inflammatory phase is lacking and collagen synthesis is activated in the early phase, but does not lead to excessive bundle formation (typical of adult scar tissue). One of the striking differences in the healing process is the continued and progressive presence of hyaluronan in foetal wound tissue<sup>[104]</sup>.

The high concentration of hyaluronan in the foetal environment, *i.e.* amniotic fluid, does not however, predispose the wound to scarless healing. Studies investigating the effects of amniotic fluid and healing have shown that simply bathing the wound in amniotic fluid does not lead to scarless healing<sup>[112]</sup>. An understanding of foetal wound healing may lead to therapeutic strategies to help avert scarring and fibrosis, which would have a tremendous impact on both medical and surgical practice.

## 1.6 AIMS OF THIS THESIS

It is the intention to investigate the use of sodium alginate as the basis of an *in-situ* gelling wound dressing. This will be achieved by the following:

- 1 Using different polymers to induce gelation and or improve water uptake capacity of sodium alginate.
- 2 Determining the ability of delta gluconolactone to be used for the gelation of an alginate wound dressing.
- 3 Characterising the absorptive capacity of the delta gluconolactone system, and the gelation time using rheological techniques.
- 4 Developing a dressing to provide a suitable environment for healing.
- 5 Characterising the absorptive capacity of dressings produced using a simple and reproducible method.

## **CHAPTER 2 EXPERIMENTAL**

Characterisation of samples was achieved using rheological methods and a new method for determining water uptake capacity using Franz cells. The Franz cell method was simple, flexible and reproducible. The aims of this chapter are to:

- i) Introduce the different methods for sample preparation.
- ii) Introduce rheology and the rheological methods used and
- iii) Introduce the Franz cell method for determining the absorptive capacity of gels and its validation.

### **2.1 SAMPLE PREPARATION**

This section is split into two parts. Section 2.1.1 details the methods used to formulate gels using polymers to modify the properties of sodium alginate (used in section 2.3 and Chapter 4) and section 2.1.2 details the methods used to formulate gels using delta gluconolactone and calcium carbonate (used in Chapters 5, 6 and 7).

#### **2.1.1 Sample Preparation Using Cellulose Based Polymers**

##### **2.1.1.1 MATERIALS**

Sodium alginate, LF 10/60, FMC Biopolymer, PO BOX494 N-3002, Drammen, Norway. Calcium chloride, AR, BDH Laboratory Supplies, Poole, Dorset, England. Aquasorb A500®, Hercules Ltd. Aqualon Division. Salford, Manchester. England. Natrosol250M Pharm®, Hercules Ltd. Aqualon Division. Salford, Manchester. England. Celquat SC240C®, National Starch and Chemical Ltd. Daventry, Northamptonshire, England.

##### **2.1.1.2 EQUIPMENT**

Oertling Balance, 4 Place, Oertling, Smethwick, West Midlands. England Carrimed Rheometer, CSL<sup>2</sup> 500, TA Instruments, Leatherhead, Surrey. England. Bench top Autoclave, Dixons Surgical Instruments Ltd. Wickford, Essex. England.



### 2.1.1.3 FORMULATION METHODS

#### 2.1.1.3.a FORMULATION OF SODIUM ALGINATE, AND SODIUM ALGINATE AND POLYMER SOLUTIONS

A 100ml beaker was tared on a balance, the correct weight of solution (water or calcium solution section 2.1.1.3.b) was added ( $\pm 0.005\text{g}$ ) and the weight recorded. The correct amount of excipient was then placed into a glass weighing boat ( $\pm 0.005\text{g}$ ) and the weight recorded. The beaker was fixed under an overhead stirrer and a vortex formed in the solution prior to the addition of excipient. The full amount of excipient was introduced before appreciable thickening had occurred. The formulation was then stirred for 20 min or until there was complete dissolution. All samples were sterilised, in screw top jars, using a bench top autoclave (parameters: 15 psi, 121°C for 15 min).

#### 2.1.1.3.b CALCIUM SOLUTION PREPARATION

The level of calcium was 0.15%w/w, 10.4mmol/L, higher than that in human serum<sup>[113]</sup> to give the dressings haemostatic properties<sup>[78]</sup>. (Calcium chloride dihydrate RMM 147.02).

### **2.1.2 Sample Preparation Using Delta Gluconolactone And Calcium Carbonate**

#### 2.1.2.1 MATERIALS

Sodium alginate, LF 10/60, LF 10/60 LS, LF 10/60 D, FMC Biopolymer, PO BOX494 N-3002, Drammen, Norway. Hyaluronic acid, MV, FMC Biopolymer, PO BOX494 N-3002, Drammen, Norway. Glycerol, Spectrophotometric Grade 99.5%, Aldrich Chemical Company, Gillingham, Dorset. England. Sodium carboxymethylcellulose, low, medium and high viscosity, Fluka Chimie, Buckinghamshire. England. Calcium carbonate, Sturcal f, Specialty Minerals, Birmingham. England. Di-sodium hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset. England. Potassium dihydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset. England. Delta-gluconolactone, GPR, Aldrich Chemical Company, Gillingham, Dorset. England. Sodium chloride, AR, BDH Laboratory Supplies, Poole, Dorset. England. Hydrochloric acid, AR, BDH Laboratory Supplies, Poole, Dorset. England.

#### 2.1.2.2 EQUIPMENT

Oertling Balance, 4 Place, Oertling, Smethwick, West Midlands. England. Overhead stirrer, Janke, BDH Laboratory Supplies, Poole, Dorset. England. I11 8579 Bench top

pH meter, Hanna instruments, BDH Laboratory Supplies, Poole, Dorset. England. Carrimed Rheometer, CSL<sup>2</sup> 500, TA Instruments, Leatherhead, Surrey. England. Thermostatically controlled water bath. Grant w38, BDH Laboratory Supplies, Poole, Dorset. England. Bench top Autoclave, Dixons Surgical Instruments Ltd. Wickford, Essex. England.

### 2.1.2.3 FORMULATION METHODS

The method outlined below is the basis of sample preparation for the experiments in Chapters 5, 6 and 7. The method outlines the process and timing for formulating a gel for water uptake and rheological evaluation as part of an experimental design. The experiments in Chapters 5, 6 and 7 differ slightly in the constituents, number of batches prepared and the tests performed for each experimental design.

#### 2.1.2.3.a SAMPLE PREPARATION FOR THE INVESTIGATION OF RHEOLOGICAL AND WATER UPTAKE PROPERTIES

The day before a water uptake and gelling experiment three separate batches of a formulation were prepared. Two batches were required for the water uptake experiment and one batch was required for the rheological evaluation. The following excipients were dissolved or dispersed in the diluent (water). The amount of diluent added varied according to the amounts of excipients (max formulation weight at this point 65g). The excipients were sodium alginate, calcium carbonate, hyaluronic acid, phosphate buffer (section 2.1.2.3.b), sodium chloride, and sodium carboxymethyl cellulose.

The mixing of excipients produced viscous solutions, which were poured into pre weighed Schott flasks. The filled Schott flasks were re-weighed and steam sterilised in a bench top autoclave (parameters: 15 psi, 121°C for 15 min). After cooling the flasks were weighed to account for any water loss and placed in a thermostatically controlled water bath for equilibration. After equilibration (1h), two Schott flasks were removed from the water bath for the water uptake experiment. Any weight difference was accounted for with distilled water with stirring for 10 min. Glycerol (if required) was added at this point. Gelation was induced by adding 5ml of freshly prepared delta gluconolactone solution (section 2.1.2.3.c) by syringe and stirring was continued for 2min (total formulation weight 70g). The solution was now gelling and was quickly transferred to pre-weighed Franz cells before setting. The Franz cells were sealed and



inverted ready for the water uptake experiment to begin the next morning. When the Franz cells were weighed (time = zero) and the water uptake experiment commenced, by the introduction of water to the upper compartment of the Franz cell. The method for assessing water uptake is described in more detail in section 2.3.4.

The third batch of alginate solution was removed from the water bath, water and glycerol were added as described above. This was then used for the gelling experiment. 32.5g was transferred to a second vessel at the correct temperature and stirred, 2.5ml of freshly prepared delta gluconolactone solution (section 2.1.2.3.c) was added and stirred for a further 2min. To observe gelation, 1ml of the now gelling solution was transferred to the rheometer and 5 min post addition of the delta gluconolactone solution the rheological investigation commenced. In the event of rapid gelling timings were reduced and a note taken of the changes. The viscosity of the sample was determined from the sample, of the third batch, left after removal of the 32.5g for gelling. Rheological methodology can be found in section 2.2.

#### 2.1.2.3.b PREPARATION OF PHOSPHATE BUFFER

The phosphate buffer solution was prepared as for phosphate buffer 0.33M pH 7.5 in the British Pharmacopoeia<sup>[114]</sup>.

#### 2.1.2.3.c PREPARATION OF FRESH DELTA-GLUCONOLACTONE SOLUTION

Throughout the experiment a ratio of 1:2 calcium:delta gluconolactone was used, to obtain a final pH 7, based on the work by Draget *et al*<sup>[74]</sup>. The delta gluconolactone solution was prepared in 10ml of deionised water. The amount of delta gluconolactone used was calculated from the amount of formulation sterilised, to ensure the 1:2 ratio calcium:delta gluconolactone was preserved.

## 2.2 RHEOLOGICAL METHODS FOR THE CHARACTERISATION OF FORMULATED GELS AND SOLUTIONS

The information that can be gained from profiling the rheological properties of formulations will help greatly in determining the suitability of a particular formulation for use as a wound gel.

### 2.2.1 A Brief Introduction To The Theory Of Rheology

Rheology can be simply defined as the study of the deformation and flow of materials. The application of a force to a sample and measurement of the deformation is a fundamental aspect of determining rheological properties. This brief introduction to the rheology of materials is not exhaustive and the reader is referred to the texts by Barnes *et al*<sup>[115]</sup>, Barnes<sup>[116]</sup> and Whorlow<sup>[117]</sup> for a more detailed discussions on the theory and application of rheological techniques. The introduction covers the concepts of viscosity and elasticity and the ideal materials that exhibit these properties: the Newtonian liquid for viscosity and the Hookean solid for elasticity. The instrumentation used throughout this project for the determination of the rheological properties, a TA Instruments Carri-Med CSL<sup>2</sup> 500 controlled stress rheometer, is described. With the basic principles introduced the discussion expands into the subject of materials that do not exhibit ideal properties and the methodology used to investigate these materials. The materials investigated throughout this project show non-ideal rheological properties. Section 2.2.2 covers the methodology used.

#### 2.2.1.1 VISCOSITY AND ELASTICITY OF MATERIALS AND IDEAL BEHAVIOUR

##### 2.2.1.1.a THE VISCOSITY OF MATERIAL AND NEWTONIAN BEHAVIOUR

Viscosity is a measure of the resistance to flow, of a substance, to an applied force<sup>[115]</sup>. The force (F) per unit area (A) of motion  $F/A$  (the shear stress  $\sigma$ ) (Figure 2.1), is proportional to the velocity gradient  $U/d$  (shear rate  $\gamma$ ). The constant of this proportionality is the viscosity coefficient ( $\eta$ ) Equation 2.1.

$$\sigma = \eta \gamma \quad (\text{Equation 2.1})$$

Materials that exhibit properties, which fit the expression above, are known as Newtonian systems. Newtonian systems can be described by the following characteristics: i) the only stress generated in simple shear flow is the shear stress  $\sigma$ , the



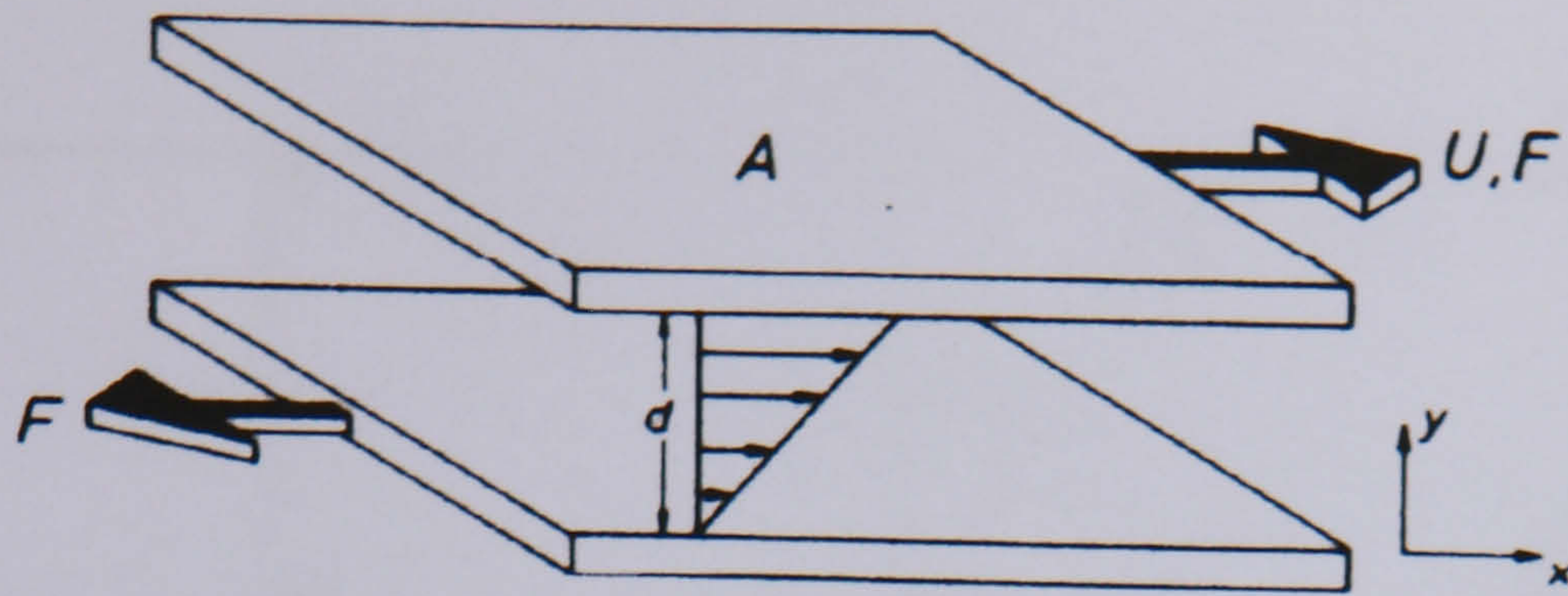


Figure 2.1 Schematic diagram of the application of shear stress<sup>[115]</sup> to a Newtonian liquid: stress  $\sigma$  applied in direction  $U$ ,  $F$  (Where  $A$  = area,  $d$  = distance,  $U$ = and  $F$  = Force.).

normal stress difference being zero. ii) The shear viscosity does not vary with shear rate. iii) The viscosity is constant with respect to time of shearing and the stress in the liquid falls to zero immediately shearing is stopped. In any subsequent shearing, however long the period of resting between measurements, the viscosity is as previously measured. An example of the rheological profile for a Newtonian liquid is given in Figure 2.2.

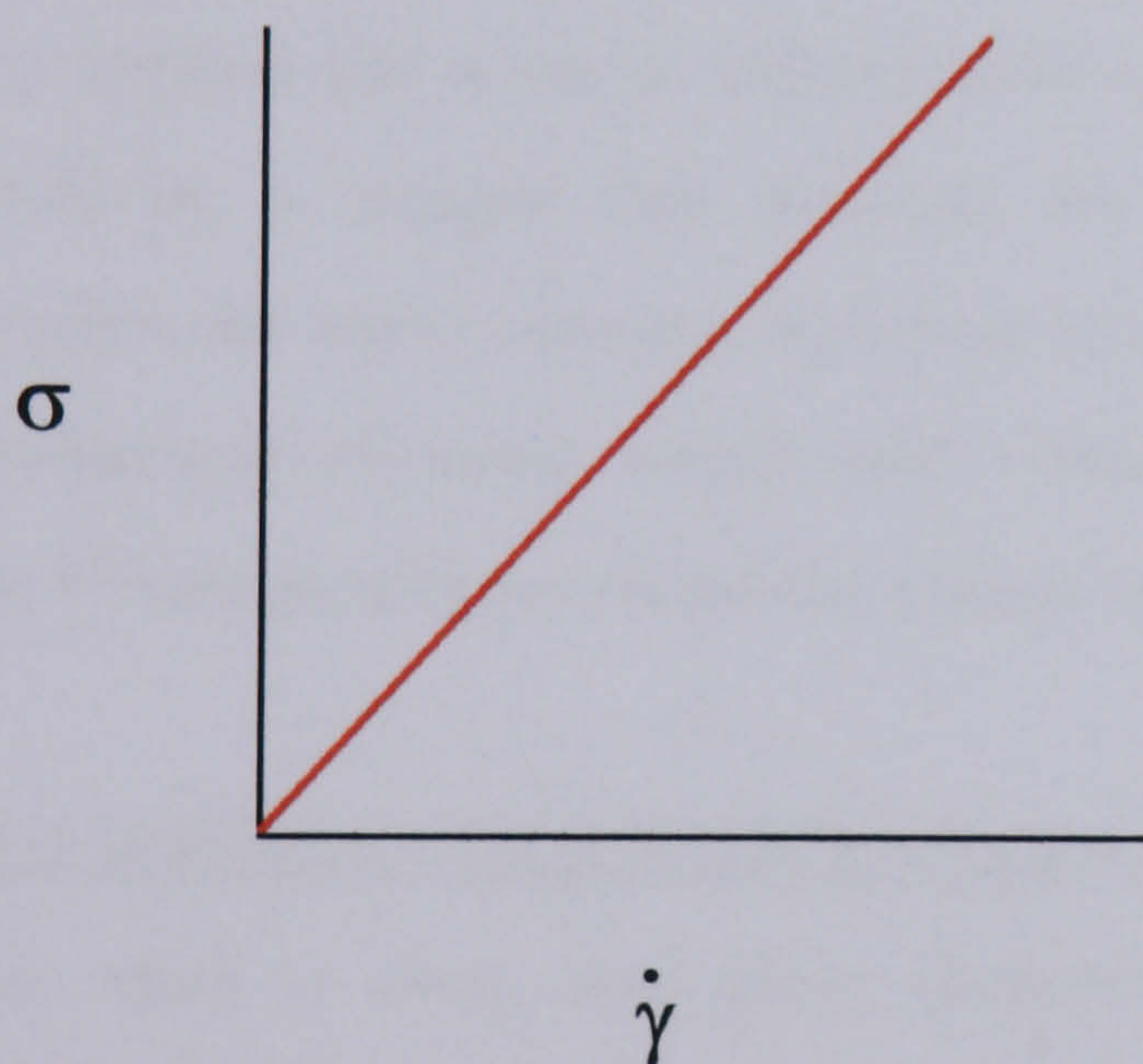


Figure 2.2 A schematic representation of the change of shear stress with increasing shear rate for a Newtonian liquid<sup>[118]</sup>.

#### 2.2.1.1.b THE ELASTICITY OF MATERIAL AND HOOKEAN BEHAVIOUR

Elasticity is a measure of the resistance to shear<sup>[116]</sup>. For a Hookean solid when a stress is applied (to the surface DC Figure 2.3), this results in an instantaneous and proportional deformation. Once deformation is complete there is no further movement of the deformed state, but this state will persist as long as stress is applied. The elasticity modulus ( $G$ ) is constant of proportionality between the strain or shear applied ( $\gamma$ ) and the resultant shear stress ( $\sigma$ ) Equation 2.2.



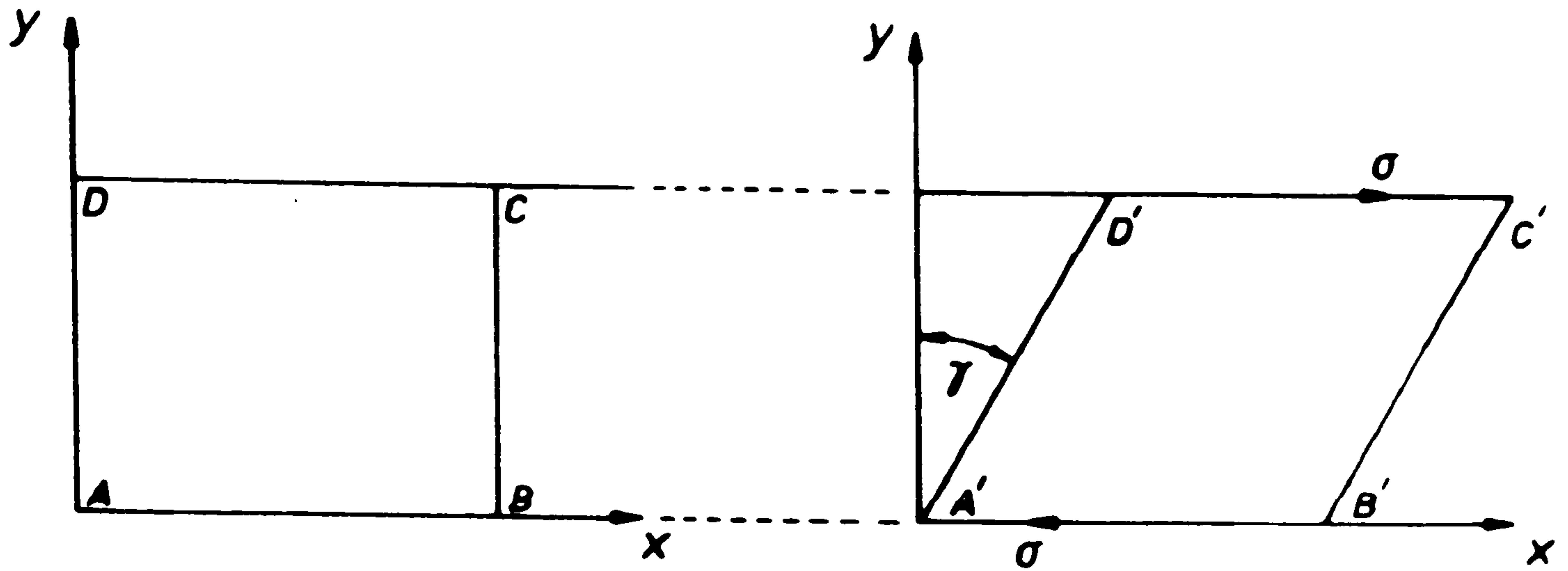


Figure 2.3 Schematic diagram of the application of shear stress<sup>[115]</sup> to a Hookean solid: stress  $\sigma$  applied to surface DC (Where A, B, C and D are the surface positions without stress applied and A', B', C' and D' are the surface positions with a stress ( $\sigma$ ) applied).

$$\sigma = G \gamma \quad (\text{Equation 2.2})$$

An example of 'material' that obeys Hooke's law is a spring; a linear elastic element. When a stress is applied the strain is instantaneously proportional. A graph of stress against strain will be a straight line through the origin, the strain must change instantaneously when the stress changes, and then remain constant until the stress again changes. The behaviour of many metals and other hard solids can be represented accurately by the Hooke model, provided the strains are small<sup>[117]</sup>.

#### 2.2.1.2 THE MEASUREMENT OF RHEOLOGICAL PROPERTIES

Throughout this study a cone and plate rheometer was used to determine the rheological properties of the materials produced: the rheometer was a TA Instruments Carri-Med CSL<sup>2</sup> 500, a controlled stress rheometer. The discussion will therefore concentrate on this instrument. The measuring geometry (cone or plate) was held on a drive shaft supported by an air bearing. A low inertia motor applied a torque to the measuring geometry, with the resultant displacement measured via an optical encoder. The torque range of the instrument was 0.0001-50 mN.m. The optical encoder measured the angular deflection (displacement) to give an absolute measure of strain. The peltier plate was located on top of a pneumatic ram, a circulating water bath was used to aid in the heating and cooling of the peltier plate. The measuring geometry, used throughout this study, was a 4cm diameter acrylic cone with a cone angle of 2° and was truncated at 57μm, Figure 2.4 shows how a cone is truncated.



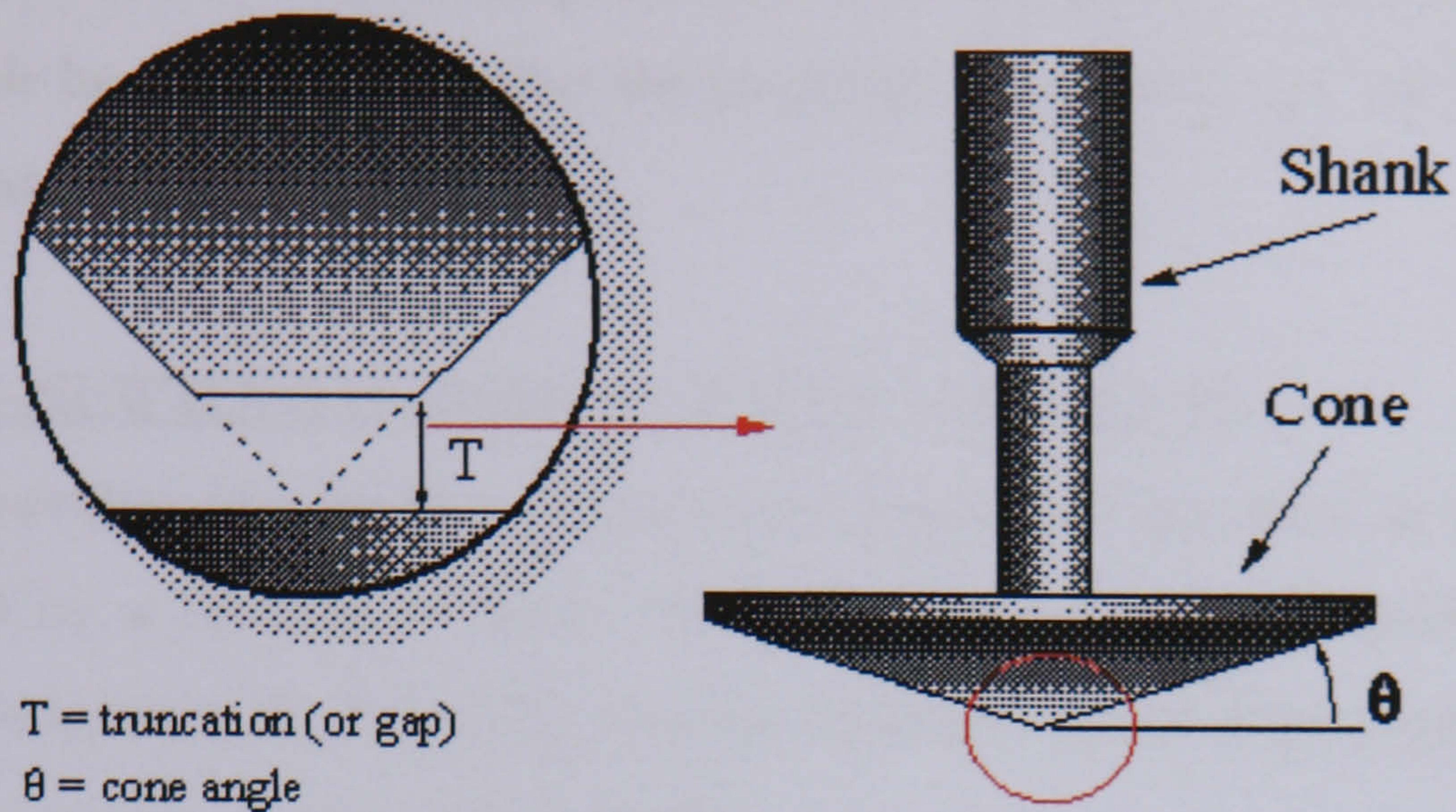


Figure 2.4 The truncation of a cone<sup>[118]</sup>.

With a cone and plate rheometer, the gap between cone and plate must be zero to ensure that the applied stress is uniform across the whole of the cone. The truncation of the cone ensures this is achieved consistently. The shear rate in a sample (Figure 2.5) for the Carri-Med CSL<sup>2</sup> 500 is calculated using Equation 2.3, where  $\omega$  is the angular frequency of the rotating geometry and  $(F\gamma) = 1/\tan \theta$ ,  $\theta$  is the gap angle (when using a cone). The shear rate is independent of sample properties.

$$\text{Shear rate} = (F\gamma) \omega \quad \text{Equation 2.3}$$

The shear stress is calculated using Equation 2.4 Where  $(F\sigma) = (3/2\pi R^3)$  and  $T$  is the torque (mN.m).

$$\text{Shear stress} = (F\sigma) T \quad \text{Equation 2.4}$$

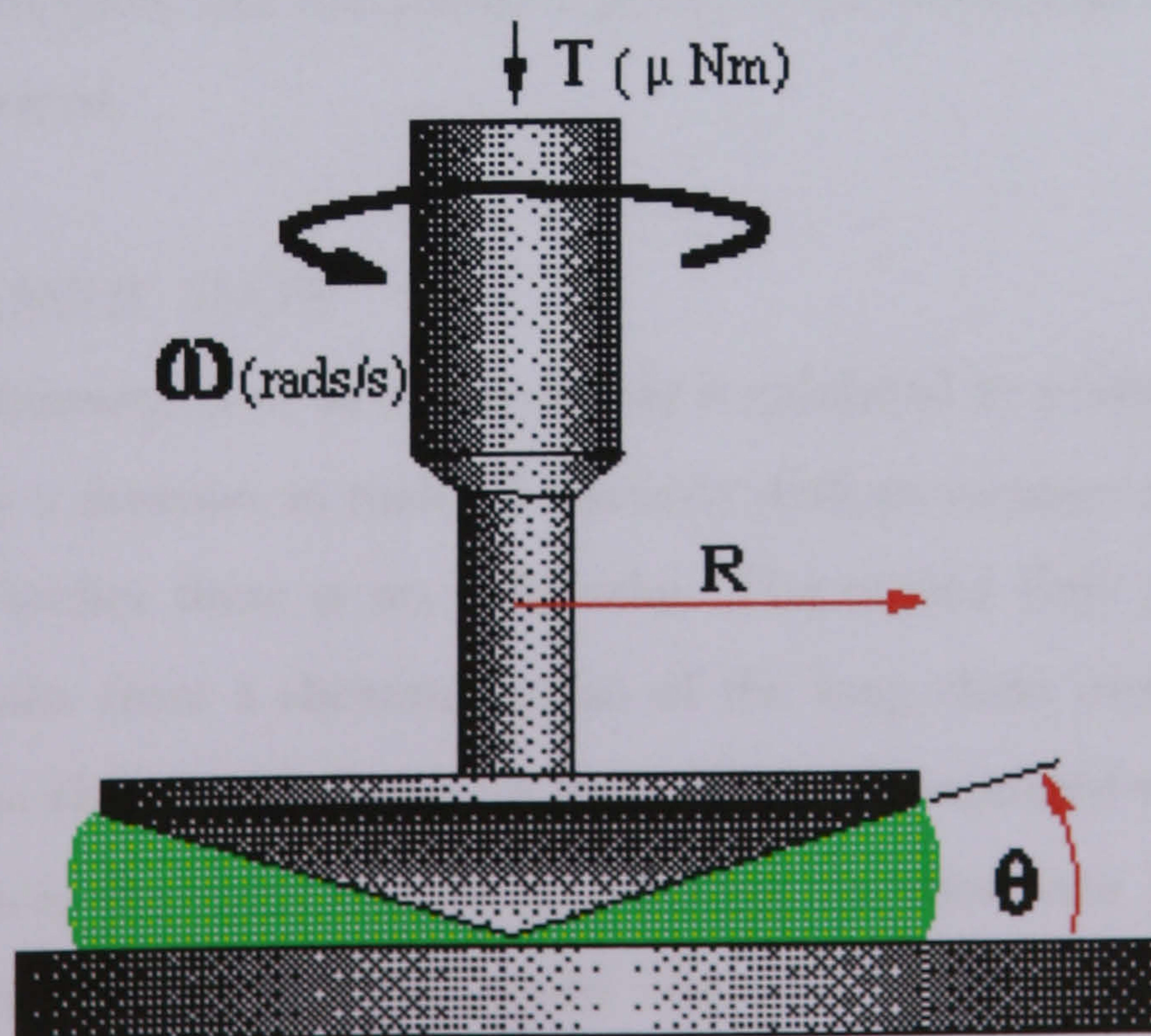


Figure 2.5 A sample under shear between a cone and plate<sup>[118]</sup>.



However, the disadvantages of the cone and plate are that: 1) at high stress values 'secondary flow' could occur exemplified by shear thickening. 2) Temperature effects, such as sample heating can also affect the properties of a sample, the use of temperature controlled plates compensate for this.

### 2.2.1.3 NON-NEWTONIAN BEHAVIOUR OF MATERIALS

The ideal properties of a liquid *i.e.*, Newtonian properties, described in section 2.2.1.1a are exhibited by a fraction of liquid materials. The majority of liquids exhibit non-Newtonian properties, the following sections introduce plastic flow, pseudoplastic flow, dilatant flow and the concept of thixotropy.

#### 2.2.1.3.a PLASTIC FLOW

Plastic flow is characterised by a yield value, materials exhibiting this property are more commonly known as Bingham bodies. A Bingham body does not begin to flow until a shearing stress, corresponding to the yield value is surpassed. The resistance to flow at low shear stress allows Bingham bodies to be classed as solids, because at stresses below the yield value, the material acts as an elastic material. Plastic flow can be described using Equation 2.5, where at stresses greater than the yield value  $\sigma_Y$ , the flow curve is linear, with an intercept of  $\sigma_Y$  on the shear stress axis.  $U$  is the plastic viscosity, and is equal to the slope of the flow curve.

$$\sigma - \sigma_Y = U\dot{\gamma} \quad \text{Equation 2.5}$$

Whorlow<sup>[117]</sup> notes that the Bingham model rarely represents the behaviour of plastics *i.e.*, solid or molten polymers, but adequately represents the behaviour of concentrated suspensions and emulsions.

#### 2.2.1.3.b PSEUDOPLASTIC FLOW

Pseudoplastic (shear thinning) flow as a general rule is exhibited by polymers in solution and is characterised by a decrease in material viscosity with an increase in shear rate. In contrast to Bingham bodies there is no yield value. The curved flow profile of shear thinning materials results from a shearing action of the long chain molecules, such as linear polymers. As the shear stress increases the normally disarranged molecules begin to align their long axes in the direction of flow, reducing the shear rate. It is possible to model the flow curve using the Cross model (Equation 2.6), which requires four parameters to describe a flow curve:



$$(\eta - \eta_{\infty})/(\eta_0 - \eta_{\infty}) = 1/(1 + (K\dot{\gamma})^m) \quad (\text{Equation 2.6})$$

$\eta_0$ , the viscosity at very low shear values,  $\eta_{\infty}$  the viscosity at very high shear values, and  $K$  which is a constant with dimensions of time;  $m$  is a dimensionless constant. The degree of shear thinning is described by the value of  $m$  from the Cross model (Equation 2.6). A value of  $m$  tending toward zero describes a more Newtonian material and  $m$  tending toward unity describes a more shear thinning material<sup>[116]</sup>. The constant  $m$  was used throughout Chapters 4, 5, 6 and 7 to assess the shear thinning nature of formulations. The models/laws must be seen as being empirical in nature and coming from curve fitting exercises, such that often only parts of these models fit a curve<sup>[115]</sup>.

#### 2.2.1.3.c DILATANT FLOW

Dilatant (shear thickening) flow describes materials that exhibit an increase in resistance to shear with an increase in shear rate. Concentrated suspensions of non-aggregating solid particles will show shear thickening. The particular circumstances and severity of shear thickening will depend on the phase volume, the particle size distribution and the continuous phase viscosity. In a flow curve a region of shear thickening usually follows a shear thinning region, brought about by two dimensional layering. The layered arrangement is unstable, and is disrupted above a critical shear stress. The ensuing random arrangement increases viscosity<sup>[117]</sup>. Barnes<sup>[116]</sup> notes that the severity of shear thickening can be reduced by widening the particle size distribution.

#### 2.2.1.3.d THIXOTROPY

A reversible time dependent decrease of viscosity is termed thixotropy. The accepted definition of thixotropy is a gradual decrease of the viscosity under shear stress followed by a gradual recovery of structure when the stress is removed<sup>[115]</sup>. Thixotropy will normally occur when a liquid is shear thinning. As the shear stress is gradually reduced (down curve) the flow curve will be displaced from the up curve, a schematic representation can be seen in Figure 2.6. Anti-thixotropy is a gradual increase in viscosity under stress followed by a gradual recovery of structure also shown in Figure 2.6.



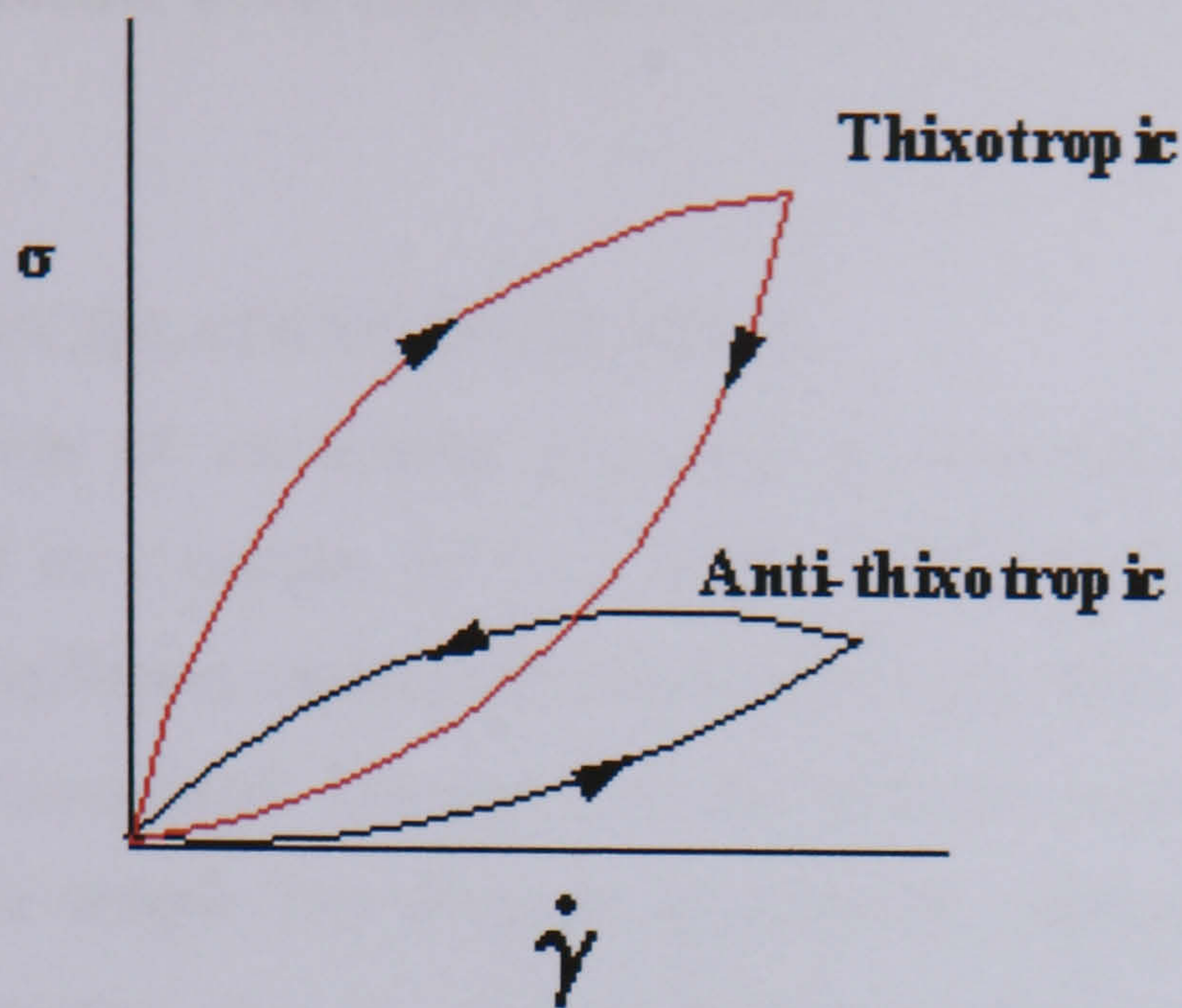


Figure 2.6 A schematic representation of the thixotropic and anti-thixotropic hysteresis loops<sup>[118]</sup>.

The finite time taken for any shear induced change in material microstructure to take place, implies that flow history must be taken into account when making predictions about flow behaviour. During shearing microstructure is brought to a new equilibrium, over a time, by competition between effects, of shear stress and Brownian motion/flow induced structure build-up. When shearing ceases, Brownian motion (the only force left) is able to slowly rebuild the microstructure, lost during shearing, which can take hours to complete<sup>[117]</sup>.

#### 2.2.1.4 LINEAR VISCOELASTICITY

Linear viscoelasticity is the simultaneous existence of viscous and elastic properties in a material<sup>[115]</sup>. It is possible to obtain information on the molecular structure, material parameters and functions from an investigation of the linear viscoelastic properties. There four types of rheological test that can be used to investigate the linear viscoelastic region, these are creep oscillation, stress relaxation and start-up<sup>[116]</sup>. The test used in this thesis were creep and oscillation, the methodology for these tests is discussed in section 2.2.2. The creep test is the immediate application of a constant stress to a sample and observing the resultant strain over time. The oscillation technique is the application of an oscillatory stress or strain as input and monitoring the resultant strain or stress as output. The linear viscoelastic region is the region over which the response to an applied stress remains linear as the stress increases. Therefore, creep and oscillation techniques



can be non destructive when carried out at stresses/strains within the linear viscoelastic region.

#### 2.2.1.4.a THE OSCILLATION TECHNIQUE

The determination of viscoelastic properties is achieved by the application of an oscillatory shear to a sample. In very simple terms the sample is ‘wobbled’. When conducting an oscillation experiment a sinusoidal stress wave applied to the sample, by the geometry, is measured. The phase of the resultant strain wave is indicative of the properties of the sample. For example, applying an oscillatory shear to a Newtonian liquid; the properties of a Newtonian liquid are discussed in section 2.2.1.1.a. A sinusoidal stress wave produces a sinusoidal strain wave that is exactly 90° out of phase, with the applied stress. Where as, for a Hookean material; properties are discussed in section 2.2.1.1.b. A sinusoidal stress wave produces a sinusoidal strain wave that is perfectly in phase, with the applied stress. The liquid like response, which is a certain amount out of phase with the input, is described by the loss modulus  $G''$ . This is a measure of the energy lost/dissipated per cycle of the input. The solid like response, which is a certain amount in phase with the input, is described by the storage modulus  $G'$ . This is a measure of the energy stored within the material and can be recovered from it per cycle of input.

The storage ( $G'$ ) and loss ( $G''$ ) modulus are the most useful parameters gained from oscillation investigations. Other parameters useful for describing a material are: 1) the complex modulus ( $G^*$ ) Equation 2.7, 2) the dynamic viscosity ( $\eta'$ ) Equation 2.8, and 3) tan delta ( $\tan \delta$ ) Equation 2.9.

$$G^* = (G'^2 + G''^2)^{1/2} \quad \text{Equation 2.7}$$

$$\eta' = G''/\omega \quad \text{Equation 2.8}$$

$$\tan \delta = G''/G' \quad \text{Equation 2.9}$$

#### 2.2.1.4.b THE CREEP TECHNIQUE

The determination of viscoelastic properties is achieved by the application of a constant stress and observing the response of the material to the applied stress and then removing the stress and observing the recovery of structure over time. The response for most viscoelastic materials to the application of a constant stress (Figure 2.7), is an immediate elastic response, then there is a delayed elastic response and then a viscous



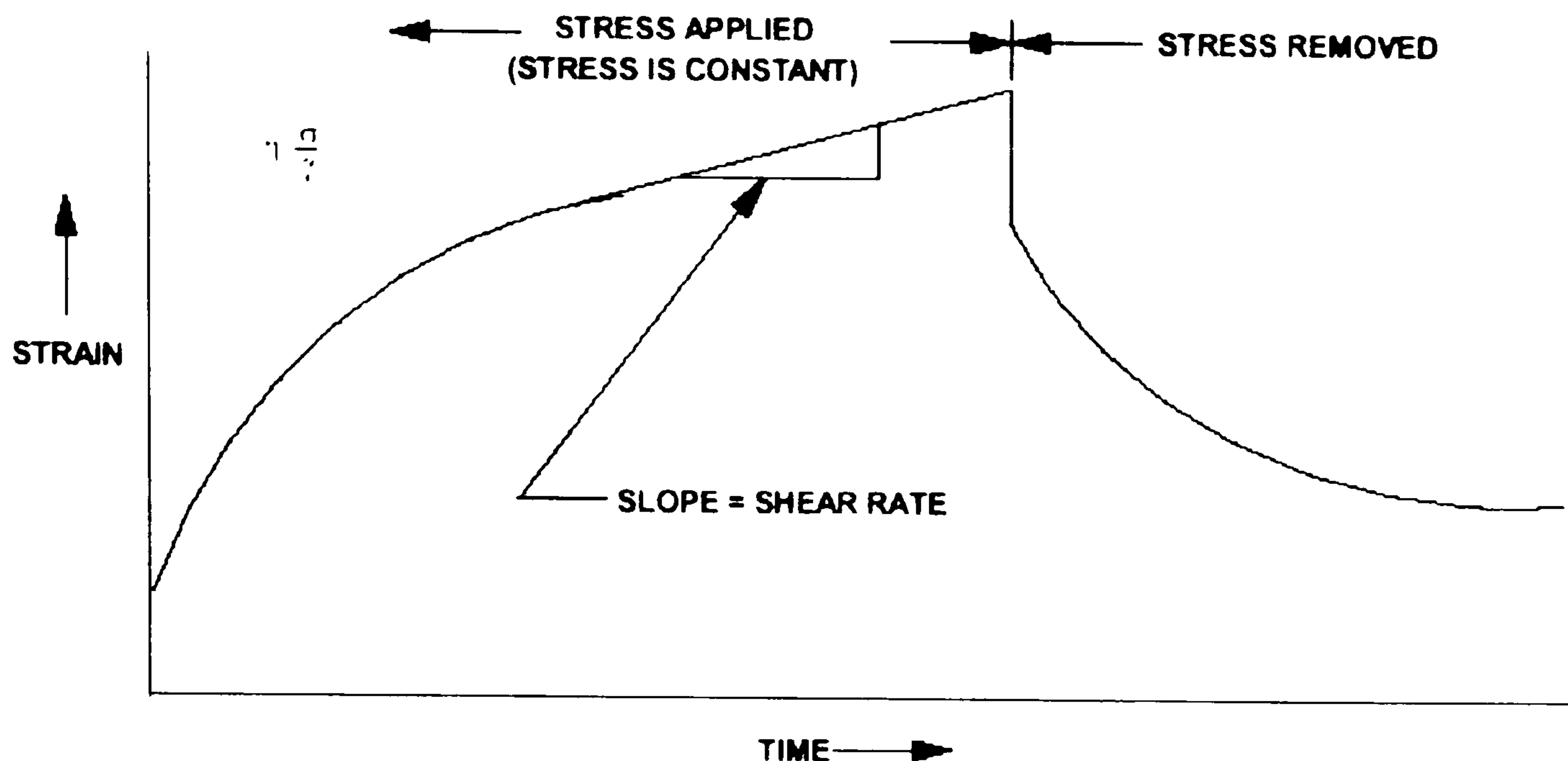


Figure 2.7 A schematic representation the response of a viscoelastic material to the application and removal of a constant stress<sup>[118]</sup>.

response as the material reaches a steady state position to the applied stress (retardation). On the removal of the applied stress, the recovery of the sample material is obtained. There is an instant elastic recovery, followed by elastic recovery region. The viscous flow region is not recovered, because reaching a steady state irreversibly destroys the structure.

The industrial use of the creep method is seen as a realistic representation of the effects of gravity on a sample. This has found wide application in the manufacture of paints and dyes<sup>[116]</sup>.

## 2.2.2 The Methods And Methodology Used To Characterise The Rheological Properties Of Formulated Gels And Solutions

This section describes the rheological methods employed to characterise formulated gels and solutions. The formulations used as examples in this section were prepared as outlined in section 2.1. The gelation of formulations prepared in section 2.1.2 was observed using a time sweep method, described in section 2.2.2.2.c.

### 2.2.2.1 EQUIPMENT

Carrimed Rheometer, CSL<sup>2</sup> 500, TA Instruments. Leatherhead, Surrey, England



2.2.2.1.a A DESCRIPTION OF THE EQUIPMENT

The Carrimed rheometer was a controlled stress cone and plate rheometer. The geometry used was an acrylic cone that had a diameter of 4cm and a cone angle of 2°. The gap between the cone and plate was set at 57µm, as specified on the cone.

2.2.2.2 OSCILLATION METHODS USED TO INVESTIGATE THE VISCOELASTICITY OF FORMULATIONS

Oscillation methods were used to investigate the extent of the viscoelastic nature of formulations and to determine the gelling time of samples.

2.2.2.2.a DETERMINATION OF THE LINEAR VISCOELASTIC REGION

The linear viscoelastic region of a formulation was determined by the application of an increasing oscillatory stress at a fixed frequency (1Hz). The method was used to find an oscillatory stress that will not damage the structure of the sample during oscillatory procedures. The linear viscoelastic region is the region over which the response to an applied oscillatory stress remains linear as the stress increases (Figure 2.8). The stress value at the centre of the linear region was chosen for use in oscillatory investigations.

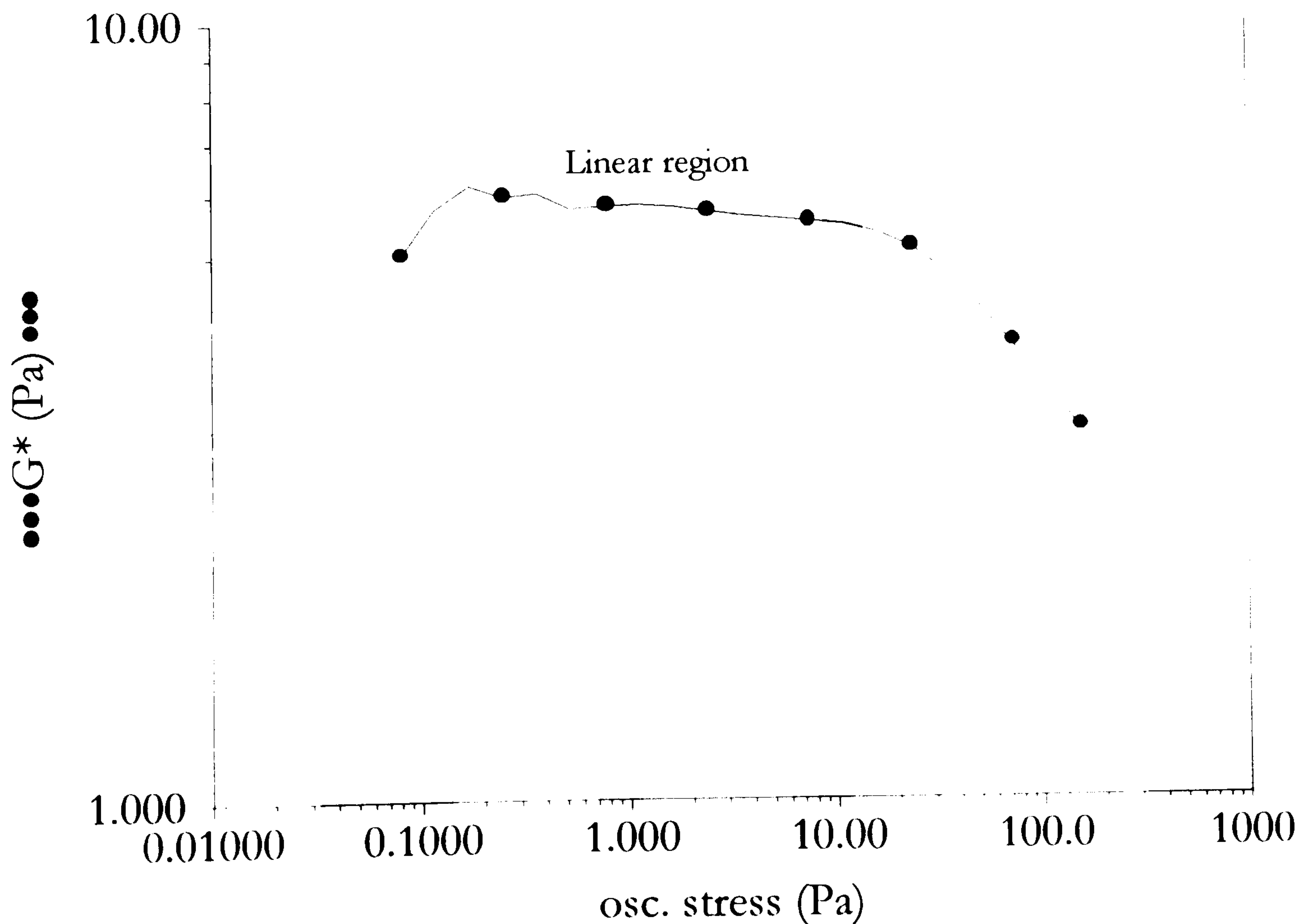


Figure 2.8 The linear viscoelastic region of a sample solution of 4% w/w sodium alginate.



The sample used in Figure 2.8 was a sterile solution of 4<sup>o</sup> w/w sodium alginate. The following parameters were used in the rheometer method file: 1) Step type, torque ramp. 2) Frequency, 1.00 Hz. 3) Ramp mode, log. 4) Temperature, 25°C. 5) Start value, 0.08Pa. 6) End value, 150Pa. 7) N° of data points, 21.

### 2.2.2.2.b INVESTIGATING FORMULATION STRUCTURE WITH A FREQUENCY SWEEP

The frequency sweep was performed to determine the response of a solution to an increasing angular frequency at a fixed oscillatory stress, within the linear viscoelastic region. The response of G' and G'' to the applied frequency gives an indication of the structure of a gel. A predominating value of G' suggests more solid like properties and a predominating value of G'' suggests a more liquid like nature.

Figure 2.9 is an example of a frequency sweep performed on a sterile solution of 4%w/w sodium alginate. The following parameters were used in the rheometer method

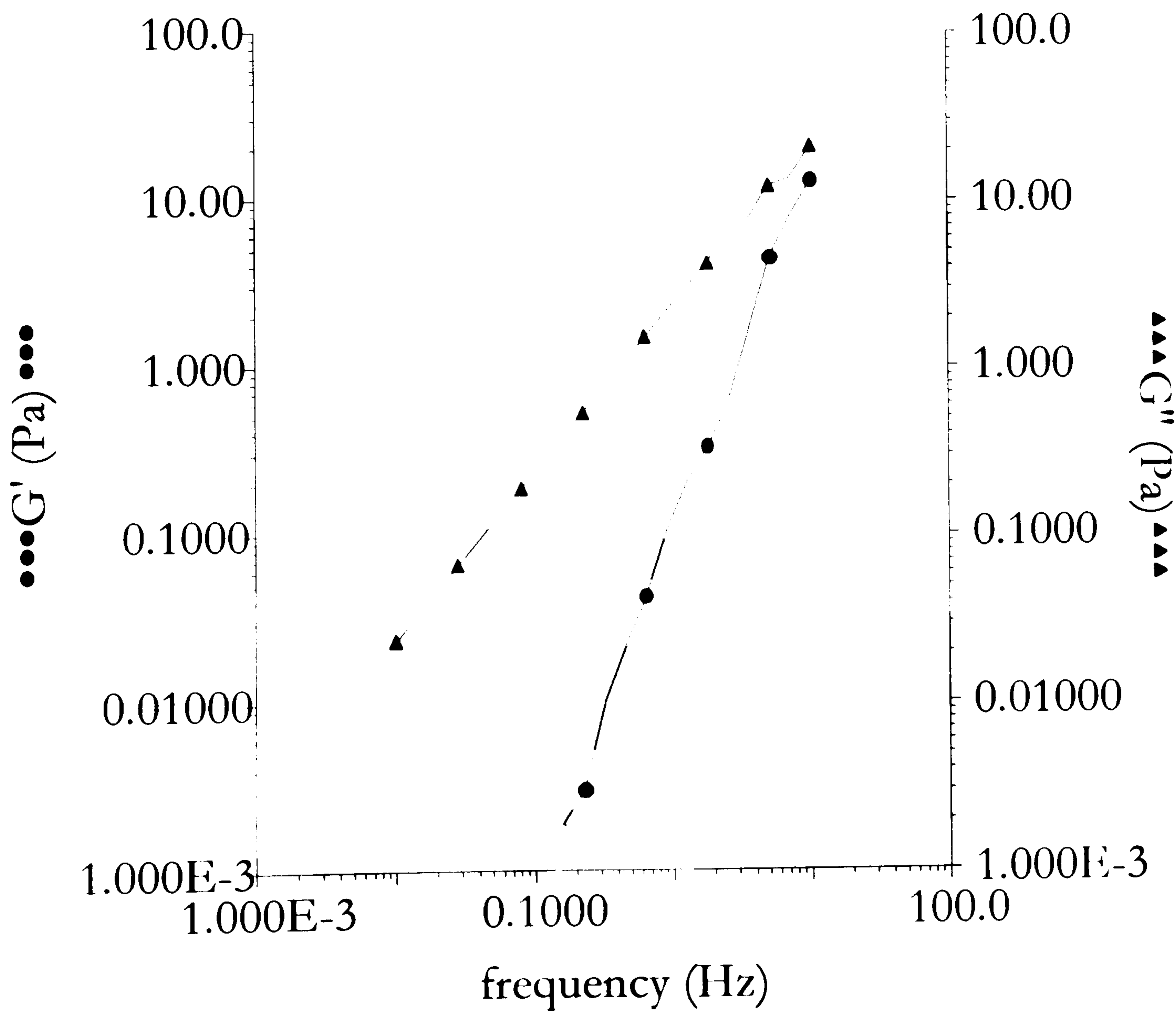


Figure 2.9 The structure of a solution of 4<sup>o</sup> w/w sodium alginate determined using a frequency sweep.



file: 1) Step type, frequency ramp. 2) Controlled variable, oscillation stress. 3) Ramp mode, log. 4) Temperature, 25°C. 5) Start frequency, 0.01 Hz. 6) End frequency, 10 Hz. 7) Applied stress value, 2.00 Pa. 8) Number of data points, 21.

#### 2.2.2.2.c INVESTIGATING STRUCTURE CHANGE WITH A TIME SWEEP

The phase transition or gelation of formulations, prepared for gelling (section 2.1.2), was observed with a time sweep. The change in the values of  $G'$  and  $G''$  was observed over time by performing repeated frequency sweep methods at fixed oscillatory shear

(2Pa) and angular frequency (1Hz) over time. The point of phase transition was calculated at the time at which  $G'$  and  $G''$  crossed (Figure 2.10). The duration of the method was 3h, as a result a combination of a plastic cover and silicone oil was used to prevent sample dehydration<sup>[118]</sup>. The effectiveness of the method is demonstrated in Figure 2.11. A 4%w/w sodium alginate solution was analysed using a time sweep for 3h 10min with and without a cover and silicone oil. Without the cover and oil the value of  $G'$  rises with time, which would interfere with the gelation profile.

The following parameters were used in the rheometer method file to investigate the gelling formulation in Figure 2.10 and the non-gelling alginate solution in Figure 2.11: 1) Step type, time ramp. 2) Controlled variable, oscillation stress. 3) Temperature, 25°C. 4) Oscillation frequency, 1.00 Hz. Applied stress value, 2.00 Pa. 5) Ramp duration, 190 min.

#### 2.2.2.3 INVESTIGATING ELASTICITY USING CREEP

Samples were investigated for their elastic nature using a creep test. Samples were tested immediately after a frequency sweep. The change of strain against time was measured, a shear stress of 2Pa was applied for 4 min. Then the recovery of the samples' structure was determined for a further 4 min, by measuring the strain with out the application of a stress. The following parameters were used in the rheometer method file: 1) Temperature, 25°C. 2) Retardation; Applied stress value, 2.00Pa and Max step time, 4.00 min. 3) Recovery; Applied stress value, 0.00 Pa and Max step time, 4.00 min.



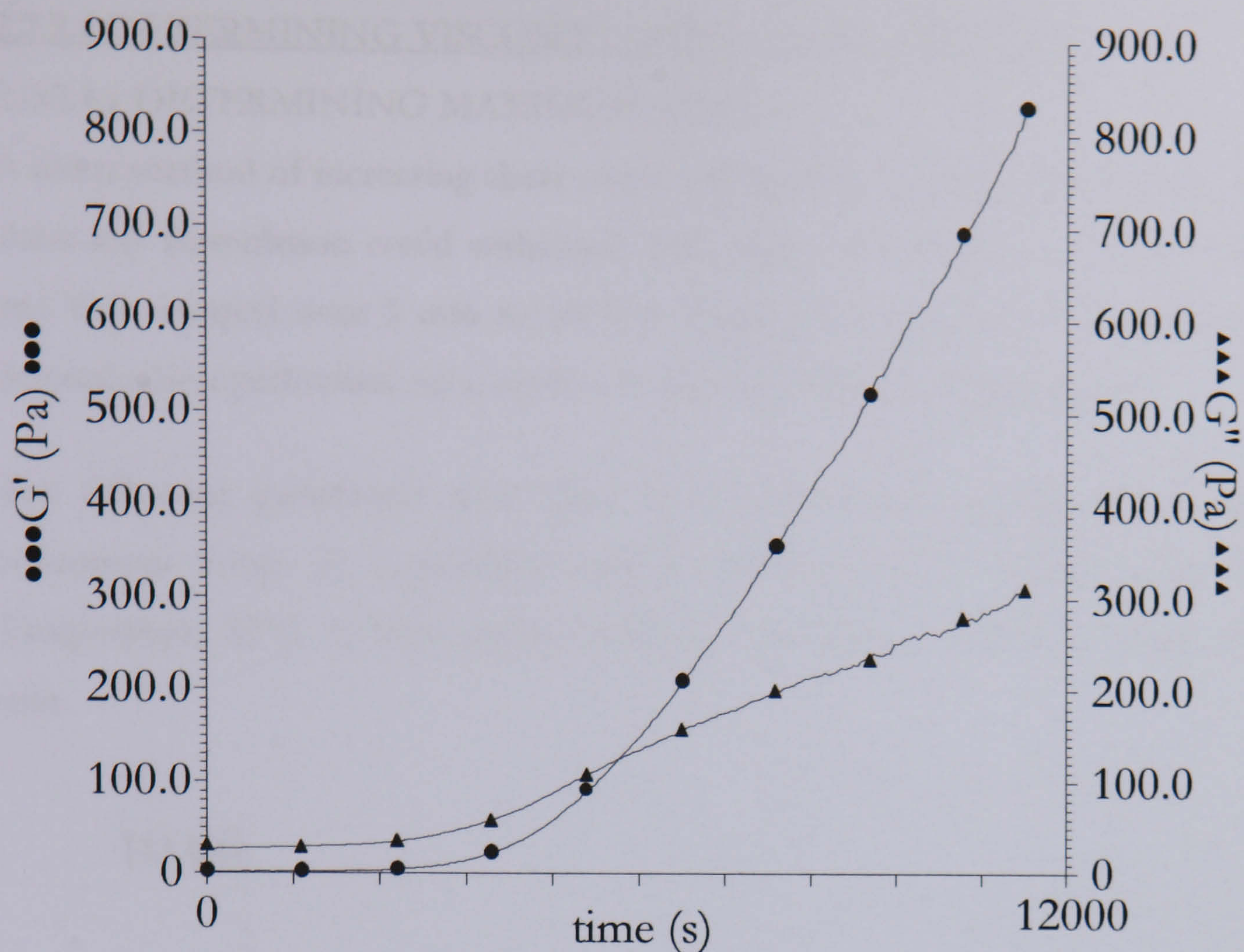


Figure 2.10 The phase transition of an 8%w/w sodium alginate solution, induced by internal release of calcium ions (section 1.4.5.2.b). The gel point is where  $G'$  and  $G''$  cross.

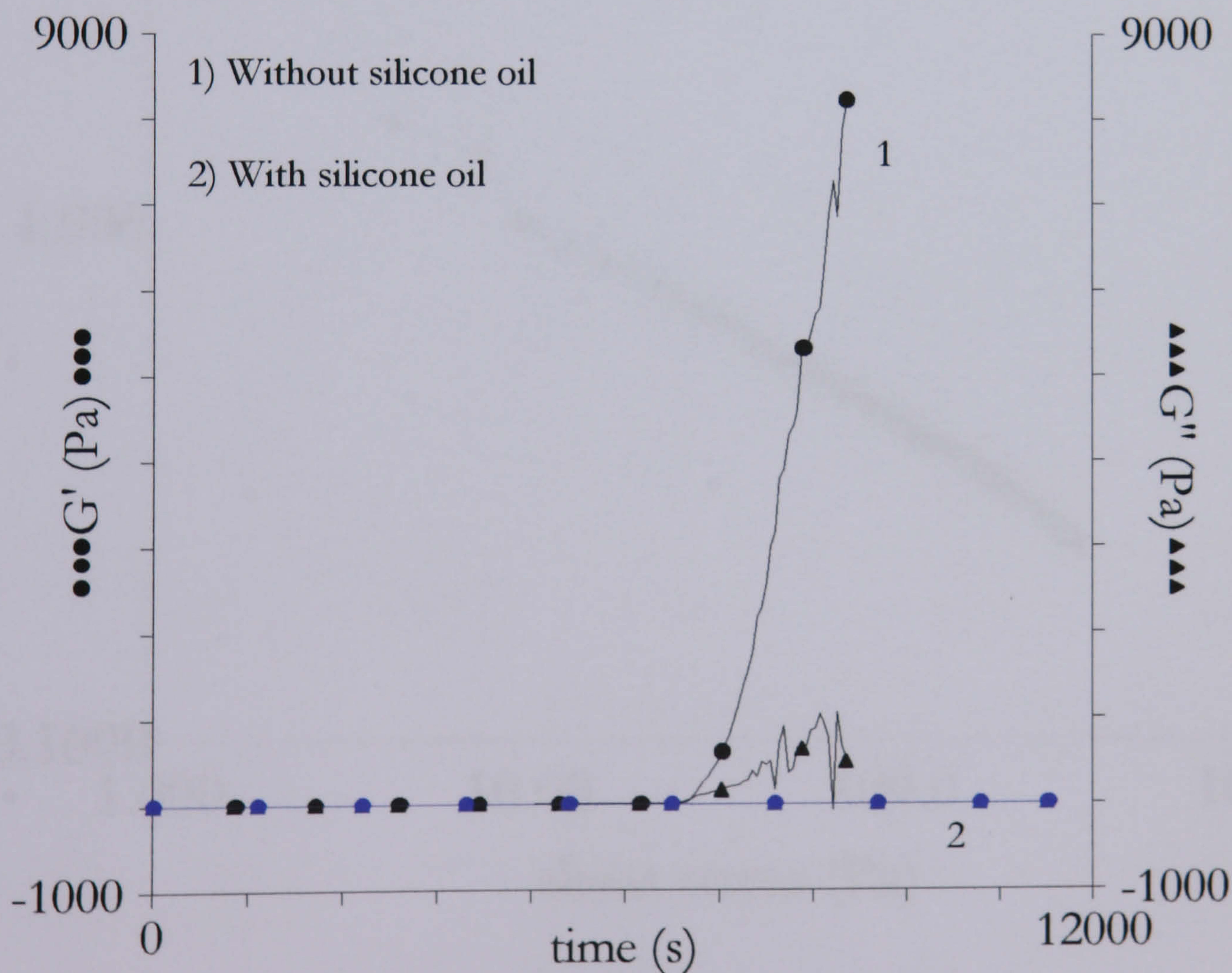


Figure 2.11 The use of a plastic cover and silicone oil to prevent sample dehydration during extended analyses. The sudden increase of  $G'$  in plot 1, is due to dehydration and not gelation.



2.2.2.4 DETERMINING VISCOSITY USING FLOW METHODS

2.2.2.4.a DETERMINING MAXIMUM STRESS

A linear method of increasing shear stress was applied to find a value for the maximum shear any formulation could withstand. The shear stress was started at a value of 2Pa and then ramped over 5 min to 1000Pa. Figure 2.12 is an example maximum stress determination performed on a sterile solution of 4%w/w sodium alginate.

The following parameters were used in the rheometer method file: 1) Step type, continuous ramp. 2) Controlled variable, shear stress. 3) Ramp mode, linear. 4) Temperature, 25°C. 5) Start stress, 2.00Pa. 6) End stress, 1000Pa. 7) Ramp duration, 5 min.

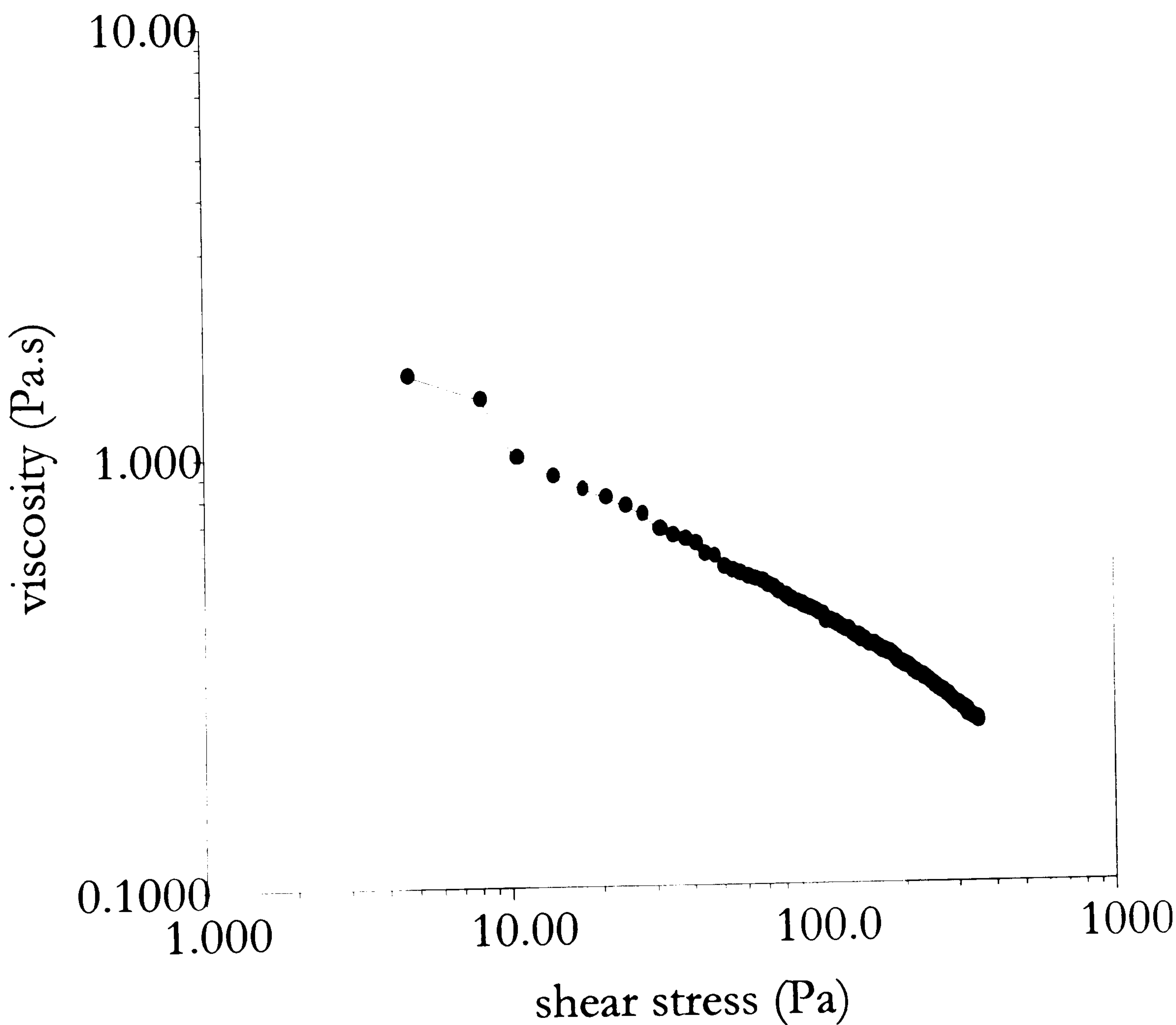


Figure 2.12 Determination of the maximum stress a 4%w/w sodium alginate solution could withstand.



2.2.2.4.b DETERMINING VISCOSITY AND RATE INDEX

A steady state method was used to investigate and determine the viscosity and shear thinning properties of a formulation. The method produces fewer, but more accurate points on the resultant curve and an example is given in Figure 2.13. The viscosity and rate index (shear thinning ability) were determined using the Cross model (section 2.2.1.3). The following parameters were used in the rheometer method file: 1) Step type, stepped ramp. 2) Controlled variable, shear stress. 3) Ramp mode, log. 4) Temperature, 25°C. 5) Start stress, 2.00 Pa. 6) End stress, 300 Pa. 7) Number of data points, 16.

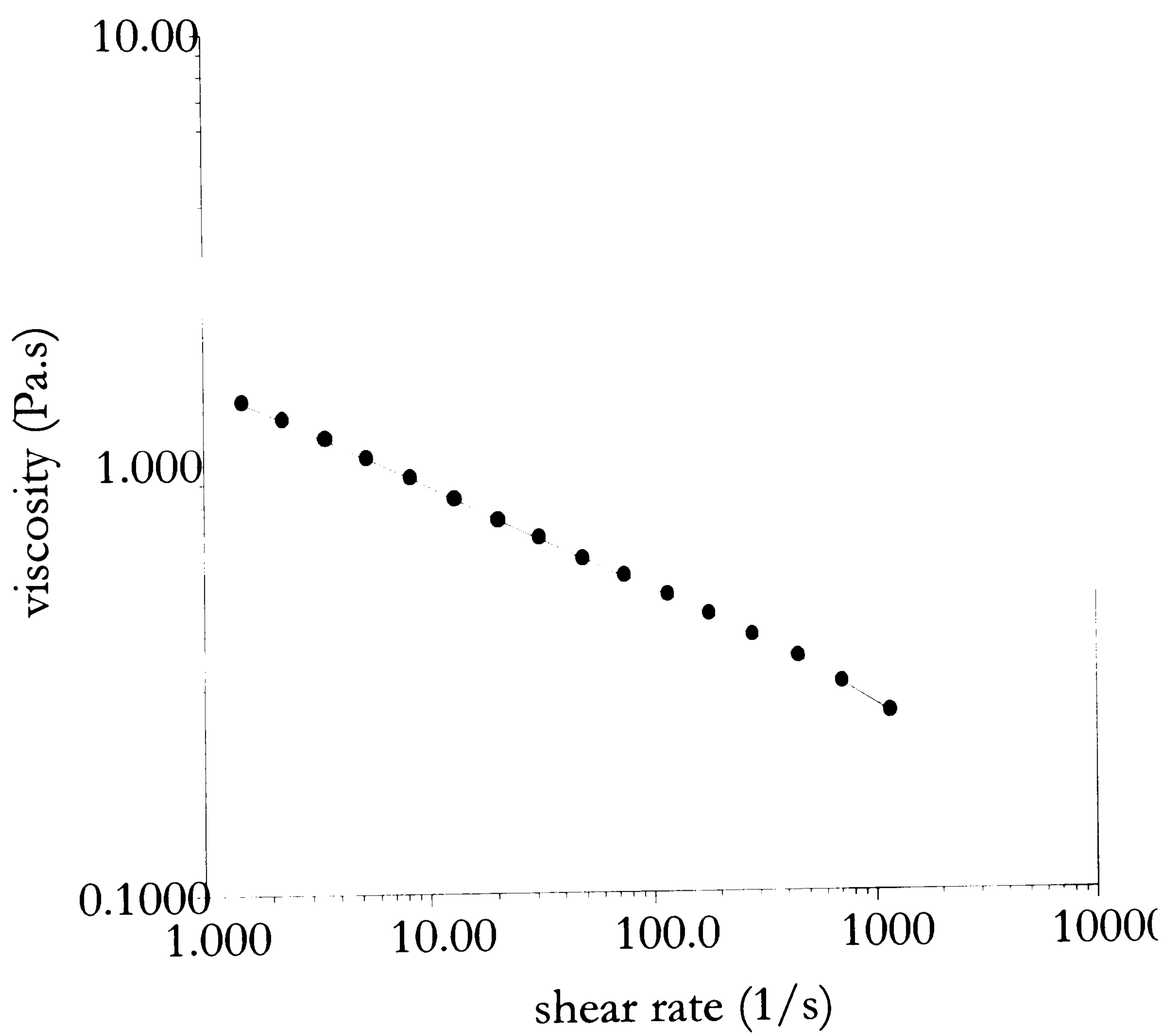


Figure 2.13 The determination of the flow profile of a 4% w/w sodium alginate solution used a steady state method.



2.3 A METHOD FOR DETERMINING THE ABSORPTIVE CAPACITY OF HYDROGELS

2.3.1 Introduction

Franz cells (Figure 2.14) are predominantly used for investigating percutaneous absorption<sup>[119, 120]</sup> where a donor solution or gel is held in the upper compartment (B) and a receptor solution is held in the lower compartment (A). Aliquots of the solution are removed and analysed to quantify absorption by the receptor solution. The gels formulated for this project were intended for use in wound management and to that end, they must be capable of absorbing fluids expelled from a wound during healing.

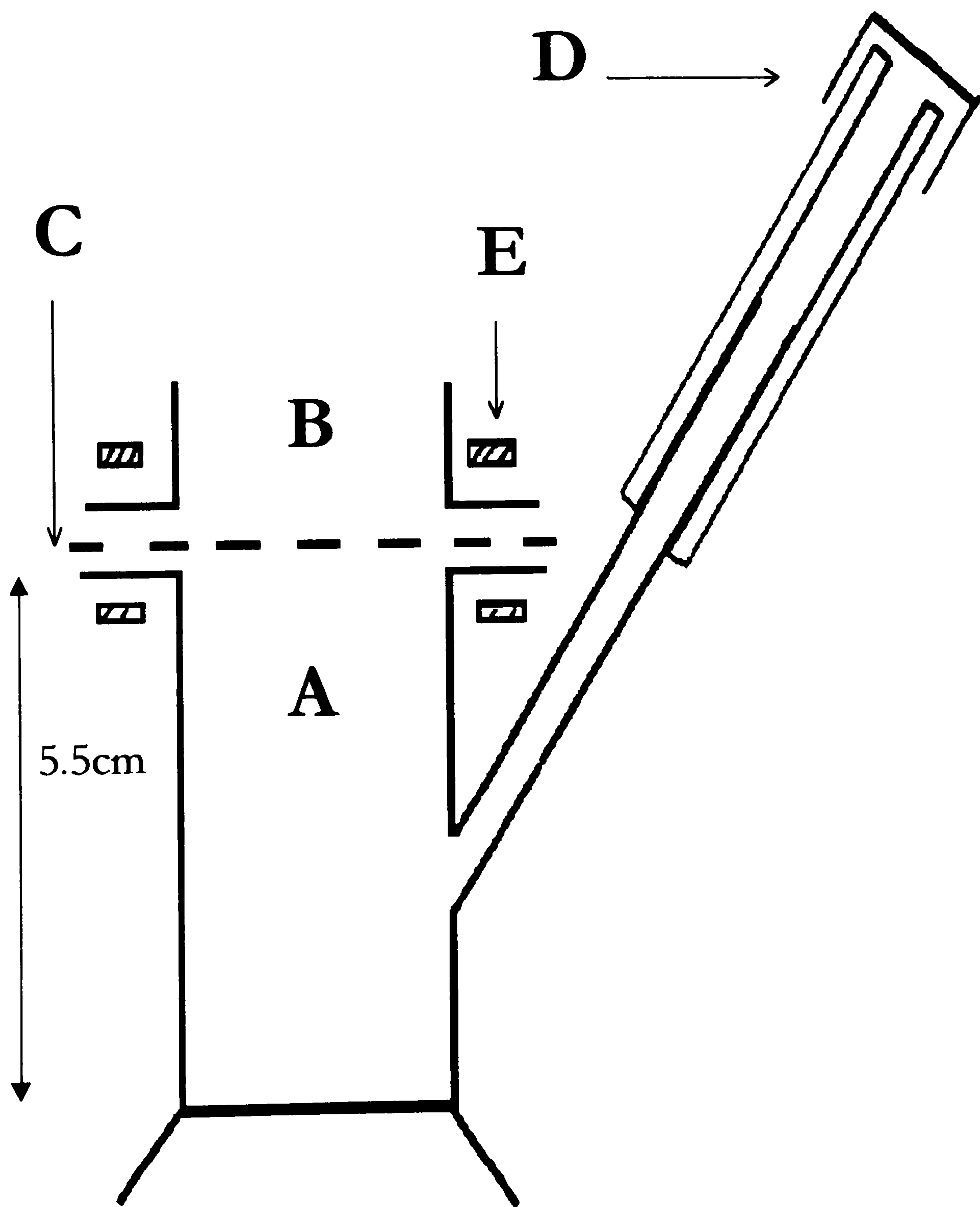


Figure 2.14 Schematic diagram of a Franz cell. Key: lower compartment A, upper compartment B, Visking tubing C, plastic tube and cover D and horseshoe clamp E.



There is a paucity of methods describing the capacity of amorphous hydrogels to absorb fluid. Shigeyama *et al*<sup>[121]</sup> used a Franz cell to investigate the absorptive capacity of a cream for the treatment of bedsores. The cream was layered on top of Visking tubing (C) (Figure 2.14). A nylon tea bag method was used to determine the absorptive capacity of gel dressings<sup>[122]</sup> and dry powders<sup>[123]</sup>. Thomas and Loveless<sup>[124]</sup> used a method based on an agar gel to assess the capacity of gels to absorb or donate liquid, but the method provided no restrictions on the gel. A Paddington cup method was applied to the determination of the absorptive capacity of sheet hydrogel dressings<sup>[122]</sup> but this method was not applicable to amorphous or *in-situ* forming hydrogels.

Unrestricted swelling of a gel during analysis does not mimic the wound environment. The tea bag method<sup>[122]</sup> was used mainly, it would seem, for the determination of the absorptive capacity of personal care products, such as nappy fillings. A gel dressing applied to a wound under a secondary dressing will absorb fluid and, as a result, the gel will swell. The swelling will impinge on both the wound and the secondary bandage. The Franz cell provides such an environment, the Visking tubing being the wound surface and the side arm representing the secondary dressing. The Visking tubing is exposed to a certain amount of pressure as the gel expands; it is not rigid and expands slightly. The side arm, on the other hand, by virtue of its diameter will provide a certain amount of hindrance to a swelling gel as would be expected by a secondary bandage or dressing. By simply filling the lower reservoir with the gel and separating the compartments with Visking tubing, it is possible to model the wound environment.

The method used in this study was validated, using three Franz cells (manufactured by Crown glass), by determining the precision, reproducibility, sensitivity and the robustness of the method. The accuracy and linearity of response were more difficult validation parameters to fulfill using this method, but were addressed. A dozen Franz cells were custom made to cope with the large number of absorptive capacity determinations required in Chapters 4, 5, 6 and 7, the robustness of the method will indicate the ability to compare results from two different types of cells. All Franz cells were labeled with a letter on the upper and lower compartments, these letters were retained throughout this project.



2.3.2 Materials

Refer to section 2.1.1.1, the solutions formulated for this section were manufactured using the methods outlined in section 2.1.

2.3.3 Equipment

Refer to section 2.1.1.2, the equipment outlined was used in combination with the following equipment: Franz cells, A-C, Crown Glass Ltd. USA. Franz cells, D-O, Mr. B. Chappell, Liverpool University, England. Vernier, 548-710RS, Mitatoyo, Japan. Balance 2, Precisia Balances, Zurich, Switzerland.

2.3.3.1 FRANZ CELL DIAMETERS

The internal diameters of all cells were calculated using a Vernier (a measuring device), to allow the calculation of the area of gel in contact with the Visking tubing and therefore determine the absorptive capacity of gels in cm<sup>2</sup> using a conversion factor. The results are summarised in Table 2.1. The average results from nine Vernier measurements are quoted because the cells were not perfectly round.

Table 2.1 The diameters of Franz cells used in this study.

Cell	Custom or Commercial	Average Diameter cm	Average Area cm <sup>2</sup>	Conversion factor
A	Commercial	15.38	1.86	0.54
B	Commercial	14.93	1.75	0.57
C	Commercial	15.67	1.93	0.52
D	Custom	16.12	2.04	0.49
E	Custom	15.53	1.89	0.53
F	Custom	15.23	1.82	0.55
G	Custom	15.58	1.91	0.52
H	Custom	16.08	2.03	0.49
I	Custom	16.65	2.18	0.46
J	Custom	17.60	2.43	0.41
K	Custom	16.13	2.04	0.49
L	Custom	16.46	2.13	0.47
M	Custom	17.00	2.27	0.44
N	Custom	16.10	2.03	0.49
O	Custom	15.98	2.01	0.50



## **2.3.4 The Franz Cell Method For Determining The Absorptive Capacity Of Hydrogels And Its Validation**

### **2.3.4.1 THE FRANZ CELL METHOD**

The following method was used on all formulations prepared for the determination of absorptive capacity. All samples were poured into the lower compartment (A) of the Franz cells (Figure 2.14). The Visking tubing (C) and upper compartment (B) were then secured in place, using a horseshoe clamp (E). To ensure no sample loss due to swelling or drying during the experiment, an extra piece of plastic tubing with a cover (D) was attached to the side arm. The cells were covered with Parafilm®, inverted and left at an angle overnight to ensure that any air bubbles at the gel/membrane interface moved into the side arm. Water absorption was observed by recording the change in weight of the Franz cell with time. Compartment (B) was emptied of distilled water, dried with tissue paper and the weight recorded at hourly intervals for 9h or 12h, then at 24h. Following the weight determination, compartment (B) was refilled with distilled water and the experiment resumed.

### **2.3.4.2 FRANZ CELL METHOD VALIDATION PARAMETERS**

The methods for formulation manufacture and absorptive capacity determinations are described in sections 2.1.1 and 2.3.4.1 respectively.

#### **2.3.4.2.a DETERMINATION OF METHOD PRECISION**

Three 120g batches (X, Y and Z) of 8% w/w sodium alginate and calcium chloride were prepared and each batch was characterised on a different day (X<sub>1</sub> and X<sub>2</sub>, Y<sub>1</sub> and Y<sub>2</sub>, and Z<sub>1</sub> and Z<sub>2</sub>) *e.g.*, X<sub>1</sub> on the day after manufacture and X<sub>2</sub> two days after manufacture with storage in a fridge.

#### **2.3.4.2.b LINEARITY OF RESPONSE**

Two 60g batches of 2% w/w (S<sub>1</sub> and S<sub>2</sub>), 5% w/w (U<sub>1</sub> and U<sub>2</sub>), 6% w/w (V<sub>1</sub> and V<sub>2</sub>) or 8% w/w (W<sub>1</sub> and W<sub>2</sub>) sodium alginate with calcium chloride were prepared. Three 60g batches of 4% w/w (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) sodium alginate and calcium chloride were similarly prepared. Each batch was prepared and characterised separately.



#### 2.3.4.2.c DETERMINATION OF METHOD SENSITIVITY

Two 60g, batches of 8% w/w sodium alginate and calcium chloride, were formulated with 0.5% w/w (P<sub>1</sub> and P<sub>2</sub>), 1% w/w (Q<sub>1</sub> and Q<sub>2</sub>) and 3% w/w (R<sub>1</sub> and R<sub>2</sub>) Aquasorb<sup>®</sup>. Each batch was prepared and characterised separately.

#### 2.3.4.2.d DETERMINATION OF METHOD ACCURACY

Three commercially available formulations were characterised for their water uptake capacity: Sterigel<sup>®</sup> from SSL International (formerly Seton Healthcare), Nugel<sup>®</sup> from Johnson and Johnson Medical and Intrasil gel<sup>®</sup> from Smith and Nephew Healthcare Ltd. The Oertling balance was calibrated at 7 points between 0.05 and 200g (Appendix 1).

#### 2.3.4.2.e DETERMINATION OF METHOD ROBUSTNESS.

The robustness of the method was determined firstly by comparing between batch and between Franz cell differences, of absorptive capacity from the precision experiment (batches X, Y and Z). Secondly by comparing the two types of Franz cell, using the data generated characterising the commercial gels from section 2.3.4.2.d. A comparison was made of the absorptive capacity determined using the commercial and custom made cells. Thirdly, the use of a second balance (Balance 2) for characterising a batch (Γ<sub>3</sub>) of 4%w/w sodium alginate (section 2.3.4.2.b) and comparing the three sets of data absorption data.

### 2.3.4.3 VALIDATION RESULTS

The results obtained in sections 2.3.4.2.a –2.3.4.2.e to determine precision, linearity, sensitivity, accuracy and robustness of the Franz cell method are found in Tables 2.2–2.5 respectively. The balance calibration data can be found in Appendix 1.

#### 2.3.4.3.a THE PRECISION OF THE FRANZ CELL METHOD

The precision of the method was determined using the data from Table 2.2. The mean absorbency of water at 24h was 0.029 g/g/cm<sup>2</sup>/24h with a standard deviation of ±0.002 g/g/cm<sup>2</sup>/24h and an RSD of 5.6% (n=18). A two way ANOVA was performed to determine any between batch or between cell differences. There were no significant differences between either the batch of alginate or the Franz cell used (p>0.05).



Table 2.2 Results determining the precision of the Franz cell method, 8<sup>0</sup> ow/w Sodium alginate 24h time point data (n=18).

Water Uptake (g/g/cm <sup>2</sup> )			
Batch	Franz cell		
	a	b	c
X <sub>1</sub>	0.026	0.031	0.030
X <sub>2</sub>	0.031	0.030	0.030
Y <sub>1</sub>	0.029	0.028	0.029
Y <sub>2</sub>	0.030	0.032	0.029
Z <sub>1</sub>	0.028	0.027	0.027
Z <sub>2</sub>	0.029	0.032	0.028

2.3.4.3.b LINEARITY OF RESPONSE OF THE FRANZ CELL METHOD

The linearity of the response of absorptive capacity to an increase in concentration was determined by regression analysis of the data shown in Table 2.3. A graphical representation comparing the applicability of linear or polynomial regression to the curve is seen in Figure 2.15. The r<sup>2</sup> values are 0.948 (SD 0.005) and 0.956 (SD 0.003) for linear and polynomial regression respectively (n=33).

Table 2.3 Different concentrations of sodium alginate, 24h time point data (g/g/cm<sup>2</sup>), used to calculate the Franz cell methods' linearity of response (n=33). Shown graphically in Figure 2.15.

Water uptake (g/g/cm <sup>2</sup> )				
Alginate		Cell		
(%ow/w)	Batch	a	b	c
2	S <sub>1</sub>	0.0105	0.0115	0.0085
2	S <sub>2</sub>	0.0106	0.0110	0.0108
4	T <sub>1</sub>	0.0235	0.0214	0.0225
4	T <sub>2</sub>	0.0248	0.0244	0.0241
4*	T <sub>3</sub>	0.0229	0.0246	0.0227
5	U <sub>1</sub>	0.0245	0.0259	0.0246
5	U <sub>2</sub>	0.0242	0.0255	0.0243
6	V <sub>1</sub>	0.0313	0.0326	0.0317
6	V <sub>2</sub>	0.0273	0.0286	0.0277
8	W <sub>1</sub>	0.0325	0.0350	0.0318
8	W <sub>2</sub>	0.0350	0.0371	0.0331

\* Extra batch used for robustness determination.



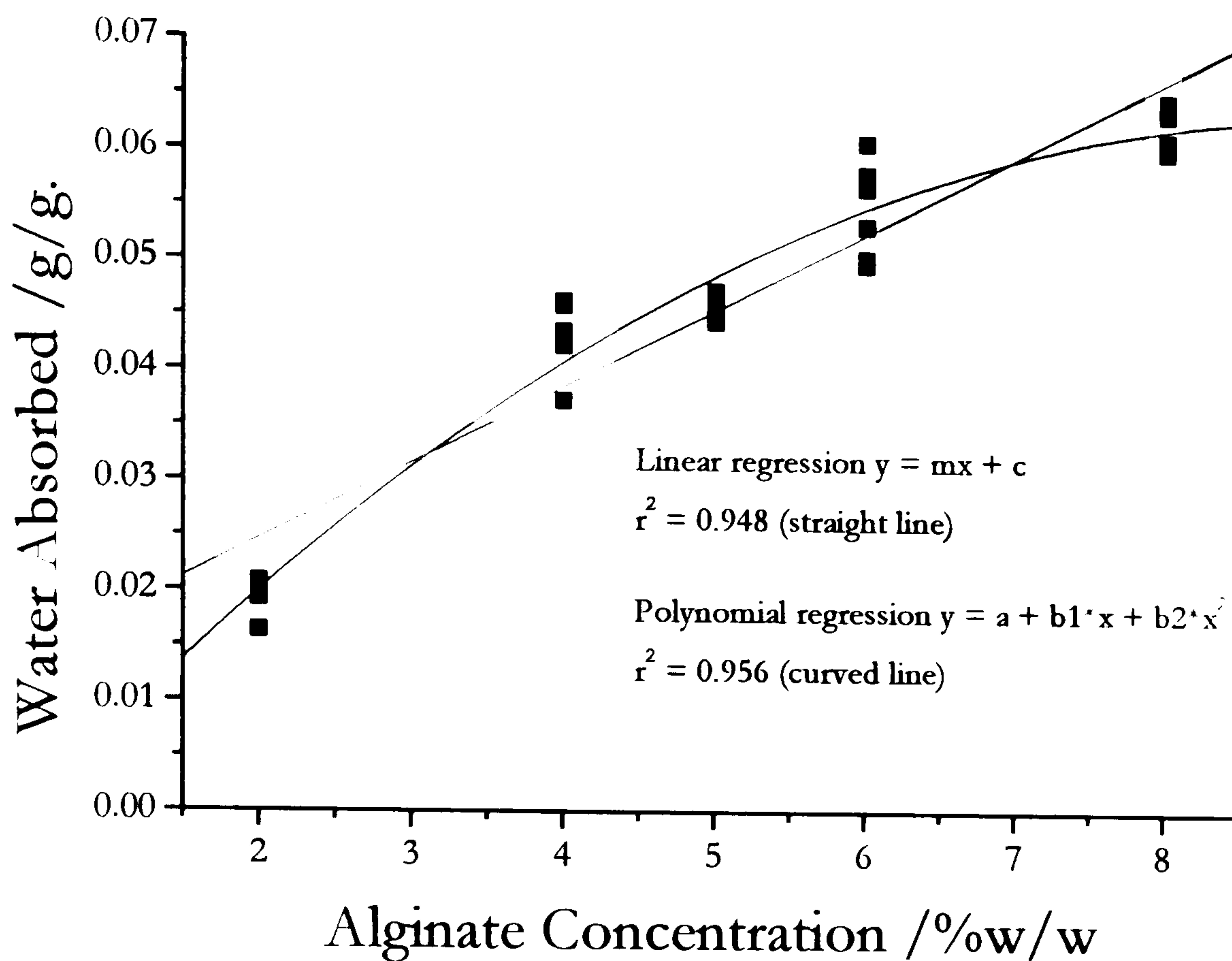


Figure 2.15 The linear (red) and polynomial (blue) regression curves of the data in Table 2.3.

#### 2.3.4.3.c THE SENSITIVITY OF THE FRANZ CELL METHOD

The ability of the method to differentiate between small changes in polymer concentration was assessed using a one way ANOVA, to analyse the data in Table 2.4. The response of the Franz cell method to small differences in added polymer (Aquasorb<sup>®</sup>) concentration were significant ( $p < 0.05$ ).

Table 2.4 8%w/w Sodium alginate with 0.5, 1 and 3%w/w Aquasorb<sup>®</sup> 24h time point data (g/g/cm<sup>2</sup>/24h), used to calculate the sensitivity of the method.

Aquasorb <sup>®</sup>		Franz cell		
(%w/w)	Batch	a	b	c
0.5	P <sub>1</sub>	0.0327	0.0342	0.0294
0.5	P <sub>2</sub>	0.0314	0.0316	0.0324
1	Q <sub>1</sub>	0.0342	0.0356	0.0340
1	Q <sub>2</sub>	0.0337	0.0360	0.0341
3	R <sub>1</sub>	0.0386	0.0397	0.0355
3	R <sub>2</sub>	0.0399	0.0413	0.0390

#### 2.3.4.3.d THE ACCURACY OF THE FRANZ CELL METHOD

The Oertling balance was calibrated at 7 points between 0.05g and 200g (using non-traceable calibration weights), the linear regression of the balance readings gave a  $r^2$  (0.999 (SD  $\pm 0.017$ g) (Appendix 1). The accuracy was also estimated by the characterisation of commercially available amorphous hydrogels. These were Sterigel<sup>®</sup>,



Nugel<sup>®</sup> and Intrasilite gel<sup>®</sup>, which absorbed 0.024, 0.035 and 0.031 g/g/cm<sup>2</sup>/24h respectively (Table 2.5).

Table 2.5 The absorptive capacity of commercial amorphous hydrogels using different glassware at the 24h time point (g/g/cm<sup>2</sup>/24h).

Hydrogel	Cell					
	a	b	c	d	e	f
Nugel <sup>®</sup>	0.0333	0.0342	0.0332	0.0367	0.0404	0.0471
	0.0370	0.0368	0.0358	d	e	f
Intrasite gel <sup>®</sup>	0.0307	0.0313	0.0303	0.0362	0.0381	0.0415
	0.0336	0.0335	0.0293	e	f	g
Sterigel <sup>®</sup>	0.0231	0.0236	0.0228	0.0254	0.0224	0.0210
	0.0246	0.0245	0.0233			

### 2.3.4.3.e THE ROBUSTNESS OF THE FRANZ CELL METHOD

The robustness of the Franz cell method was tested in three ways. 1) The two way ANOVA treating the batch of alginate (X, Y and Z) and the Franz cell used proved there were no significant differences between the absorption results for either the batch of alginate or the Franz cell(P>0.05). 2) The accuracy investigation characterised the commercial amorphous hydrogels using both the custom made (D, E, F and G) and the commercial (Crown glass) Franz cells (A, B and C). The absorption results were compared using a one way ANOVA treating the type of glassware (section 2.3.4.3.e, Table 2.5). For both Intrasilite gel<sup>®</sup> and Nugel<sup>®</sup> there was a significant difference seen between the two sets of glassware (P<0.05). However, for Sterigel<sup>®</sup> there was no significant difference (P>0.05). 3) 3 batches of 4%w/w sodium alginate (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) were characterised in section 2.3.4.2.b. The robustness of the method was tested by characterising batch T<sub>3</sub> using a second balance. A one way ANOVA treating the absorption results generated using two different balances, proved there was no significant difference (P>0.05).

### 2.3.4.4 DISCUSSION

The Franz cell method for determining water uptake capacity gave good precision, the relative standard deviation for the mean result of three different batches (X, Y and Z) of alginate was 5.6%. A two way ANOVA treatment of the data (section 2.3.5.2.a Table 2.2) showed no significant difference between the batch of alginate (X, Y and Z) and the Franz cells (A, B and C) used (p>0.05). The sensitivity of the method was also acceptable with the differentiation of small changes in the polymer concentration



possible. The one way ANOVA performed on the data in Table 2.4 (section 2.3.5.2.c) showed that all three polymer concentrations provided responses which were significantly different. However, the absorption response of the Franz cell method to an increase in polymer concentration (section 2.4.3.3.b) was not linear. The accuracy was assessed on the basis of the balance calibration, which gave a  $r^2$  value 0.999 for the response of the balance to 7 different calibration weights

The robustness of the Franz cell method was tested in three ways. Firstly, a two way ANOVA was applied and gave a very good indication of the effects of different batches and cells, with no significance attributed to either. A second test was applied by using a second balance during the response linearity experiment (section 2.3.4.2.b), which proved there was no significant difference between the balances used ( $P>0.05$ ).

The third test of robustness was the comparison of the source of glassware used to characterise the absorptive capacity of the commercial amorphous hydrogels. A one way ANOVA treating the source of glassware proved that for Intrasil gel<sup>®</sup> and Nugal<sup>®</sup> there was a significant difference between the absorption levels in different glassware ( $P<0.05$ ). With this in mind the only conclusion to be drawn was that the Franz cell method was not robust enough to compare absorption results from glassware of different sources.

#### 2.3.4.5 SUMMARY

The method was validated with respect to precision, accuracy and sensitivity. The relation between alginate concentration and water uptake capacity was not linear. The level of the robustness was adequate for the purposes of the project at hand, but this did not extend to the use of different glassware (Crown or Custom) within a particular experiment. The causes of the difference between the glassware were not elucidated.



## CHAPTER 3 INTRODUCTION TO EXPERIMENTAL DESIGN IN FORMULATION DEVELOPMENT

### 3.1 INTRODUCTION

The process of experimentation can be divided into a number of stages<sup>[125]</sup>:

- i) Choice of experimental factors (variables) and their levels.
- ii) Selection of a response from which to estimate the effects of factors.
- iii) Choice of suitable experimental design to efficiently investigate the factors chosen.
- iv) Experimentation and data analysis
- v) Conclusions

The choice of an experimental design is an important part of the experimental process. The planning stages (i and ii above) and experimentation carried out previously will influence design choice. The summary below can be used to aid the choice of experimental designs<sup>[126]</sup>. Only four specific designs are mentioned, there is not the space here to cover the large number of designs available. A more detailed discussion on the subject and the types of experimental designs and their applications can be found in the texts by Clarke and Kempson<sup>[127]</sup> and, Lewis *et al*<sup>[128]</sup>.

- i) Large number of factors: an experiment with a large number of factors requires a screening design that will identify factors that influence or may influence a process or formulation.
- ii) Small number of factors: an experiment with a small number of factors or when the results of a screening procedure identify influential factors. A factorial or fractional factorial experimental design can be used to estimate the effects and interactions of factors.
- iii) Predicting response: Response surface methodology can then be used to predict the response of the formulation or process over the experimental domain.
- iv) Optimisation: To find the best combination of factors in a system, graphical algebraic or mathematical methods can be used, in conjunction with the models from response surface methodology studies. Sequential simplex optimisation can also be used.



General principles that should be applied to the chosen design are that factor ranges should span the domain of interest (or design space). The design should be balanced *i.e.*, all factors should have the same number of levels (amounts). The factors chosen and the factors with interactions of interest should have minimal or zero correlation between them *i.e.*, they are independent.

The advantages of using the types of experimental design (reduced factorial) for this project are 1) fewer number experiments are required to complete a task, 2) greater confidence in the results and 3) an ability to determine the effect and the interactions of factors (variables). However, the disadvantages of using experimental designs are that it is not possible to predict the results for a formulation outside the design domain. Replication of the experiments is required to improve the data quality and the choice of the experimental design is crucial in preventing the wrong information from being generated.

## **3.2 EXPERIMENTAL DESIGNS**

Experimental design can be defined as the strategy for setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible<sup>[128]</sup>. The sections below introduce three experimental designs: full factorial (section 3.2.1), fractional factorial (section 3.2.2) and, Plackett and Burman screening designs (section 3.2.3).

### **3.2.1 Full Factorial Experimental Designs**

A factorial experimental design is the investigation of factors at different levels to see whether the factors operate independently of one another or whether there is interaction between them<sup>[127]</sup>. Factorial experimental designs are, in some ways, seen as inefficient because all factors in a design are tested at each level. As a result, the number of experiments increases to the power of the number of factors. An example of this would be a two level experiment with four factors ( $2^4$ ) requiring 16 experiments. Simply by adding an extra factor would increase the number of experiments to 32. Similarly investigating four factors at three levels ( $3^4$ ) would require 81 experiments. As outlined in section 3.1 factorial experiments are useful for small numbers of factors. An example follows to introduce the process of estimating the effects and interactions of factors in a factorial experimental design.



### 3.2.1.1 AN EXAMPLE OF A FULL FACTORIAL EXPERIMENT

A theoretical investigator looked at the effect of two factors on the breaking strength of a gel. The factors were type of natural polymer included and the gel thickness. The levels of the factors were as follows: a) the polymers were cellulose and alginate and b) the gel thickness was 2mm and 5mm. The experimental design domain is shown in Table 3.1. Table 3.2 represents the same design domain using coding for the levels *i.e.*, -1 represents the low level of a factor and +1 represents the high level of a factor. The coding can also be represented as simply a + or a – sign. Experimentation took place in a random order. The breaking strength was determined (with an arbitrary unit X) and the results are recorded in Table 3.3

To estimate the effects of factors on breaking strength, the results in Table 3.3 were analysed by analysis of variance (ANOVA) using Minitab®. A copy of the processed results is given in Figure 3.1, the results can be explained more easily by following through the manual calculation of the results. The main effects of factors are calculated in section 3.2.1.1.a and the interactions of factors are calculated in section 3.2.1.1.b.

#### 3.2.1.1.a CALCULATING THE MAIN EFFECTS OF FACTORS

The effect of a variable is represented by its main effect and this distinguishes it from the effects of interactions. The main effects of factors are calculated using the following principal (represented graphically in Figure 3.2):

$$\text{Main effect of a Variable} = \text{Mean response, high level} - \text{Mean response, low level} \quad (\text{Equation 3.1})$$

Polymer effect determined by using the row means Table 3.3:

$$4.88 \text{ (Breaking strength mean with alginate)} - 4.85 \text{ (Breaking strength mean with cellulose)} = 0.03X \quad (\text{Equation 3.2})$$

Thickness effect determined by using the column means Table 3.3:

$$5.5 \text{ (Breaking strength mean at high thickness)} - 4.23 \text{ (Breaking strength mean at low thickness)} = 1.27X \quad (\text{Equation 3.3})$$

The mean of all the results (4.86) is used as the point from which the factor effects can be shown to exist. The dotted line crossing the graph horizontally in Figure 3.2.



Table 3.1 The experimental design domain, giving the four experiments that are required to investigate the system.

Experiment	polymer	gel thickness /mm
1	cellulose	2
2	cellulose	5
3	alginate	2
4	alginate	5

Table 3.2 The use of coded variables to represent the levels of each factor in Table 3.1. The results from the breaking strength tests are included.

Experiment	polymer	gel thickness	breaking strength /X
1	-1	-1	3.85
2	-1	+1	5.85
3	+1	-1	4.60
4	+1	+1	5.15

Table 3.3 The mean breaking strength values for determining the effects of factors: mean values of row, column and all cells.

Factors Polymer	Gel thickness		Mean
	2mm	5mm	
Cellulose	3.85	5.85	4.85
Alginate	4.60	5.15	4.88
Mean	4.23	5.5	4.86



Factorial Fit

Estimated Effects and Coefficients for Strength

Term	Effect	Coef
Constant		4.8625
polymer	0.0250	0.0125
thickness	1.2750	0.6375
polymer*thickness	-0.7250	-0.3625

Analysis of Variance for Strength (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	1.6263	1.6263	0.8131	*	*
2-Way Interactions	1	0.5256	0.5256	0.5256	*	*
Residual Error	0	0.0000	0.0000	0.0000		
Total	3	2.1519				

Figure 3.1 The results from Table 3.2 processed using an analysis of variance in Minitab® to calculate the main effects and interactions of factors. The F and P values are blank (\*) because there was not enough data to calculate them.

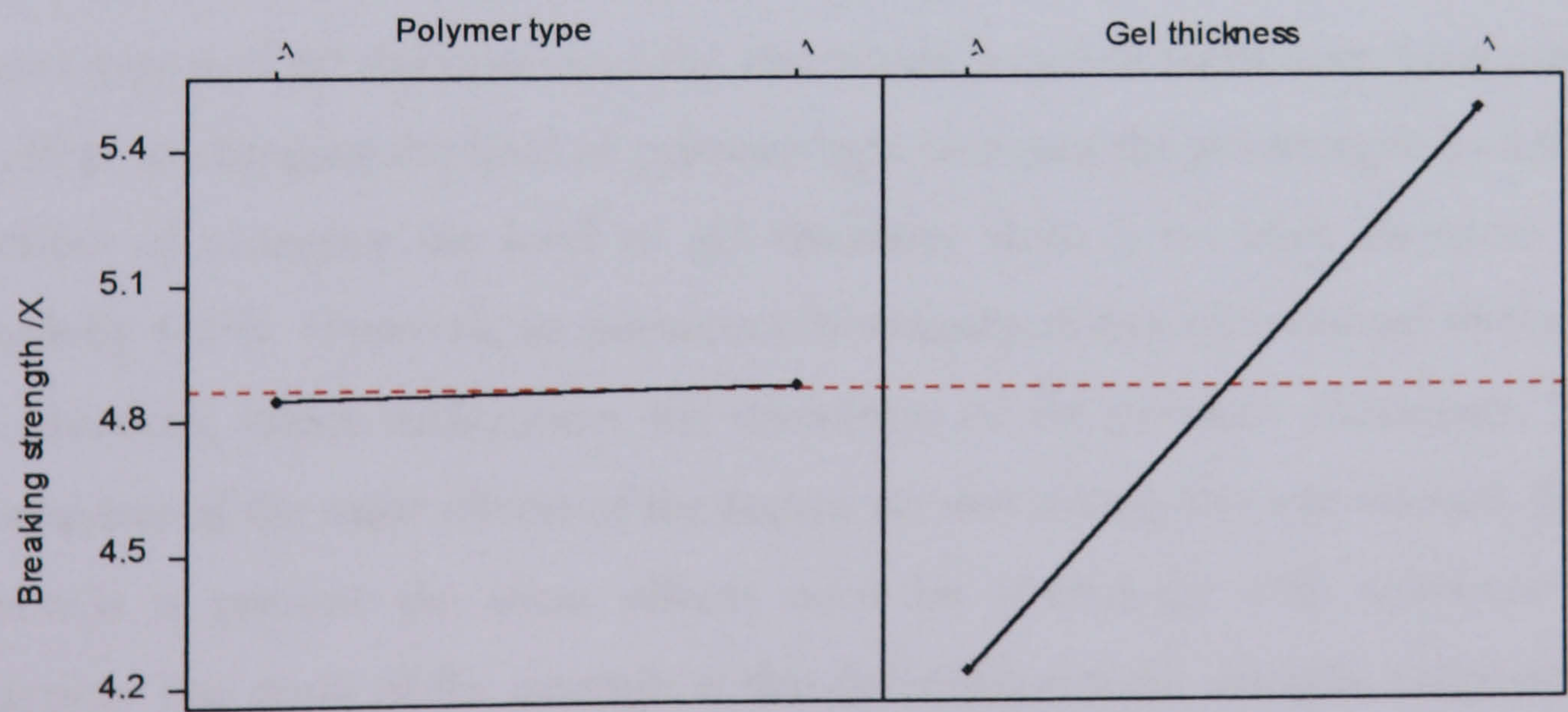


Figure 3.2 The main effects profile of factors, a graphical representation from Minitab®.



3.2.1.1.b CALCULATING INTERACTIONS

The possibility of an interaction being present can be determined by comparing the values of the cells, not the means, as follows. Increasing the gel thickness from 2 to 5mm when using cellulose increases the breaking strength by 2.0X. Whereas, by increasing the gel thickness from 2 to 5mm using alginate the breaking strength increased by only 0.55X. This inconsistency is seen as an interaction between polymer type and gel thickness, *i.e.*, the effect of gel thickness depends on the type of polymer used. A graphical representation of these interactions can be found in Figure 3.3. The calculations are given below.

Interactions are calculated as follows:

$$\text{Interaction} = \frac{1}{2}(\text{Effect factor x at high level of y} - \text{effect of factor x at low level of y}) \tag{Equation 3.4}$$

The interaction of gel thickness and polymer is as follows using Equation 3.4  
 $\text{Interaction} = \frac{1}{2} ((3.85-4.60)-(5.85-5.15)) = -0.725$ . The interaction can also be calculated as the difference between the means of the diagonal cells in Table 3.3.

3.2.1.1.c INTERPRETING THE MAIN EFFECTS AND INTERACTIONS

From these calculations (section 3.2.1.1.a and 3.2.1.1.b) the main effects of the factors polymer type and gel thickness and the interaction between them have been estimated. The effect of changing the level of polymer type increases the gel strength by 0.03X and the effect of changing the level of gel thickness from 2 to 5mm increases the gel strength by 1.27X. However, an interaction between polymer type and gel thickness has been observed, which undermines the usefulness of the previous statements. Though the estimates of the main effects of the factors are not wrong they can mislead. When an interaction is present the main effects must be considered with reference to the interaction. The result of the example is that for maximum gel strength, cellulose should be used at a high gel thickness.



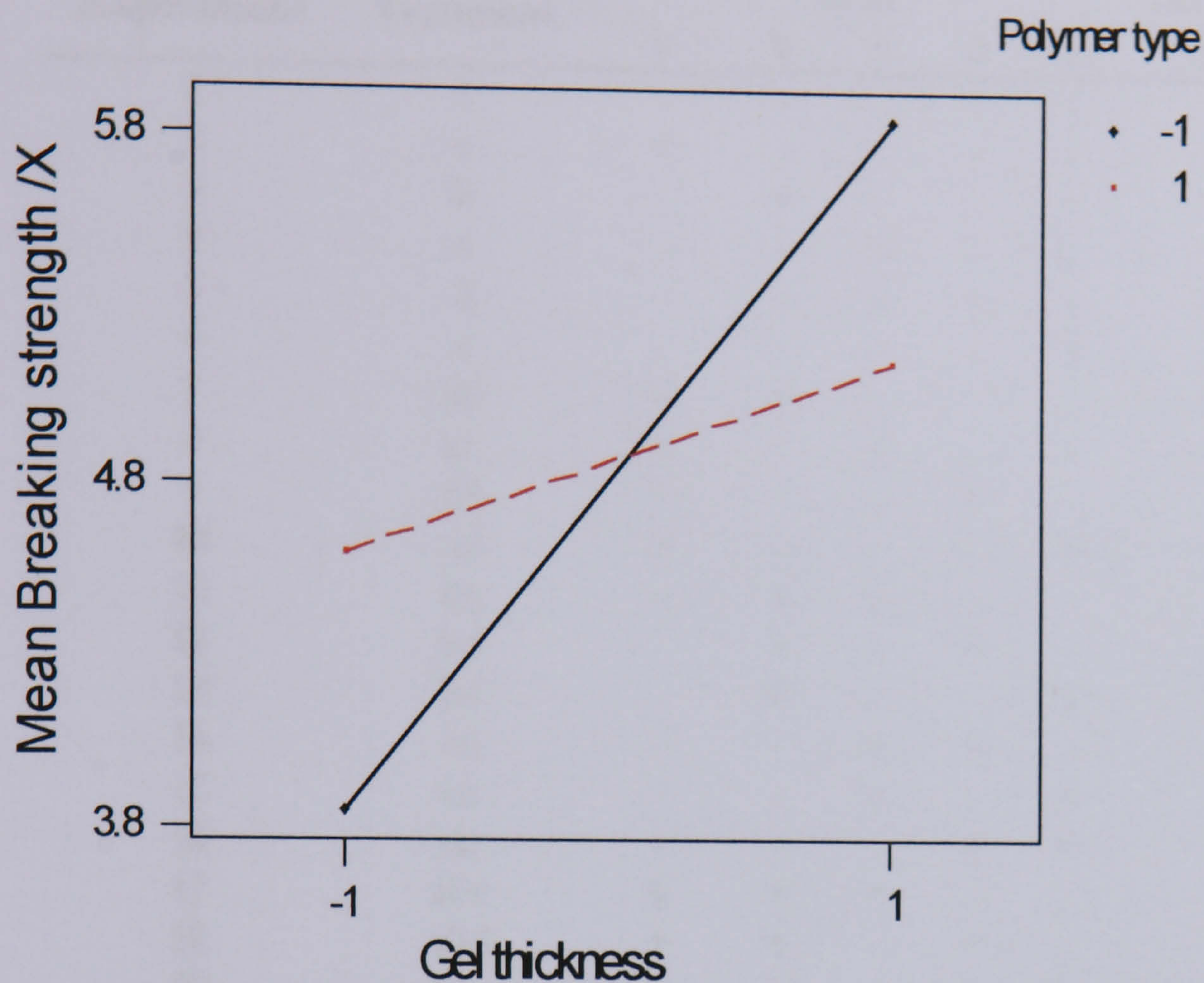


Figure 3.3 The interaction profile of factors, a graphical representation from Minitab®.

### 3.2.2 Fractional Factorial Experimental Design

The inefficiency of factorial designs can be combated by the use of fractional factorial designs<sup>[1227,128]</sup>. By taking advantage of confounding, the number of experiments can be reduced. Confounding is where the effect of a factor or interaction cannot be fully resolved from another. Ideally, the highest resolution should be used in an investigation to ensure the quality of the design. However, it is possible to use designs of lower resolution. A five factor design at two levels, for example, requires  $2^5$  (32) experiments to completely resolve the main effects and interactions. However, with the use of a fractional factorial design, the main effects and first order interactions of the same five factors, can be estimated in half the experiments. The  $2^{5-1}$  experimental design is partitioned at the highest order of interaction, a  $2^{5-1}$  experiment, Table 3.4 summarises how the design is partitioned. A more detailed discussion can be found in Clarke and Kempson<sup>[127]</sup> and Lewis *et al*<sup>[128]</sup>.



Table 3.4 A  $2^{5-1}$  experiment, by partitioning the design i.e., performing the experiments, where abcde is positive allows the main effect and first order interactions of factors to be estimated.

A $2^{5-1}$ experimental design.							
Experiment	Treatment	level					Partitioning abcde
		a	b	c	d	e	
1	1	-	-	-	-	-	-
2	a	+	-	-	-	-	+
3	b	-	+	-	-	-	+
4	c	-	-	+	-	-	+
5	d	-	-	-	+	-	+
6	e	-	-	-	-	+	+
7	ab	+	+	-	-	-	-
8	ac	+	-	+	-	-	-
9	ad	+	-	-	+	-	-
10	ae	+	-	-	-	+	-
11	bc	-	+	+	-	-	-
12	bd	-	+	-	+	-	-
13	be	-	+	-	-	+	-
14	cd	-	-	+	+	-	-
15	ce	-	-	+	-	+	-
16	de	-	-	-	+	+	-
17	abc	+	+	+	-	-	+
18	abd	+	+	-	+	-	+
19	abe	+	+	-	-	+	+
20	acd	+	-	+	+	-	+
21	ace	+	-	+	-	+	+
22	ade	+	-	-	+	+	+
23	bcd	-	+	+	-	+	+
24	bce	-	+	+	-	+	+
25	bde	-	+	-	+	+	+
26	cde	-	-	+	+	+	+
27	abcd	+	+	+	+	-	-
28	abce	+	+	+	-	+	-
29	abde	+	+	-	+	+	-
30	acde	+	-	+	+	+	-
31	bcde	-	+	+	+	+	-
32	abcde	+	+	+	+	+	+

### 3.2.2.1 CALCULATING THE MAIN EFFECTS AND INTERACTIONS

It is possible to analyse the data from fractional factorial designs using: 1) the long hand methods outlined in section 3.2.1, 2) the use of ANOVA or mathematical models (such as multi linear regression and first order additive models<sup>[128]</sup>) produce the same results<sup>[126]</sup> and 3) The use of statistical software packages, one example is Minitab<sup>®</sup> (used throughout this project), allowing rapid data processing. The example design in



section 3.2 highlights the benefits of using a statistical package for calculating and representing the main effects and interactions of factors.

### 3.2.3 The Use Of Experimental Design To Screen Large Numbers Of Factors

The often large number of factors identified for investigation at the planning stage of an experiment can be incorporated into a screening design to rapidly assess factors for potential effects in a given system. A low resolution fractional factorial design can be used to screen large numbers of factors. The design described here and used in Chapter 6 is a design developed by Plackett and Burman<sup>[126]</sup>. These are efficient low resolution designs, which allow for example, ten factors to be screened at two levels in sixteen experiments: this includes four repeated experiments at the centre of the design. The four repeated experiments allow an estimate to be made of the standard error for the design, a limiting value and therefore by direct comparison the significance of any factor effects<sup>[128]</sup>. By varying the levels of all factors at the same time, the influence of a factor is determined with greater precision because the result of each experiment enters equally into the calculation of the estimate of factor activity. Thus the error for each experiment is spread across twelve experiments, minimising its influence. The factor effects are estimated using a first order additive model.

#### 3.2.3.1 CALCULATING FACTOR EFFECTS IN A PLACKETT AND BURMAN SCREENING EXPERIMENTAL DESIGN

A Plackett and Burman design of ten factors, a to j, is given in Table 3.5 with accompanying coded variables. There are a total of sixteen experiments, which are completed in a random order. Experiments 1-12 estimate the factor effects and 13-16 are used to estimate the precision. The long hand calculation is given below to demonstrate the use of a first order additive model. The use of Minitab® is much faster, the only manual calculation is that of the limiting value.

Using a first order additive model for ten variables,  $y_0$  = result,  $\beta$  = estimate of factor effect and  $c$  = error (Equation 3.5)<sup>[128]</sup>.

$$y_0 = \beta_0 + \beta_a x_a + \beta_b x_b \dots + \beta_j x_j + c \quad (\text{Equation 3.5})$$



The results from each experiment can be described by combining the factor effects as in Equation 3.6 (refer to Table 3.5), where the estimates of the effect of each factor are combined taking into consideration their sign as in Table 3.5.

$$y_1 = \beta_0 - \beta_a - \beta_b \dots - \beta_j + \epsilon_1$$

(Equation 3.6)

$$\dots\dots\dots\text{to}\dots\dots\dots$$

$$y_{12} = \beta_0 - \beta_a + \beta_b + \beta_c + \beta_d \dots - \beta_j + \epsilon_{12}$$

Table 3.5 Plackett and Burman experimental design for 10 factors. Coded variables + = high level, - = low level and 0 = central point.

Experiment	Factors										Result
	X <sub>a</sub>	X <sub>b</sub>	X <sub>c</sub>	X <sub>d</sub>	X <sub>e</sub>	X <sub>f</sub>	X <sub>g</sub>	X <sub>h</sub>	X <sub>i</sub>	X <sub>j</sub>	y
1	-	-	-	-	-	-	-	-	-	-	y <sub>1</sub>
2	+	+	-	+	+	+	-	-	-	+	y <sub>2</sub>
3	-	+	+	-	+	+	+	-	-	-	y <sub>3</sub>
4	+	-	+	+	-	+	+	+	-	-	y <sub>4</sub>
5	-	+	-	+	+	-	+	+	+	-	y <sub>5</sub>
6	-	-	+	-	+	+	-	+	+	+	y <sub>6</sub>
7	-	-	-	+	-	+	+	-	+	+	y <sub>7</sub>
8	+	-	-	-	+	-	+	+	-	+	y <sub>8</sub>
9	+	+	-	-	-	+	-	+	+	-	y <sub>9</sub>
10	+	+	+	-	-	-	+	-	+	+	y <sub>10</sub>
11	-	+	+	+	-	-	-	+	-	+	y <sub>11</sub>
12	+	-	+	+	+	-	-	-	+	-	y <sub>12</sub>
13	0	0	0	0	0	0	0	0	0	0	y <sub>13</sub>
14	0	0	0	0	0	0	0	0	0	0	y <sub>14</sub>
15	0	0	0	0	0	0	0	0	0	0	y <sub>15</sub>
16	0	0	0	0	0	0	0	0	0	0	y <sub>16</sub>

Therefore to estimate the effect of each factor (β<sub>a</sub>), a linear combination of the values of the results in column y may be taken with the same signs for the column X<sub>a</sub> (Equation 3.7):

$$\beta_a + \frac{1}{10}(-\epsilon_1 + c_2 \dots + \epsilon_{12}) = \frac{1}{10}(-y_1 + y_2 - y_3 \dots + y_{12})$$

(Equation 3.7)

The combination of responses on the left hand side of Equation 3.7 is represented by b<sub>a</sub>, which is an unbiased estimate of the effect β<sub>a</sub>. The random errors c<sub>a,j</sub> are normally distributed about zero and therefore b<sub>a</sub> = β<sub>a</sub><sup>[128]</sup>. The effects can be calculated for each factor as in Equation 3.7a and presented graphically as an effect profile. An example effects profile is shown in Figure 3.4.

$$b_a = \frac{1}{10}(-y_1 + y_2 - y_3 + y_4 - y_5 - y_6 - y_7 + y_8 + y_9 + y_{10} - y_{11} + y_{12})$$

(Equation 3.7a)



The significance of these effects can be calculated as an estimate of the precision of the experiment based on the coded 0 variables<sup>[128]</sup>. Thus, by calculating the standard deviation ( $\sigma$ ) of results  $y_{13}$ ,  $y_{14}$ ,  $y_{15}$  and  $y_{16}$ , an estimate of the standard error for each coefficient can be calculated: ( $\sigma/\sqrt{10} = \alpha$ ). The value of Student's  $t$  for a probability of 95% is 3.18 at three degrees of freedom<sup>[129]</sup>. Thus effects exceeding a limiting value (Figure 3.4) of ( $\alpha * 3.182$ ) are considered significant at 95% confidence limit.

The disadvantages of using Plackett and Burman screening designs are that the designs are limited to factors at two levels, contain considerable confounding<sup>[126]</sup> and do not indicate the existence of interactions between factors. It is also possible to screen large numbers of factors with fractional factorial designs. The advantages of screening with fractional factorial designs are that designs of lower resolution (fewer experiments) can be supplemented to give full factorial information on key factors. Potential interactions can also be investigated by the selection of a suitable alias pattern (partitioning).

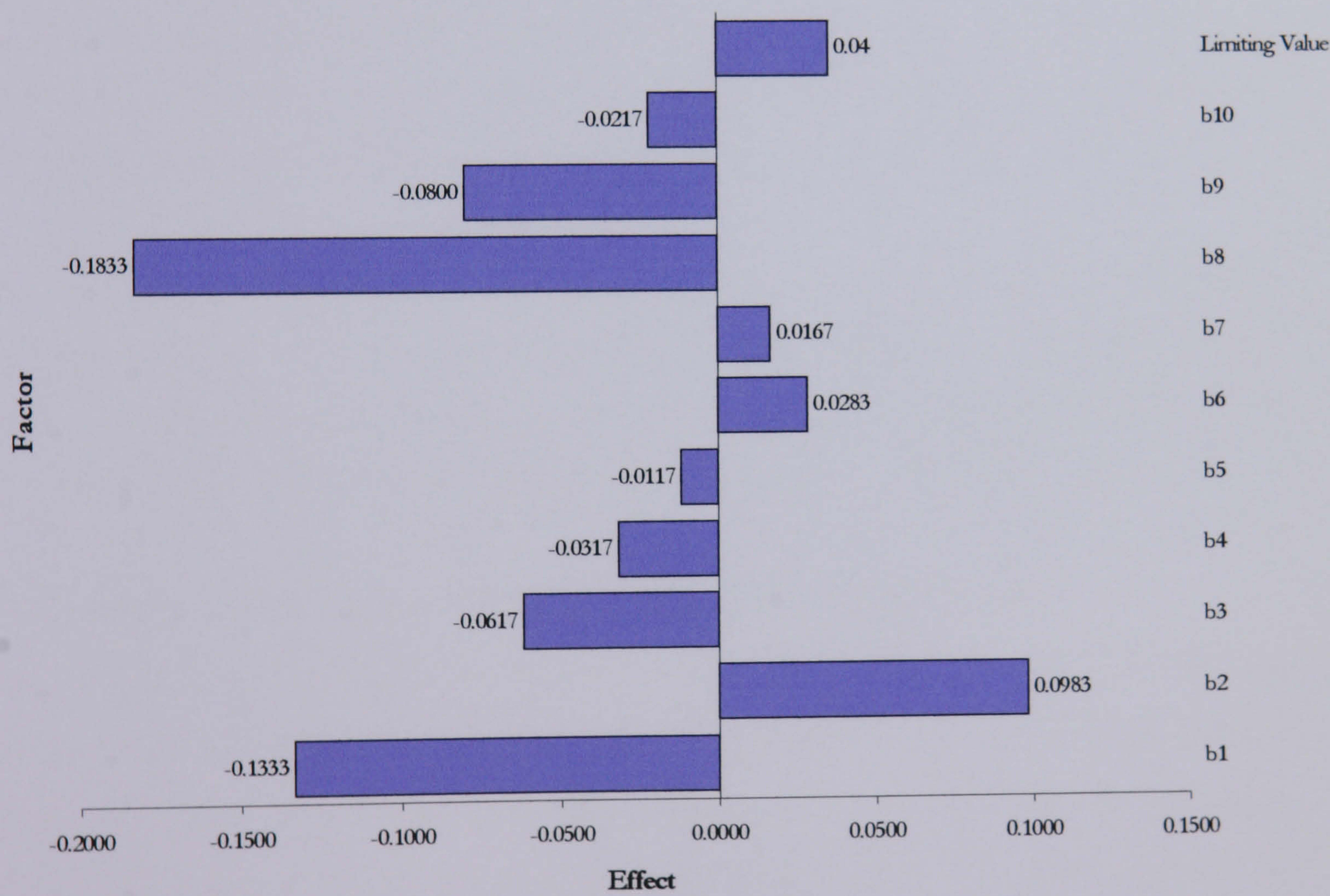


Figure 3.4 Example effects profile for a 10 factor Plackett and Burman design. The effect can be presented with the units of the test carried out.



### **3.3 SUMMARY OF THE EXPERIMENTAL DESIGNS USED IN THIS PROJECT**

The experimental designs described in section 3.2 are utilised in Chapters 4, 5, 6 and 7 of this thesis, in an effort to improve data quality and the efficiency of research with expensive excipients. The full factorial experimental designs were used for investigating small numbers of factors in Chapters 4 and 5. A Plackett and Burman screening design was used to investigate 10 factors in Chapter 6. This investigation was followed by the use of a fractional factorial design (Chapter 7), to determine the main effects and interactions of the factors found to have the most significant effects in Chapter 6.



## **CHAPTER 4 THE FORMULATION OF SODIUM ALGINATE GELS WITH CELLULOSE BASED POLYMERS**

### **4.1 INTRODUCTION**

The ideal properties of a wound dressing (section 1.3, Table 1.5) are well documented<sup>[1,20]</sup>, however, the ideal physical properties of wound gels are more difficult to determine. In order to compare the gels formulated in this chapter and assess their potential for development as wound dressings, three commercially available hydrogel (physical) dressings were characterised (section 4.2). Ideal properties for hydrogel (physical) dressings can be considered as ease of application, conformation to the wound surface and an ability to absorb exudate.

The *in-situ* gelation of a wound gel *i.e.*, the formation of a gel state by the wound dressing within the wound space, has a number of benefits for wound healing. The wound is completely covered by the wound dressing, before gelation occurs, and therefore the dressing conforms intimately to the wound surface providing a uniform, absorptive and occlusive environment for healing.

The aim of this section is to characterise three commercially available amorphous hydrogel dressings and to formulate alginate gels, using calcium ions and three different cellulose based polymers. The ability of sodium alginate to form a physical gel in the presence of divalent metal ions was discussed previously in section 1.4.

### **4.2 COMMERCIAL AMORPHOUS HYDROGEL DRESSINGS**

The commercial hydrogels characterised were: Sterigel<sup>®</sup> from SSL International (formerly Seton Healthcare. NB the original formulation not the reformulated product released in 2000), Nugel<sup>®</sup> from Johnson and Johnson Medical and Intrasil gel<sup>®</sup> from Smith and Nephew Healthcare Ltd. The aim was to use the same methods to characterise the commercial gels that were used to characterise the manufactured gels. The properties used to compare the gels were water absorptive capacity, viscosity and elasticity.



4.2.1 Experimental

Gels were characterised for their rheological and water uptake properties using the methods outlined in sections 2.2 and 2.3 respectively.

4.2.2 Results And Discussion

The water uptake capacity of Sterigel<sup>®</sup> was 0.023g/g/cm<sup>2</sup>/24h, lower than both Nugel<sup>®</sup> and Intrasilite<sup>®</sup>, which had water uptake capacities of 0.041g/g/cm<sup>2</sup>/24h and 0.039g/g/cm<sup>2</sup>/24h respectively. The rheological properties of Intrasilite<sup>®</sup>, Nugel<sup>®</sup> and Sterigel<sup>®</sup> are presented in Figures 4.1 and 4.2. The viscosity of Intrasilite<sup>®</sup> and Nugel<sup>®</sup> were similarly high (Figure 4.1). A high viscosity means application to the wound would be much more difficult, though conformation to the wound surface would be achieved, albeit slowly because of the high viscosity.

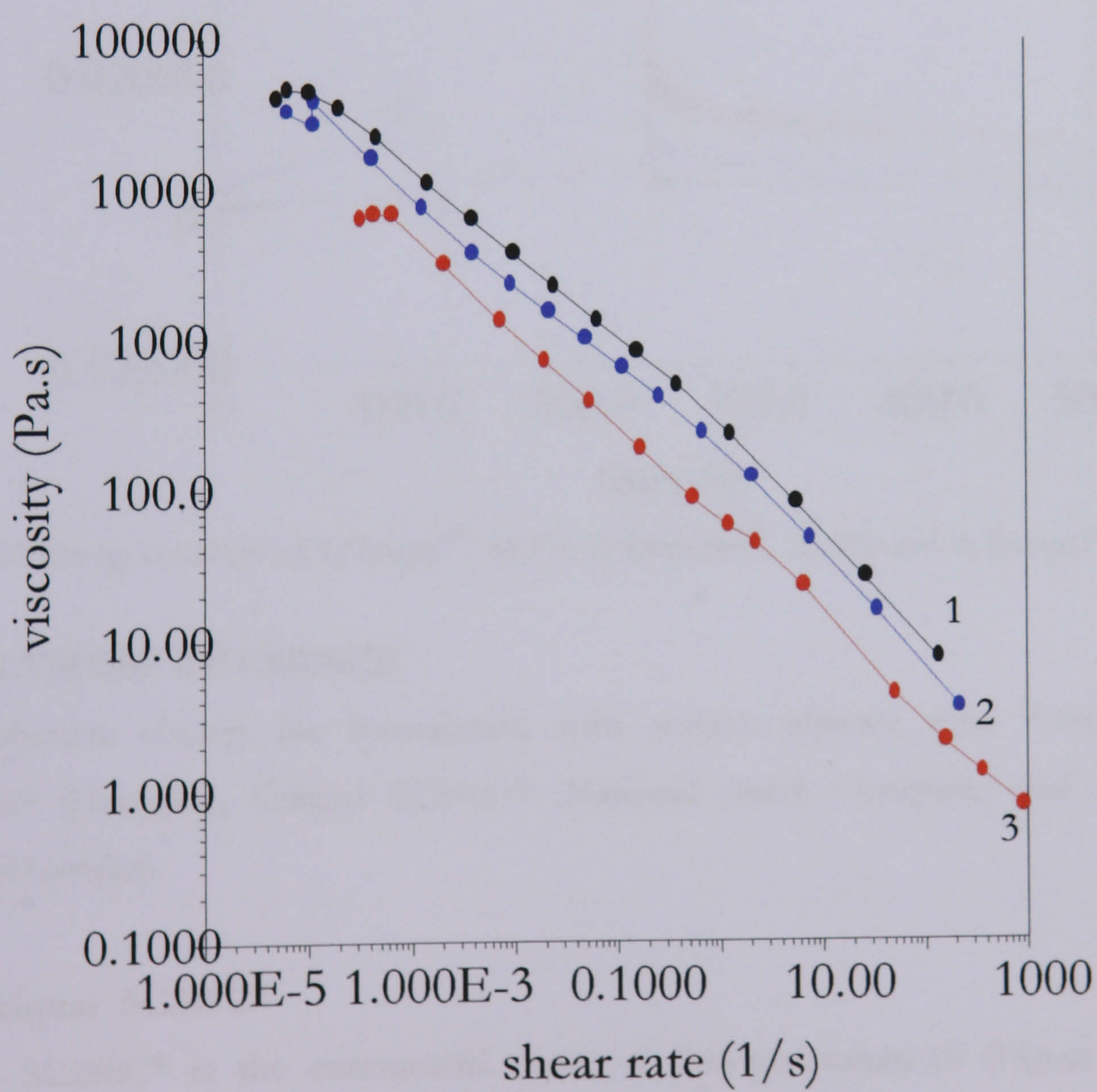


Figure 4.1 Viscosity (flow) profiles of 1) Nugel<sup>®</sup> 2) Intrasilite<sup>®</sup> and 3) Sterigel<sup>®</sup>.



The high elastic nature of Sterigel<sup>®</sup> (Figure 4.2) may reduce conformation to the wound surface, *i.e.*, the recovery of structure is such that the gel may not mould to the wound surface. The relatively low viscosity (Figure 4.1) of Sterigel<sup>®</sup> on the other hand, enables ease of application. Nugel<sup>®</sup> and Intrasilite<sup>®</sup> were far more viscous making application difficult. The viscous nature and low structure recovery would ensure conformation to the wound surface.

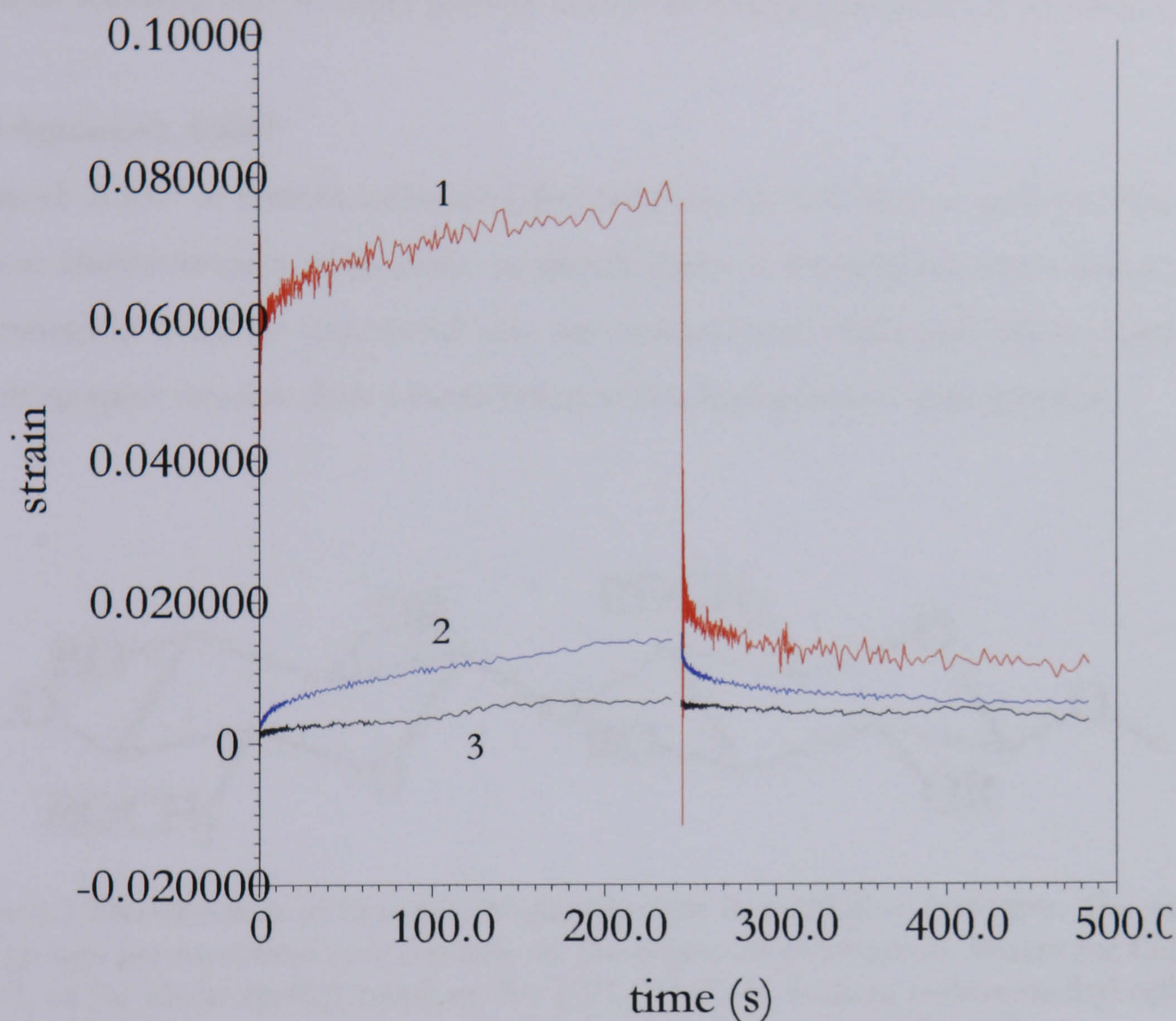


Figure 4.2 Creep recovery of 1) Nugel<sup>®</sup>, 44.3% 2) Intrasilite<sup>®</sup>, 59.3% and 3) Sterigel<sup>®</sup>, 84.3%.

### 4.3 POLYMERS OF CHOICE

The polymers chosen for formulation with sodium alginate were Natrosol 250 MPharm<sup>®</sup> (Hercules), Celquat SC240C<sup>®</sup> (National Starch Company) and Aquasorb A500<sup>®</sup> (Hercules).

#### 4.3.1 Celquat SC240C<sup>®</sup>

Celquat SC240C<sup>®</sup> is the commercial name of Polyquaternium-10 (Figure 4.3). Its cationic nature has potentially beneficial consequences when mixed with sodium alginate. The large number of positive charges have, theoretically, the potential to



interact with the anionic binding sites on the alginate polymer *i.e.*, forming a gel when mixed.

#### 4.3.2 Natrosol 250 MPharm<sup>®</sup>

Natrosol 250 MPharm<sup>®</sup> is hydroxyethyl cellulose and was chosen for its ability to shear thin<sup>[130]</sup>. Its structure is given in Figure 4.3. Therefore a gel prepared with Natrosol<sup>®</sup> would be relatively easy to expel into the wound and allow exudate to be absorbed.

#### 4.3.3 Aquasorb A500<sup>®</sup>

Aquasorb A500<sup>®</sup> is sodium carboxymethyl cellulose. Its structure is given in Figure 4.3 and was chosen because of its ability to absorb water. A formulation with a low polymer concentration could be introduced into the wound more easily and still be capable of absorbing more exudate than a formulation with a high polymer concentration.

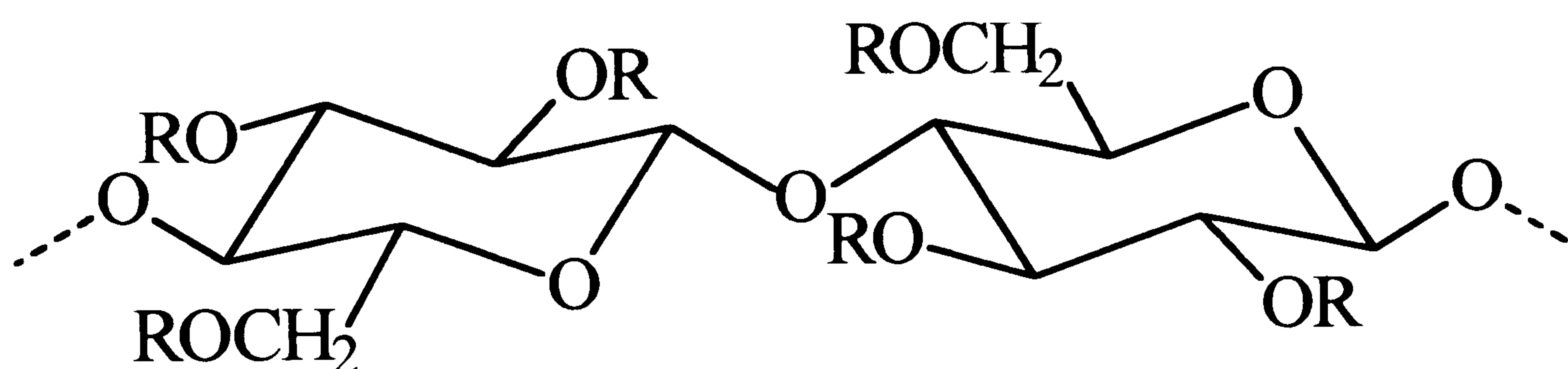


Figure 4.3 The structure of two anhydroglucose units in a cellulose derivative. The number of R groups per monomer unit depends on the degree of substitution. Where for Cellulose  $R = H$ , or for Hydroxyethyl cellulose,  $R = (CH_2CH_2O)_nH$ , Sodium carboxymethyl cellulose,  $R = CH_2COO^- Na^+$  and Polyquaternium-10,  $R = (CH_2CH_2O)_n R'N^+(CH_3)_3Cl$ .

### 4.4 EXPERIMENTAL

#### 4.4.1 Materials

Sodium alginate, LF 10/60, FMC Biopolymer, PO BOX494 N-3002, Drammen, Norway. Calcium chloride, AR, BDH Laboratory Supplies, Poole, Dorset, England. Natrosol<sup>®</sup>250MPharm, Hercules Ltd., Aqualon Division. Salford, Manchester. England. Cclquat<sup>®</sup> SC240C, National Starch & Chemical Ltd., Daventry, Northamptonshire, England. Aquasorb<sup>®</sup> A500, Hercules Ltd., Aqualon Division. Salford, Manchester. England.



4.4.2 Methods

The factorial experimental designs for investigating sodium alginate, Celquat<sup>®</sup>, Natrosol<sup>®</sup> and Aquasorb<sup>®</sup> are given in sections 4.4.3, 4.4.4, 4.4.5 and 4.4.6 respectively.

4.4.2.1 WATER UPTAKE DETERMINATION

The levels of the factors to be investigated are given in Table 4.1. The methods for gel manufacture and water uptake determination can be found in sections 2.1 and 2.2 respectively.

Table 4.1 Water uptake experiment factors and factor levels. For the investigation of sodium alginate alone, the levels of calcium were 0%<sub>w/w</sub> (-1) and 0.15%<sub>w/w</sub> (1).

Level	Concentration % <sub>w/w</sub>			
	Alginate	Celquat <sup>®</sup>	Aquasorb <sup>®</sup>	Natrosol <sup>®</sup>
-1	4	3	1	1
1	8	6	3	3

4.4.2.2 RHEOLOGICAL EVALUATION

The levels of the factors to be investigated are given in Table 4.2. The methods for gel manufacture and rheological evaluation can be found in sections 2.1 and 2.3 respectively.

Table 4.2 The factors and factor levels for the rheological evaluation.

Level	Concentration % <sub>w/w</sub>				
	Alginate	Calcium	Celquat <sup>®</sup>	Natrosol <sup>®</sup>	Aquasorb <sup>®</sup>
-1	4	0	3	1	1
1	8	0.15	6	3	3

The elastic nature of the formulations was assessed using creep profiles. The percentage of structure recovery was calculated using Equation 4.1:

$$\% \text{ structure recovered} = (\text{max strain} - \text{recovered strain}) / \text{max strain} \times 100\%$$

(Equation 4.1)

The closer % structure recovered was to 100% (Equation 4.1) the more elastic the formulation. The rate index (shear thinning ability) was calculated using the Cross model (section 2.2.1.3).



4.4.3. The Formulation Of Sodium Alginate And Calcium Chloride

4.4.3.1 EXPERIMENTAL DESIGN

The effect of the presence of calcium on the uptake capacity of sodium alginate was investigated, using the factorial experimental design in Table 4.3. The rheological evaluation was carried out with the factorial experimental design shown in Table 4.4.

Table 4.3 Water uptake: factorial experimental design domain for sodium alginate and calcium.

Experiment		1	2	3	4
Level	Alginate	-1	1	-1	1
	Calcium	-1	-1	1	1

Table 4.4 Rheological evaluation: factorial experimental design for sodium alginate and calcium.

Experiment		1	2	3	4
Level	Alginate	-1	1	-1	1
	Calcium	-1	-1	1	1

4.4.3.2 RESULTS

4.4.3.2.a WATER UPTAKE

The water uptake experiment investigated the effects of increasing alginate concentration and adding calcium, on the ability to absorb water. The results of the factorial experimental design (Table 4.3) are tabulated (Table 4.5). These data were processed using the statistical program Minitab® to calculate the main effects and interactions of factors.

Table 4.5 Results for the sodium alginate and calcium chloride water uptake experiments ( $\bar{X}$ , n = 6 ( $\pm$  standard deviation)).

Experiment	1	2	3	4
Water uptake g/g/cm <sup>2</sup>	0.028 ( $\pm 0.003$ )	0.043 ( $\pm 0.002$ )	0.030 ( $\pm 0.002$ )	0.042 ( $\pm 0.003$ )

The main effect of increasing alginate concentration (Figure 4.4) was to increase the absorptive capacity. The addition of calcium ions had a small positive effect compared to the effect of alginate (Figure 4.4). The parallel profiles of Figure 4.5 suggest that no interactions exist between the factors with regard to water uptake.



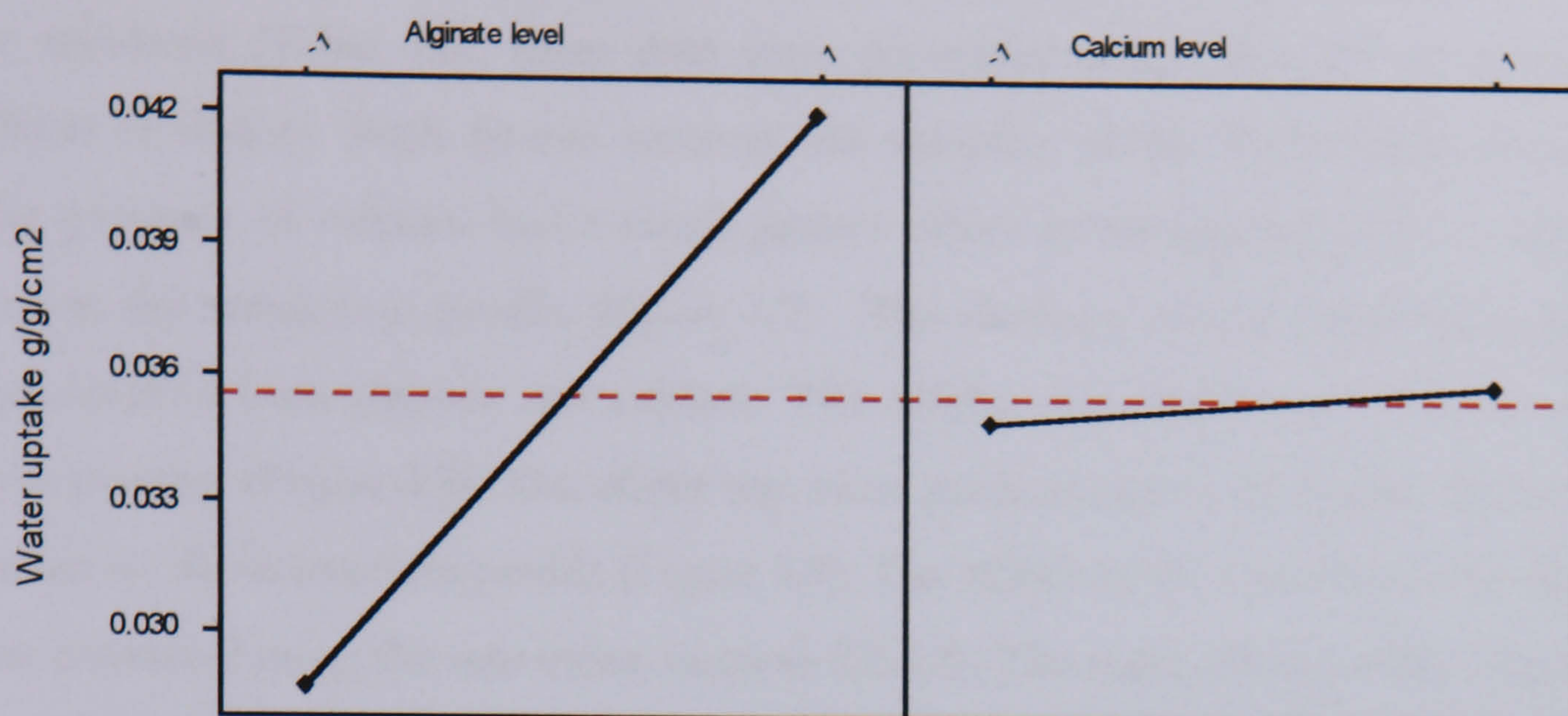


Figure 4.4 The main effects of sodium alginate and calcium concentration on water uptake.

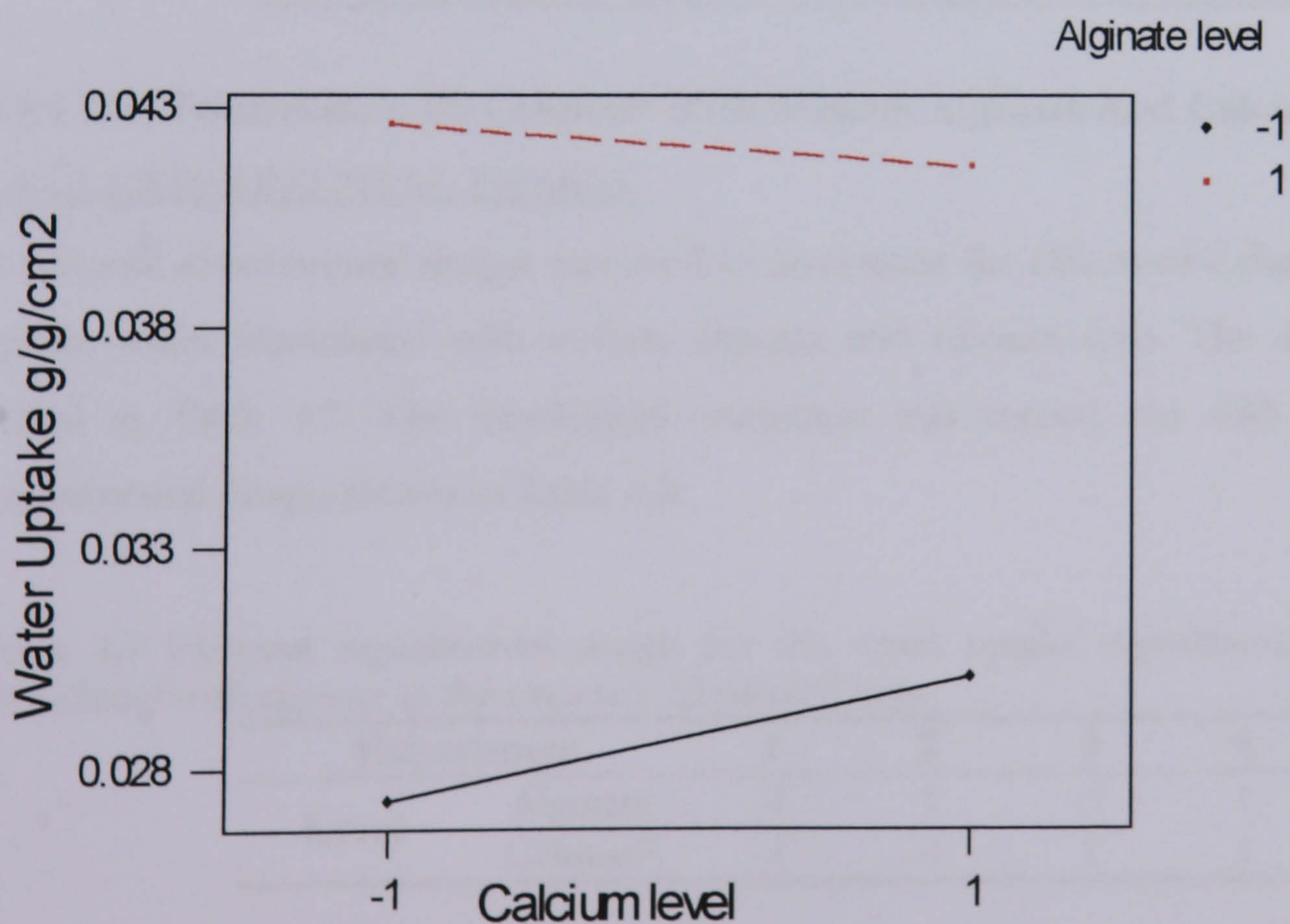


Figure 4.5 The interaction profile of alginate and calcium on water uptake.



4.4.3.2.b RHEOLOGICAL EVALUATION

The rheological evaluation estimated the effects of alginate and calcium concentration on the viscosity (flow and rate index) and elastic properties (creep recovery). The results are tabulated (Table 4.6), these data were processed using Minitab® to calculate the effects of factors. Both factors increase the viscosity of the formulation (Figure 4.6). The presence of calcium had a much greater effect at the highest level of alginate, as seen in the interaction profile (Figure 4.7). The elasticity of the formulation increased with level of both alginate and calcium. The effects of both factors on creep recovery were positive (Figure 4.8). The effect was more pronounced at the higher factor level as shown by the interaction profile (Figure 4.9). The ability of the formulation to shear thin was estimated using the rate index (section 2.2.1.3). The main effect profile (Figure 4.10) shows that alginate slightly increases the rate index whereas, calcium reduces this ability. There were no interactions between factors for rate index(Figure 4.11).

Table 4.6 Results for the rheological evaluation of sodium alginate and calcium (n = 1).

Experiment	1	2	3	4
Viscosity (Flow)/Pa.s	0.38	6.07	1.39	123
% Structure Recovered	-0.49	-2.16	-1.73	36.26
Rate Index	0.82	0.92	0.27	0.44

4.4.4 The Formulation Of Celquat® With Sodium Alginate And Calcium Chloride

4.4.4.1 EXPERIMENTAL DESIGN

A factorial experimental design was used to investigate the effects of Celquat® on water uptake when formulated with sodium alginate and calcium ions. The design can be found in Table 4.7. The rheological evaluation was carried out with the factorial experimental design shown in Table 4.8.

Table 4.7 Factorial experimental design for the water uptake experiments of Celquat® formulated with alginate in the presence of calcium ions.

Experiment		1	2	3	4
Level	Alginate	-1	1	-1	1
	Celquat®	-1	-1	1	1



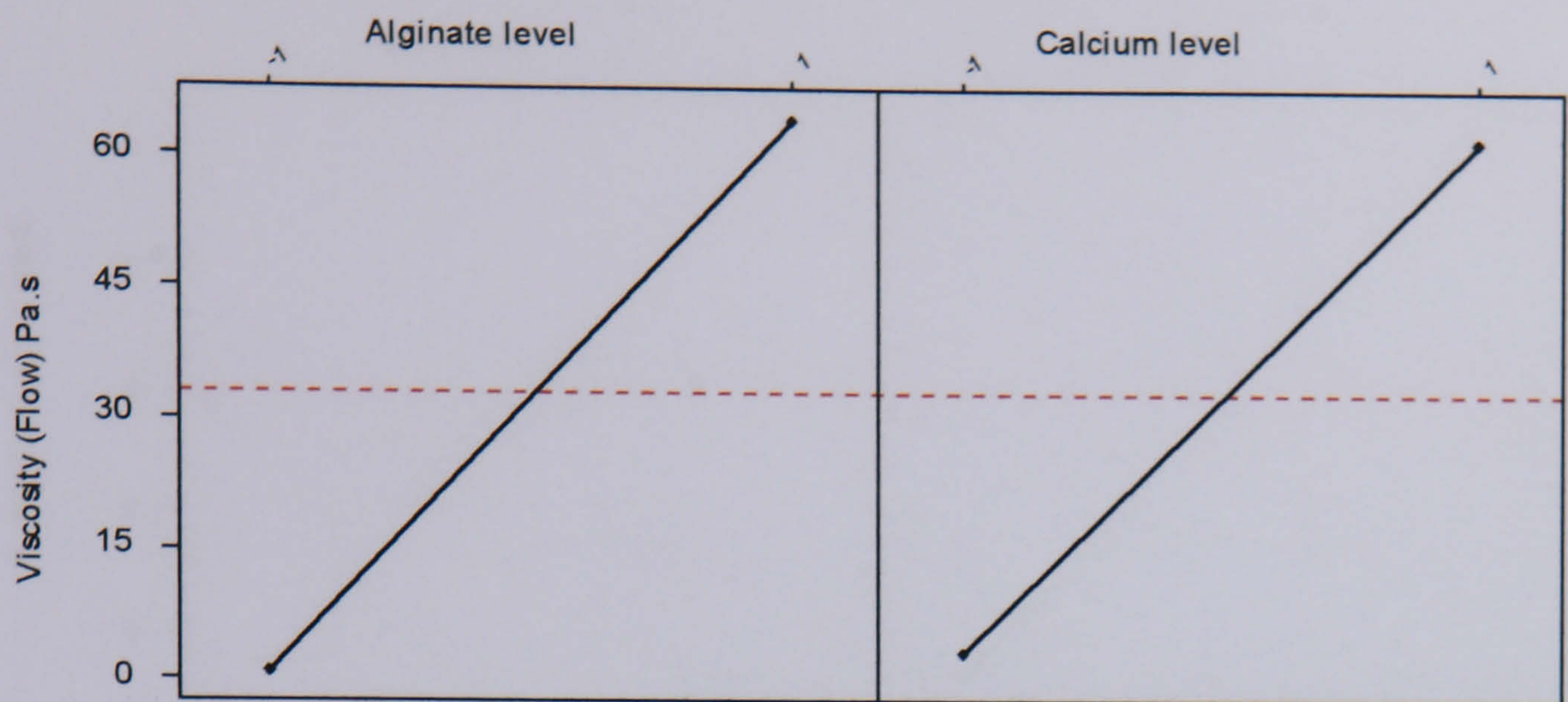


Figure 4.6 The main effects of sodium alginate and calcium concentration on viscosity.

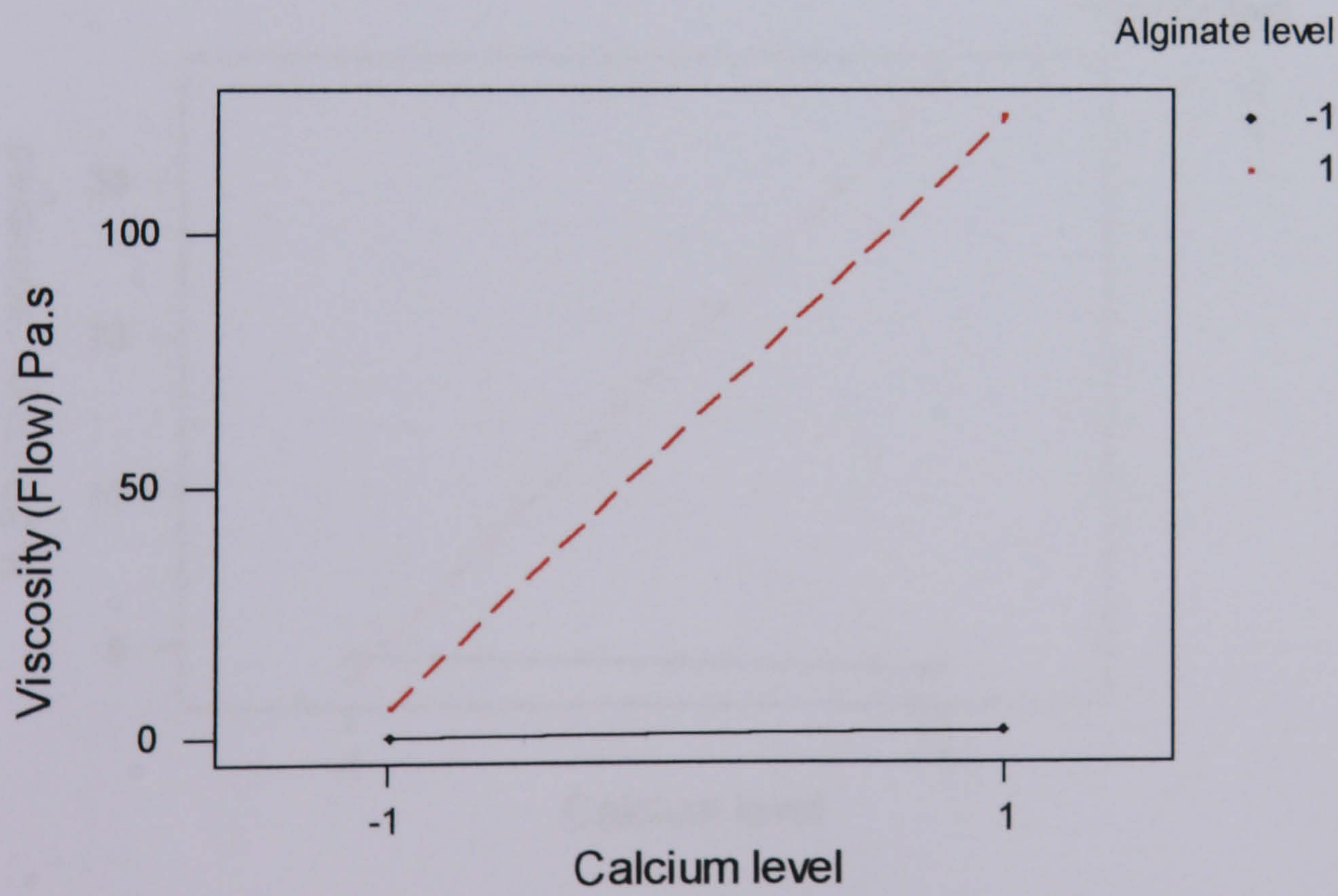


Figure 4.7 The interactions of sodium alginate and calcium concentration on viscosity.



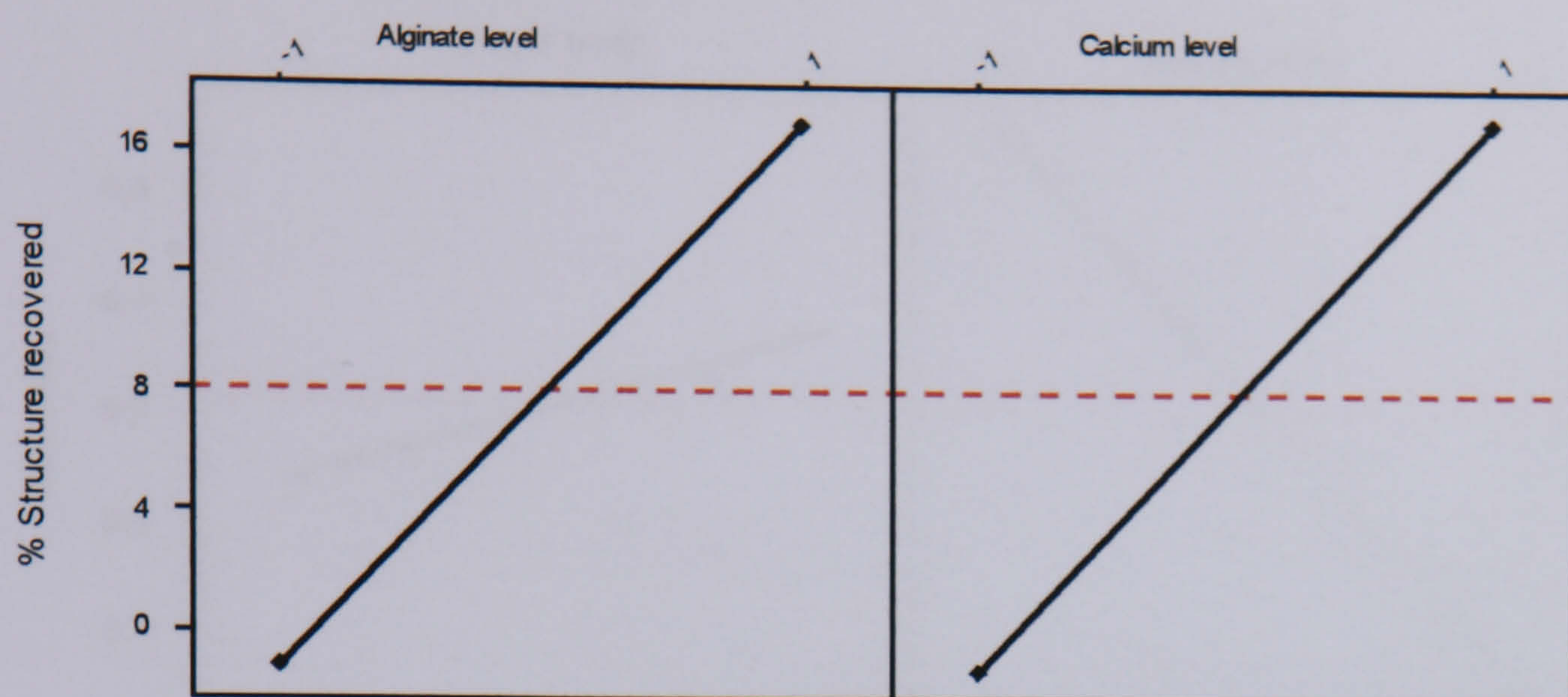


Figure 4.8 The main effects of sodium alginate and calcium concentration on creep recovery.

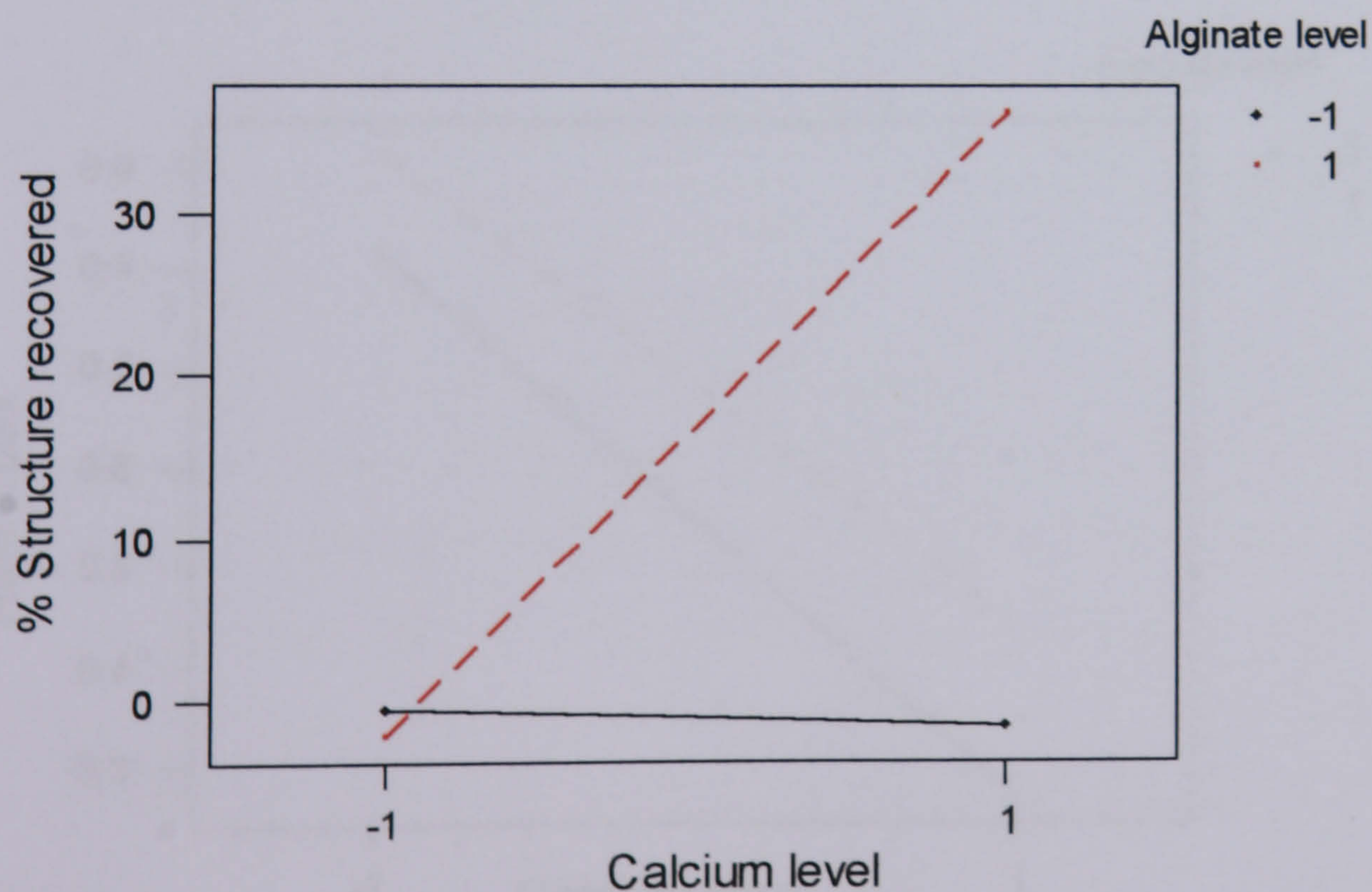


Figure 4.9 The interactions of sodium alginate and calcium concentration on creep recovery.



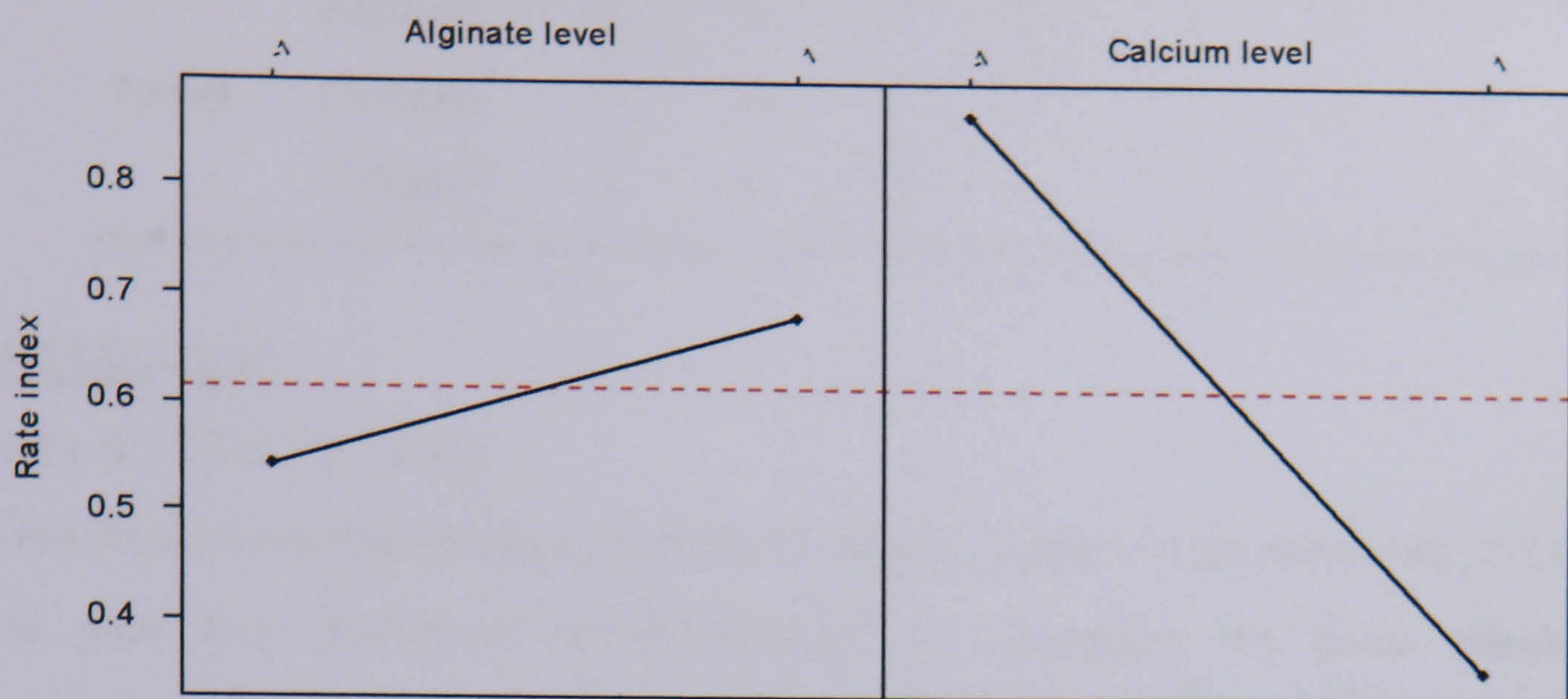


Figure 4.10 The main effects of sodium alginate and calcium concentration on rate index.

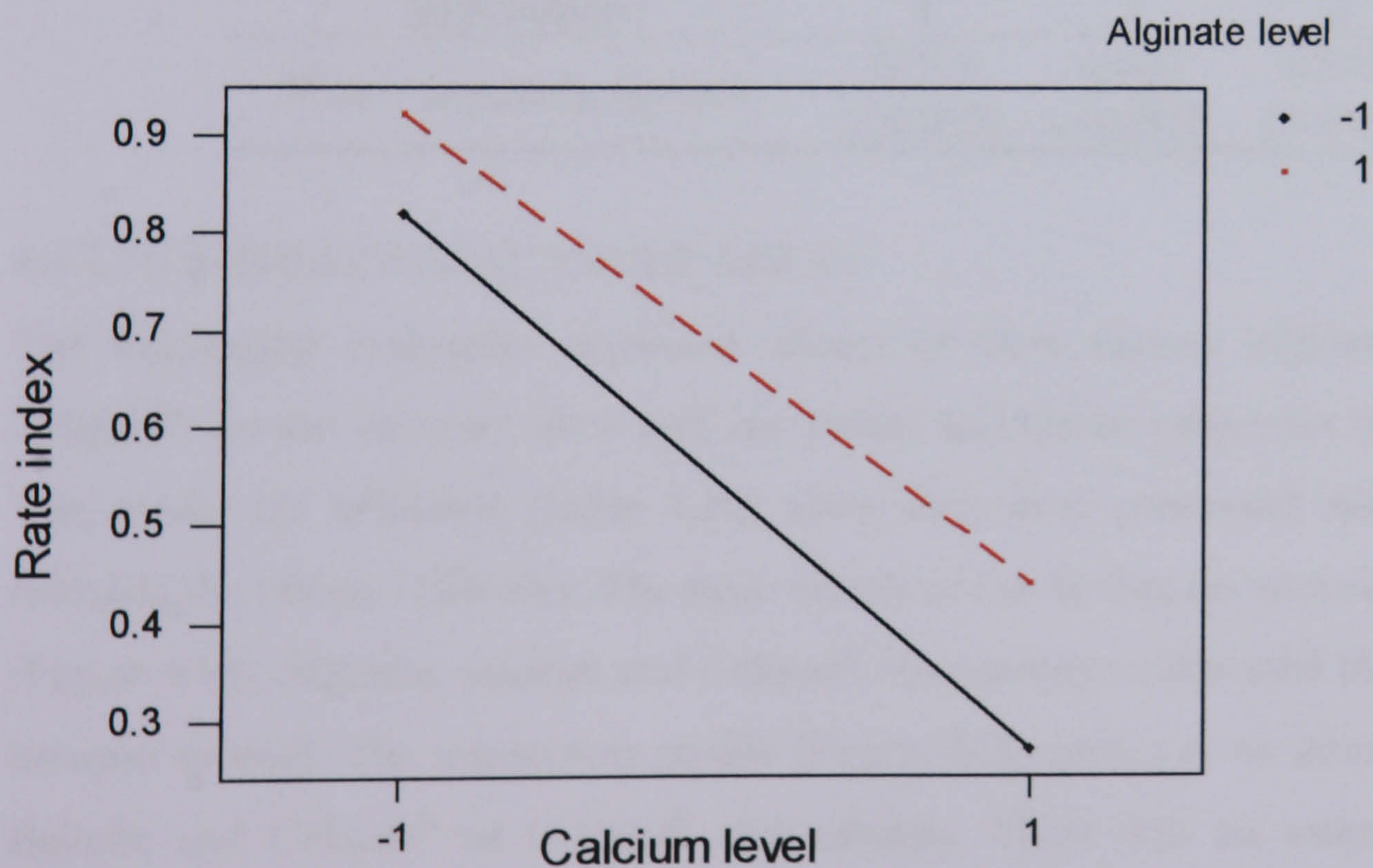


Figure 4.11 The interactions of sodium alginate and calcium concentration on rate index.



Table 4.8 Factorial experimental design for the rheological evaluation of Celquat<sup>®</sup> formulated with alginate and calcium.

Experiment	1	2	3	4	5	6	7	8
Alginate	-1	-1	-1	-1	1	1	1	1
Level Calcium	-1	-1	1	1	-1	-1	1	1
Celquat <sup>®</sup>	-1	1	-1	1	-1	1	-1	1

4.4.4.2 RESULTS

4.4.4.2.a WATER UPTAKE

The results of the water uptake experimental design (Table 4.7) are tabulated (Table 4.9). These data were processed using Minitab<sup>®</sup> to determine the main effects and interactions of factors; alginate, calcium and Celquat<sup>®</sup>. The effects of alginate concentration on water uptake were positive (Figure 4.12), increasing absorptive capacity with increasing level. By contrast Celquat<sup>®</sup> had a negative effect reducing absorptive capacity with increasing level. There were no interactions between factors (Figure 4.13), neither factor having a more significant effect in the presence of the other.

Table 4.9 Water uptake results for the Celquat<sup>®</sup>, alginate and calcium formulation ( $\bar{x}$ , n = 6 ( $\pm$  standard deviation)).

Experiment	1	2	3	4
Water uptake g/g/cm <sup>2</sup>	0.013 ( $\pm 0.013$ )	0.030 ( $\pm 0.002$ )	0.004 ( $\pm 0.001$ )	0.021 ( $\pm 0.002$ )

4.4.4.2.b RHEOLOGICAL EVALUATION

The rheological evaluation estimated effects of three factors (alginate, calcium and Celquat<sup>®</sup>) on the viscosity (flow and rate index) and elastic properties (creep recovery). The results are tabulated (Table 4.10), these data were processed using Minitab<sup>®</sup> to calculate the effects of factors. The main effects of the factors on viscosity were positive (Figure 4.14). Alginate, calcium and Celquat<sup>®</sup> concentration increased the viscosity with increase in level. The interaction profile (Figure 4.15) indicates no interaction between alginate and Celquat<sup>®</sup> or Celquat<sup>®</sup> and calcium. There was an interaction between alginate and calcium; calcium increased the viscosity more at higher levels of alginate. The elasticity of the formulation appeared to be unaffected by the increase in level of alginate or Celquat<sup>®</sup> (Figure 4.16). Calcium, by contrast had a very positive effect, increasing elasticity with an increase in level. The interaction profiles (Figure 4.17) suggest that an interaction does exist between alginate and calcium. Calcium increased



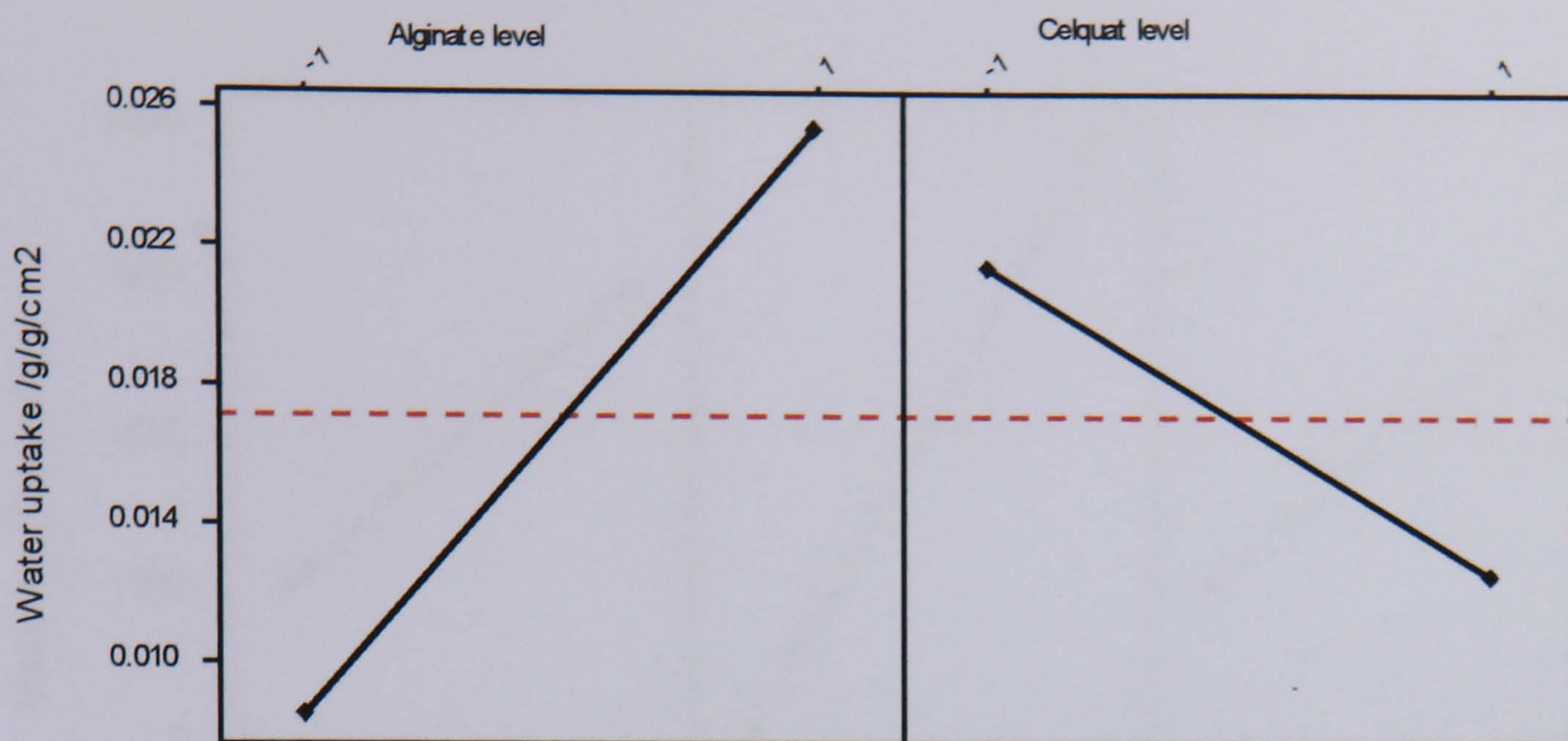


Figure 4.12 The main effects of factors sodium alginate and Celquat<sup>®</sup> on water uptake.

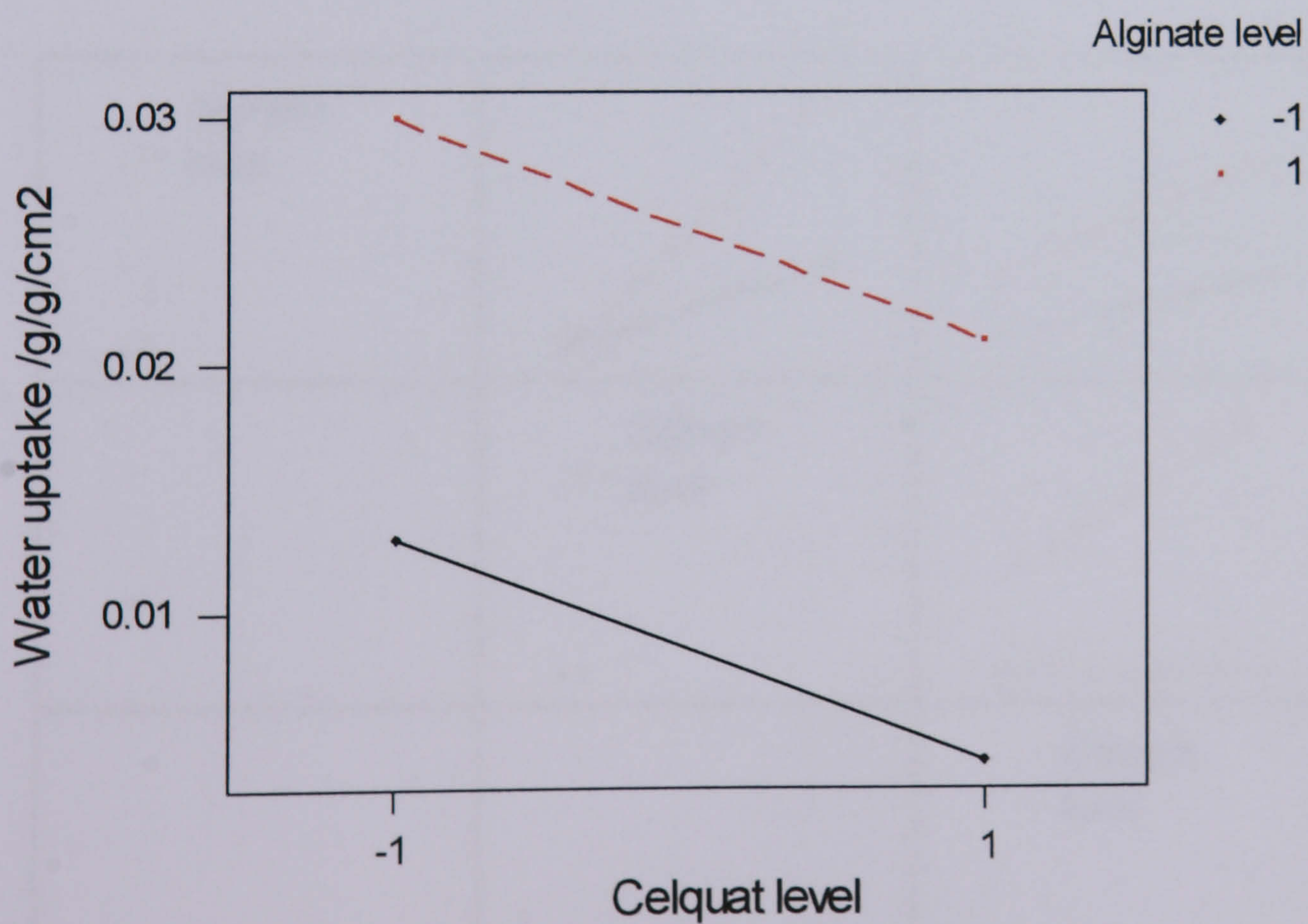


Figure 4.13 The interactions profile of factors Celquat<sup>®</sup> and alginate on water uptake.



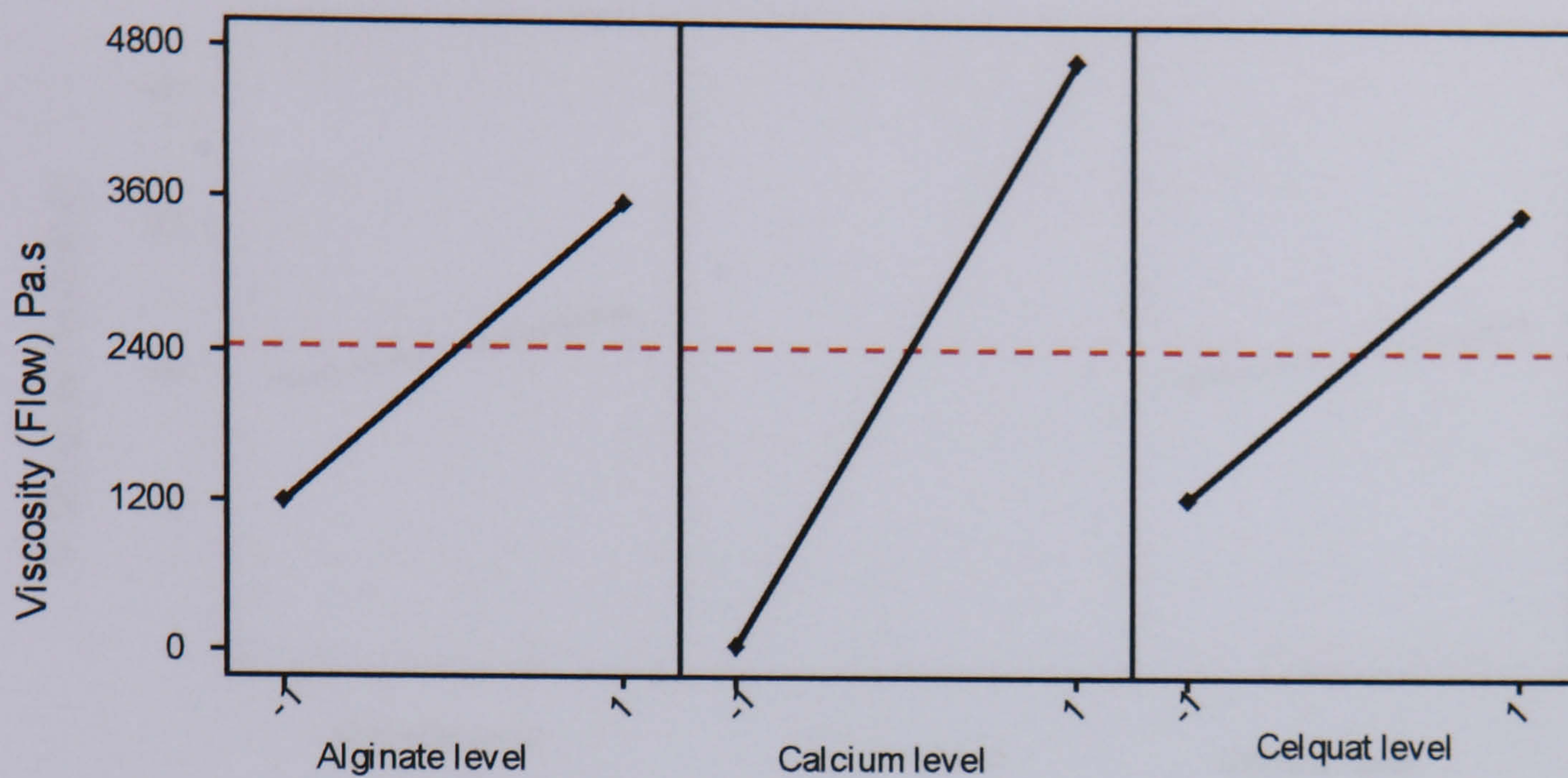


Figure 4.14 The main effects of factors Celquat<sup>®</sup>, alginate and calcium on viscosity.

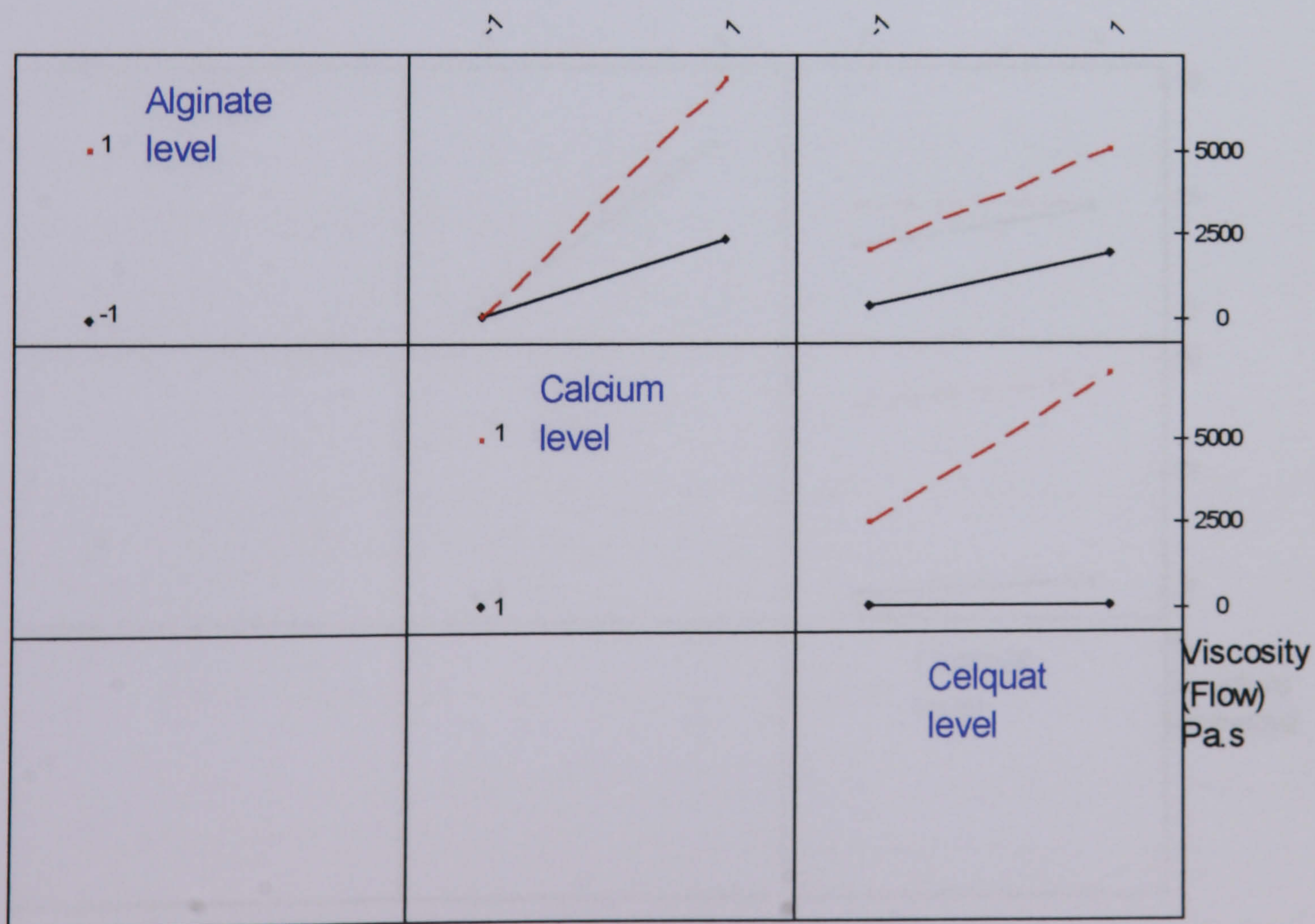


Figure 4.15 The interactions profile of factors Celquat<sup>®</sup>, alginate and calcium on viscosity.



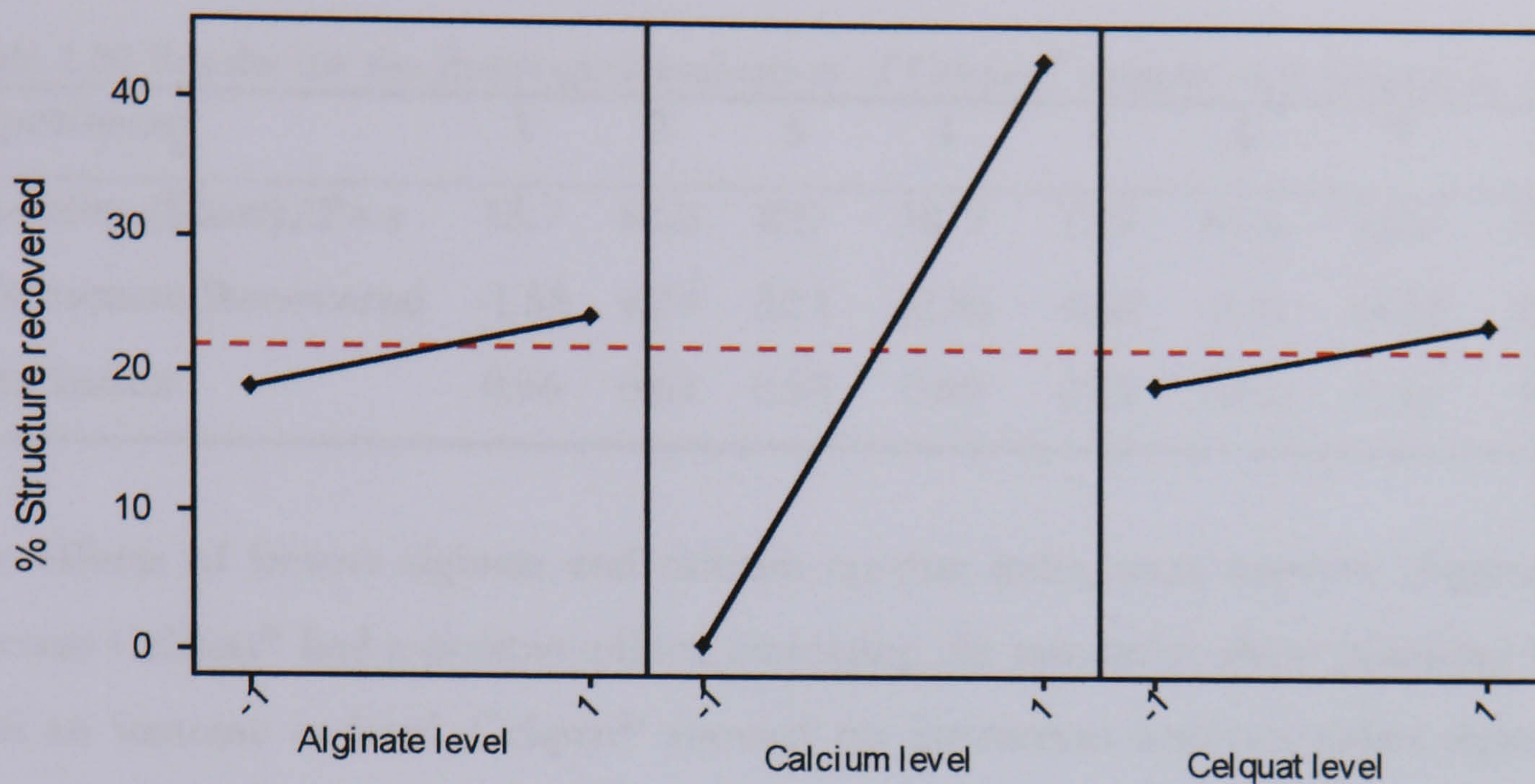


Figure 4.16 The main effects of factors Celquat<sup>®</sup>, alginate and calcium on % structure recovered.

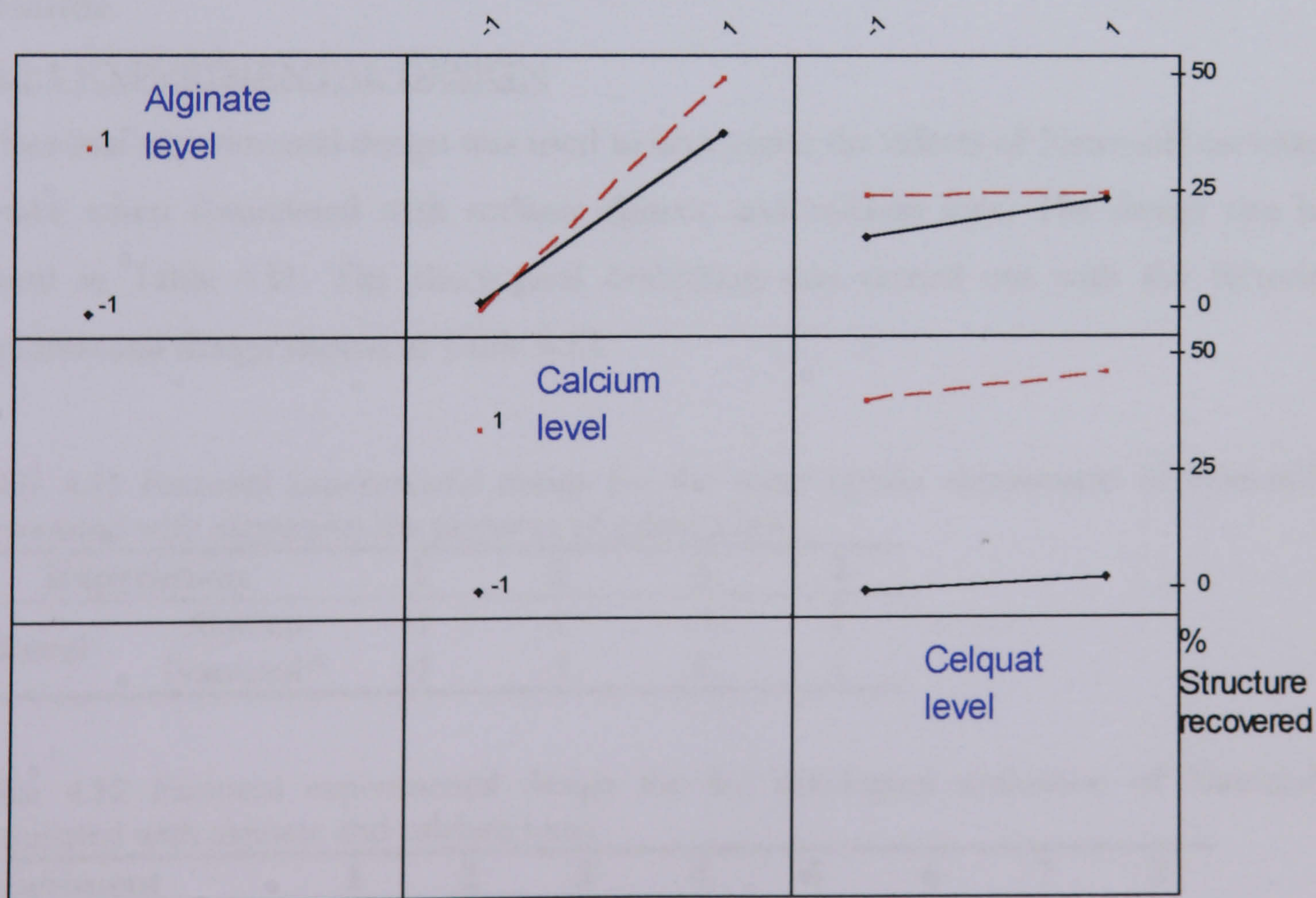


Figure 4.17 The interactions profile of factors Celquat<sup>®</sup>, alginate and calcium on % structure recovered.



elasticity more at higher levels of alginate. There is only a slight interaction between alginate and Celquat®, Celquat® having a more significant effect on elasticity at lower levels of alginate. No interaction was evident between calcium and Celquat®.

Table 4.10 Results for the rheological evaluation of Celquat<sup>®</sup> alginate and calcium (n = 1).

Experiment	1	2	3	4	5	6	7	8
Viscosity (Flow)/Pa.s	13.7	88.0	827	3877	15.9	67.4	4187	10190
% Structure Recovered	-1.58	4.04	32.1	42.81	-0.62	-0.31	48.53	49.89
Rate Index	0.56	0.61	0.53	0.60	0.55	0.62	0.50	0.56

The effects of factors alginate and calcium on rate index were negative (Figure 4.18) whereas Celquat® had a positive effect, increasing the rate index shear (thinning ability) with an increase in level. Celquat® showed no interaction between either alginate or calcium (Figure 4.19). Alginate and calcium appear to have a slight interaction, where at higher levels of alginate the presence of calcium has a greater effect reducing the shear thinning ability of the formulation.

4.4.5 The Formulation Of Natrosol® With Sodium Alginate And Calcium Chloride

4.4.5.1 EXPERIMENTAL DESIGN

A factorial experimental design was used to investigate the effects of Natrosol® on water uptake when formulated with sodium alginate and calcium ions. The design can be found in Table 4.11. The rheological evaluation was carried out with the factorial experimental design shown in Table 4.12.

Table 4.11 Factorial experimental design for the water uptake experiments of Natrosol<sup>®</sup> formulated with alginate in the presence of calcium ions.

Experiment	1	2	3	4
Level				
Alginate	-1	1	-1	1
Natrosol <sup>®</sup>	-1	-1	1	1

Table 4.12 Factorial experimental design for the rheological evaluation of Natrosol<sup>®</sup> formulated with alginate and calcium ions.

Experiment	1	2	3	4	5	6	7	8
Level								
Alginate	-1	-1	-1	-1	1	1	1	1
Calcium	-1	-1	1	1	-1	-1	1	1
Natrosol <sup>®</sup>	-1	1	-1	1	-1	1	-1	1



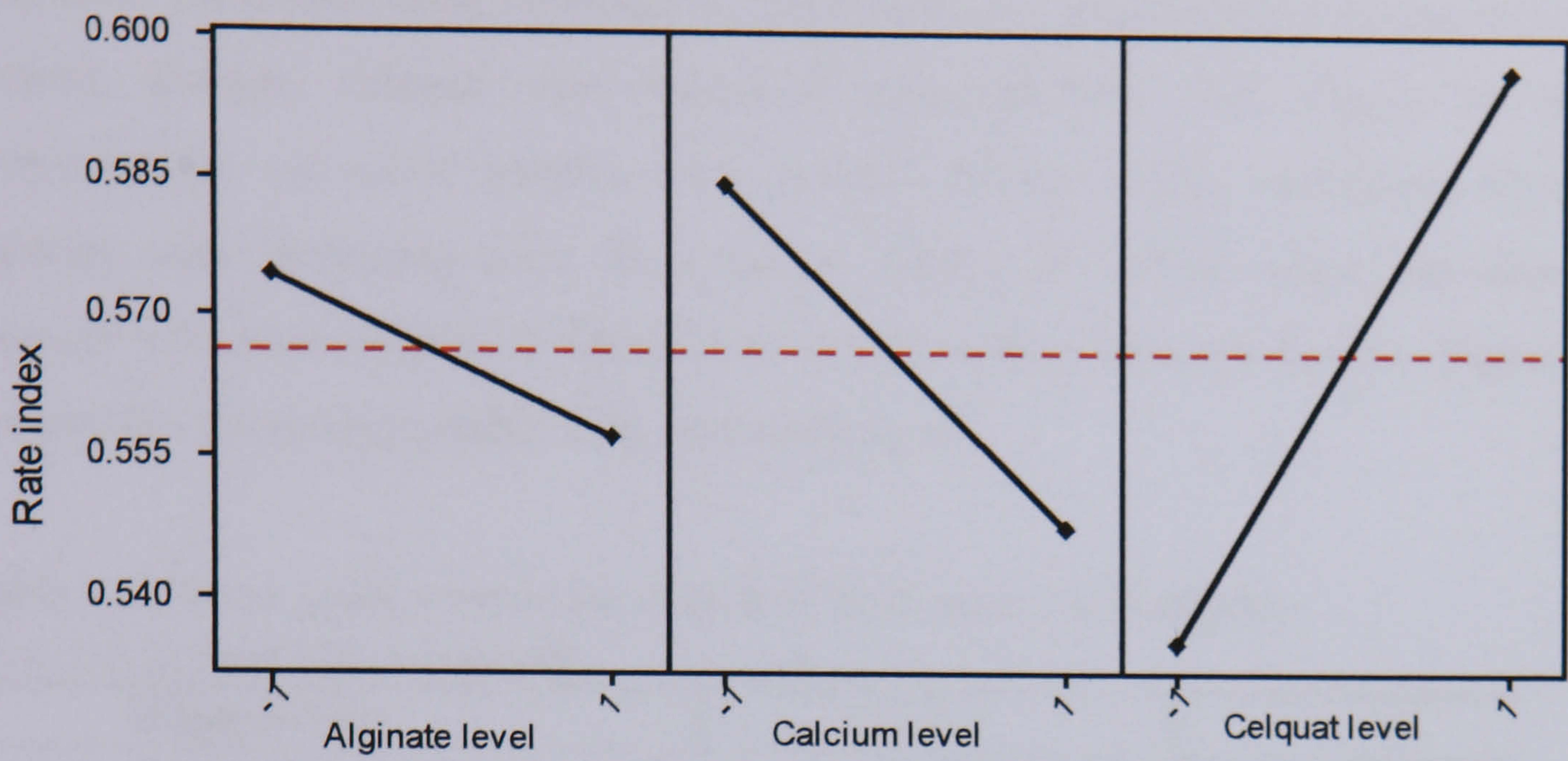


Figure 4.18 The main effects of factors Celquat®, alginate and calcium on rate index.

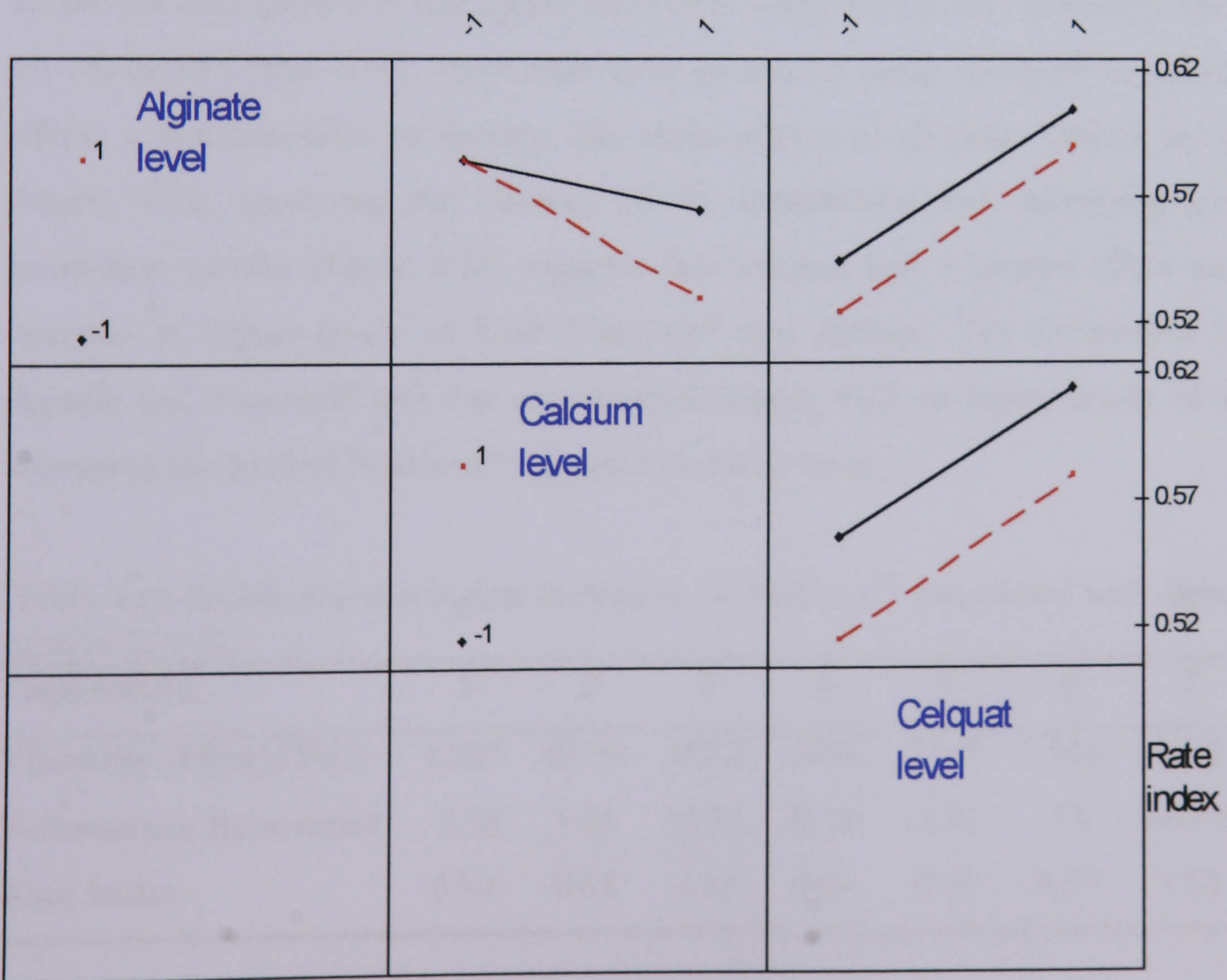


Figure 4.19 The interactions profile of factors Celquat®, alginate and calcium on rate index.



4.4.5.2 RESULTS

4.4.5.2.a WATER UPTAKE

The results of the experimental design (Table 4.11) are tabulated (Table 4.13). These data were processed using Minitab® to determine the main effects and interactions of factors; alginate, calcium and Natrosol® concentration. The effects of alginate concentration on water uptake were positive (Figure 4.20), increasing absorptive capacity with increasing level. By contrast, Natrosol® had no effect on absorptive capacity with increasing level. There were no interactions between factors (Figure 4.21), the profiles remaining parallel with increasing level.

Table 4.13 Water uptake results for Natrosol® formulated with alginate ( $\bar{X}$ , n = 6 ( $\pm$  standard deviation)).

Experiment	1	2	3	4
Water Uptake g/g/cm <sup>2</sup>	0.026 ( $\pm 0.002$ )	0.040 ( $\pm 0.002$ )	0.026 ( $\pm 0.003$ )	0.040 ( $\pm 0.003$ )

4.4.5.2.b RHEOLOGICAL EVALUATION

The rheological evaluation estimated the effects of alginate and calcium concentration on the viscosity (flow and rate index) and elastic properties (creep recovery). The results are tabulated (Table 4.14), these data were processed using Minitab® to calculate the effects and interactions of factors. The main effects of all three factors are positive (Figure 4.22), increasing the viscosity of the formulation with increasing level. The interaction profile (Figure 4.23) suggests that calcium had a greater effect increasing viscosity at higher levels of both Natrosol® and alginate. The interaction between alginate and Natrosol® was less pronounced (Figure 4.23) at higher levels of alginate, increasing the level of Natrosol® increased viscosity more.

Table 4.14 Results for rheological evaluation of Natrosol® formulated with alginate and calcium (n = 1).

Experiment	1	2	3	4	5	6	7	8
Viscosity (Flow)/Pa.s	12.93	83.34	481.2	1164	29.43	153.6	1502	5186
% Structure Recovered	-2.38	1.01	15.73	31.10	-3.16	7.5	60.62	32.65
Rate Index	0.50	0.65	0.53	0.61	0.45	0.57	0.53	0.61



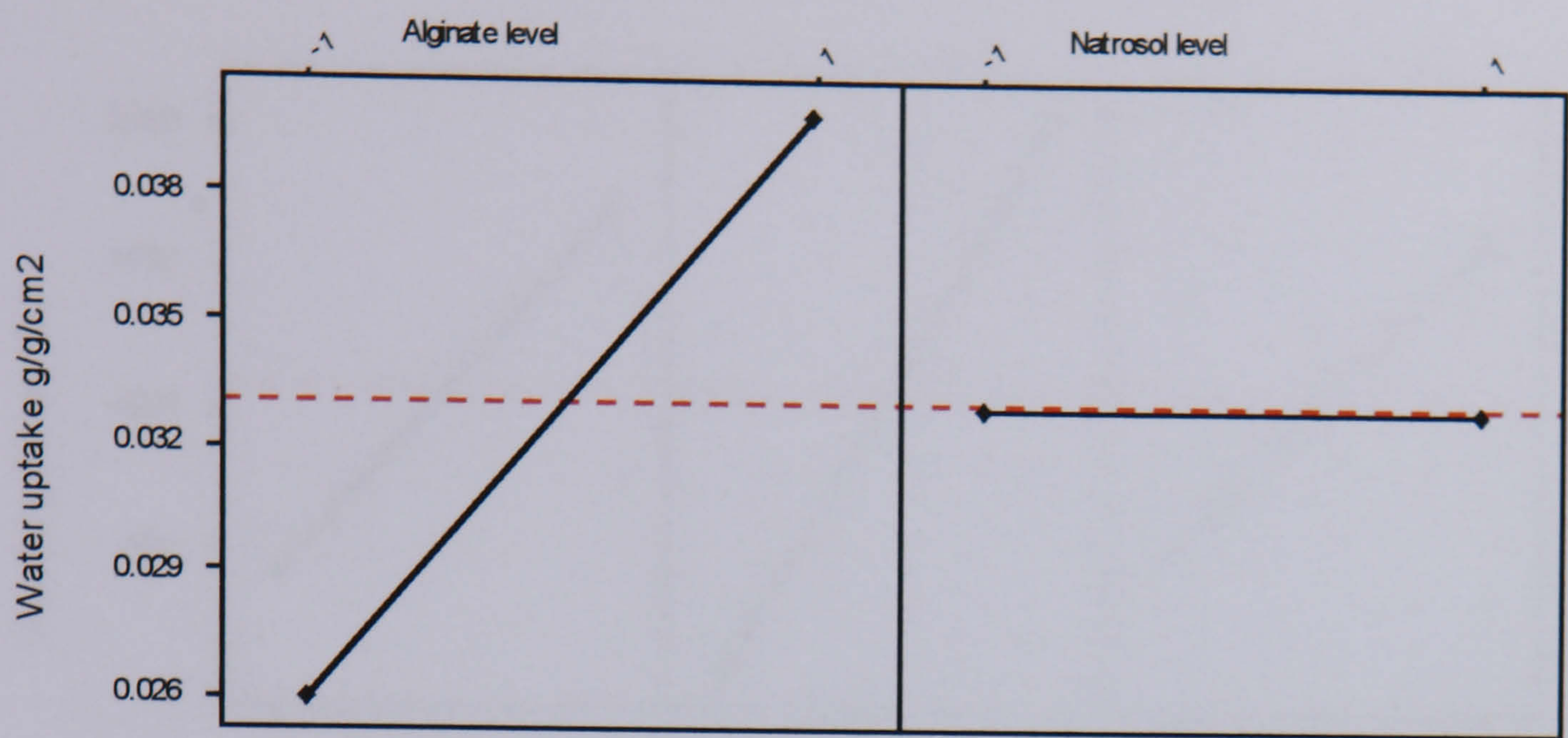


Figure 4.20 The main effects profile of factors alginate and Natrosol<sup>®</sup> on water uptake.

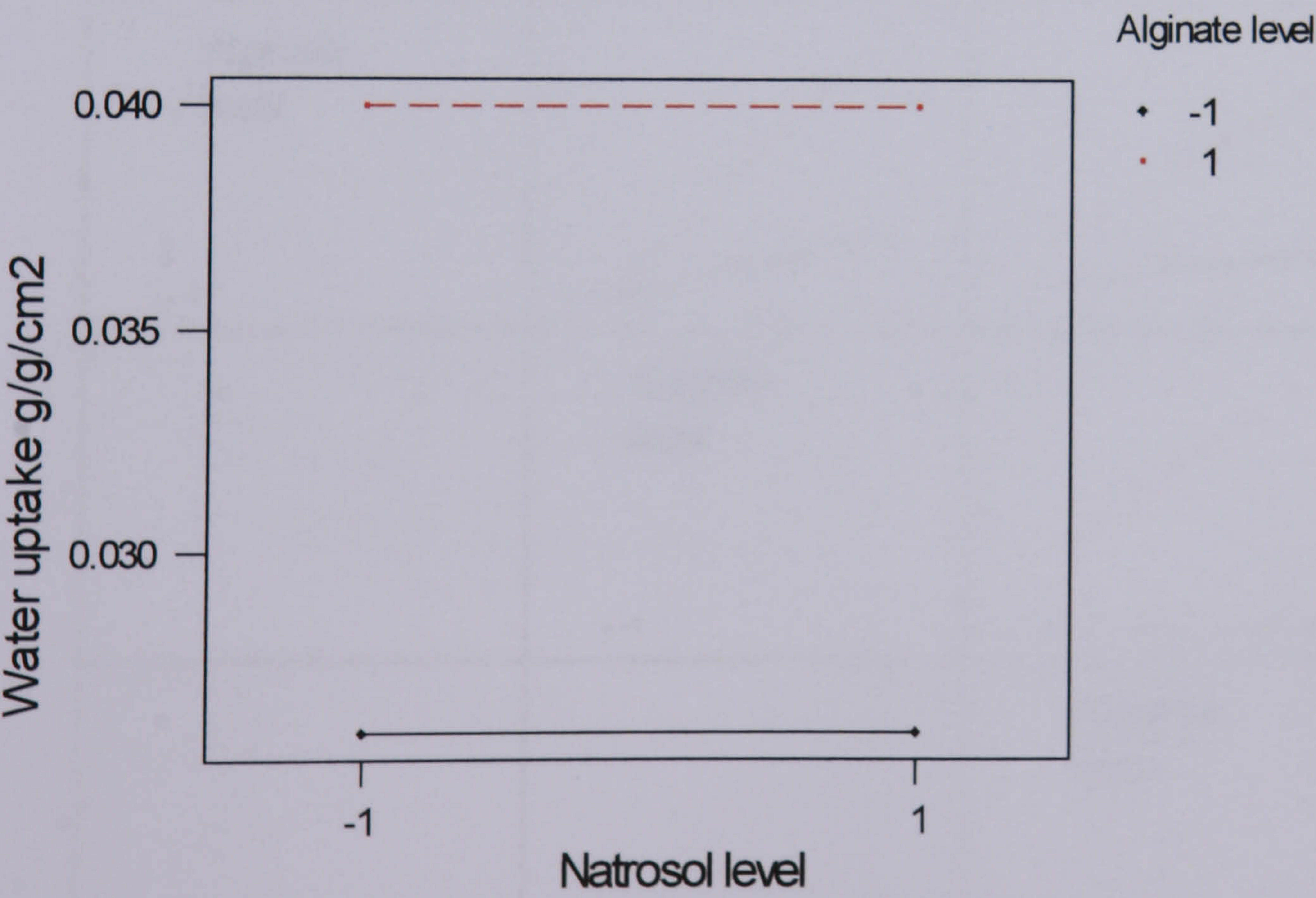


Figure 4.21 The interactions profile of factors alginate and Natrosol<sup>®</sup> on water uptake.



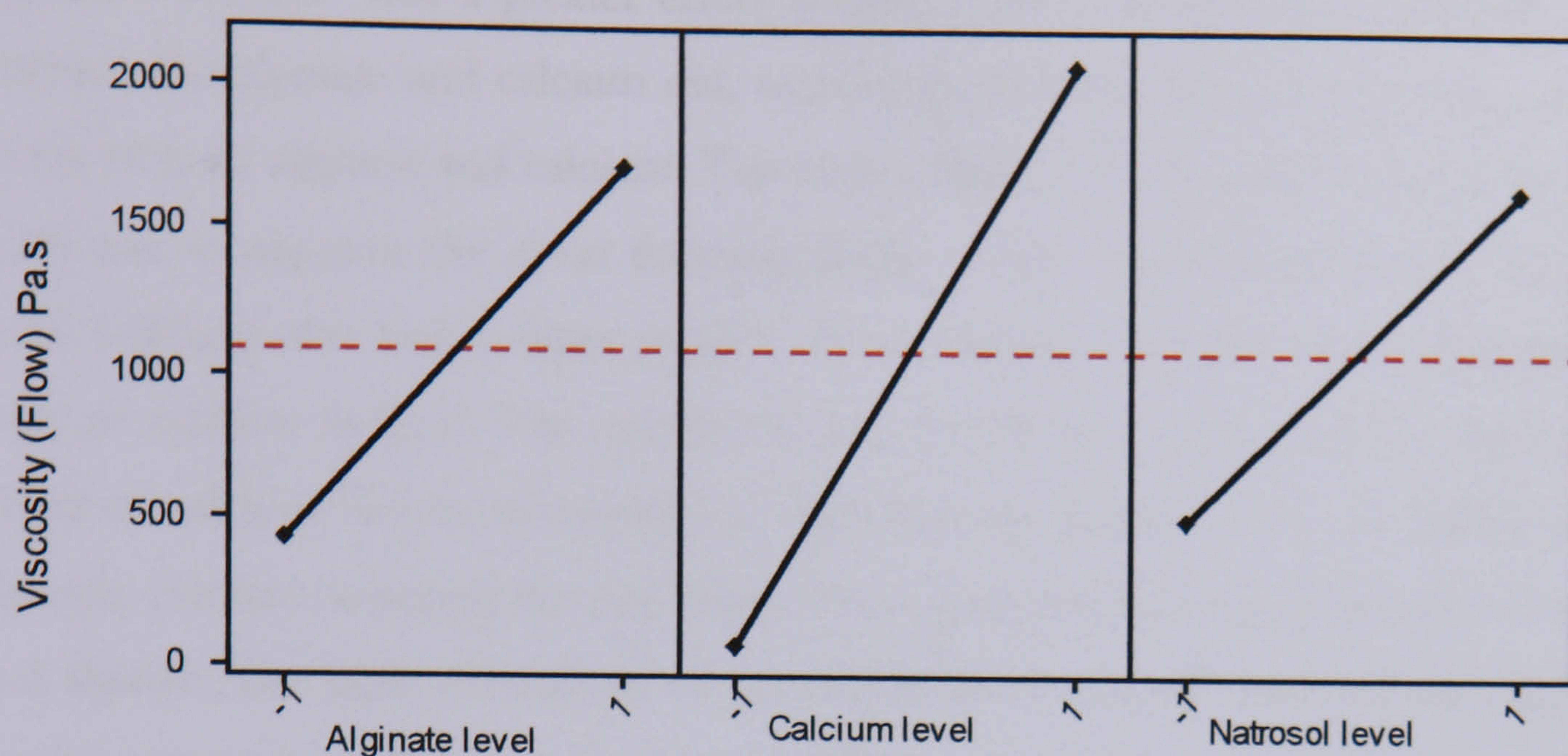


Figure 4.22 The main effects profile of factors Natrosol<sup>®</sup>, alginate and calcium on viscosity.

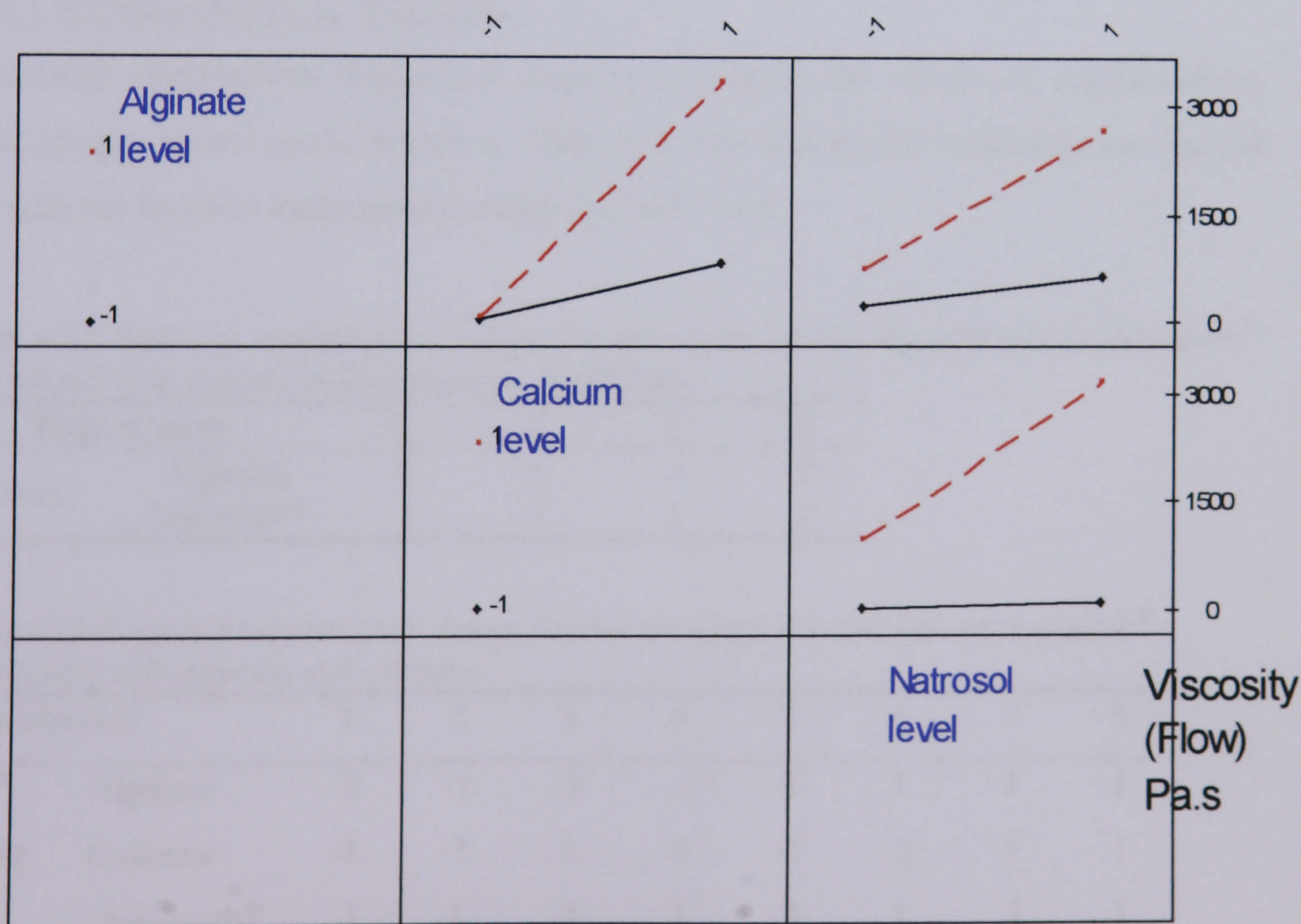


Figure 4.23 The interactions profile of factors Natrosol<sup>®</sup>, alginate and calcium on viscosity.



The presence of Natrosol® had little effect on the elastic nature of the formulation (Figure 4.24), but both alginate and calcium increased elasticity with increasing level. There was a slight interaction between alginate and Natrosol®, where, at higher levels of alginate Natrosol® had a greater effect (Figure 4.25). Calcium and Natrosol® did not interact, but alginate and calcium did, increasing elasticity to a greater extent at higher levels of both alginate and calcium. The main effect of Natrosol® on rate index (Figure 4.26) was to improve the shear thinning ability of the formulation with an increase in level. Calcium also had a slight positive effect, whereas alginate had a negative effect with an increase in level. The interaction profile provides evidence of a slight positive effect of calcium due to an interaction with alginate (Figure 4.27). At higher levels of alginate, calcium increased the rate index. There were no interactions between Natrosol® and alginate, the slight divergence of the profile for Natrosol® and calcium suggests an interaction exists; at high levels of Natrosol® the rate index was much greater with less calcium present.

#### 4.4.6 The Formulation Of Aquasorb® With Sodium Alginate And Calcium Chloride

##### 4.4.6.1 EXPERIMENTAL DESIGN

A factorial experimental design was used to investigate the effects of Aquasorb® on water uptake which can be found in Table 4.15. The rheological evaluation was carried out with the factorial experimental design in Table 4.16.

Table 4.15 Factorial experimental design for the water uptake experiments of Aquasorb® formulated with alginate in the presence of calcium.

Experiment		1	2	3	4
Level	Alginate	-1	1	-1	1
	Aquasorb®	-1	-1	1	1

Table 4.16 Factorial experimental design for the rheological evaluation of Aquasorb® formulated with alginate and calcium.

Experiment		1	2	3	4	5	6	7	8
Level	Alginate	-1	-1	-1	-1	1	1	1	1
	Calcium	-1	-1	1	1	-1	-1	1	1
	Aquasorb <sup>®</sup>	-1	1	-1	1	-1	1	-1	1



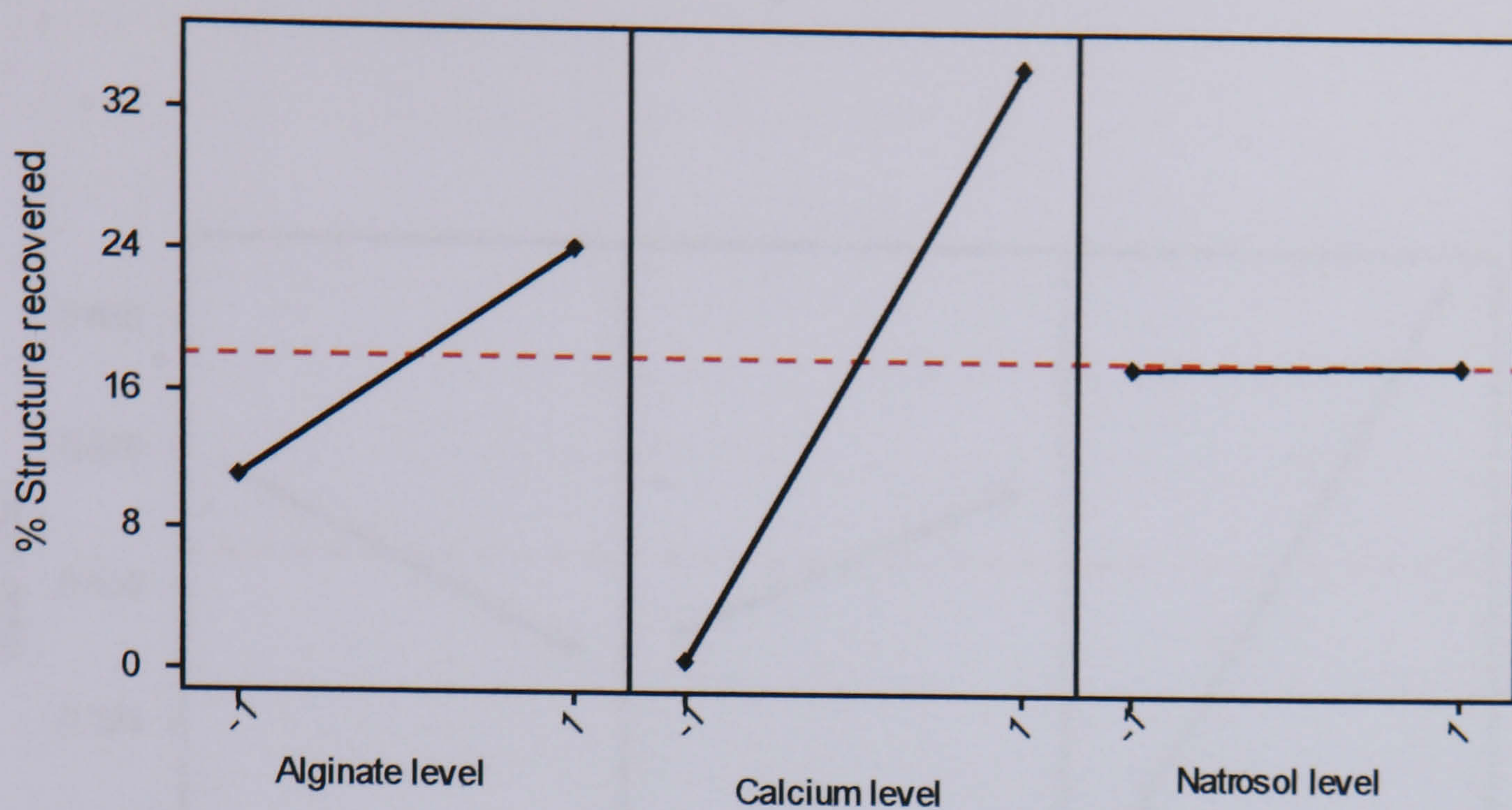


Figure 4.24 The main effects of factors Natrosol®, alginate and calcium on % structure recovered.

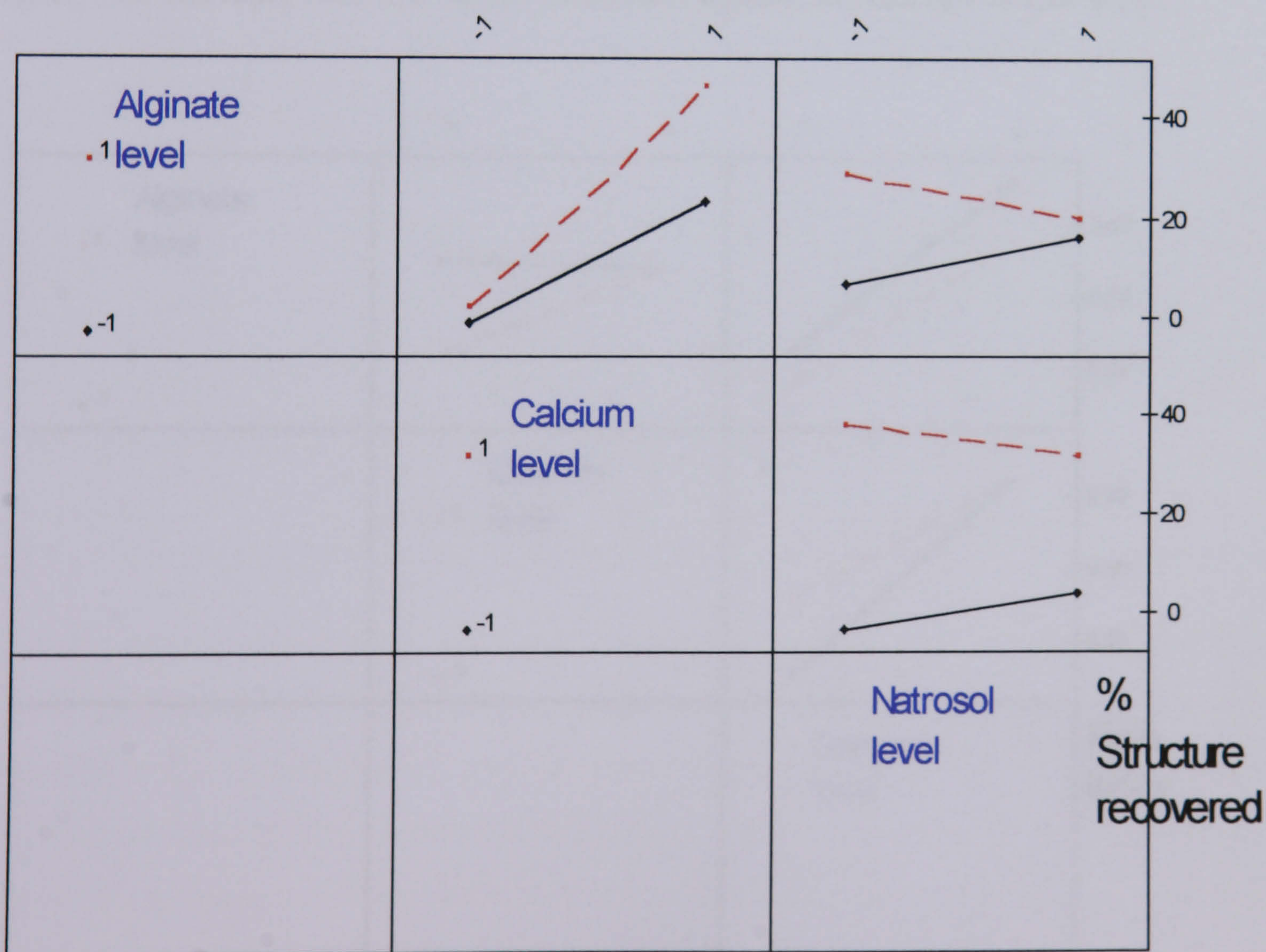


Figure 4.25 The interactions profile of factors Natrosol®, alginate and calcium on % structure recovered.







4.4.6.2 RESULTS

4.4.6.2.a WATER UPTAKE

The results of the experimental design (Table 4.15) are tabulated (Table 4.17). These data were processed using Minitab® to determine the main effects and interactions of factors; alginate, calcium and Aquasorb® concentration. The main effect of factors; alginate and Aquasorb®, was to increase the absorptive capacity with an increase in level (Figure 4.28). Interaction between factors did not occur with the interaction profile remaining parallel (Figure 4.29).

Table 4.17 Water uptake results for Aquasorb® formulated with alginate in the presence of calcium ( $\bar{x}$ , n = 6 ( $\pm$  standard deviation)).

Experiment	1	2	3	4
Water Uptake g/g/cm <sup>2</sup>	0.030 ( $\pm 0.002$ )	0.038 ( $\pm 0.003$ )	0.038 ( $\pm 0.004$ )	0.043 ( $\pm 0.003$ )

4.4.6.2.b RHEOLOGICAL EVALUATION

The rheological evaluation estimated the effects of alginate and calcium concentration on the viscosity (flow and rate index) and elastic properties (creep recovery). The results are tabulated (Table 4.18), these data were processed using Minitab® to calculate the effects and interactions of factors.

Table 4.18 Results for rheological evaluation of Aquasorb® formulated with alginate and calcium (n = 1).

Experiment	1	2	3	4	5	6	7	8
Viscosity (Flow)/Pa.s	4.6	396	320	6870	24.7	520	1828	18850
% Structure Recovered	-0.97	23.3	60.60	83.49	-1.18	15.37	64.91	70.0
Rate Index	0.54	0.38	0.50	0.67	0.34	0.31	0.54	0.76

All three factors had a positive effect on viscosity. The main effects profile (Figure 4.30) shows an increase in viscosity with an increase in level for alginate, calcium and Aquasorb®. There were also interactions between all three factors (Figure 4.31). Alginate and calcium interacted at a higher level of alginate, where increasing the level of calcium had a much greater effect, increasing viscosity. This effect was mirrored by the interaction of Aquasorb® and calcium, at higher levels of calcium Aquasorb® had a much greater effect on viscosity. Alginate and Aquasorb® interact, such that at higher levels of alginate, Aquasorb® had a much greater effect increasing viscosity.



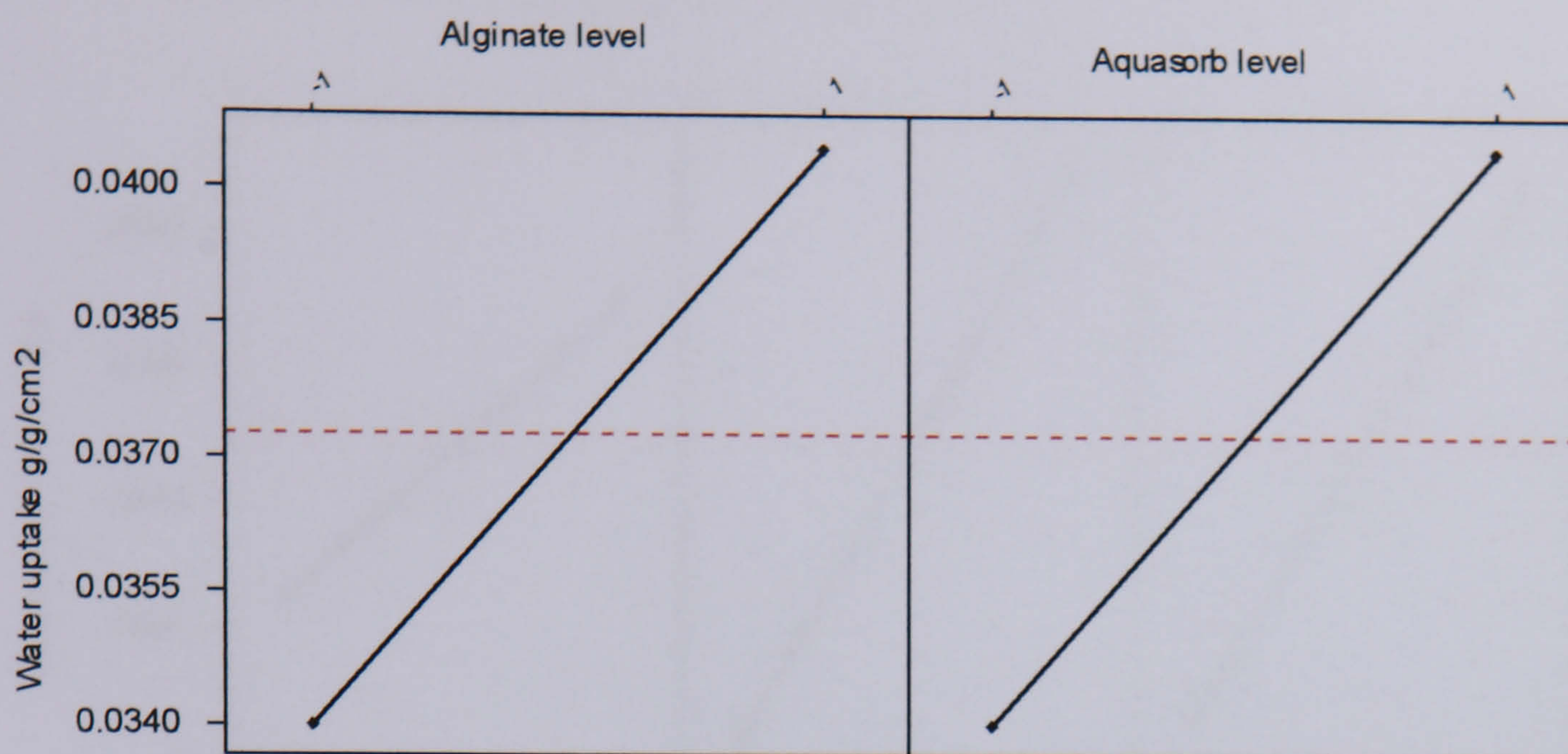


Figure 4.28 The main effects of factors alginate and Aquasorb<sup>®</sup> on water uptake.

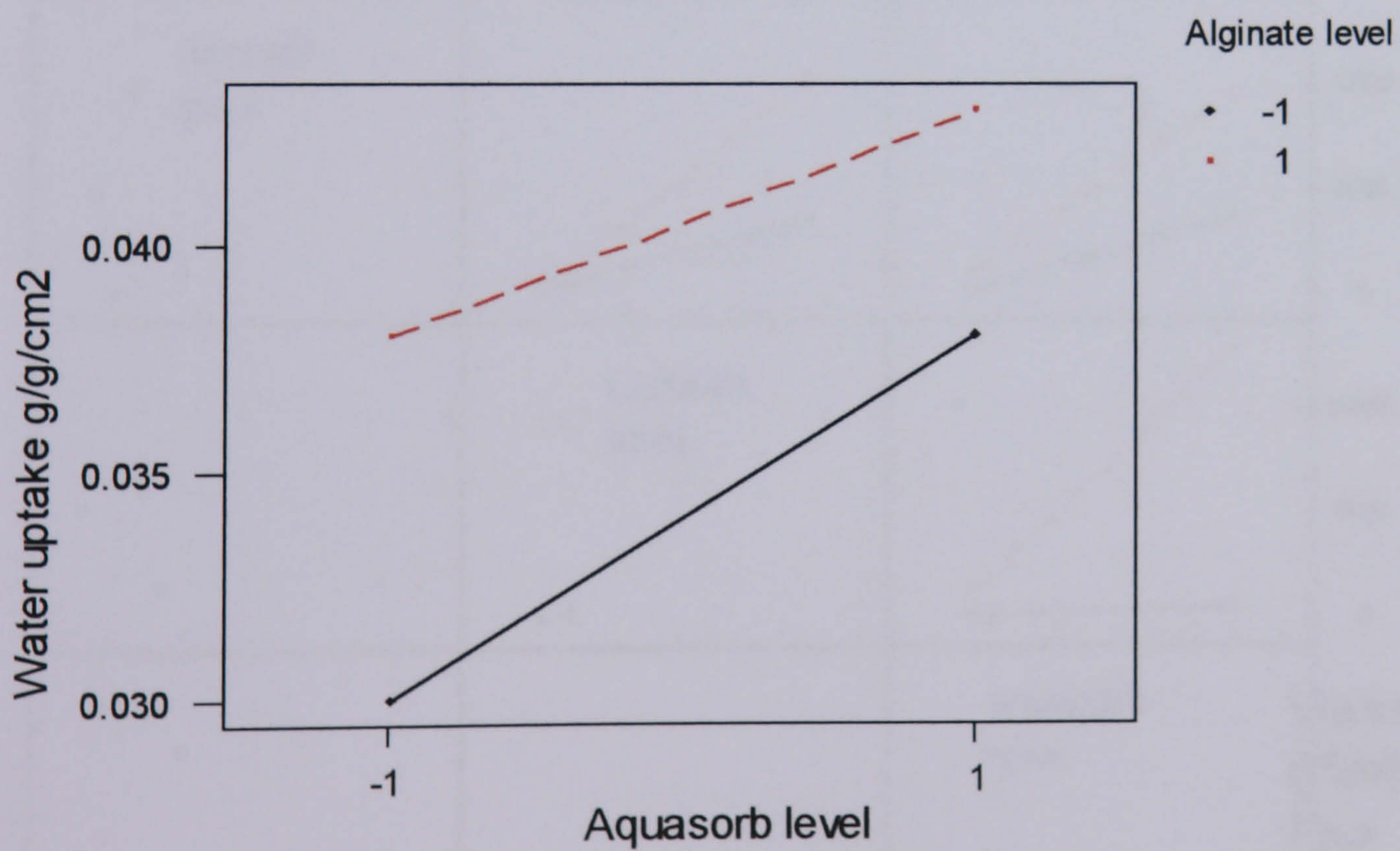


Figure 4.29 The interaction profile of factors alginate and Aquasorb<sup>®</sup> on water uptake.



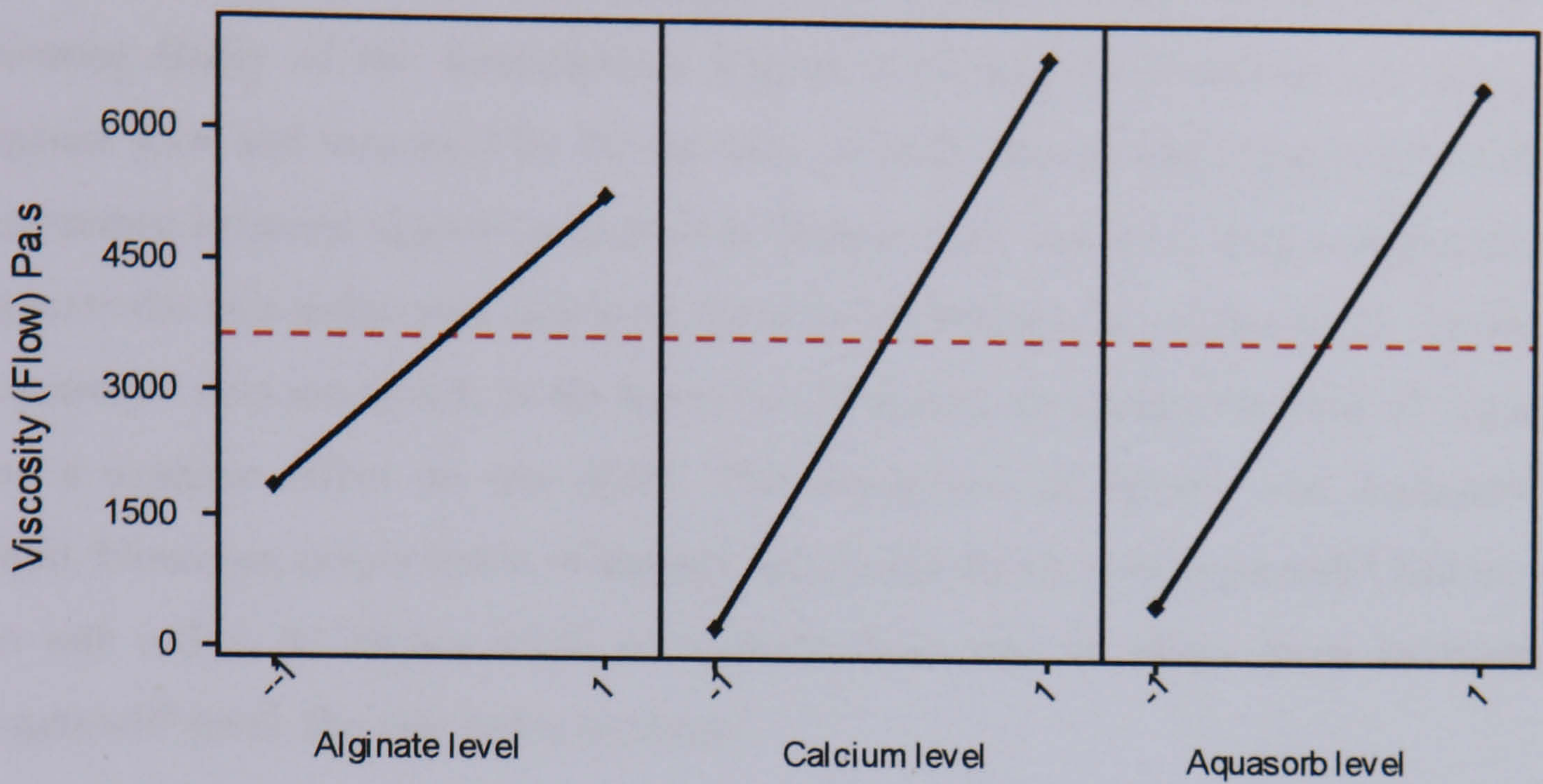


Figure 4.30 The main effects of factors Aquasorb<sup>®</sup>, alginate and calcium on viscosity.

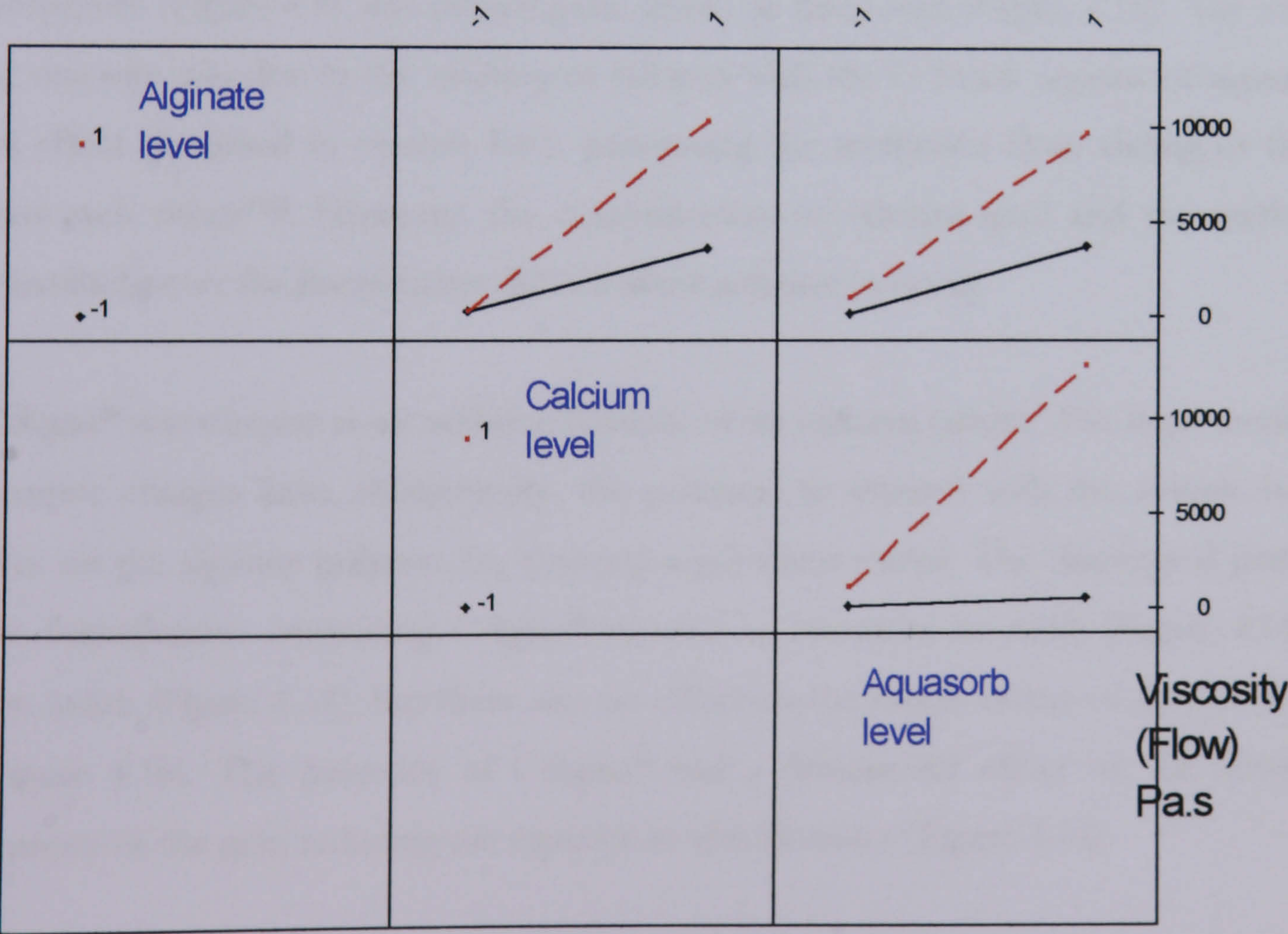


Figure 4.31 The interactions profiles of factors Aquasorb<sup>®</sup>, alginate and calcium on viscosity.



The elasticity of the formulations was affected differently by all three factors (Figure 4.32). Alginate had a slight negative effect on elasticity with increasing level. However calcium, and to a lesser extent Aquasorb®, increased elasticity with increasing level. There were no large interactions between factors (Figure 4.33). The rate index or shear thinning ability of the formulations (Figure 4.34) was decreased by the increase of alginate level and increased by the increase of both calcium and Aquasorb® levels. The interaction between alginate and calcium (Figure 4.35) was such that at higher levels of alginate the rate index was increased more by an increase in calcium level. Calcium and Aquasorb® also interacted, at the lower level calcium, increasing the level of Aquasorb® had a negative effect on rate index. The interaction of alginate and Aquasorb® was slight. However, at low levels of alginate increasing the level of Aquasorb® had no effect on rate index. At higher levels of alginate there was an effect from increasing the Aquasorb® level, the rate index increased.

## 4.5 DISCUSSION

The experiments of section 4.4 showed that the presence of calcium affects the rheological properties of the formulations, increasing viscosity (Figure 4.6) and elastic properties (Figure 4.8), but reducing the ability to shear thin (Figure 4.10). The increase in viscosity was due to the binding of calcium with the G block regions of alginate<sup>[131]</sup>, an effect discussed in section 1.4.1, preventing the molecules from sliding or flowing past each other<sup>[116]</sup>. However, the concentration of calcium used and the method of introduction to the formulation did not allow gelation to occur.

Celquat® was chosen as an additive because of its cationic nature. The large number of positive charges have, theoretically, the potential to interact with the anionic binding sites on the alginate polymer *i.e.*, forming a gel when mixed. The rheological profile of the formulations containing Celquat® showed an increased viscosity (Figure 4.14) and rate index (Figure 4.18), but there was no effect on the elastic nature of the formulation (Figure 4.16). The presence of Celquat® had a detrimental effect on the absorptive capacity of the gels, reducing the capacity to absorb water (Figure 4.12).



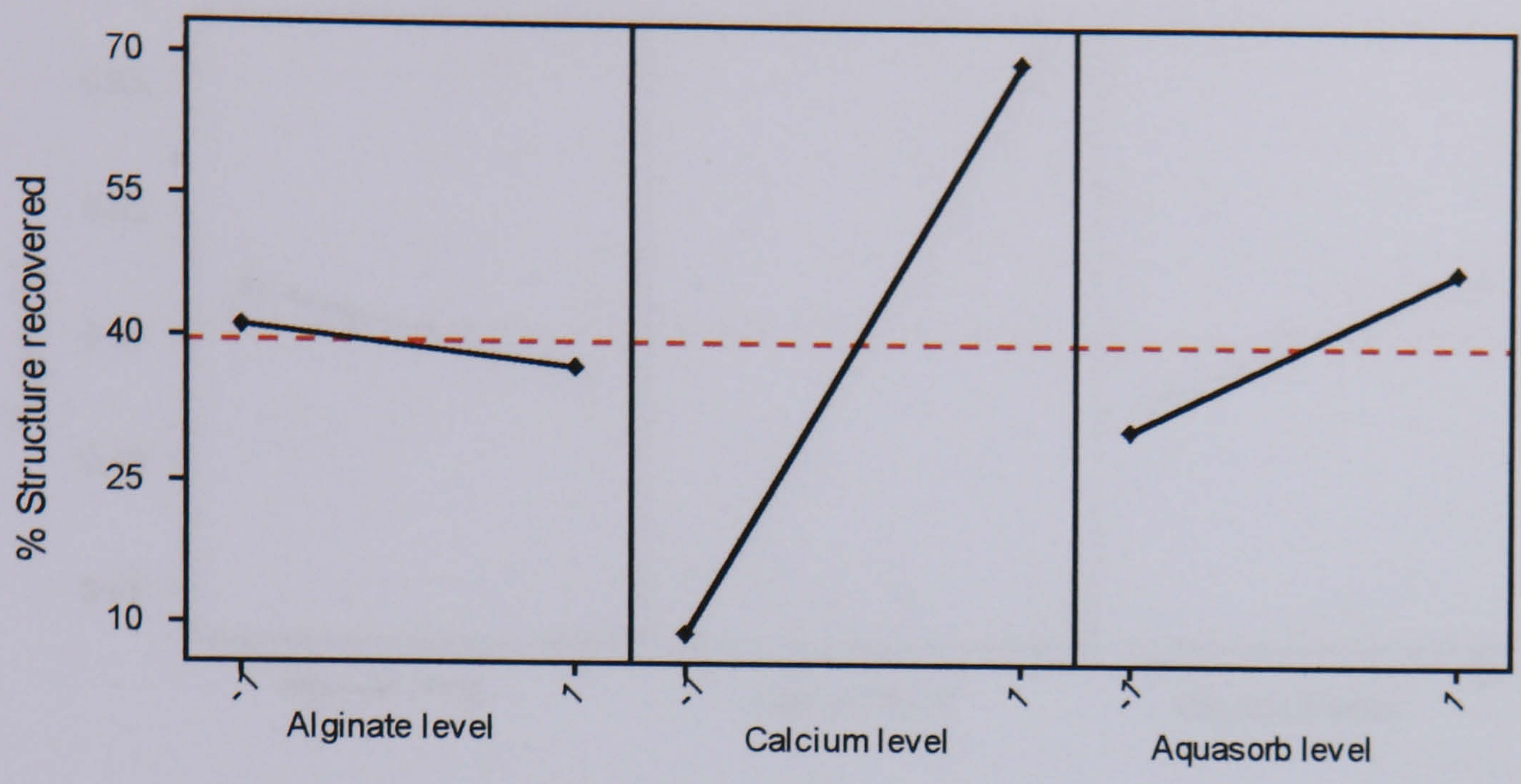


Figure 4.32 The main effects of factors Aquasorb<sup>®</sup>, alginate and calcium on % structure recovered.

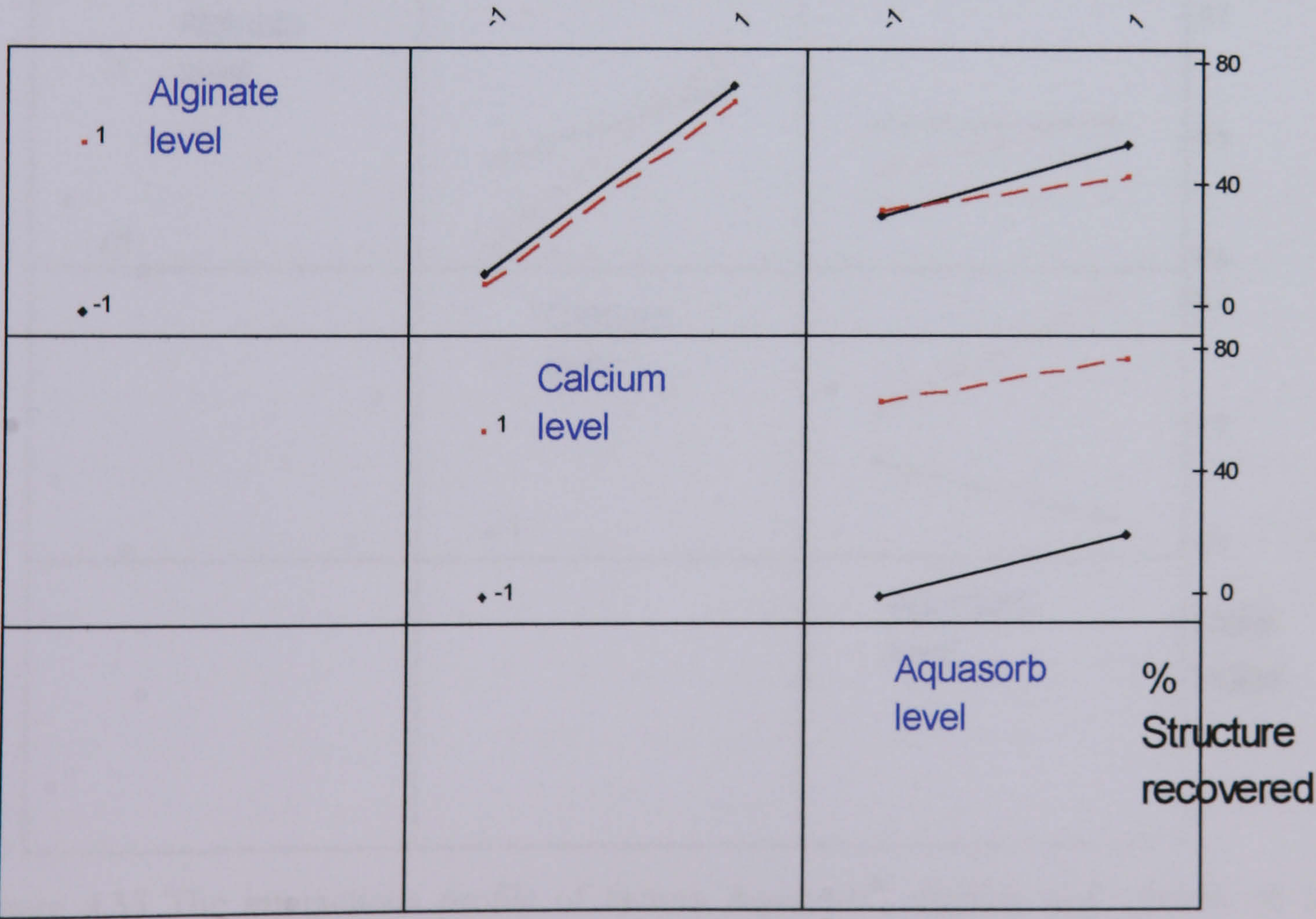


Figure 4.33 The interactions profile of factors Aquasorb<sup>®</sup>, alginate and calcium % structure recovered.



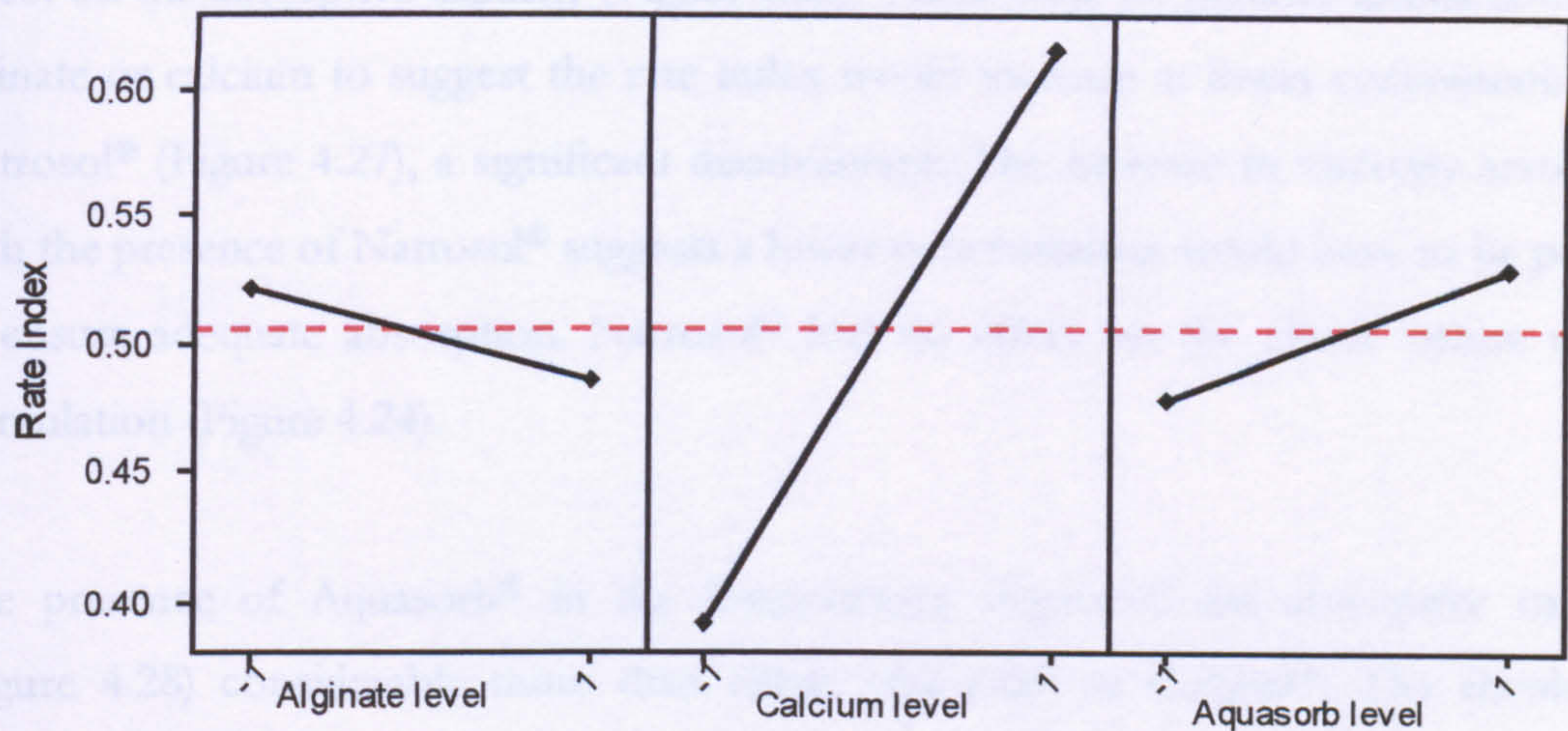


Figure 4.34 The main effects of factors Aquasorb<sup>®</sup>, alginate and calcium on rate index.

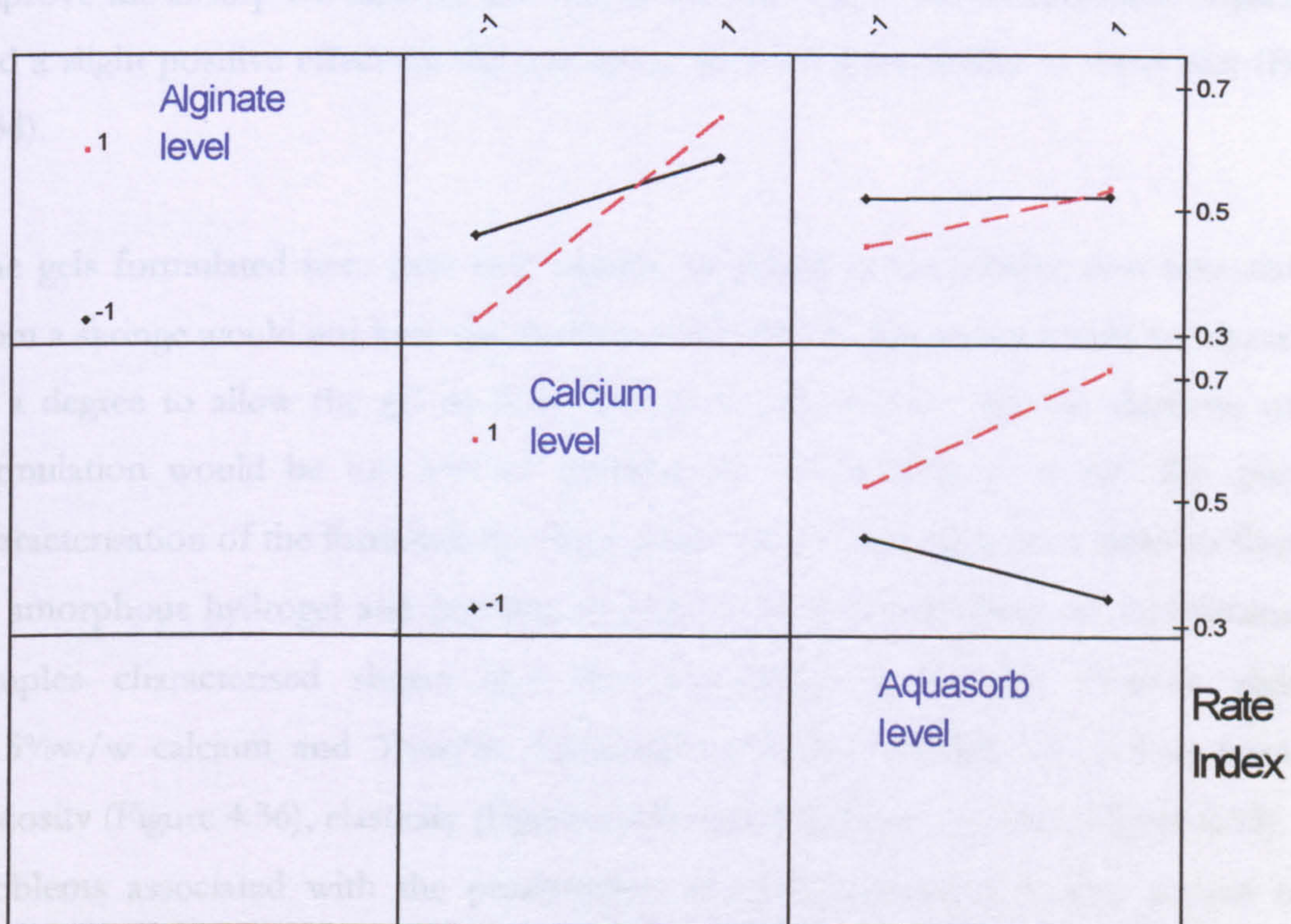


Figure 4.35 The interactions profile of factors Aquasorb<sup>®</sup>, alginate and calcium on rate index.



Natrosol® was chosen for its ability to shear thin, the rheological profiles show that Natrosol® had a positive effect on rate index increasing the shear thinning ability with an increase in level (Figure 4.26). However, this benefit was tempered by the lack of effect on the absorptive capacity (Figure 4.20). There were no positive interactions with alginate or calcium to suggest the rate index would increase at lower concentrations of Natrosol® (Figure 4.27), a significant disadvantage. The increase in viscosity associated with the presence of Natrosol® suggests a lower concentration would have to be present to ensure adequate absorption. Natrosol® had no effect on the elastic nature of the formulation (Figure 4.24).

The presence of Aquasorb® in the formulations improved the absorptive capacity (Figure 4.28) considerably more than either Natrosol® or Celquat®. The rheological profile was potentially beneficial, though the viscosity did increase with Aquasorb® level (Figure 4.30), an effect seen with Natrosol® and Celquat®. The presence of Aquasorb® had a positive effect on the elastic nature of the formulation, increasing the % structure recovered (Figure 4.32). This would potentially allow less calcium to be used and further improve the absorptive capacity and reduce the viscosity of the formulation. Aquasorb® had a slight positive effect on the rate index, increasing the ability to shear thin (Figure 4.34).

The gels formulated here were not capable of gelling *in-situ*, mixing two formulations from a syringe would not have the desired effect. The formulations would not shear thin to a degree to allow the gel to flow easily onto the wound and the elasticity of the formulation would be too low to consider the formulation as a gel. The physical characterisation of the formulations showed that the performance best matches those of an amorphous hydrogel and not that of a gel. A direct comparison of the commercial samples characterised shows that the formulation containing 8%w/w alginate, 0.15%w/w calcium and 3%w/w Aquasorb® performed equally as well in terms of viscosity (Figure 4.36), elasticity (Figure 4.37) and absorptive capacity (Figure 4.38). The problems associated with the presentation of the formulation to the patient could present a difficult selling point. The large amount of alginate in the formulation conveys a dark green colour, which could have potentially poor patient acceptability compared to the clear Intrasisite® gel.



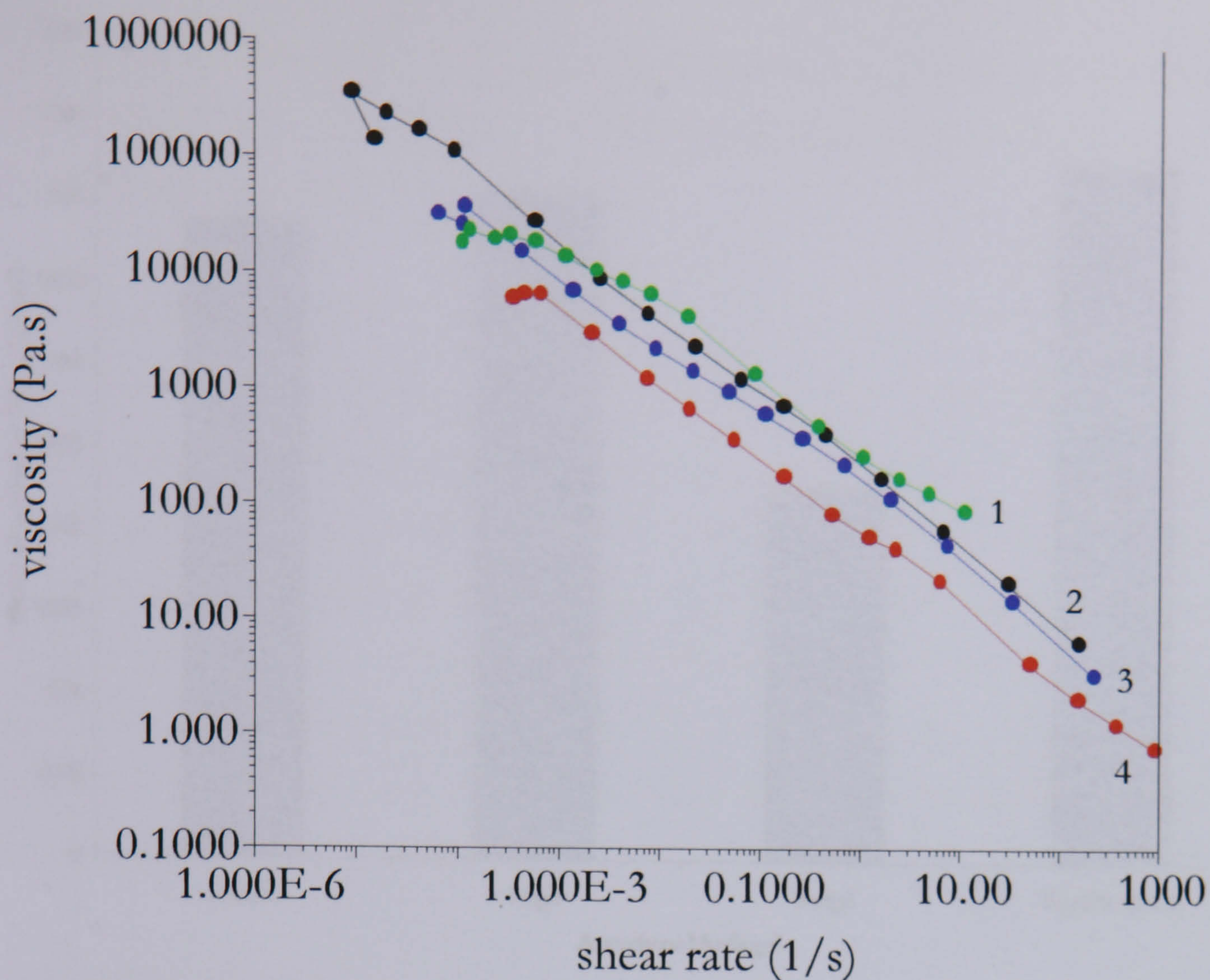


Figure 4.36 Comparing the viscosity profile of the top formulation 1) 8%w/w sodium alginate, 3%w/w Aquasorb® and 0.15%w/w calcium with 2) Nugel®, 3) Intrasilite® and 4) Sterigel®.

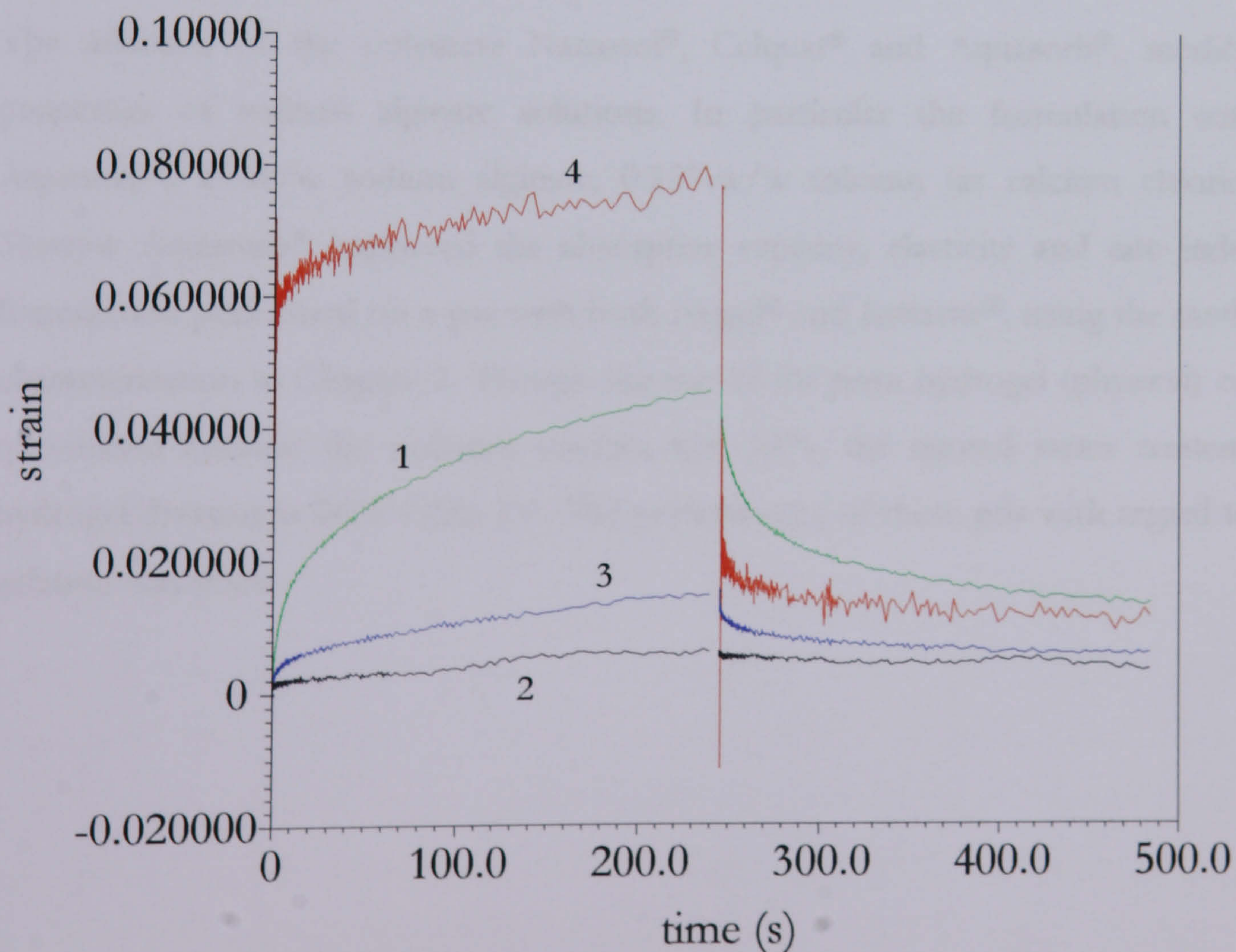


Figure 4.37 Comparing the elasticity of the top formulation 1) 8%w/w sodium alginate, 3%w/w Aquasorb® and 0.15%w/w calcium with 2) Intrasilite®, 3) Sterigel® and 4) Nugel® using creep.



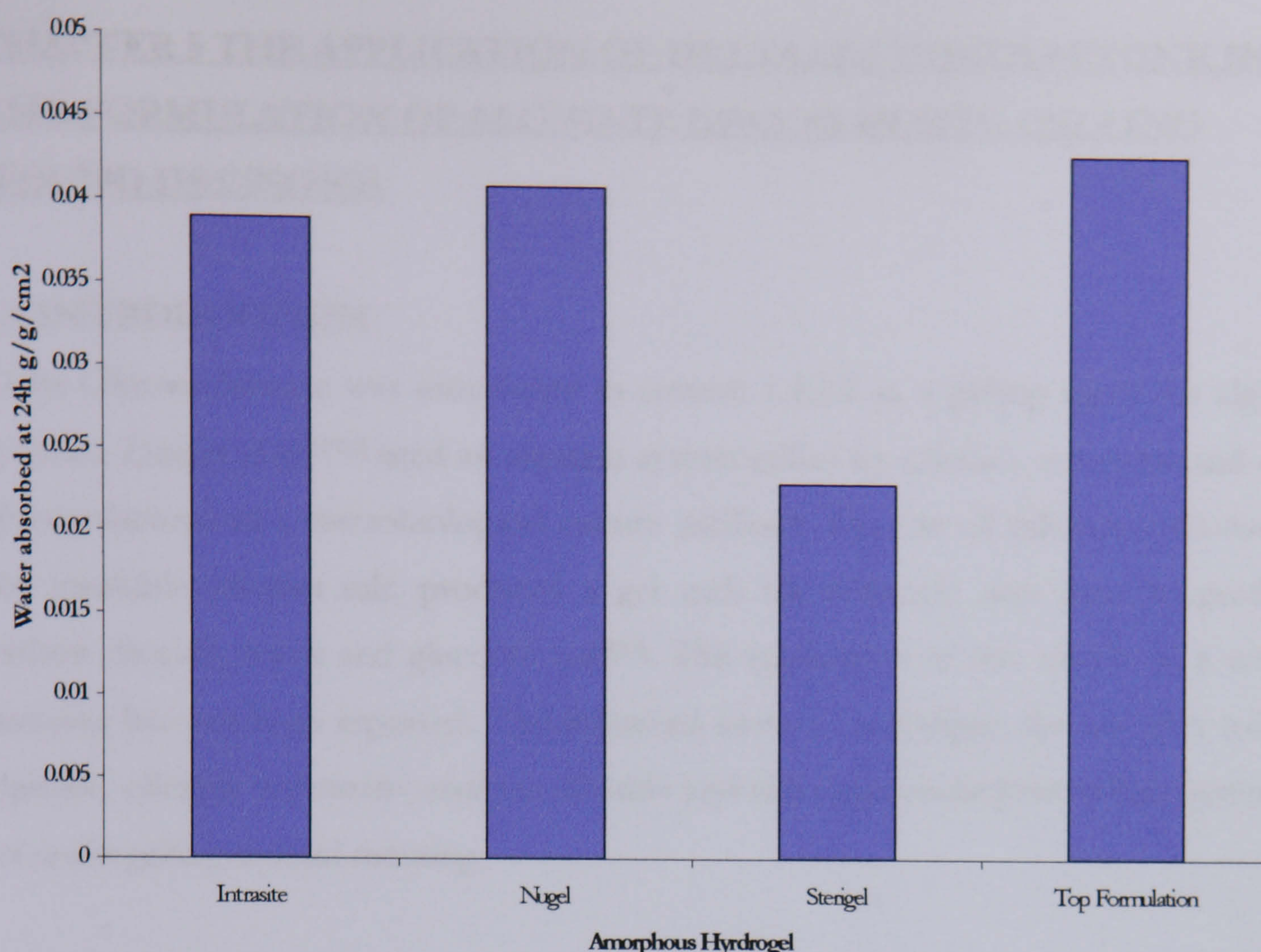


Figure 4.38 Comparing the water uptake performance of the top formulation 8%w/w alginate, 3%w/w Aquasorb<sup>®</sup> and 0.15%w/w calcium with Intrasilite<sup>®</sup>, Sterigel<sup>®</sup> and Nugel<sup>®</sup>.

## 4.6 CONCLUSION

The addition of the polymers Natrosol<sup>®</sup>, Celquat<sup>®</sup> and Aquasorb<sup>®</sup>, modified the properties of sodium alginate solutions. In particular the formulation containing Aquasorb<sup>®</sup>: 8%w/w sodium alginate, 0.15%w/w calcium (as calcium chloride) and 3%w/w Aquasorb<sup>®</sup> improved the absorptive capacity, elasticity and rate index. The formulation performed on a par with both Nugel<sup>®</sup> and Intrasilite<sup>®</sup>, using the methods of characterisation in Chapter 2. Though the use of the term hydrogel (physical) could be questioned because the polymer content was 12%, the quoted water content for a hydrogel dressing is 96% Table 1.7. The performance of these gels with regard to *in-situ* gelation was poor.



## **CHAPTER 5 THE APPLICATION OF DELTA GLUCONOLACTONE IN THE FORMULATION OF ALGINATE GELS AS IN-SITU GELLING WOUND DRESSINGS**

### **5.1 INTRODUCTION**

Delta Gluconolactone was introduced in section 1.4.5.2 as a gelling agent for alginate systems. Draget *et al*<sup>[74,91]</sup> used an alginate system gelled by calcium carbonate and delta gluconolactone as a microbiological culture medium. The use of calcium carbonate as the insoluble calcium salt, produced a gel with the relatively non toxic by-products carbon dioxide, water and gluconic acid<sup>[74]</sup>. The application of this system as a wound dressing has not been reported. The following sections investigate the use of a sodium alginate, calcium carbonate, sodium chloride and delta gluconolactone gelling system as an *in-situ* gelling wound dressing.

### **5.2 EXPERIMENTAL**

#### **5.2.1 Materials**

Sodium alginate, LF 10/60, FMC Biopolymer, PO BOX494 N-3002, Drammen, Norway. Calcium carbonate, Sturcal f, Specialty Minerals, Birmingham. England. Disodium hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset. England. Potassium di-hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset. England. Delta gluconolactone, GPR, Aldrich Chemical Company, Gillingham, Dorset. England. Sodium chloride, AR, BDH Laboratory Supplies, Poole, Dorset. England. Hydrochloric acid, AR, BDH Laboratory Supplies, Poole, Dorset. England.

#### **5.2.2 Equipment**

Oertling Balance, 4 decimal places, Oertling, Smethwick, West Midlands, England. Carrimed Rheometer, CSL<sup>2</sup> 500, TA Instruments Ltd. Surrey, England. Bench top Autoclave, Dixons Surgical Instruments Ltd. Wickford, Essex, England. Glass Franz cells, Custom made by Mr. B. Chappel, Glass blower. Liverpool University, Liverpool, England. Overhead stirrer, Janke, BDH Laboratory Supplies, Poole, Dorset, England. Bench pH meter, HI 8579 Hanna instruments. BDH Laboratory Supplies, Poole, Dorset, England.



5.2.3 Methods

Experimental designs were used to investigate a sodium alginate based system as an *in-situ* gelling wound dressing. A two factor, two level, full factorial design was used to investigate gelling with i) calcium carbonate and delta gluconolactone (section 5.2.3; and ii) sodium chloride, calcium carbonate and delta gluconolactone (section 5.2.4).

5.2.3.1 WATER UPTAKE DETERMINATION

The methods for gel manufacture and water uptake determination are described in sections 2.1 and 2.2 respectively.

5.2.3.2 RHEOLOGICAL EVALUATION

The methods for gel manufacture and rheological evaluation are described in sections 2.1 and 2.3 respectively.

5.2.3.3 pH DETERMINATION

The methods for determining formulation pH are described in section 2.1.

5.2.4 The gelling of alginate with calcium carbonate and delta gluconolactone.

5.2.4.1 EXPERIMENTAL DESIGN

The pH, rheology of the pre-gel solution and the gelling of formulations prepared from sodium alginate, calcium carbonate and delta gluconolactone were investigated using a two factor, two level experimental design (Factors and levels are given in Table 5.1 and the experimental design in Table 5.2).

Table 5.1 Factors and levels for the experimental design investigating the gelation of sodium alginate and calcium carbonate.

Level	Concentration %w/w	
	Sodium Alginate	Calcium Carbonate
-1	2	0.16
1	5	0.32



Table 5.2 Two factor, two level experimental design investigating the gelation of sodium alginate and calcium carbonate.

Factor	Experiment			
	1	2	3	4
Alginate	-1	+1	-1	+1
Calcium	-1	-1	+1	+1

5.2.4.2 RESULTS

The results of the physical evaluation are presented collectively in Table 5.3 and are discussed individually below. In more detail, the tests performed were rheological evaluation of gelling time, gel strength, zero rate viscosity, rate index and the pH (post sterilisation and post gelation).

Table 5.3 Results of the physical tests performed on each formulation, given in Table 5.2.

Test	Experiment			
	1	2	3	4
Gelling time /min	22.7	310.8	6.3	27.7
Gel Strength (G') /Pa	183	121	1663	2429
pH Post sterilisation	9.2	8.8	9.2	8.9
pH 48h post gelation	6.5	5.8	7.1	6.9
Zero rate viscosity /Pa.s	0.1	1.8	0.1	1.6
Rate index	0.05	0.75	0.06	0.80

5.2.4.2.a GELLING TIME

Increasing the level of sodium alginate increased gelling time; increasing the level of calcium decreased gelling time (Figure 5.1). The interaction profile (Figure 5.2) indicates an interaction existed between alginate and calcium carbonate, such that at higher levels of alginate, calcium carbonate had a greater effect on gelling time.

5.2.4.2.b GEL STRENGTH

The main effects of factors were to increase gel strength (Figure 5.3). Calcium carbonate had greater effect increasing gel strength compared to sodium alginate. The interaction profile suggests a slight interaction existed between sodium alginate and calcium carbonate (Figure 5.4), calcium carbonate had a greater effect on gel strength at higher levels of alginate.



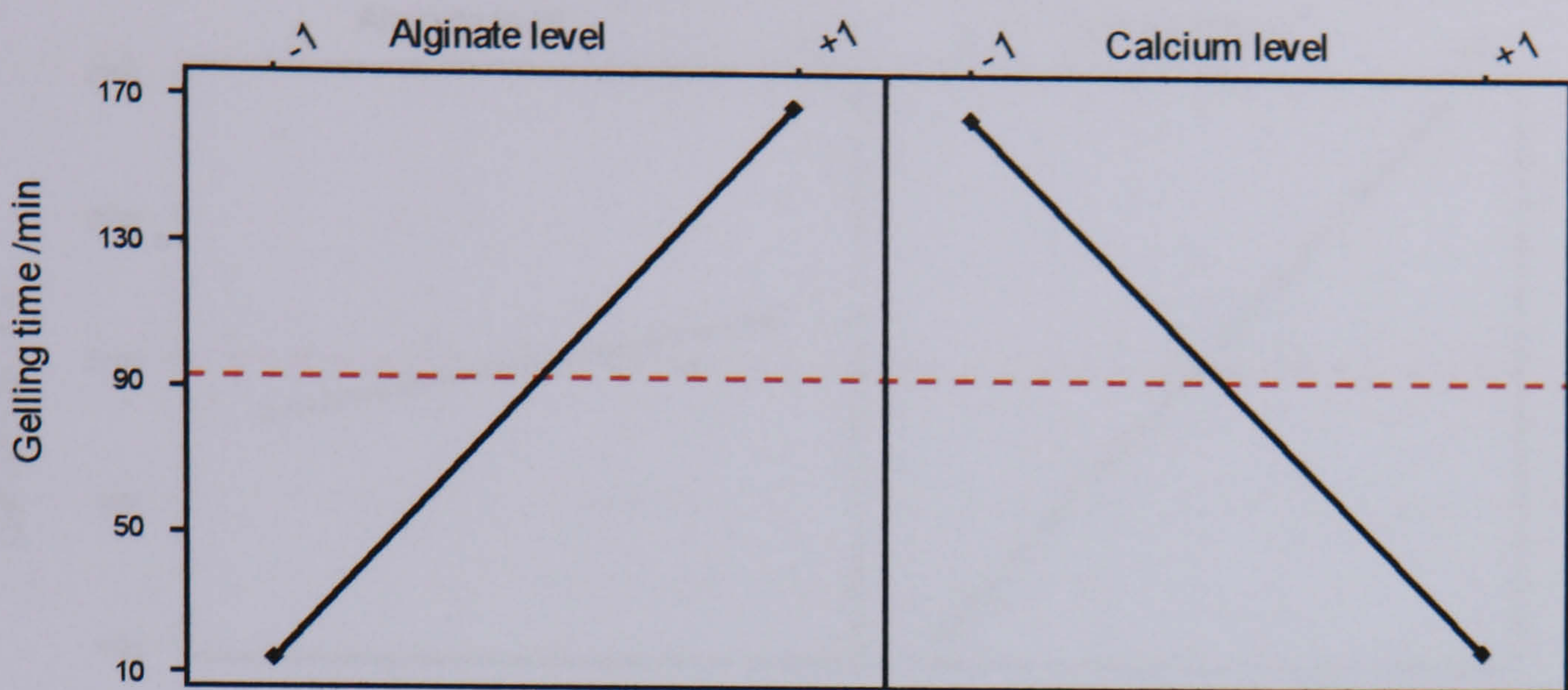


Figure 5.1 The main effects of sodium alginate and calcium concentration on gelling time.

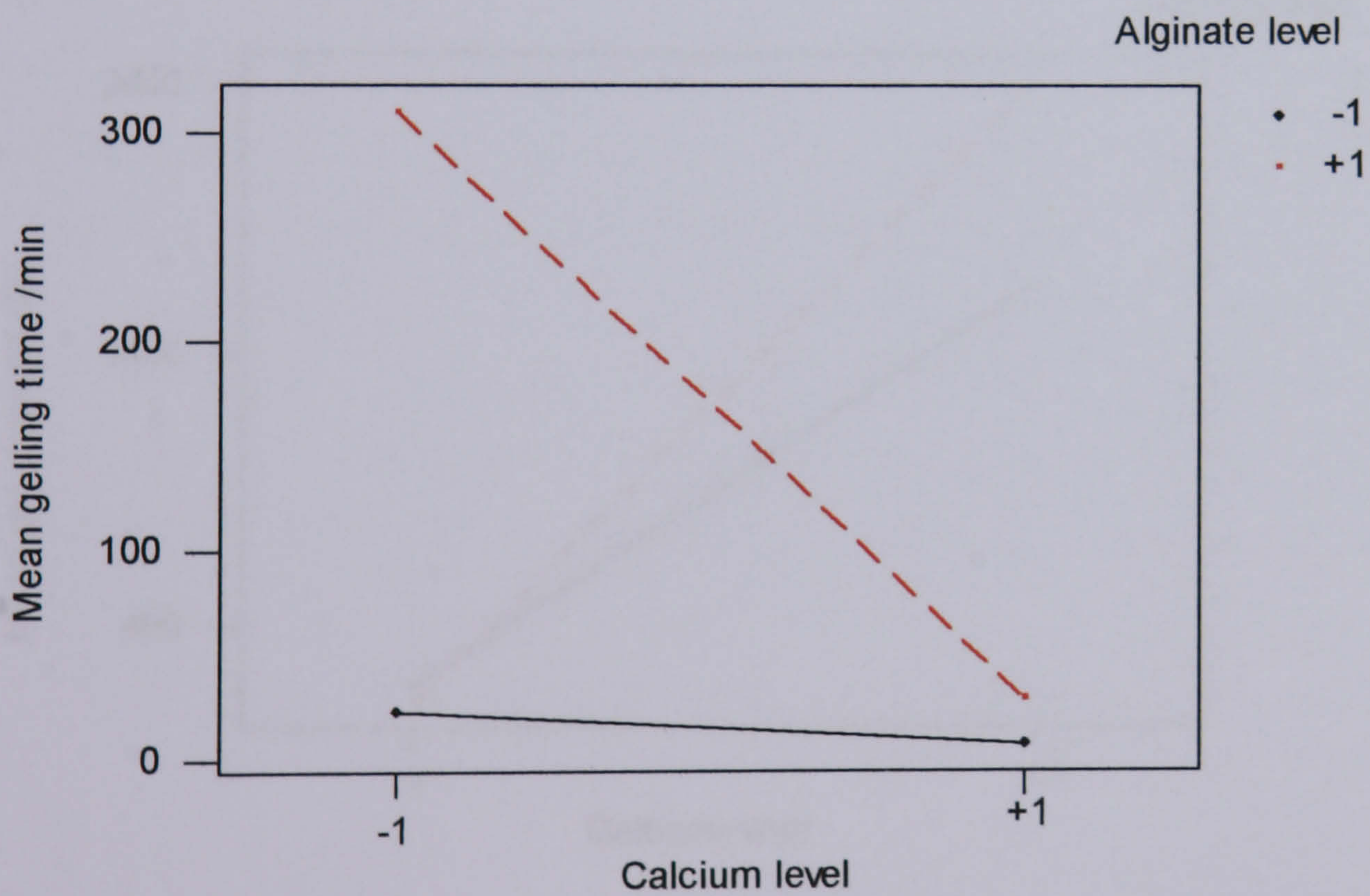


Figure 5.2 The interaction profile of sodium alginate and calcium concentration on gelling time.



### 5.2.4.2 POST STERILISATION pH

The pH post sterilisation was measured by colourimetric method by using pH indicator (Figure 5.5). The mean was given below.

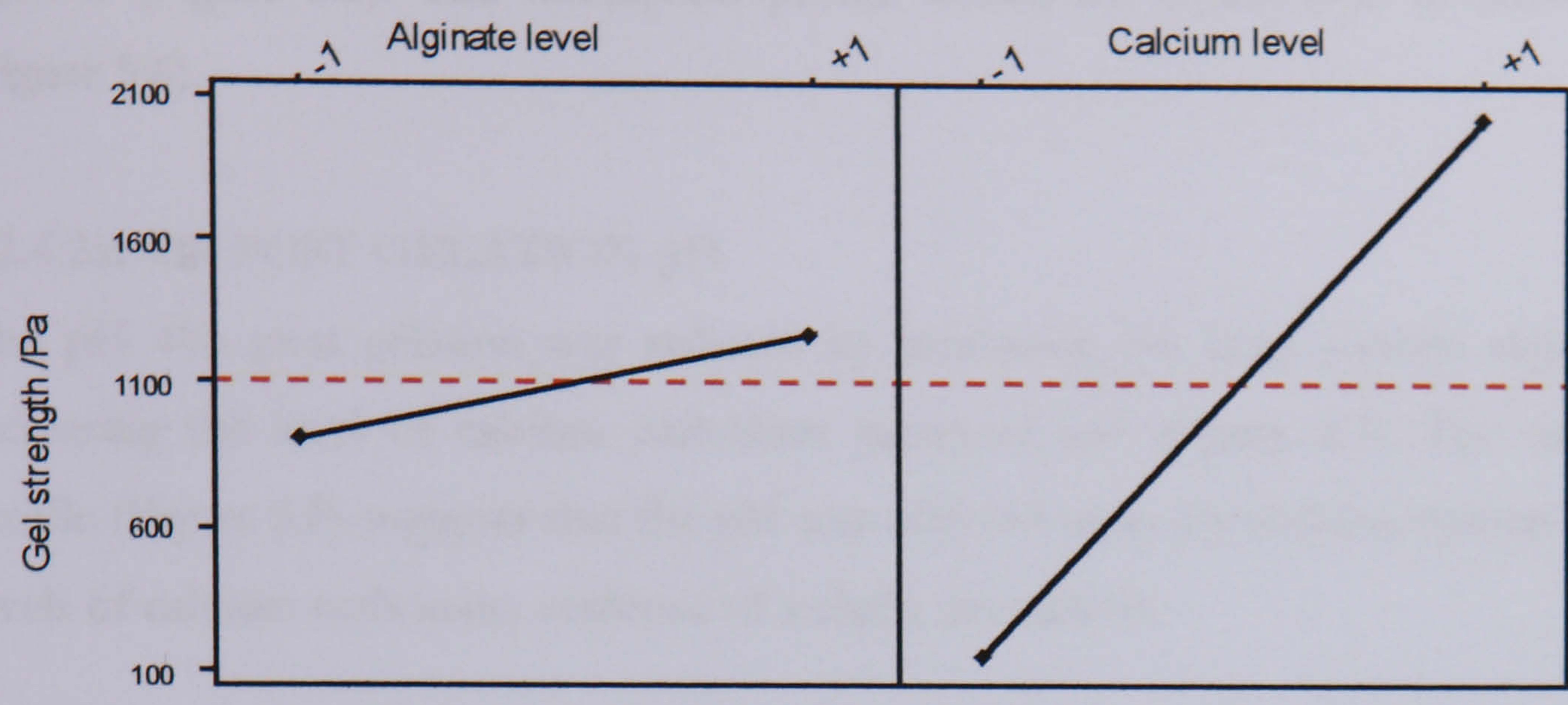


Figure 5.3 The main effects of sodium alginate and calcium concentration on gel strength.

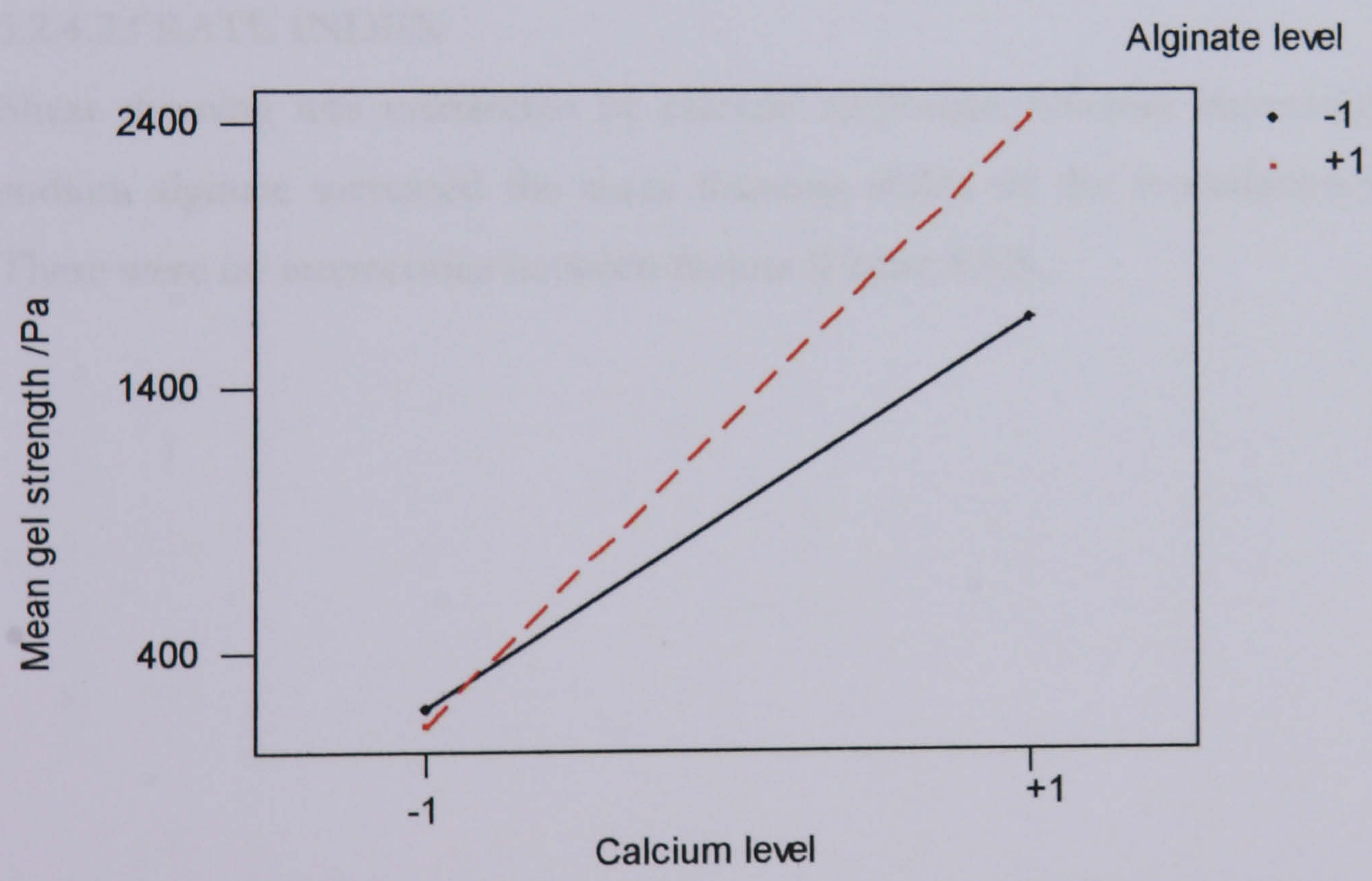


Figure 5.4 The interaction profile of sodium alginate and calcium concentration on gel strength.



#### 5.2.4.2.c POST STERILISATION pH

The pH post sterilisation was increased by calcium carbonate and decreased by sodium alginate (Figure 5.5). The interaction profile shows no interactions between factors (Figure 5.6).

#### 5.2.4.2.d 48h POST GELATION pH

The pH 48h post gelation was reduced by increasing the level sodium alginate and increasing the level of calcium carbonate increased pH (Figure 5.7). The interaction profile (Figure 5.8) suggests that the pH was affected more by sodium alginate at lower levels of calcium carbonate; evidence of a slight interaction.

#### 5.2.4.2.e ZERO RATE VISCOSITY

Calcium carbonate had no effect on the zero rate viscosity (Figure 5.9). Increasing the level of sodium alginate increased viscosity. There were no interactions between factors (Figure 5.10).

#### 5.2.4.2.f RATE INDEX

Shear thinning was unaffected by calcium carbonate, whereas increasing the level of sodium alginate increased the shear thinning ability of the formulation (Figure 5.11). There were no interactions between factors (Figure 5.12).



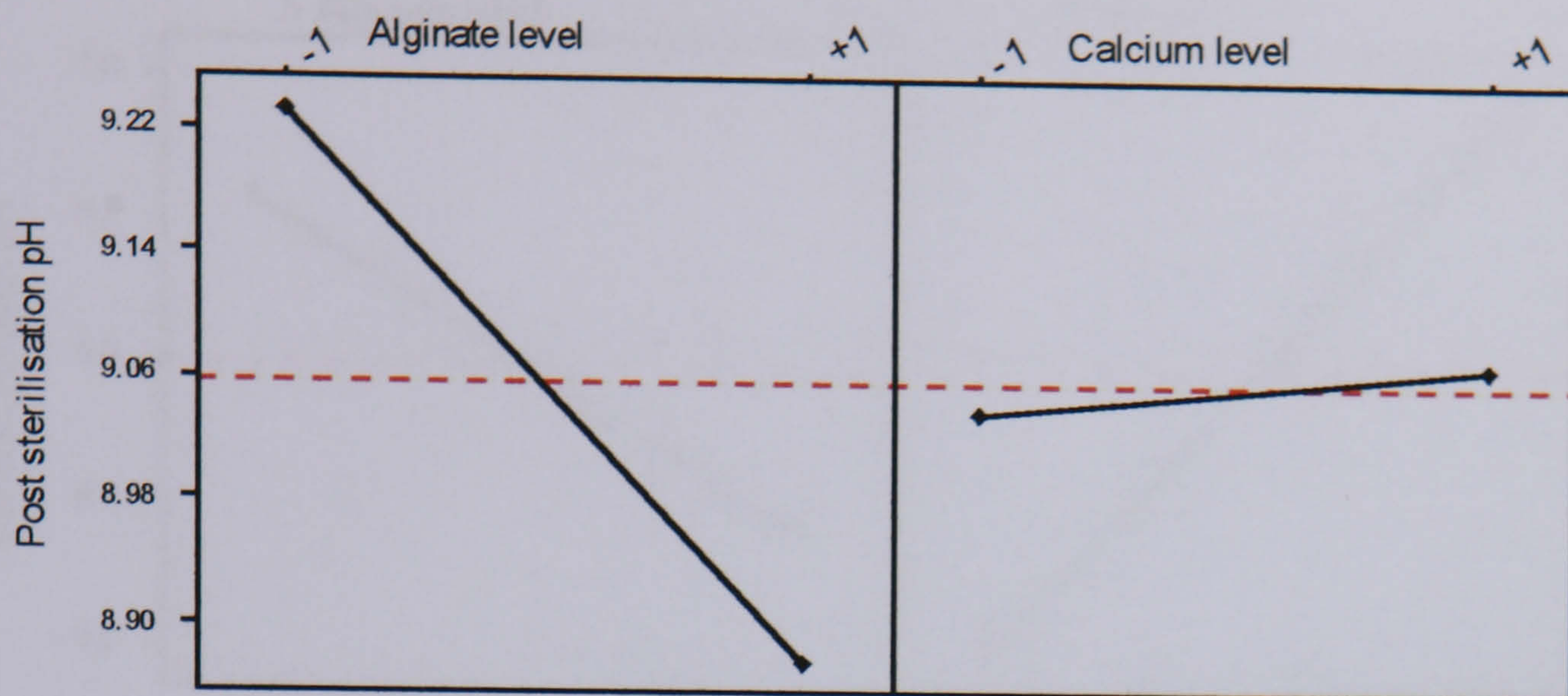


Figure 5.5 The main effects of sodium alginate and calcium concentration on the post sterilisation pH.

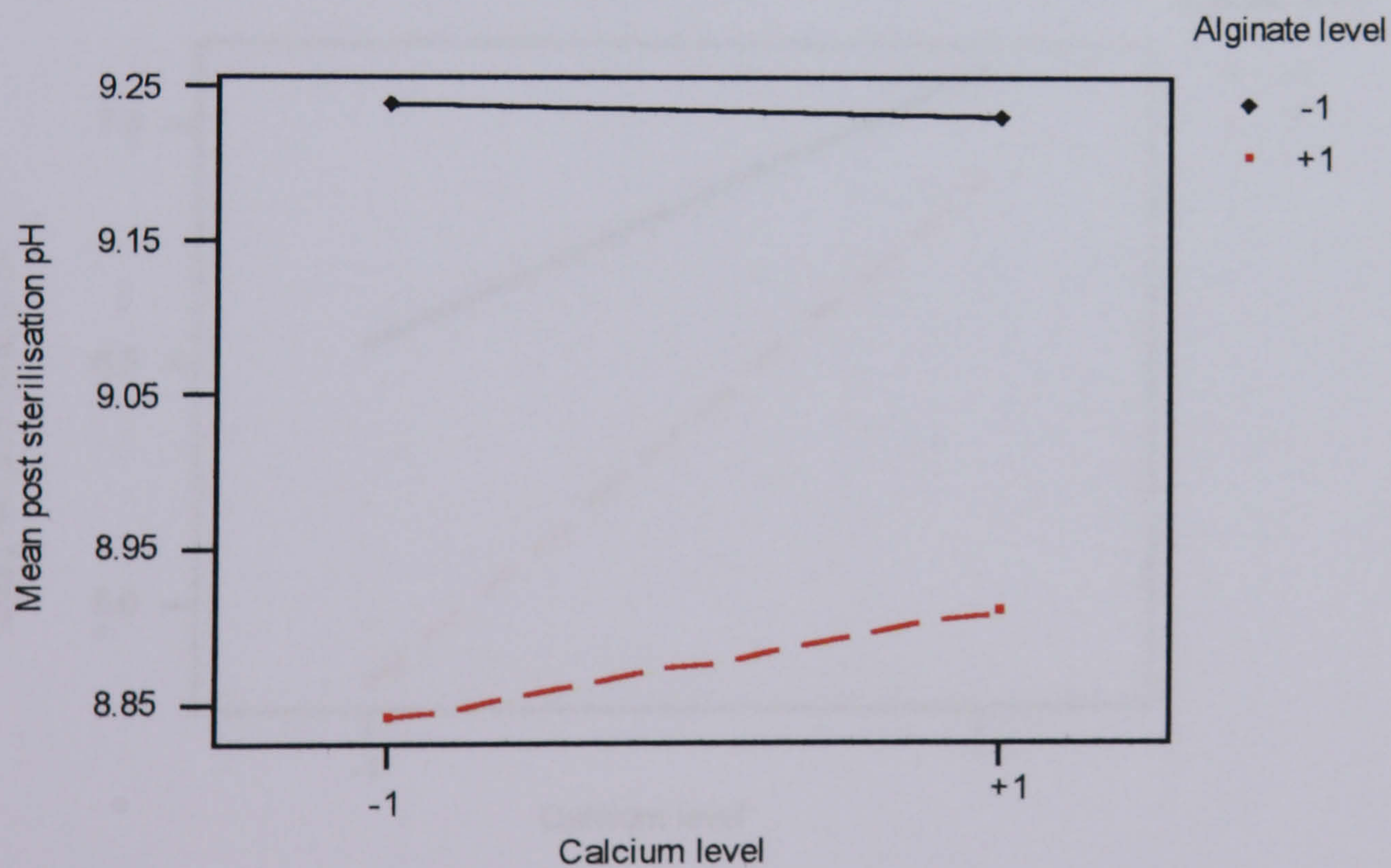


Figure 5.6 The interaction profile of sodium alginate and calcium concentration on the post sterilisation pH.



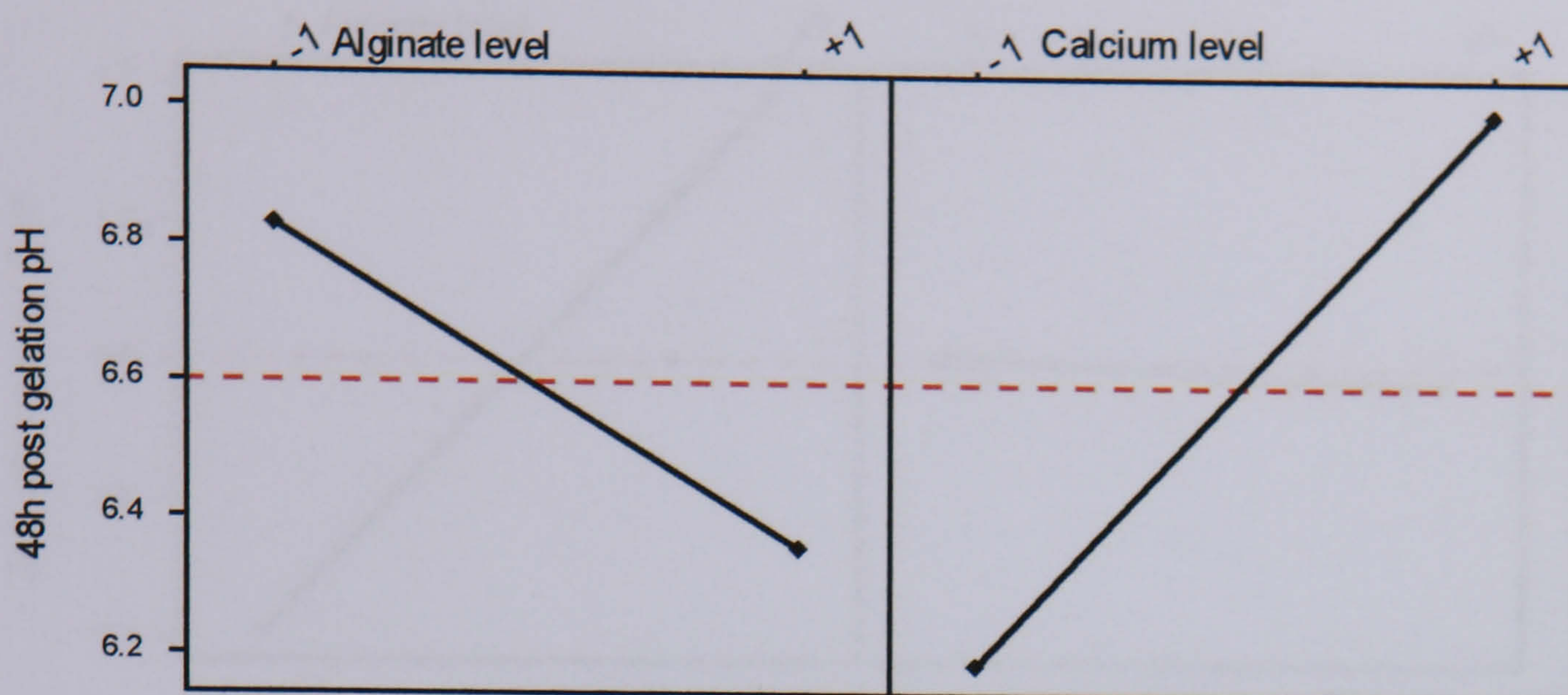


Figure 5.7 The main effects of sodium alginate and calcium carbonate on the 48h post gelation pH.

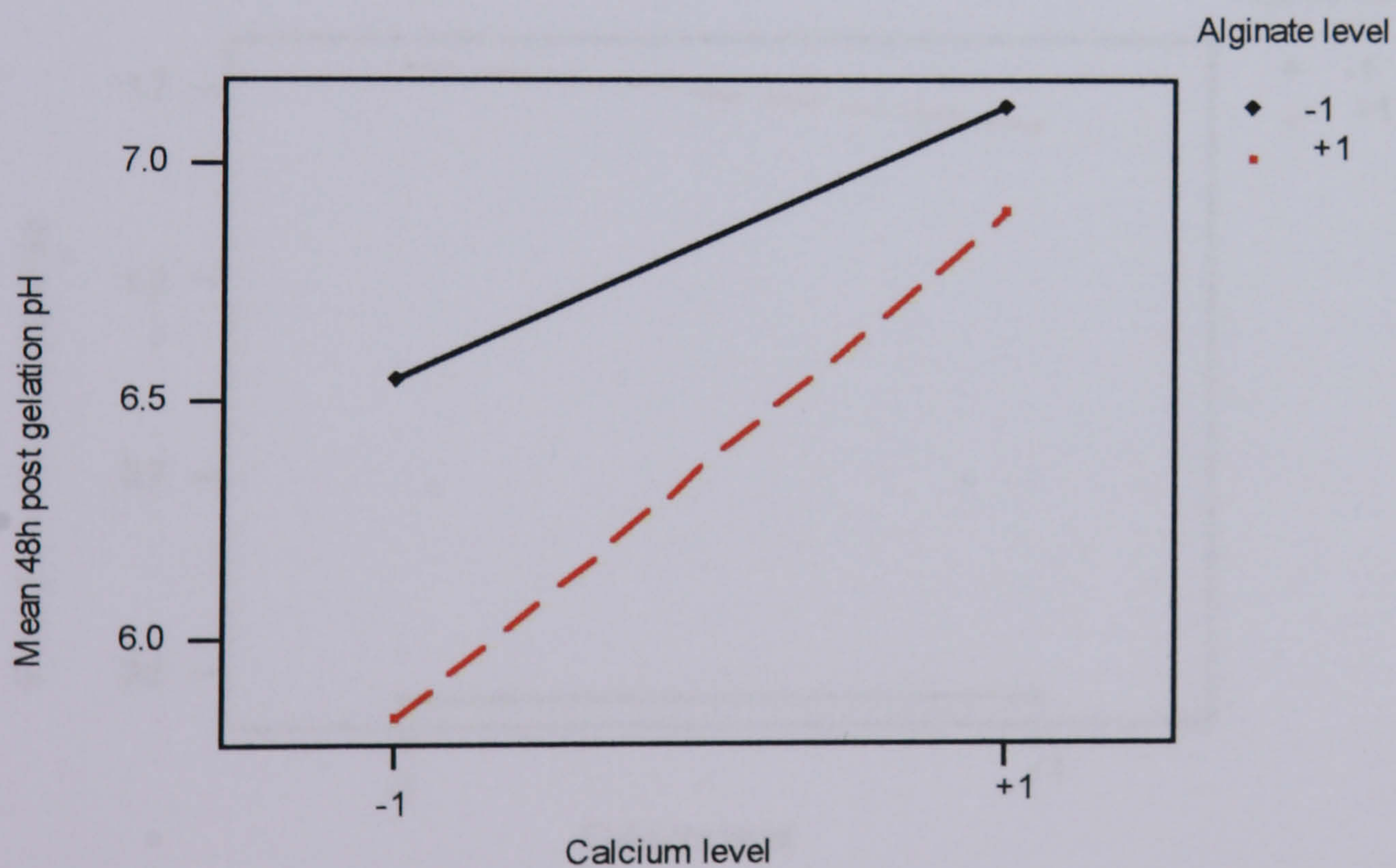


Figure 5.8 The interaction profile of sodium alginate and calcium carbonate on 48h post gelation pH.



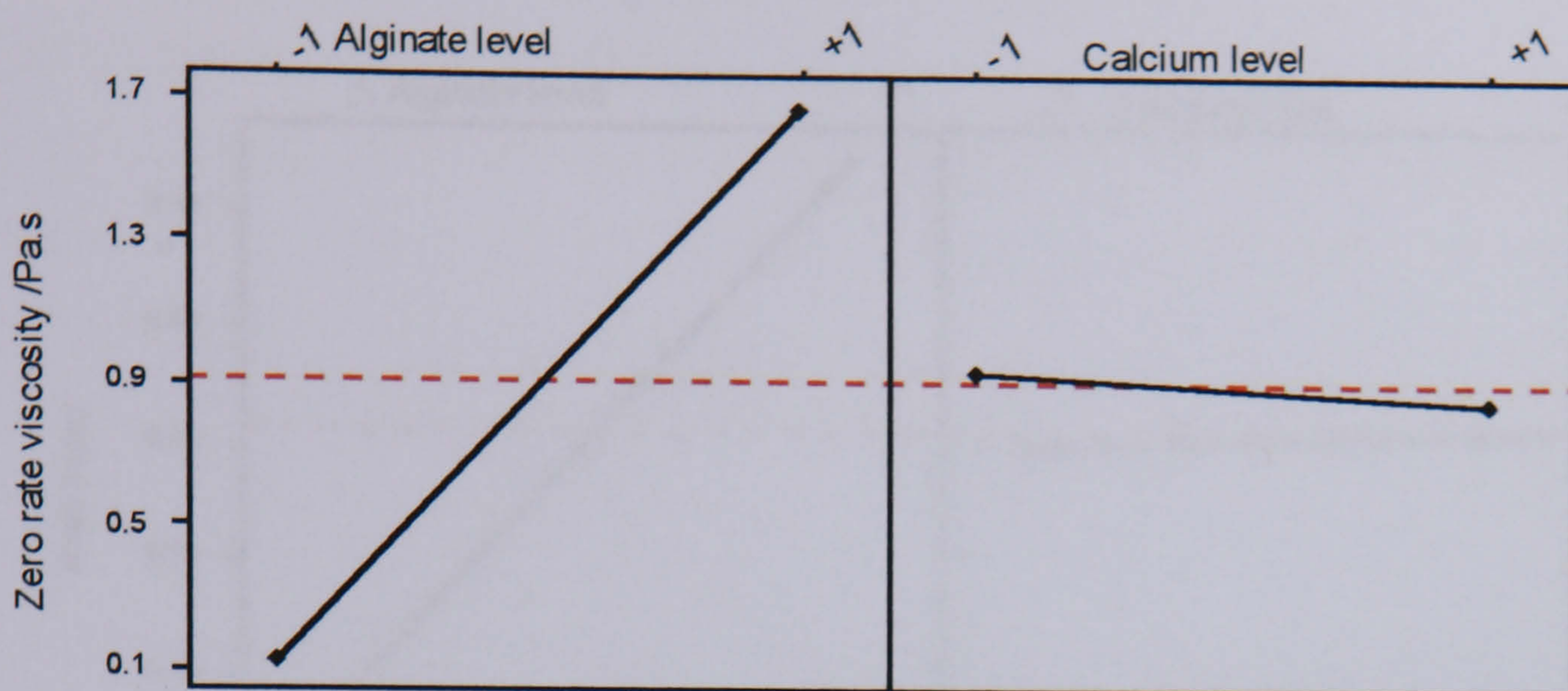


Figure 5.9 The main effects of sodium alginate and calcium carbonate on zero rate viscosity.

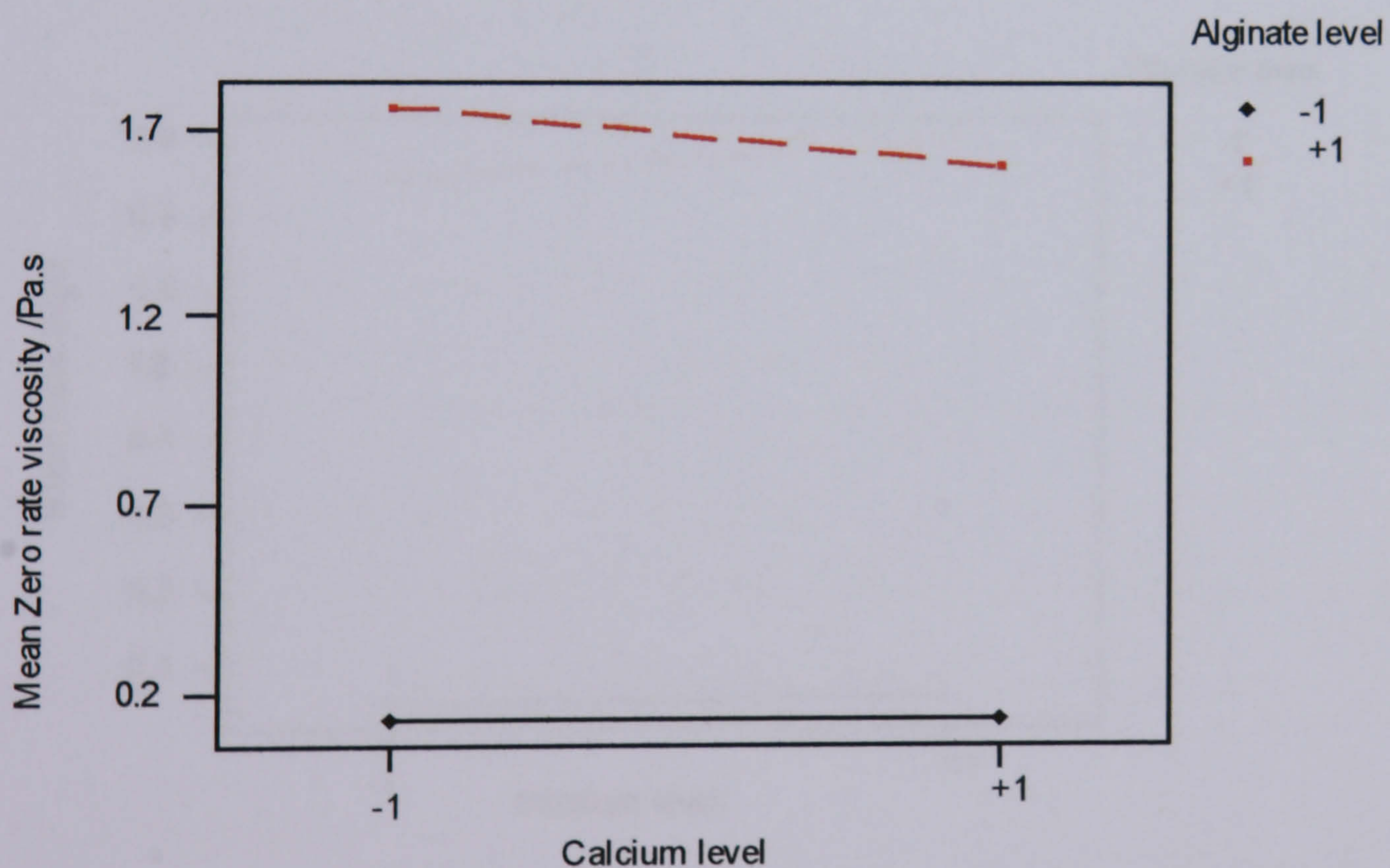


Figure 5.10 The interaction profile of sodium alginate and calcium carbonate on zero rate viscosity.



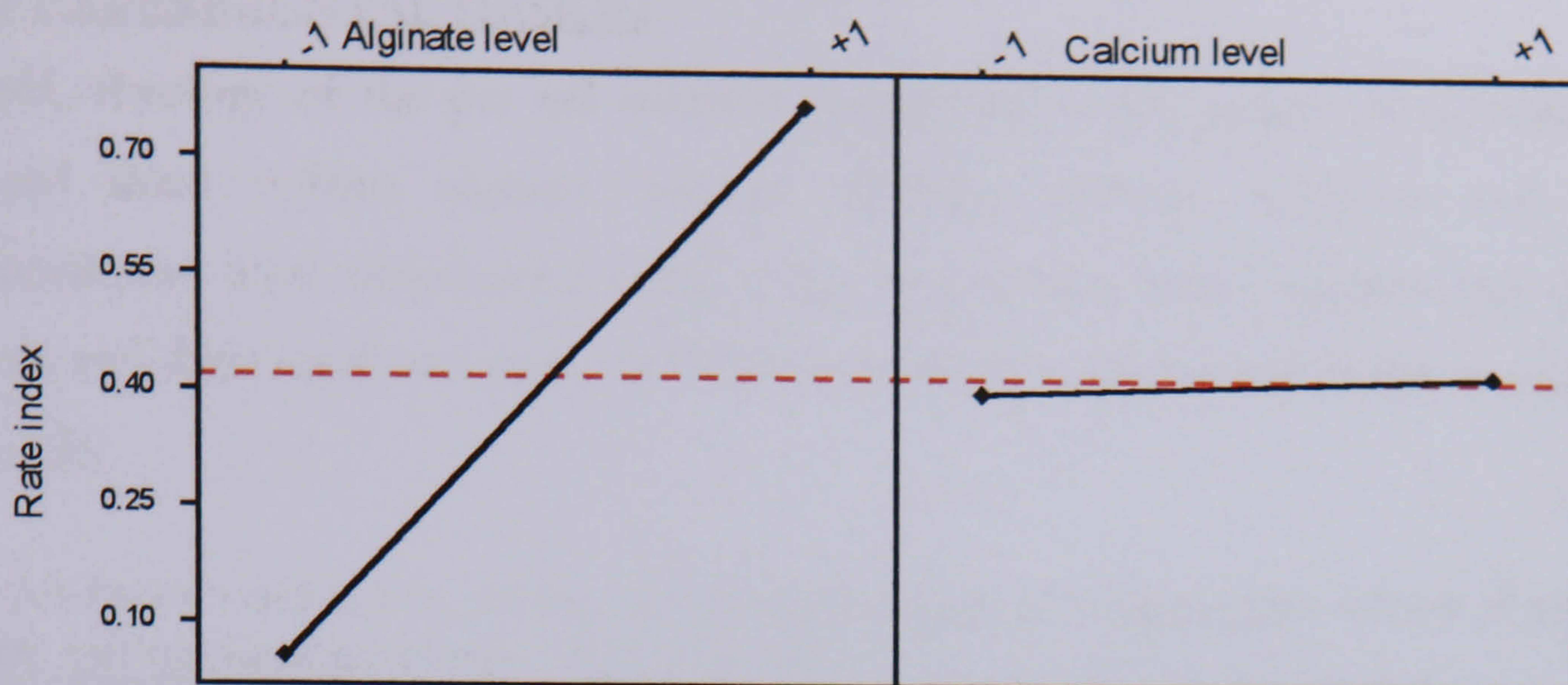


Figure 5.11 The main effects of sodium alginate and calcium carbonate on rate index.

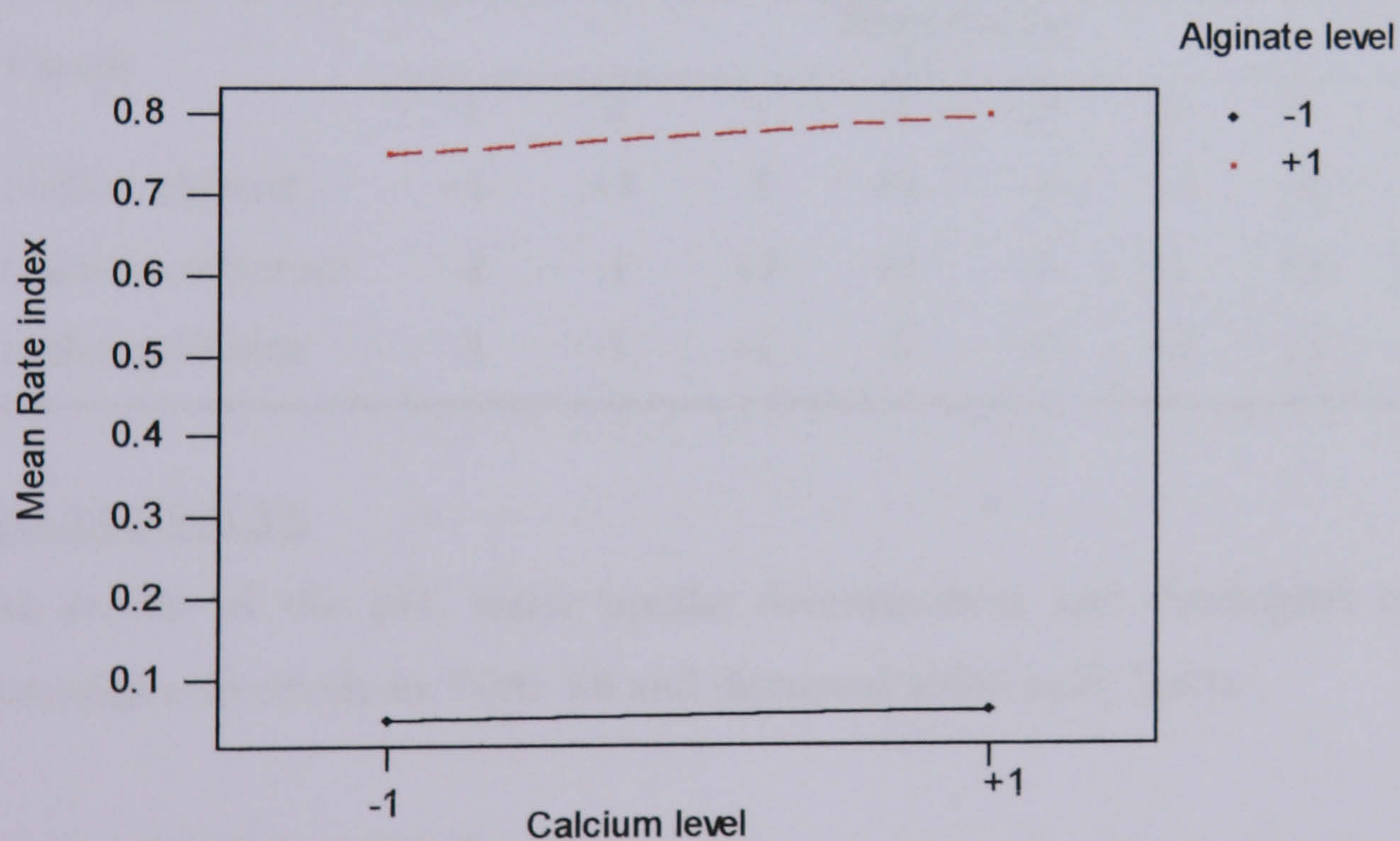


Figure 5.12 The interaction profile of sodium alginate and calcium carbonate on rate index.



5.2.5 The Gelling Of Alginate In The Presence Of Sodium Chloride, Calcium Carbonate And Delta-Gluconolactone

5.2.5.1 EXPERIMENTAL DESIGN

The pH, rheology of the pre gel solution, gelling and water uptake of formulations prepared from sodium alginate, sodium chloride, calcium carbonate and delta gluconolactone were investigated using a two factor, two level, experimental design (Factors and their levels are given in Table 5.4 and the experimental design is given in Table 5.5).

Table 5.4 Factors and Levels for the experimental design investigating the effects of sodium alginate, calcium carbonate and sodium chloride.

Level	Concentration %w/w		
	Sodium Alginate	Calcium Carbonate	Sodium Chloride
-1	2	0.20	0
1	5	0.28	0.9

Table 5.5 Three factor, two level experimental design investigating the effects of sodium alginate, calcium carbonate and sodium chloride.

Factor	Experiment							
	1	2	3	4	5	6	7	8
Sodium alginate	-1	+1	-1	+1	-1	+1	-1	+1
Calcium carbonate	-1	-1	+1	+1	-1	-1	+1	+1
Sodium chloride	-1	-1	-1	-1	+1	+1	+1	+1

5.2.5.2 RESULTS

The results of the pH, water uptake determination and rheological evaluation are presented collectively in Table 5.6 and discussed individually below.

5.2.5.2.a GELLING TIME

The gelling time of the formulation was increased by increasing the level of alginate (Figure 5.13). Whereas increasing the level of both calcium carbonate and sodium chloride reduced gelling time. The profiles for main effects and interactions (Figure 5.14) remain unchanged for alginate and calcium when compared to Figures 5.1 and 5.2. There was an interaction between sodium chloride and calcium carbonate; at low levels



of calcium carbonate increasing the level of sodium chloride reduced the gelling time more than with higher levels of calcium carbonate.

Table 5.6 Results of the physical tests performed on each formulation, given in Table 5.5.

Test	Experiment							
	1	2	3	4	5	6	7	8
Gelling time /min	15.9	81.0	7.2	36.6	17.7	41.3	12.3	25.0
Gel Strength (G') /Pa	704	341	1106	1858	1534	714	1649	978
pH Post sterilisation	9.4	9.2	9.5	9.0	8.9	8.7	8.7	8.6
pH 48h post gelation	6.9	6.2	6.8	6.6	6.6	6.1	6.7	6.7
Zero rate viscosity /Pa.s	0.09	1.74	0.14	1.73	0.15	3.17	0.14	3.43
Rate index	0.08	0.79	0.05	0.76	0.09	0.39	0.08	0.39
Water uptake /g/g/cm <sup>2</sup>	0.008	0.024	0.004	0.021	0.004	0.011	0.000	0.008

#### 5.2.5.2.b GEL STRENGTH

Increasing the level of sodium alginate lead to a decrease in gel strength (Figure 5.15). The effects of increased levels of calcium carbonate or sodium chloride were to increase the strength of the gel. The interaction profile (Figure 5.16) highlights interactions between factors. Sodium chloride increased gel strength at lower levels of calcium carbonate and decreased the gel strength at higher levels of calcium carbonate. The effect was repeated at high levels of alginate. Gel strength was reduced by increasing the level of sodium chloride. The interaction between sodium alginate and calcium carbonate shows that calcium carbonate increased gel strength more at higher levels of sodium alginate compared to the lower levels. However, overall gel strength was lower at higher levels of sodium alginate.

#### 5.2.5.2.c pH POST STERILISATION

The main effect of sodium alginate was to reduce the pH of the formulation post sterilisation, with an increase in level (Figure 5.17). Calcium carbonate also had a negative effect on pH with an increase in level. The presence of sodium chloride in the formulation had a large negative effect compared to sodium alginate or calcium carbonate. There were no interactions present between factors (Figure 5.18).



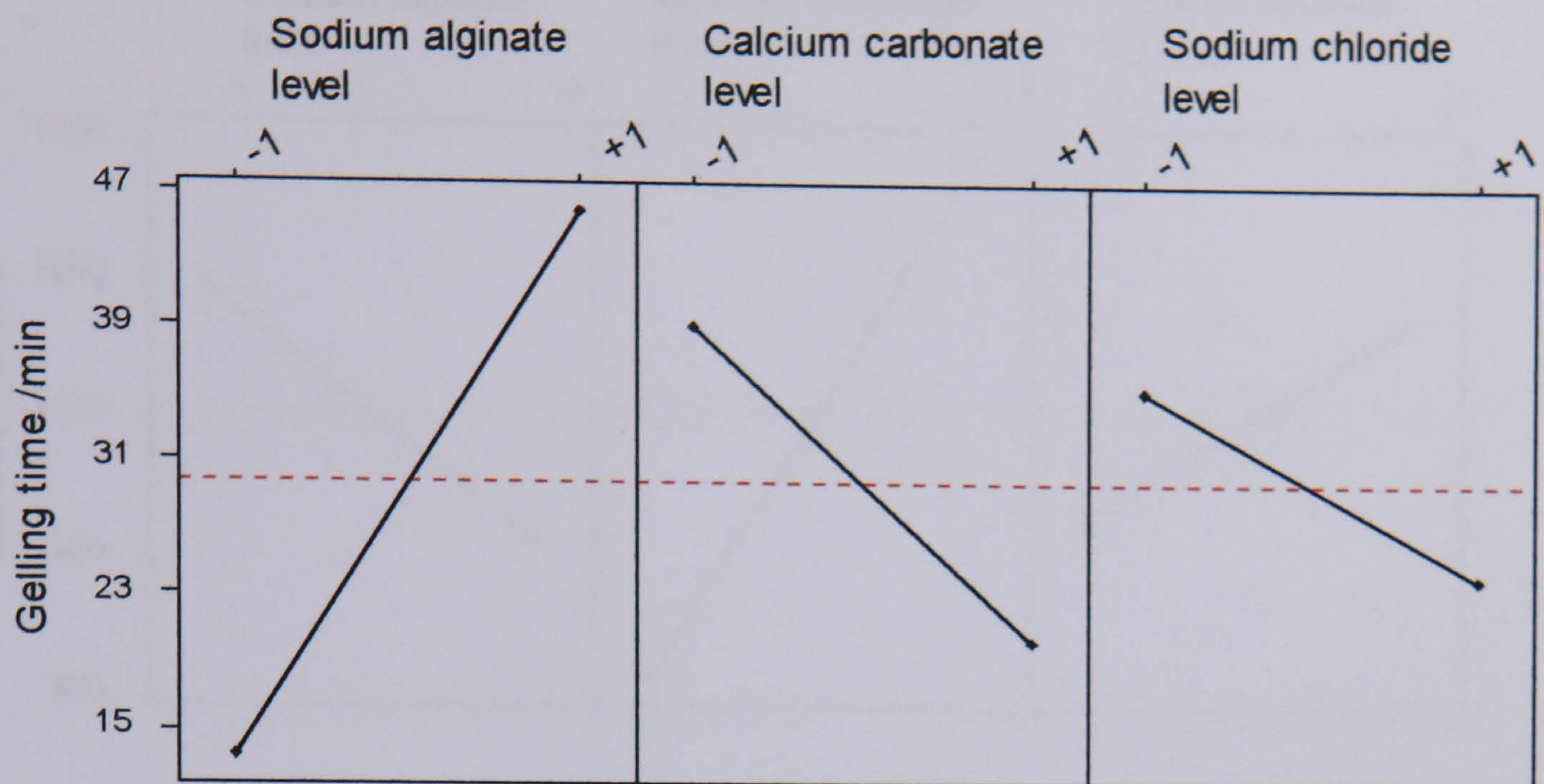


Figure 5.13 Main effects of sodium alginate, sodium chloride and calcium carbonate on gelling time.

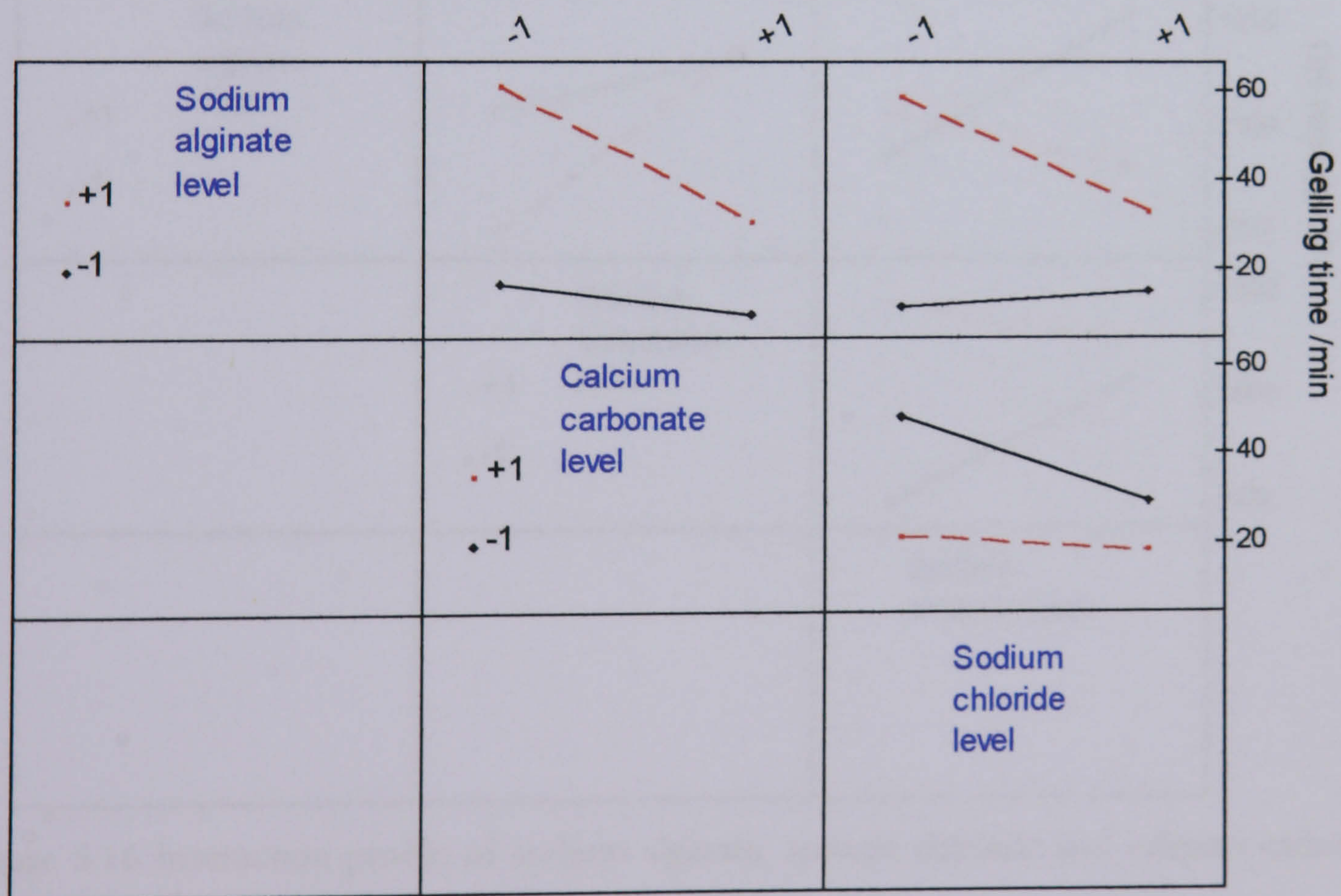


Figure 5.14 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on gelling time.



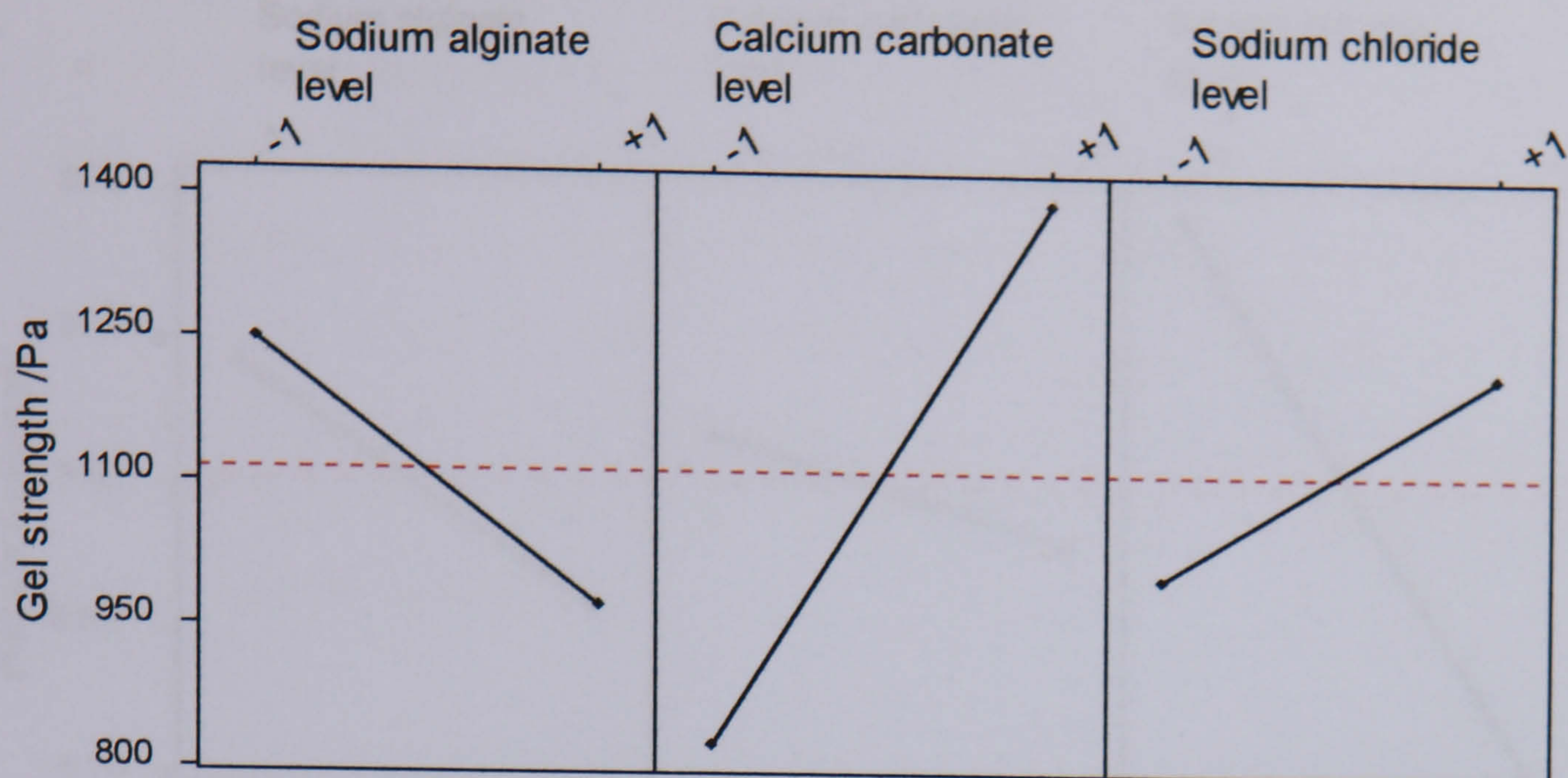


Figure 5.15 Main effects of sodium alginate, sodium chloride and calcium carbonate on gel strength.

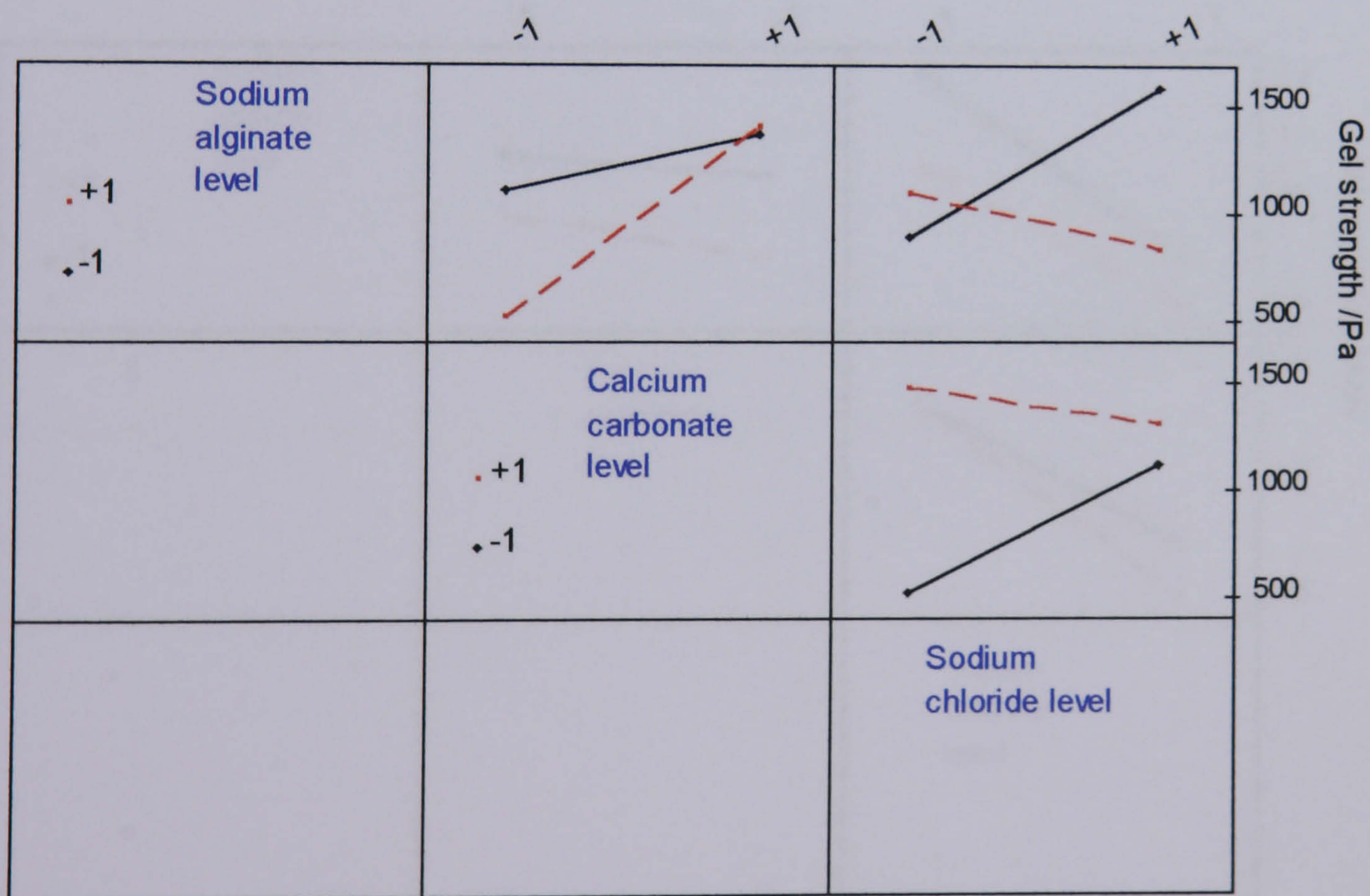


Figure 5.16 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on gel strength.



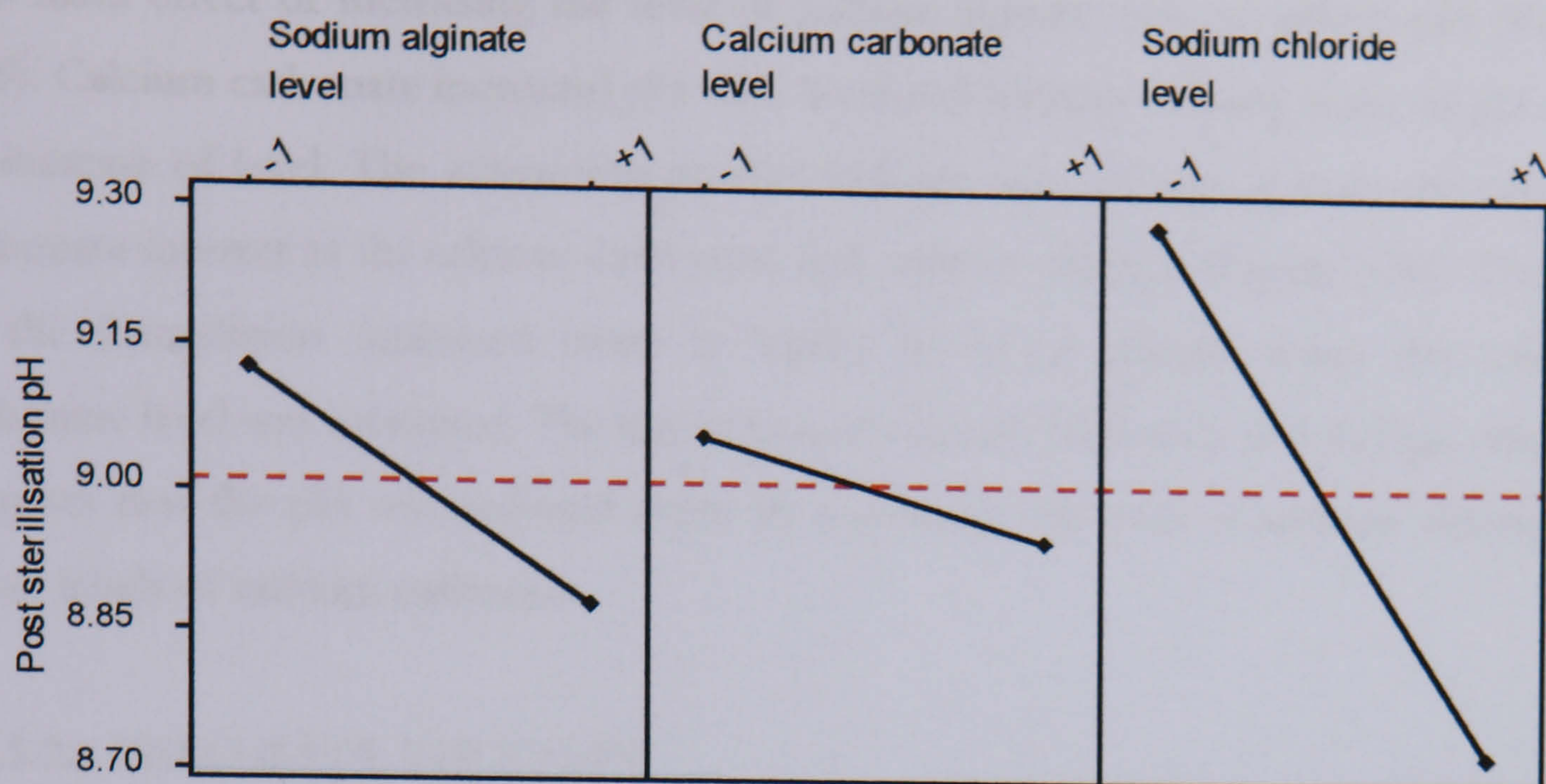


Figure 5.17 Main effects of sodium alginate, sodium chloride and calcium carbonate on post sterilisation pH.

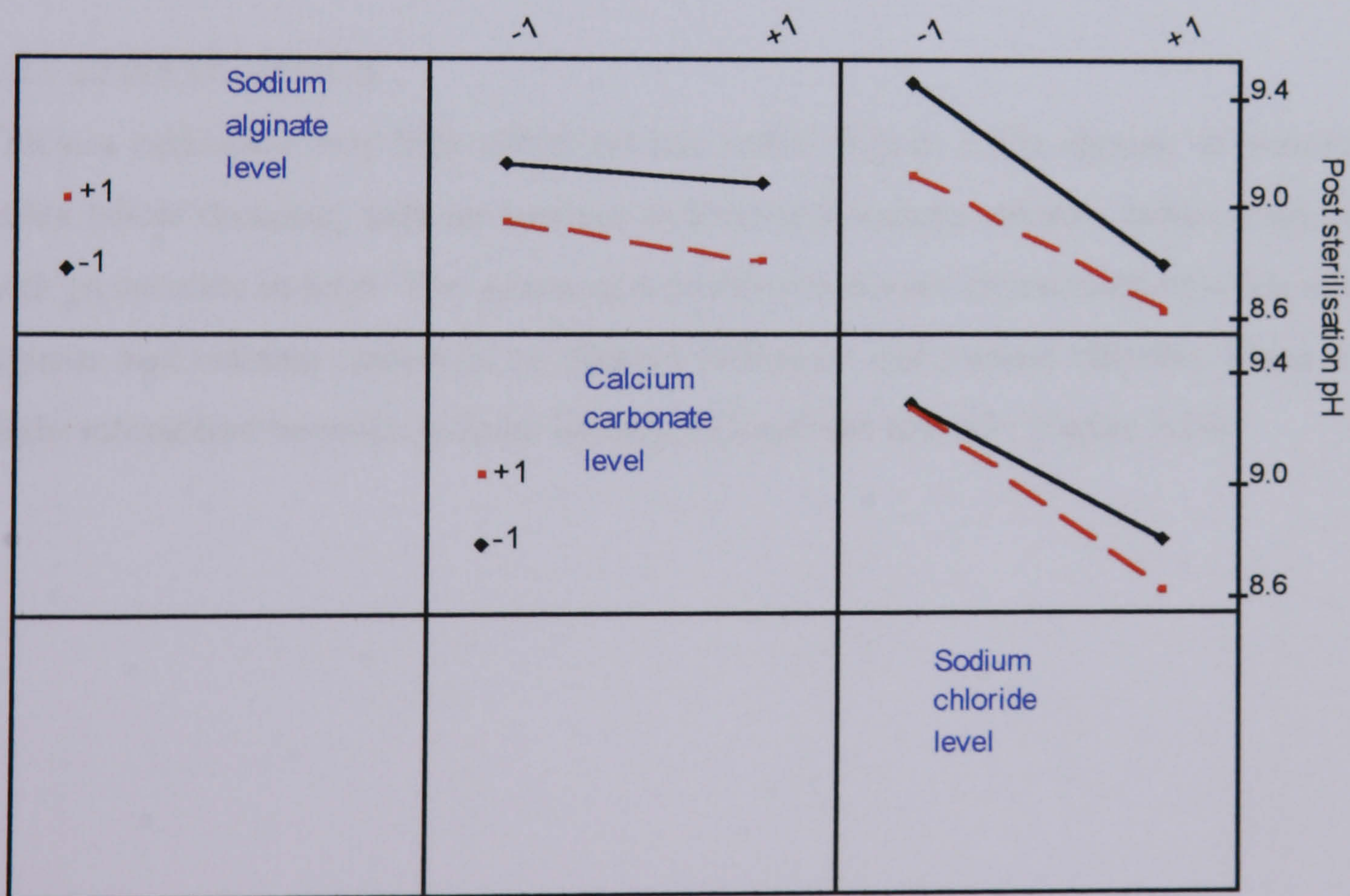


Figure 5.18 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on post sterilisation pH.



#### 5.2.5.2.d pH 48h POST GELATION

The main effect of increasing the level of sodium alginate was to reduce pH (Figure 5.19). Calcium carbonate increased pH with level and sodium chloride reduced pH with an increase of level. The interaction profiles indicate that sodium alginate and calcium carbonate interact as do calcium carbonate and sodium chloride (Figure 5.20). The pH of the formulation increased more at higher levels of alginate when the calcium carbonate level was increased. The interaction of calcium carbonate and sodium chloride suggests that the pH was reduced more by increasing the level of sodium chloride at lower levels of calcium carbonate.

#### 5.2.5.2.e ZERO RATE VISCOSITY

Calcium carbonate had a very slight effect on the viscosity of the formulation (Figure 5.21). Alginate and sodium chloride increased viscosity with an increase in level. The interaction profile shows a slight interaction between sodium alginate and sodium chloride. There were no interactions between calcium carbonate and sodium alginate or sodium chloride (Figure 5.22).

#### 5.2.5.2.f RATE INDEX

Calcium carbonate very little effect on rate index (Figure 5.23), alginate increased rate index (shear thinning) with an increase in level and sodium chloride reduced rate index with an increase in level. The interaction profile returns no interactions between sodium alginate and calcium carbonate or calcium carbonate and sodium chloride. There was a slight interaction between sodium alginate and sodium chloride (Figure 5.24).



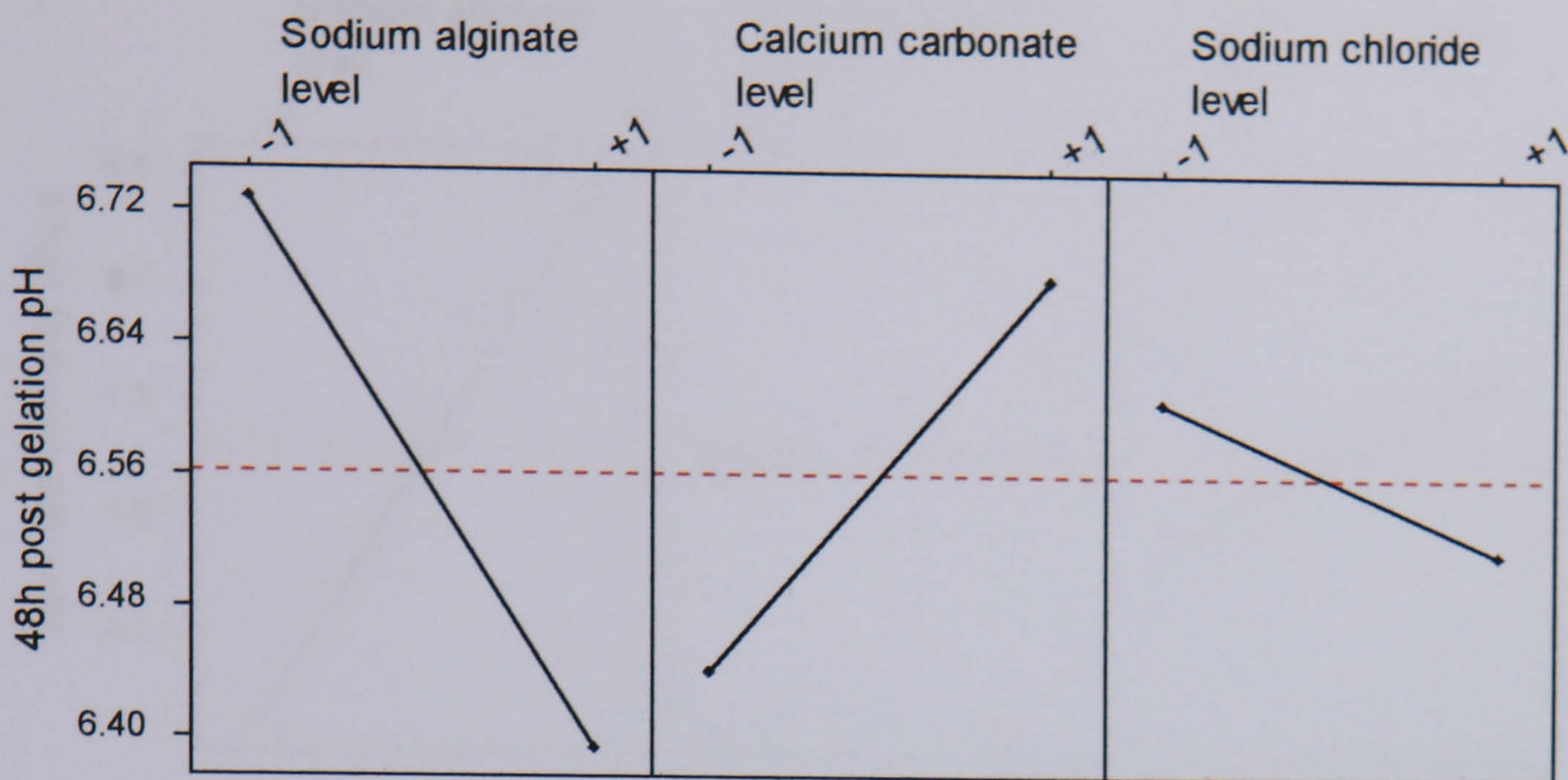


Figure 5.19 Main effects of sodium alginate, sodium chloride and calcium carbonate on 48h post gelation pH.

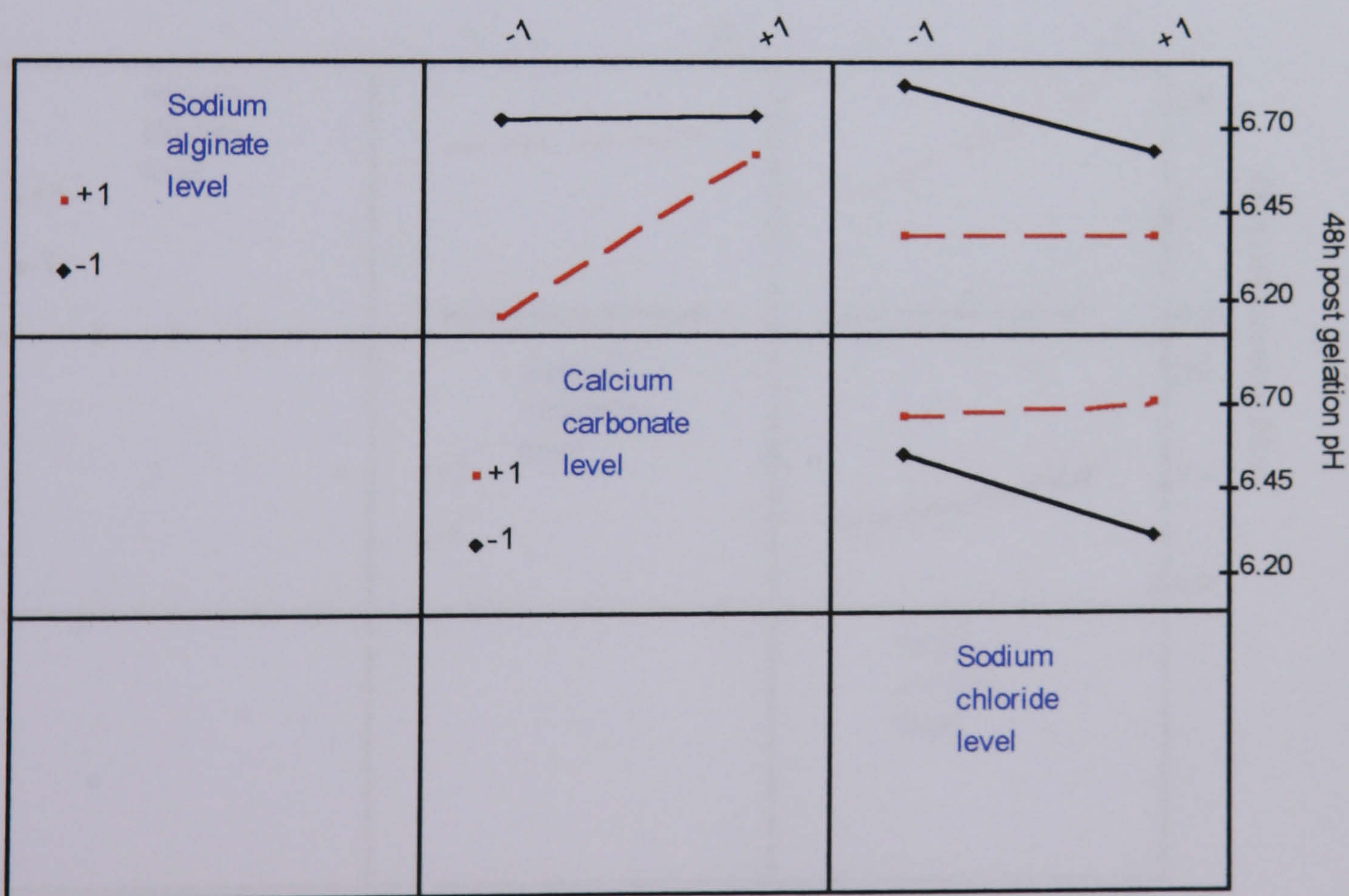


Figure 5.20 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on 48h post gelation pH.



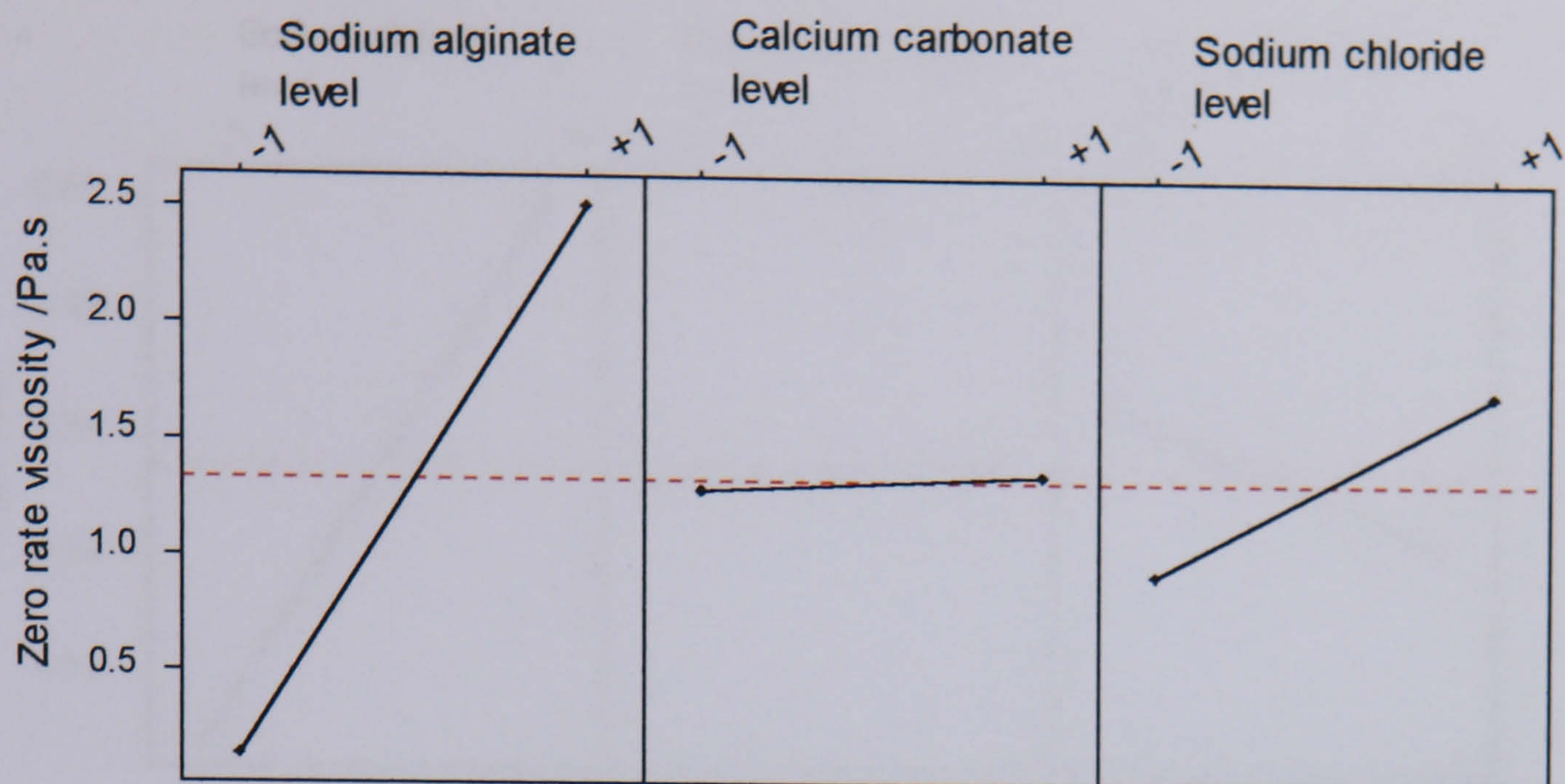


Figure 5.21 Main effects of sodium alginate, sodium chloride and calcium carbonate on zero rate viscosity

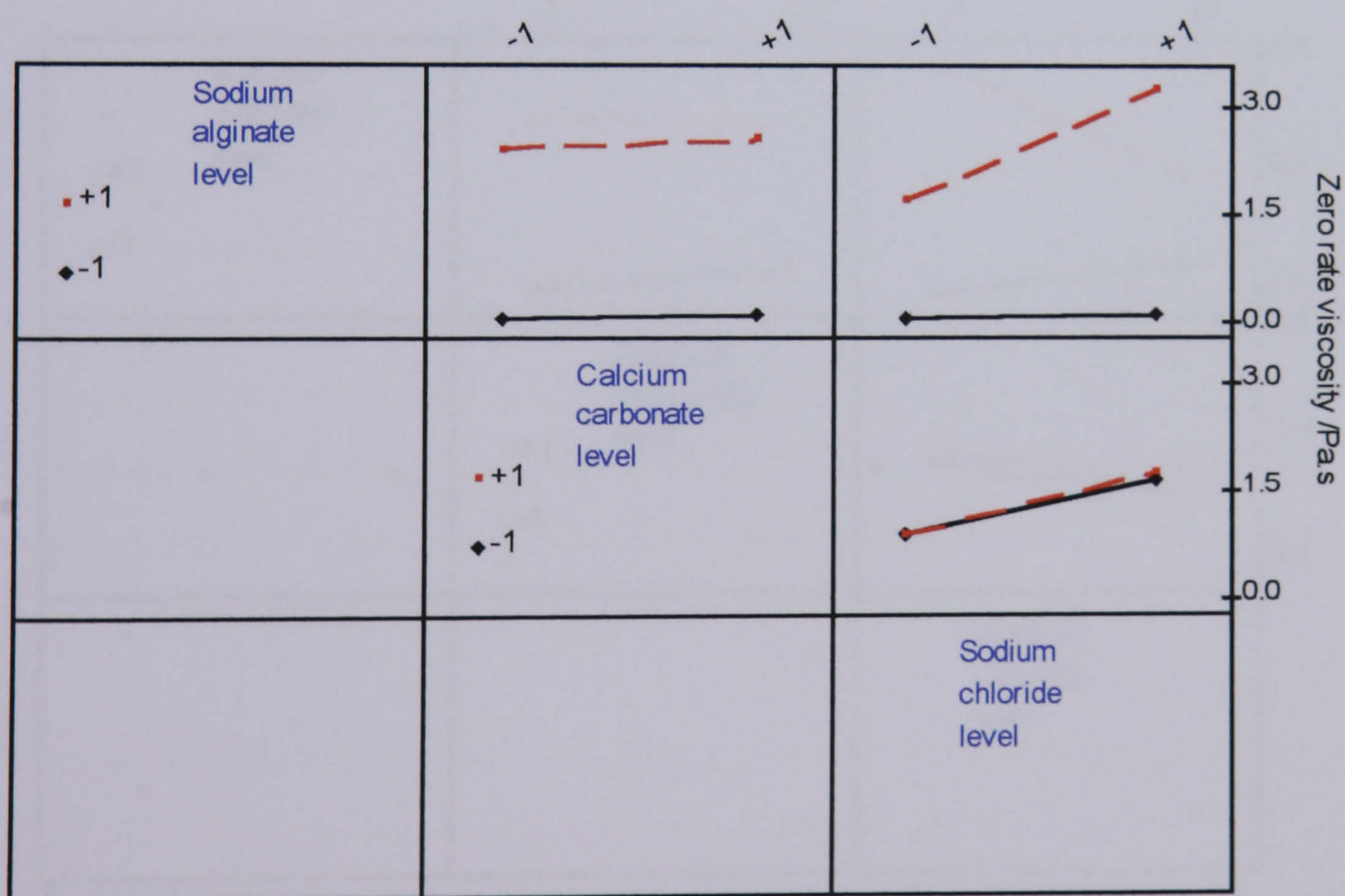


Figure 5.22 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on zero rate viscosity.



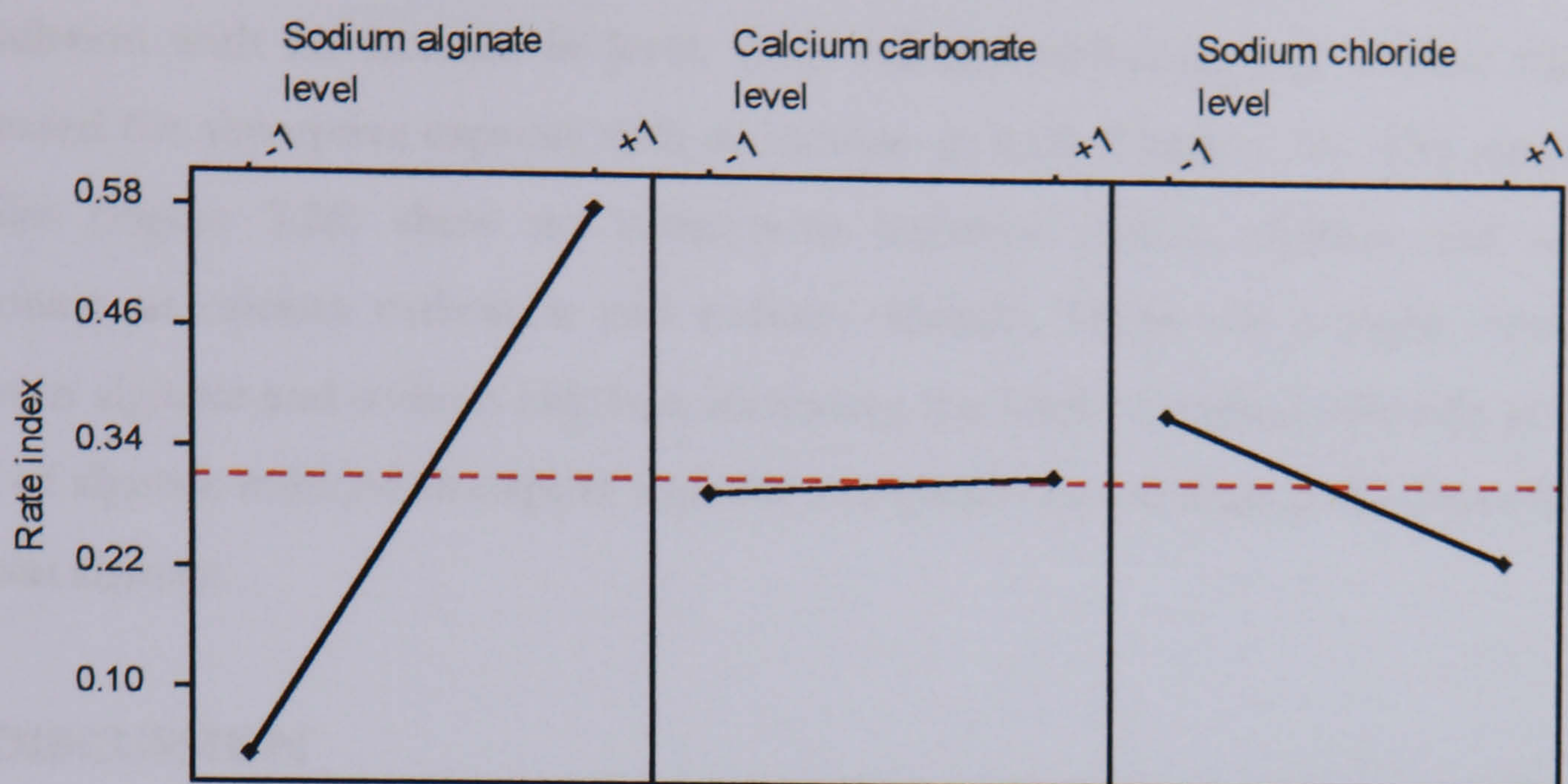


Figure 5.23 Main effects of sodium alginate, sodium chloride and calcium carbonate on rate index.

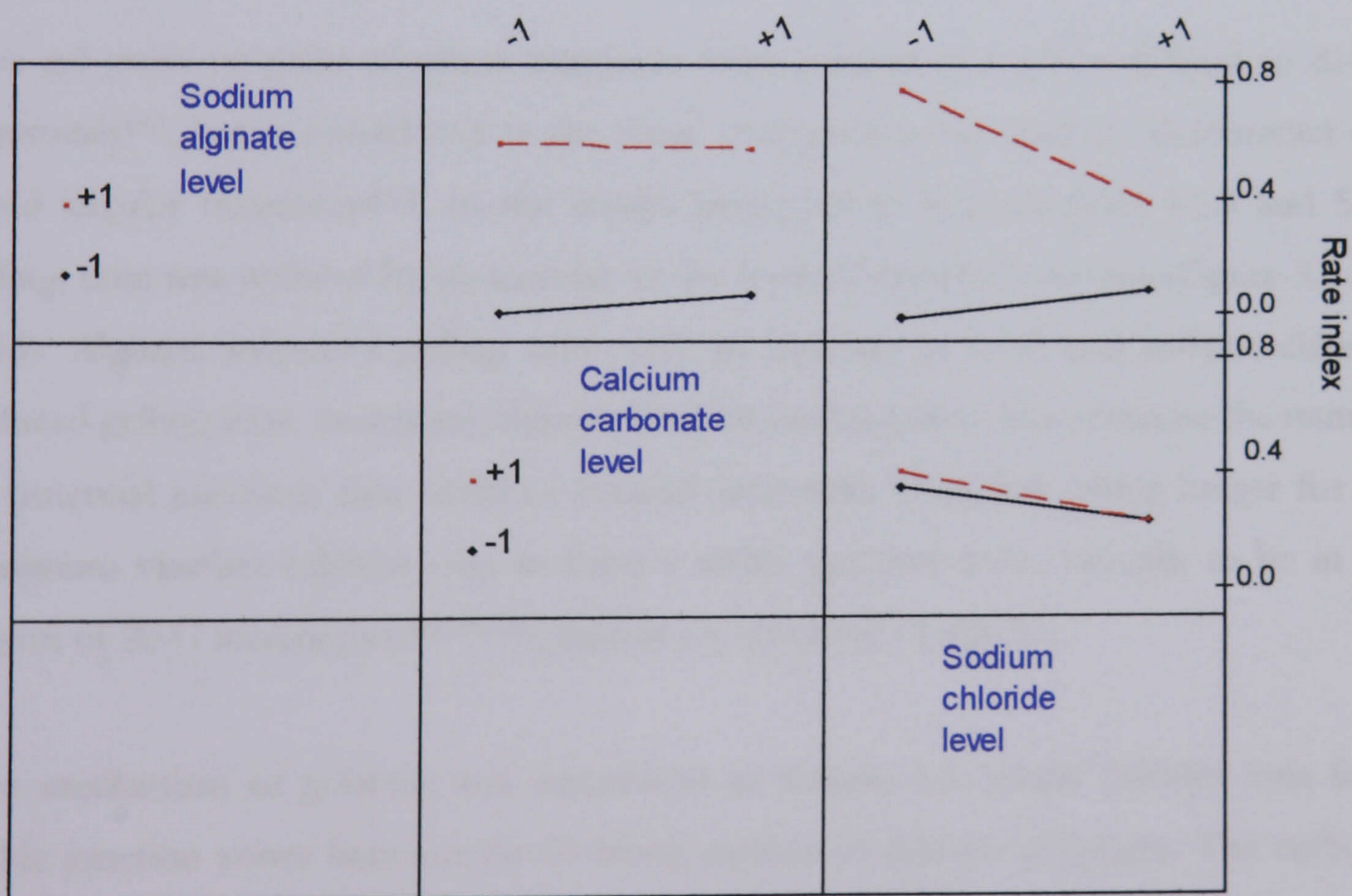


Figure 5.24 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on rate index.



#### 5.2.5.3.g WATER UPTAKE

The main effect of sodium alginate was to increase the absorptive capacity of the formulation with an increase in level. Both calcium carbonate and sodium chloride decreased the absorptive capacity with an increase in level (Figure 5.25). The interaction profiles (Figure 5.26) show no interactions between sodium alginate and calcium carbonate or calcium carbonate and sodium chloride. There was a slight interaction between alginate and sodium chloride, increasing the level of sodium chloride at higher level of alginate reduced absorptive capacity to a greater extent than at the lower level of sodium alginate.

### 5.3 DISCUSSION

This study investigated the formulation of sodium alginate, calcium carbonate, sodium chloride and delta gluconolactone as an *in-situ* gelling wound dressing. The discussion covers the investigation of sodium alginate gelled by calcium carbonate and delta gluconolactone (section 5.2.4) and the investigation of sodium alginate, calcium carbonate, sodium chloride and delta gluconolactone (section 5.2.5).

The gel point or point of phase transition from a liquid to a gel is difficult to define rigorously<sup>[89]</sup>, but is considered as the cross over point of  $G'$  and  $G''$  determined at a fixed angular frequency<sup>[132]</sup>. In the results presented in both sections 5.2.4 and 5.2.5 gelling time was reduced by an increase in the level of calcium carbonate (Figure 5.1 and 5.13). Alginate increased gelling time with an increase in level and sodium chloride reduced gelling time. Increasing alginate level increased gelling time, because the number of potential junctions that could be formed increased. Therefore taking longer for the minimum number calcium ions to form a stable junction zone, thought to be in the region of 20 G monomers <sup>[63,133,134]</sup>, than at a lower level of alginate.

The mechanism of gelation was introduced in section 1.4, where calcium ions form stable junction zones between the G block regions of alginate polymers. The carboxyl groups present on the monomers coordinate calcium ions<sup>[135]</sup>. The type of alginate used was Protanal LF 10/60 (FMC Biopolymer) which contained approximately 69% G monomers<sup>[99]</sup>. With such a high level of G monomers there was the potential to form gels rapidly. The shortest gelling time for a 2%w/w solution was 6.3 min (Table 5.3).



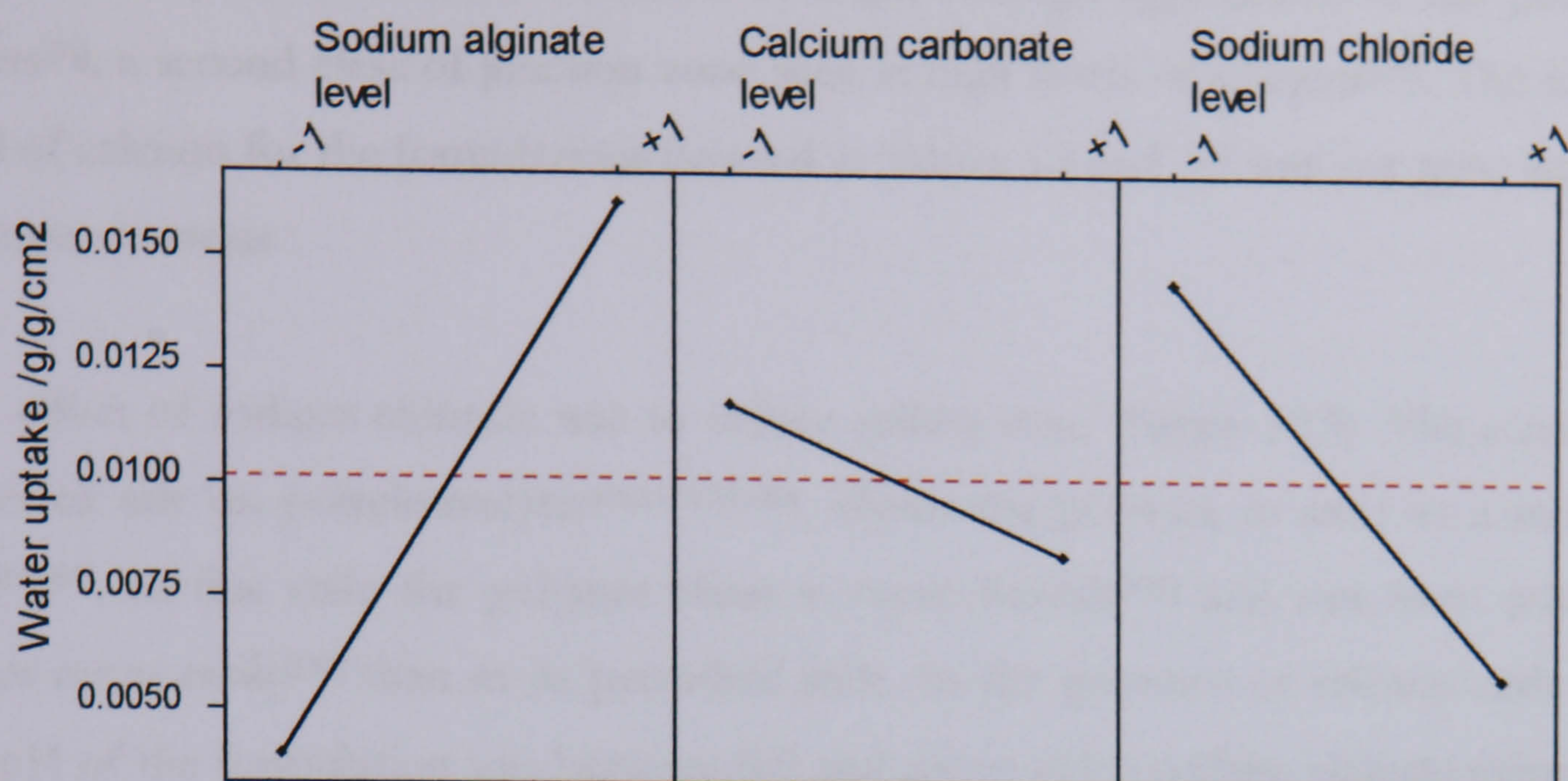


Figure 5.25 Main effects of sodium alginate, sodium chloride and calcium carbonate on water uptake.

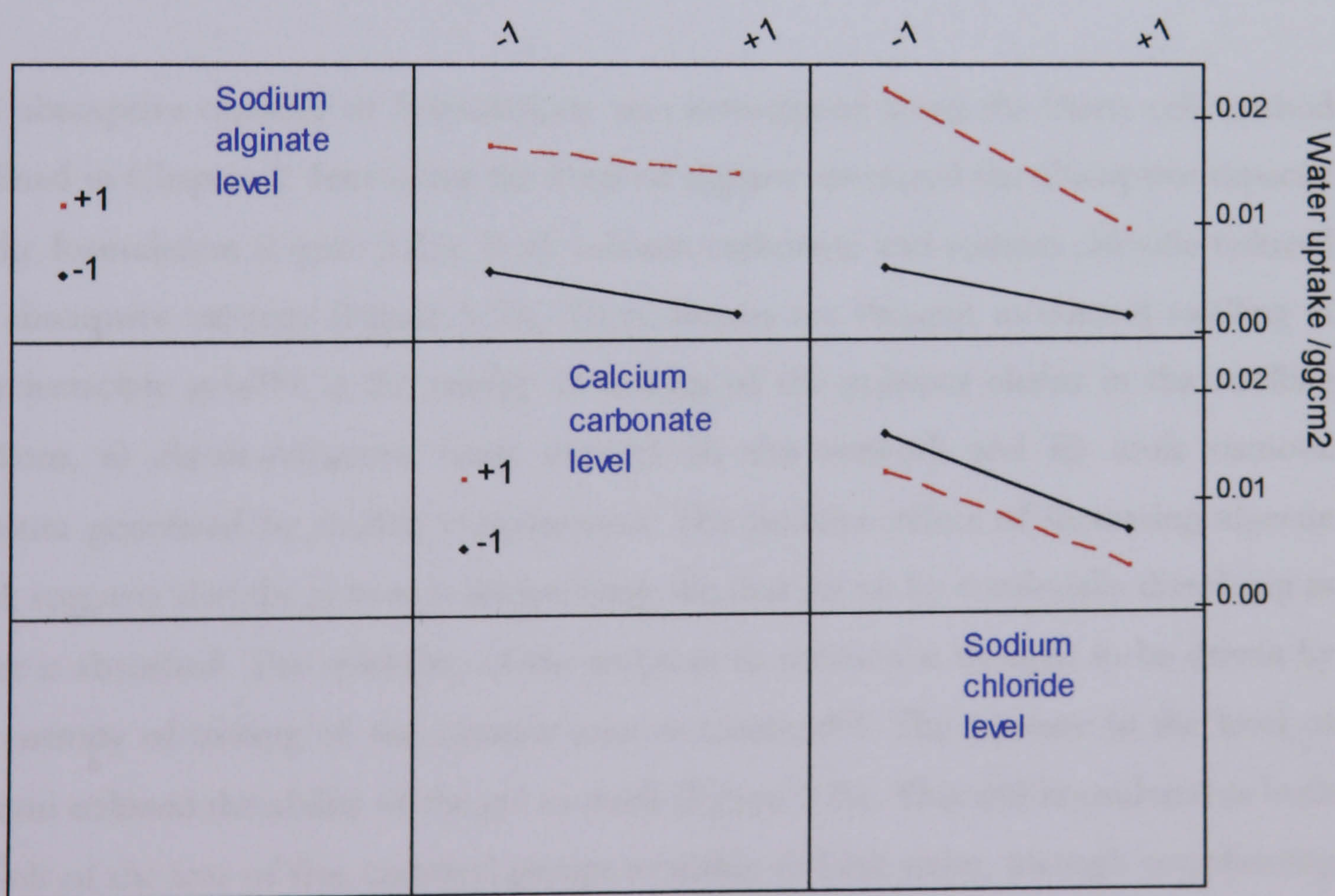


Figure 5.26 Interaction profile of sodium alginate, sodium chloride and calcium carbonate on water uptake.



The risk of using a high G block content alginate was syneresis occurring at high levels of calcium. Syneresis is the contraction of a gel, through aggregation of the polymer chains<sup>[81]</sup>, a second class of junction zone seen at high levels of calcium<sup>[136]</sup>. The higher level of calcium for the formulations detailed in Tables 5.2 and 5.5 was not great enough to cause syneresis.

The effect of sodium chloride was to reduce gelling time (Figure 5.13). The screening effect of salt on polyelectrolytes<sup>[68,137,138,139]</sup>, allows the polymer to exist as a random coil<sup>[140,66]</sup>. In this state the polymer chain is more flexible<sup>[141]</sup> and can form junction zones more easily<sup>[142]</sup> than in its perturbed state. In the presence of calcium carbonate the pH of the formulation was between 8-9 and above pH 5 sodium alginate exists as a negatively charged polyelectrolyte<sup>[86]</sup>. The presence of sodium chloride in the formulation therefore, effectively screens the negative charge and allows the polymer to maintain its form as a random coil. Sodium ions are also thought to compete with calcium ions for binding sites<sup>[135,143]</sup>, which would increase gelling time. However, the concentration where an effect occurs is thought to be approximately 0.5M<sup>[144]</sup>, which was higher than in these formulations.

The absorptive capacity of formulations was investigated using the Franz cell method outlined in Chapter 2. Increasing the level of alginate increased the absorptive capacity of the formulation (Figure 5.25). Both calcium carbonate and sodium chloride reduced the absorptive capacity (Figure 5.25). Three factors are thought to control swelling in polyelectrolyte gels<sup>[143]</sup>: i) the energy of mixing of the polymer chains in the swelling medium, ii) elastic-retractive force exerted on the network and iii) ionic osmotic pressure generated by mobile counter ions. The positive effect of increasing alginate level, suggests that the system is maintaining the first factor by continually dissolving as water is absorbed. The solubility of the polymer in solution is thought to be driven by the entropy of mixing of the counter ions in solution<sup>[62]</sup>. The increase in the level of calcium reduced the ability of the gel to swell (Figure 5.25). This was considered as both a result of the loss of free carboxyl groups available to bind water; through coordinating calcium ions and the physical constriction of the increased structure of the gel as the level of calcium carbonate was increased. The solubility of alginate and therefore its ability to absorb, could be affected by the presence of sodium chloride reducing the entropy of mixing thought to drive the solubility of alginate in aqueous systems<sup>[62]</sup>.



The strength of the gels in this study were determined by obtaining the maximum  $G'$  value during the gelation experiment. The  $G'$  value or storage modulus (section 2.2.1.4) is associated with the elastic nature of a system. Hence, the higher the value of  $G'$  the more structure in the system and therefore the stronger the system. The type of alginate used was Protanal LF10/60, an alginate containing 69% G monomers<sup>[99]</sup>. It is well documented that a high G content alginate is capable of forming strong gels<sup>[62, 74,91,145]</sup>.

In sections 5.2.4 and 5.2.5 calcium increased the strength of the gel as the level increased (Figures 5.3 and 5.15), a result of the larger number of stable junction zones forming with the increased level of calcium carbonate, therefore gel strength increased. The effect of alginate was positive in section 5.2.4 (Figure 5.3) and negative in section 5.2.5 (Figure 5.15). The presence of sodium ions in section 5.2.5 can account for this discrepancy of properties. Examining the gel strength interaction profile (Figure 5.16), for alginate, calcium carbonate, sodium chloride and their effect on gel strength immediately suggests that sodium chloride was adversely effecting gel strength. The interactions of both sodium alginate and calcium carbonate with sodium chloride had a negative profile at the higher level of alginate. Sodium ions are known to competitively bind in the G block regions of the alginate chain<sup>[135,146]</sup>. This would effect the system in two ways, firstly binding of the non-elastic chain ends. Secondly the screening effect of sodium ions increases the flexibility of polyelectrolyte chains, leading to a reduction in gel strength.

The Cross model (section 2.2.1.3) was used to determine the zero rate viscosity and the rate index of formulations. The main effect of alginate in section 5.2.4 and 5.2.5 was positive (Figures 5.9 and 5.21 respectively), increasing viscosity with an increase in level. This effect was explained in terms of polymer entanglements. As polymer concentration increased entanglement between chains increased<sup>[65,116,147,148]</sup>. As a result the viscosity increased because the force required to push polymer chains past each other increased. Calcium carbonate exhibited very little effect on viscosity (section 5.2.4 and 5.2.5).

The effect of sodium chloride on viscosity (Figure 5.21) was to increase viscosity with an increase in level. The interaction profile provides further evidence of this (Figure 5.22). The viscosity increases more at higher levels of sodium alginate in the presence of higher levels of sodium chloride.



The rate index was used to estimate the shear thinning ability of formulations. In both sections 5.2.4 and 5.2.5 increasing alginate level increased the shear thinning ability (Figure 5.23). The formulations prior to gelation were at a pH of between 8-9 pH units, the alginate polymer was therefore fully charged, as a result the molecular conformation would tend to the perturbed state (rod like). As shear increases the polymer molecules move past each other more easily and the viscosity reduces (shear thinning). Calcium carbonate had no effect, whereas the effect of sodium chloride reduced rate index (Figure 5.23), reducing the ability to shear thin. This was explained in terms of the increased flexibility of the molecules as a result of charge screening<sup>[68,137,139]</sup> and as a result of the binding of the alginate molecules by sodium ions<sup>[135,146]</sup>.

After sterilisation, formulation pH was in the pH region of 8.8-9.9. In both sections 5.2.4 and 5.2.5, alginate and calcium decreased and increased the pH respectively, in keeping with their respective pKa values: 3.5-4 sodium alginate and 9.5 calcium carbonate. The effect of sodium chloride was to reduce the pH of the formulation.

## 5.5 SUMMARY

The gelation system of sodium alginate, calcium carbonate and delta gluconolactone proved to be an excellent system that could be used as an *in-situ* gelling wound dressing. The presence of sodium chloride in the system had detrimental effects as regard to water uptake. The high pH of the system, as a result of calcium carbonate, could be detrimental to long term stability. Investigations by Haug *et al*<sup>[71]</sup> observed a decrease in alginate solution viscosity at very high and very low pH values, a result of chain cleavage. However, in the region pH 5-10 the rate of degradation was seen as slow. Further work will be required to develop this system as a usable *in-situ* gelling wound dressing.



## **CHAPTER 6 THE USE OF A SCREENING EXPERIMENTAL DESIGN TO INVESTIGATE FACTORS IN THE DEVELOPMENT OF AN *IN-SITU* GELLING ALGINATE FORMULATION**

### **6.1 INTRODUCTION**

The investigations detailed in Chapter 5 proved that the calcium carbonate, delta gluconolactone gelling system had potential as an *in-situ* gelling wound dressing. To further investigate and improve the formulation, a Plackett and Burman experimental design was used to screen ten factors at two levels. Experimental design was introduced in Chapter 3. The factors investigated were 1) Sodium alginate concentration, 2) Sodium alginate type, 3) Calcium carbonate concentration, 4) Sodium carboxymethyl cellulose viscosity, 5) Sodium carboxymethyl cellulose concentration, 6) Glycerol concentration, 7) Hyaluronic acid concentration, 8) Sodium chloride concentration, 9) Buffer concentration and 10) Gelling temperature. The ratio of calcium carbonate to delta gluconolactone was kept at 1:2, with the intention of keeping the pH of the formulation at or below 7<sup>[74]</sup>.

### **6.2 EXPERIMENTAL**

#### **6.2.1 Materials**

Sodium alginate, LF 10/60, LF 10/60LS and LF 10/60D, FMC Biopolymer, Drammen, Norway. Calcium carbonate, Sturcal f, Specialty Minerals, Birmingham, England. Glycerol, Spectrophotometric grade (99.5%), Sigma Aldrich, Gillingham, Dorset, England. Hyaluronic acid, MV, FMC Biopolymer, Drammen, Norway. Di-sodium hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset, England. Potassium di-hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset, England. Sodium Chloride, AR, BDH Laboratory Supplies, Poole, Dorset, England. Sodium carboxymethyl cellulose, low, medium and high viscosity, Fluka Chimie AG, Buckinghamshire, England. Hydrochloric acid, AR, BDH Laboratory Supplies, Poole, Dorset, England. Delta gluconolactone, Aldrich Chemical Company, Gillingham, Dorset, England.

#### **6.2.2 Equipment**

Oertling Balance, 4 decimal places, Oertling, Smethwick, West Midlands, England. Carrimed Rheometer, CSI<sup>2</sup> 500, TA Instruments Ltd. Surrey, England. Bench top



concentration, b4) Carboxymethyl cellulose viscosity, b5) Carboxymethyl cellulose concentration, b6) Glycerol concentration, b7) Hyaluronic acid concentration, b8) Sodium chloride concentration, b9) Buffer concentration and b10) Gelling temperature.

Table 6.2 Plackett and Burman screening experimental design for 10 factors at 2 levels, with a central domain (coded 0) to estimate the precision of the experiment.

Factor → Experiment N°↓	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>
1	-	-	-	-	-	-	-	-	-	-
2	+	+	-	+	+	+	-	-	-	+
3	-	+	+	-	+	+	+	-	-	-
4	+	-	+	+	-	+	+	+	-	-
5	-	+	-	+	+	-	+	+	+	-
6	-	-	+	-	+	+	-	+	+	+
7	-	-	-	+	-	+	+	-	+	+
8	+	-	-	-	+	-	+	+	-	+
9	+	+	-	-	-	+	-	+	+	-
10	+	+	+	-	-	-	+	-	+	+
11	-	+	+	+	-	-	-	+	-	+
12	+	-	+	+	+	-	-	-	+	-
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0

Table 6.3 Results of the characterisation tests carried out on each of the sixteen formulations in the Plackett and Burman experimental design. The sterile pH was obtained from the liquid formulation at 0h and the gel 48h post gelation.

Experiment	Gelling	Water uptake	Viscosity		Gel Strength	Sterile pH	
	Time /min	24h (g/g/cm <sup>2</sup> 24h)	Sterile /Pa.s	Rate index	Max G' /Pa	0h	48h
1	15.642	0.018	0.145	0.268	91.6	9.03	6.63
2	67.200	0.038	11.23	0.596	979.3	8.89	6.43
3	6.118	0.011	0.428	0.547	6401	9.24	6.61
4	15.394	0.014	102.9	0.366	1480	8.30	6.37
5	45.492	0.006	3.416	0.488	367.7	8.65	6.37
6	5.618	0.010	0.565	0.478	203.1	8.37	6.71
7	19.736	0.021	1.400	0.459	352.2	8.92	6.47
8	79.867	0.015	29.34	0.339	225.5	8.41	6.20
9	89.817	0.018	4.993	0.562	293.6	8.56	6.35
10	23.782	0.021	3.861	0.653	2190	8.67	6.92
11	2.905	-0.002	0.368	0.457	1643	8.70	6.73
12	30.417	0.027	26.95	0.441	1488	8.46	6.99
13	21.378	0.015	3.207	0.464	583.5	8.31	6.40
14	20.143	0.013	2.651	0.455	417.8	8.23	6.33
15	19.067	0.013	3.889	0.430	491.0	8.30	7.02
16	17.713	0.015	3.236	0.436	523.6	8.31	6.44



Table 6.4 The effects of factors (b1-b10) for each characterisation test, calculated using Minitab®.

Factor Effect	Gelling	Water uptake	Viscosity		Gel strength	Sterile pH	
	Time /min	24h (g/g/cm <sup>2</sup> 24h)	Sterile /Pa.s	Rate index	Max G' /Pa	0h	48h
b1	17.581	0.006	14.413	0.022	-200.183	-0.135	-0.022
b2	-0.612	-0.001	-9.482	0.068	821.875	0.098	0.038
b3	-19.46	-0.003	7.046	0.019	924.600	-0.060	0.157
b4	-3.308	0.001	8.911	-0.004	-257.883	-0.030	-0.005
b5	5.620	0.001	-3.478	0.010	301.183	-0.013	-0.013
b6	0.482	0.002	4.786	0.030	308.617	0.030	-0.075
b7	-1.767	-0.002	8.091	0.004	526.483	0.015	-0.075
b8	6.350	-0.006	8.131	-0.023	-607.433	-0.185	-0.110
b9	2.311	0.001	-8.602	0.042	-493.817	-0.078	0.070
b10	-0.314	0.001	-7.672	0.026	-377.400	-0.023	0.012
Limiting value	1.433	0.001	0.303	0.015	77.070	0.035	0.051

6.3.1 The Effects Profile Of Factors Influencing Gelling Time

The factors with the greatest influence over gelling time (Figure 6.1) were alginate concentration (b1) increasing gelling time and calcium concentration (b3) decreasing gelling time, as expected. The effect of sodium chloride (b8) was to increase gelling time, whereas in section 5.2.4 sodium chloride reduced gelling time. Sodium chloride effects polyelectrolytes by reducing the long range Coulombic interactions, allowing the polymer molecule to form its unperturbed state<sup>[149]</sup>. The increased gelling time could be a result of competitive binding between calcium and sodium ions or the result interactions in a more complicated system. The level of buffer (b9) was active, retarding<sup>[93]</sup> but not preventing gelation. The reduction of gelling time with Hyaluronic acid level (b7) and carboxymethyl cellulose viscosity (b4) could be associated with the effect of calcium, to which both are known to be sensitive<sup>[75,150]</sup>.

6.3.2 The Effects Profile Of Factors Influencing Water Uptake

Sodium alginate (b1) had a large positive effect, increasing water uptake as seen in the effects profile (Figure 6.2). The effect of calcium (b3) was to reduce absorptive capacity. The high activity of sodium chloride (b8) reducing the absorptive capacity, was possibly a result of a combination of factors. Sodium chloride reduces the ability of polyelectrolyte gels to swell<sup>[151,152]</sup> this would reduce the absorptive capacity. Sodium chloride may also reduce the solubility<sup>[62]</sup> of sodium alginate, by affecting the entropy of mixing<sup>[62]</sup>, also reducing the absorptive capacity. Hyaluronic acid (b7) had a negative



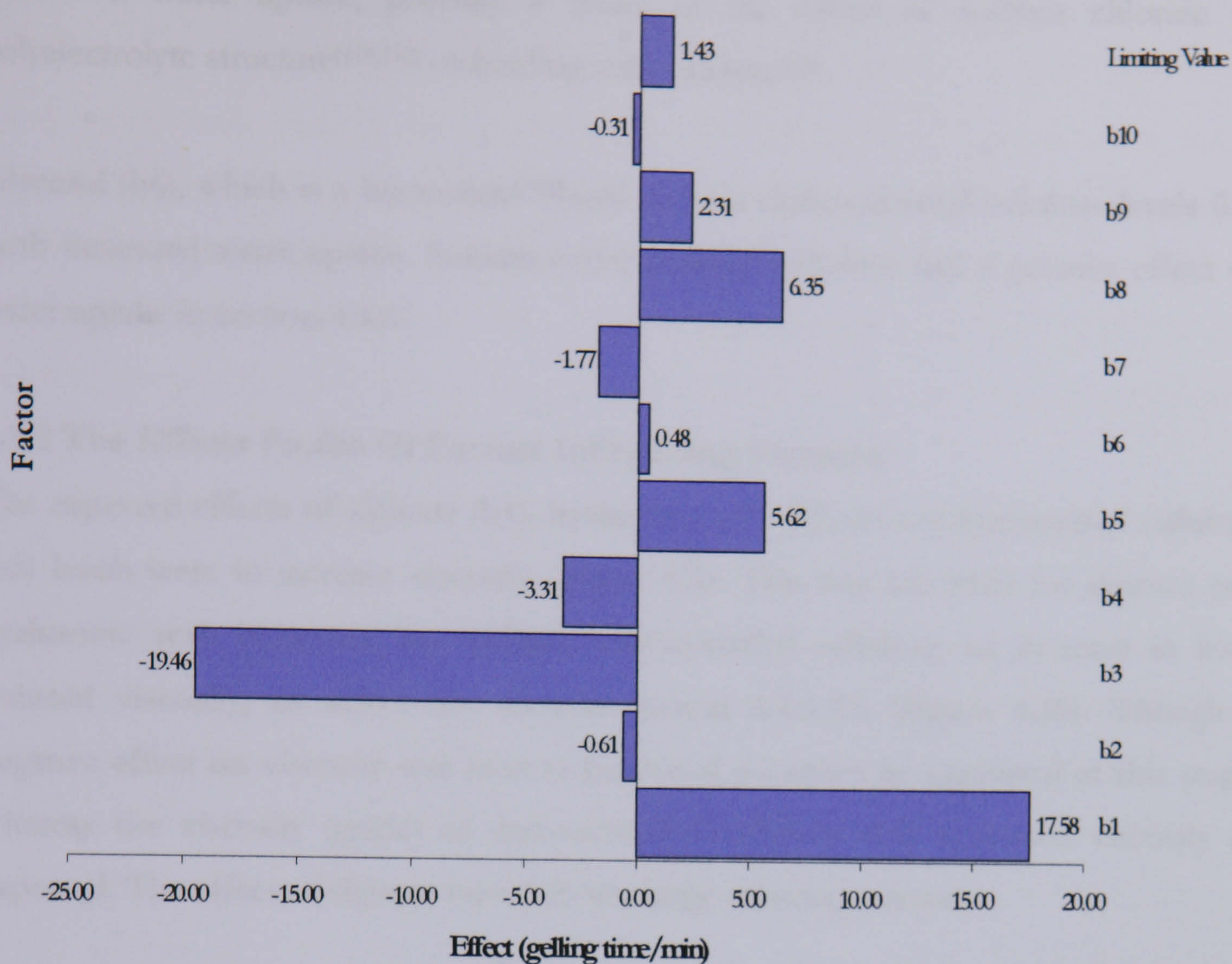


Figure 6.1 The effects profile of factor influence over gelling time (factors b1-b10).

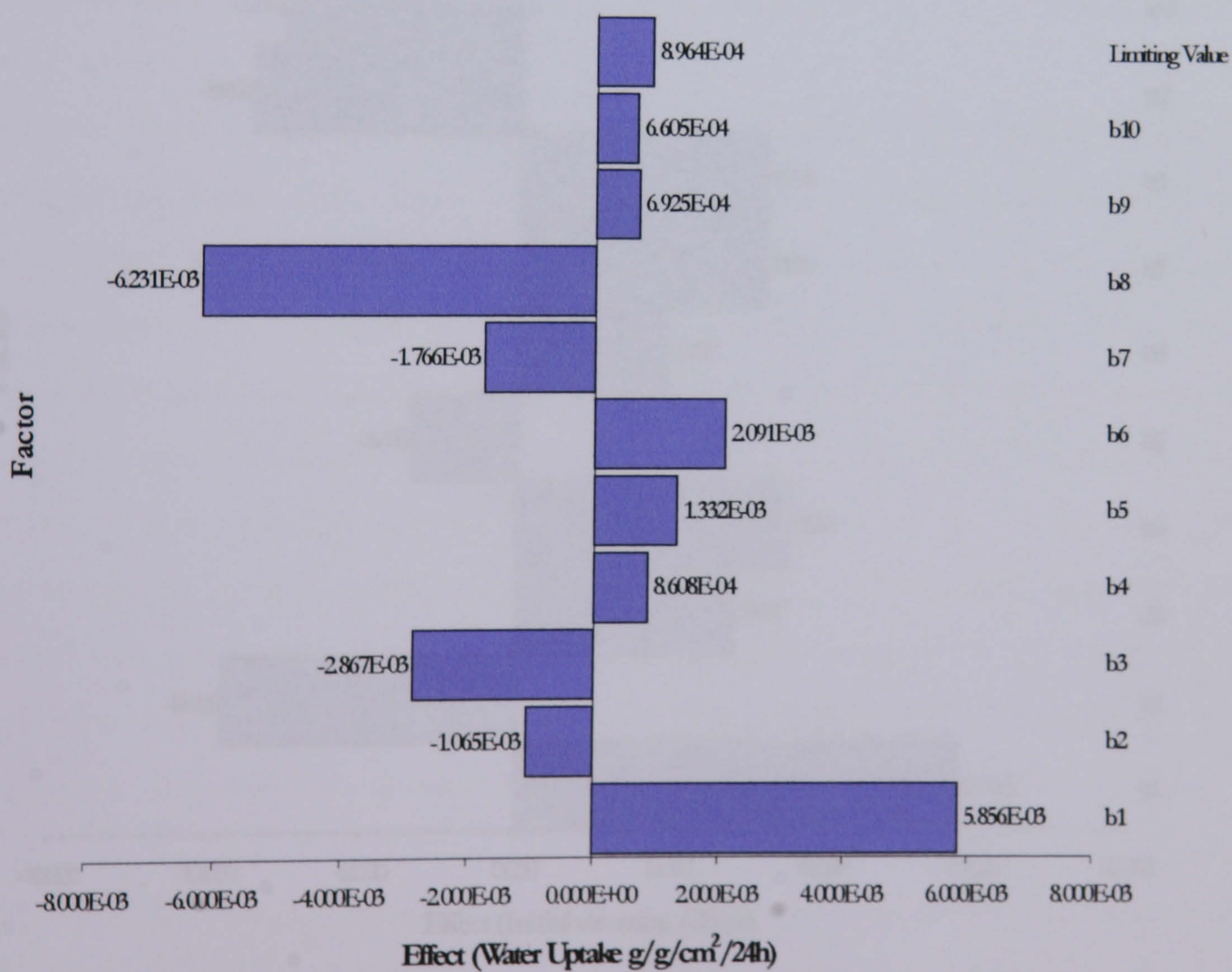


Figure 6.2 The effects profile of factor influence over water uptake (factors b1-b10).  
 $XE-y = X \times 10^{-y}$ .



effect on water uptake, possibly a result of the effect of sodium chloride on polyelectrolyte structure<sup>[149,152]</sup> or binding with calcium<sup>[150]</sup>.

Glycerol (b6), which is a humectant<sup>[153]</sup> and sodium carboxymethyl cellulose levels (b5) both increased water uptake. Sodium carboxymethyl cellulose had a positive effect on water uptake in section 4.4.6.

6.3.3 The Effects Profile Of Factors Influencing Viscosity

The expected effects of alginate (b1), hyaluronic acid (b7) and carboxymethyl cellulose (b5) levels were to increase viscosity (Figure 6.3). This was recorded for alginate and hyaluronic acid. However, for sodium carboxymethyl cellulose, an increase in level reduced viscosity, an effect not seen in section 4.4.6.2.b (Figure 4.30). Though a negative effect on viscosity was seen as beneficial it cannot be explained at this stage, whereas the viscosity (grade) of carboxymethyl cellulose (b4) increased viscosity as expected. The effect of alginate type (b2) was large reducing viscosity.

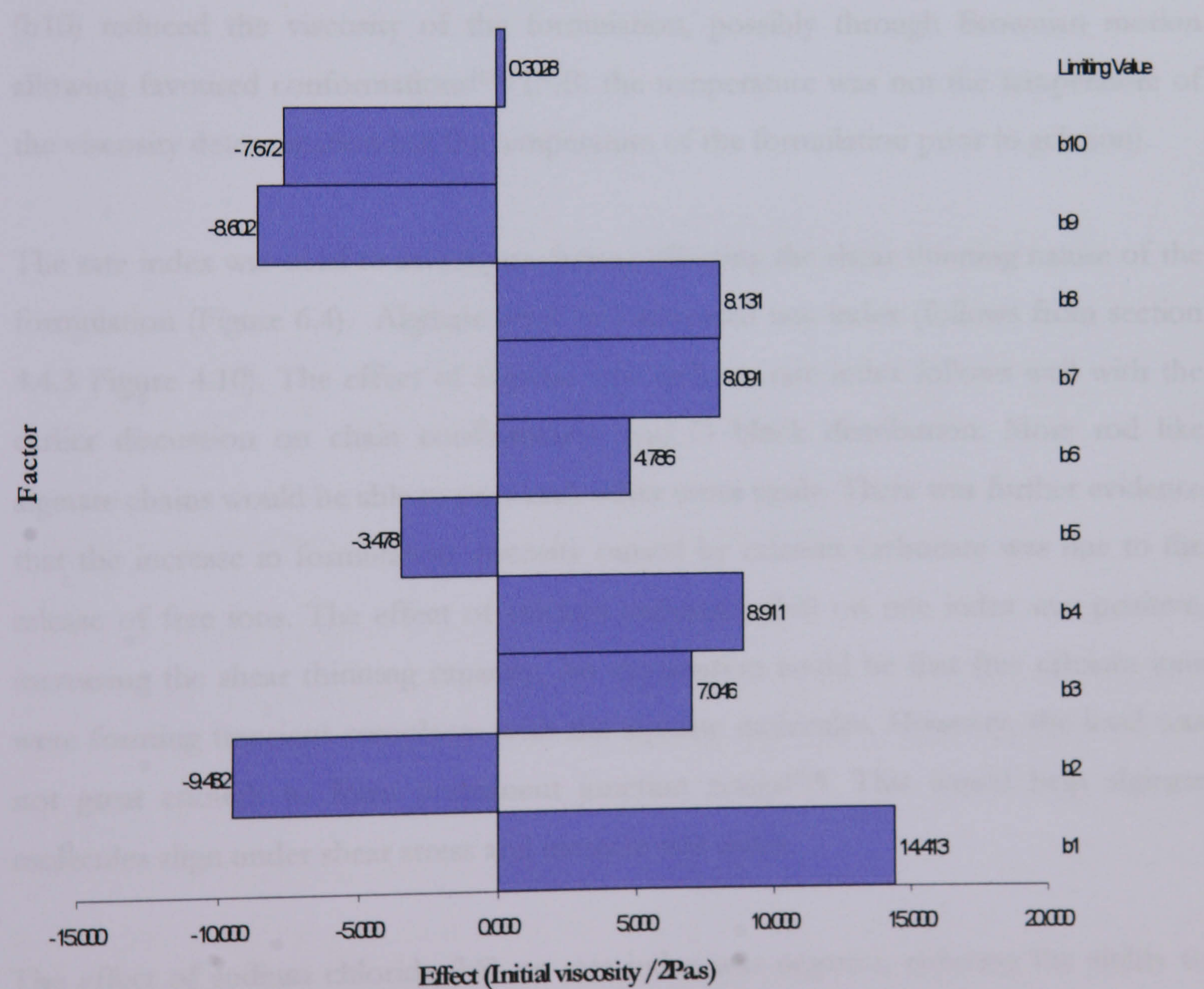


Figure 6.3 The effects profile of factor influence over viscosity (factors b1-b10).



The molecular weights were of equivalent magnitude and were not expected to affect the effects profile. The ratios of different block regions in the polymer may be the cause, restricted rotation about the glycosidic bond<sup>[154,155]</sup> induces a rod like conformation in alginates of high gulopyranuronic acid (G) content<sup>[62,140,145]</sup>. The effects of sodium chloride on conformation are to encourage the unperturbed conformation<sup>[62,149]</sup> causing the viscosity to decrease, through charge shielding. The effect of sodium chloride (b8) was positive, increasing viscosity with an increase in level.

The effect of insoluble calcium carbonate (b3) increasing viscosity was unexpected and was not thought to be due to its effect on pH (section 6.3.4). Calcium carbonate (b3) reduced pH (section 6.3.4 Figure 6.5) as did buffer (b9), but buffer reduced viscosity. The positive effect caused by calcium carbonate could therefore be due to the release of calcium ions. The effect of buffer (b9) reduced viscosity, and was explained by the variation of counter ion concentration with pH. As pH is lowered the distance between fixed charges increases<sup>[156]</sup> encouraging the perturbed conformation<sup>[116]</sup>. Temperature (b10) reduced the viscosity of the formulation, possibly through Brownian motion allowing favoured conformations<sup>[134]</sup> (NB: the temperature was not the temperature of the viscosity determination but the temperature of the formulation prior to gelation).

The rate index was used to investigate factors effecting the shear thinning nature of the formulation (Figure 6.4). Alginate level (b1) increased rate index (follows from section 4.4.3 Figure 4.10). The effect of alginate type (b2) on rate index follows well with the earlier discussion on chain conformation and G block distribution. More rod like alginate chains would be able to pass each other more easily. There was further evidence that the increase in formulation viscosity caused by calcium carbonate was due to the release of free ions. The effect of calcium carbonate (b3) on rate index was positive, increasing the shear thinning capacity. An explanation could be that free calcium ions were forming transient complexes with the alginate molecules. However, the level was not great enough to form permanent junction zones<sup>[134]</sup>. This would help alginate molecules align under shear stress and increase rate index.

The effect of sodium chloride (b8) on rate index was negative, reducing the ability to shear thin. Which suggests that a polyelectrolyte tending toward the unperturbed dimensions, *i.e.*, from the effects of sodium chloride, reduced the capacity of the



formulation to shear thin. The effects of temperature (b10) increasing shear thinning were explained from the earlier discussion about increasing alignment. Buffer (b9) increased rate index, the reduction of pH improved chain alignment (see earlier discussion in this section). Alginate molecules would therefore be able to slide past each other more easily under increased shear stress, increasing rate index.

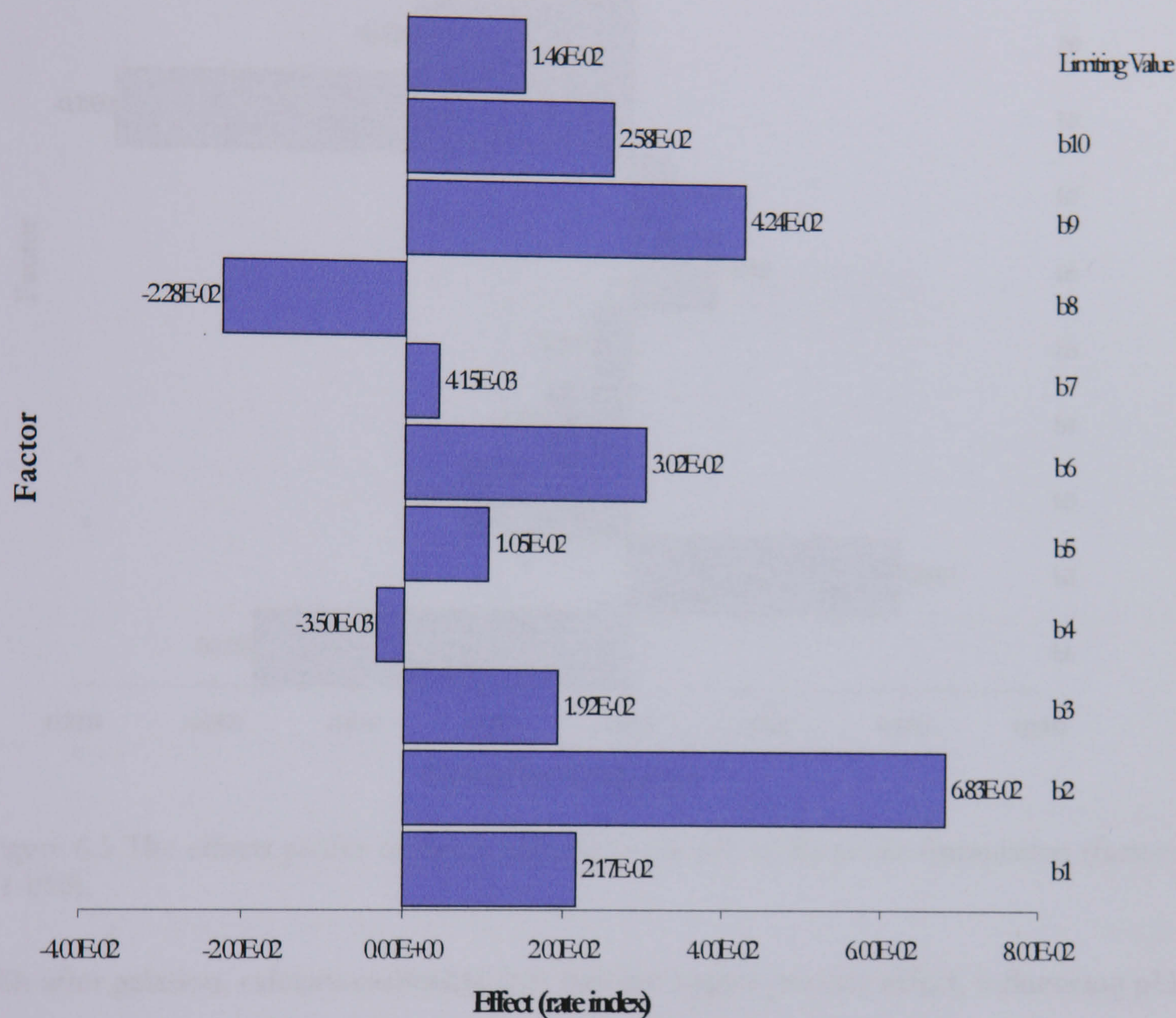


Figure 6.4 The effects profile of factor influence over rate index (factors b1-b10).

6.3.4 The Effects Profile Of Factors Influencing pH

The influence of factors on the pH of the sterile formulations were as follows. Sodium chloride (b8) reduced pH (Figure 6.5). The reasons for this remain unknown, sodium chloride is not normally associated with pH effects. Calcium carbonate (b3) reduced pH, also unexpected because calcium carbonate has a pKa of 9.5<sup>[91]</sup>. The presence of the buffer (b9), reducing pH, moderated the effects of any impurities in the AR grade sodium chloride. The reduction in pH caused by increasing sodium alginate level (b1) relates directly to the presence of more uronic acid groups: glucuronic acid (G) and mannuronic acid (M), pKa of between 3-4<sup>[62]</sup>. The effect of alginate type (b2) increasing



pH was unclear, because the pKa's of gulcuronic acid (G) and mannuronic acid (M) are comparable<sup>[62, 157]</sup>.

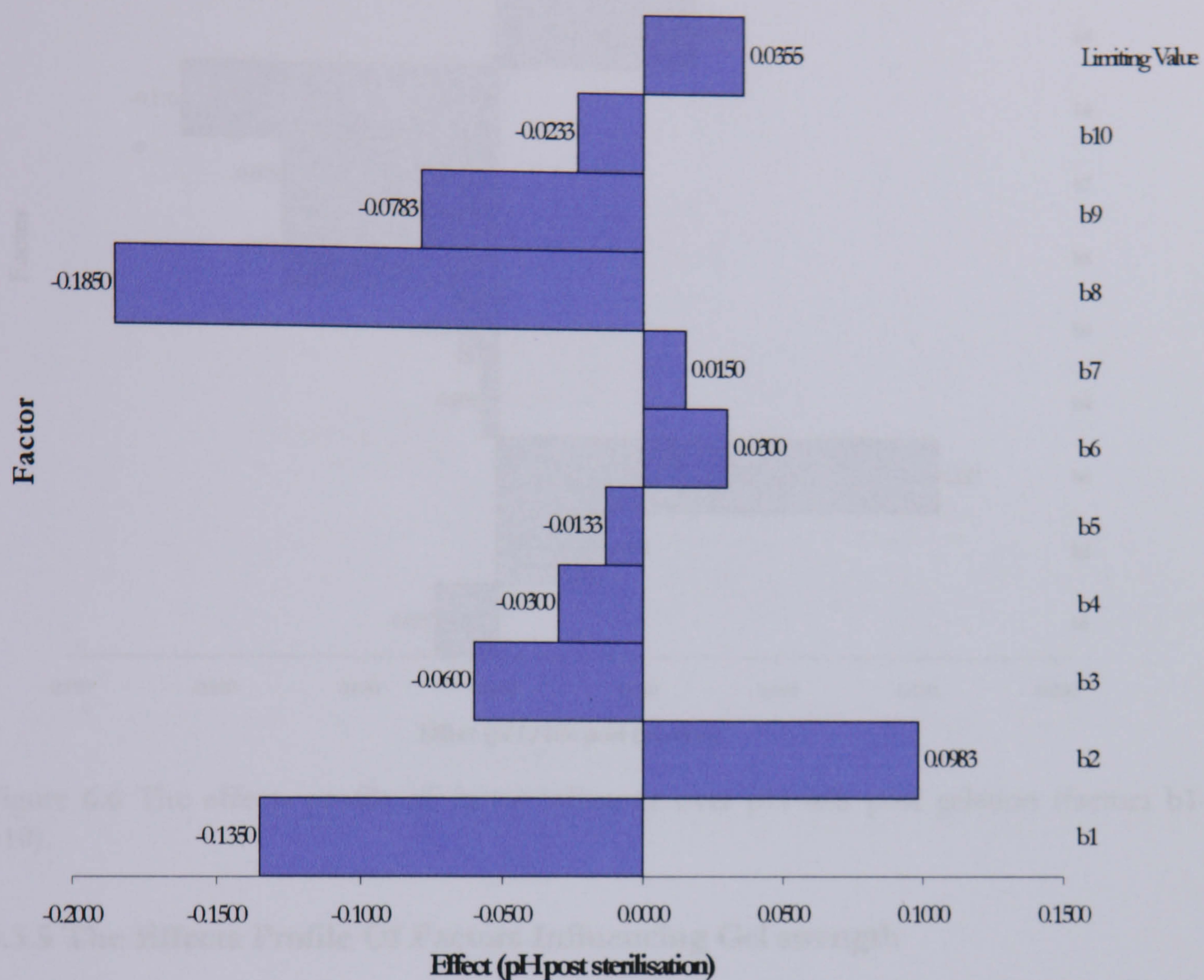


Figure 6.5 The effects profile of factor influence over pH of the sterile formulation (factors b1-b10).

48h after gelation, calcium carbonate (b3) had the largest positive effect, influencing pH (Figure 6.6). Delta gluconolactone was linked to the calcium carbonate level in a ratio of 2:1 to ensure the complete reaction of calcium carbonate to carbon dioxide and water. The reaction not going to completion and leaving the slightly basic hydrogen carbonate anion in solution<sup>[62,136]</sup> would explain the effect increasing pH. Hyaluronic acid (b7), glycerol (b6) and sodium chloride (b8) reduced pH. Buffer (b9) increased pH, counteracting the effects of any free protons and preventing the pH dropping to levels below 5.



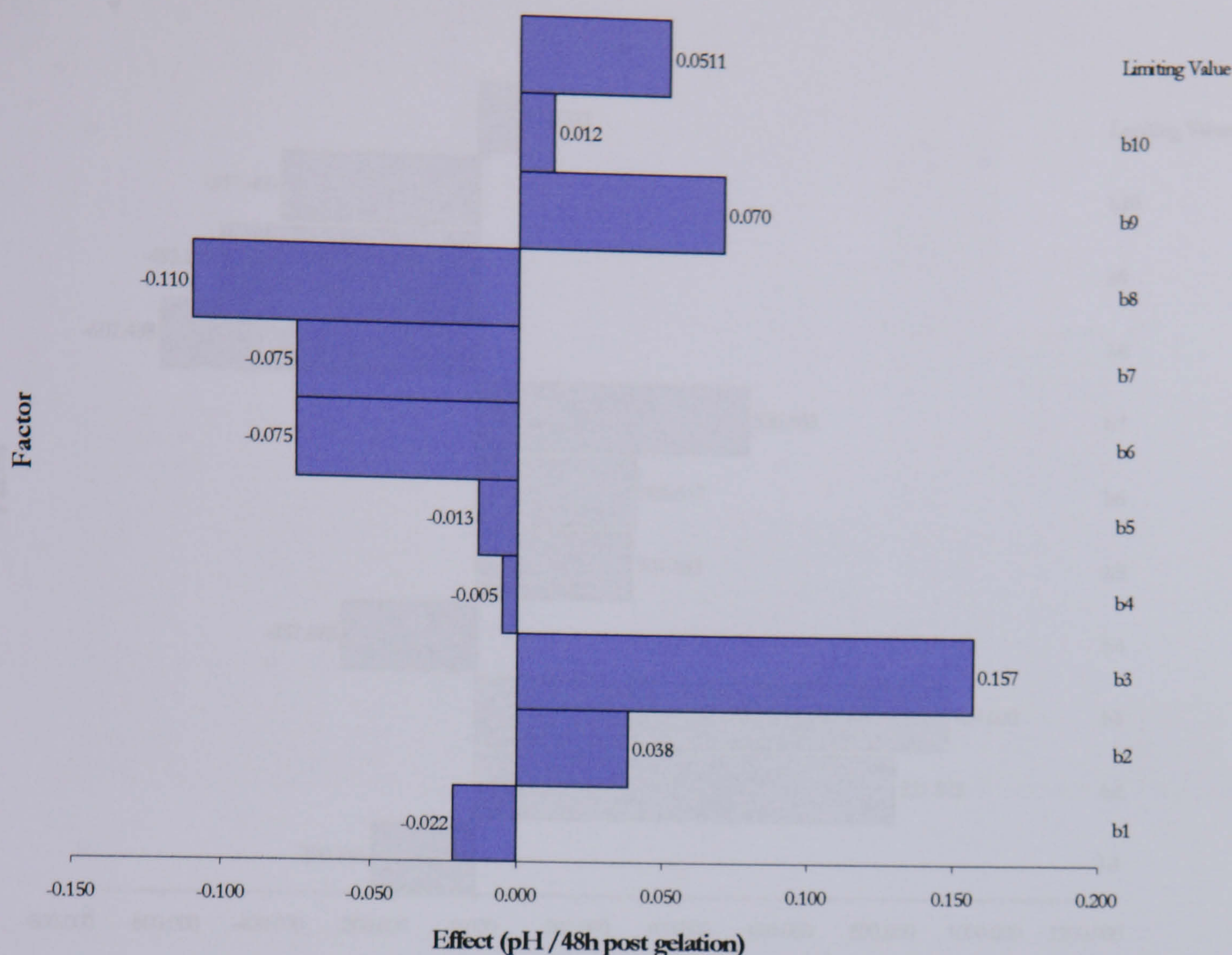


Figure 6.6 The effects profile of factor influence over pH 48h post gelation (factors b1-b10).

### 6.3.5 The Effects Profile Of Factors Influencing Gel strength

The effect of calcium carbonate (b3) and alginate type (b2) are clearly positive (Figure 6.7) and defined by relating the abundance of G block regions to the concentration of sodium alginate and calcium carbonate<sup>[99]</sup>. The negative effect exhibited by sodium alginate (b1) was a result of the fixed calcium carbonate level and the further addition of polymer reducing gel strength<sup>[74]</sup>. Both sodium chloride (b8) and buffer (b9) reduce gel strength, the role of sodium ions in alginate gelation remains unclear<sup>[135]</sup>, but is thought to compete with calcium in the gelling process<sup>[143,146,158]</sup>. The positive effect of hyaluronic acid (b7) was large considering the relatively low level, evidence of a possible binding interaction with calcium carbonate<sup>[150]</sup>. The effect of glycerol (b6) increasing gel strength was also beneficial. The effect of sodium carboxymethyl cellulose viscosity (b4) was to reduce gel strength, sodium carboxymethyl cellulose level (b5) increased gel strength, the positive effect on gel structure was also seen in Chapter 4 (Figure 4.32). The negative effect of temperature (b10) was explained by a change in gel structure as a result of higher temperature<sup>[159]</sup>, allowing normally energetically poor interactions to occur, reducing gel strength.



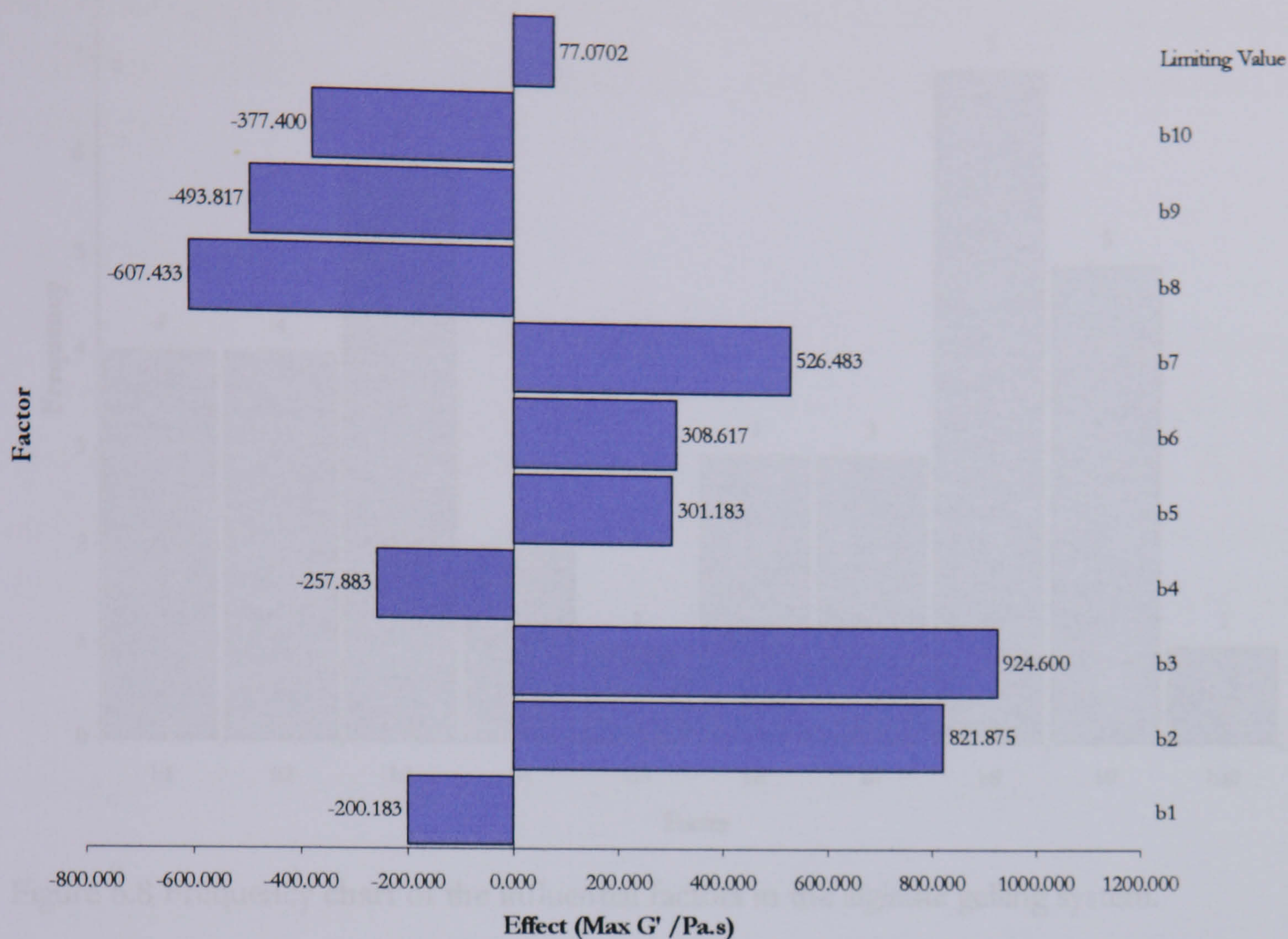


Figure 6.7 The effects profile of factor influence over G' max (gel strength) (factors b1-b10).

### 6.3.6 Choice Of The Most Active Factors

The large number of characterisation methods used to investigate the *in-situ* gelling system made the choice of factors for further investigation difficult. This was resolved by ordering the factor effects for each characterisation method. The five most active factors for each method were tabulated (Table 6.5) and a frequency chart was produced (Figure 6.8) making a tally of the appearance of each factor. The factors appearing the most frequently were then chosen for further study.

Table 6.5 Tally of the five most active factors for each characterisation method, to determine the most influential for further investigation.

Effect Order	Gelling time	Water uptake	Viscosity		Gel Strength		pH	
		24h	Sterile	Rate index	Max G'	Sterile	48h	
A	1	1	1	2	3	8	3	
B	3	8	2	9	2	1	8	
C	8	3	9	6	8	2	7	
D	5	6	4	10	7	9	6	
E	4	7	8	8	9	3	9	



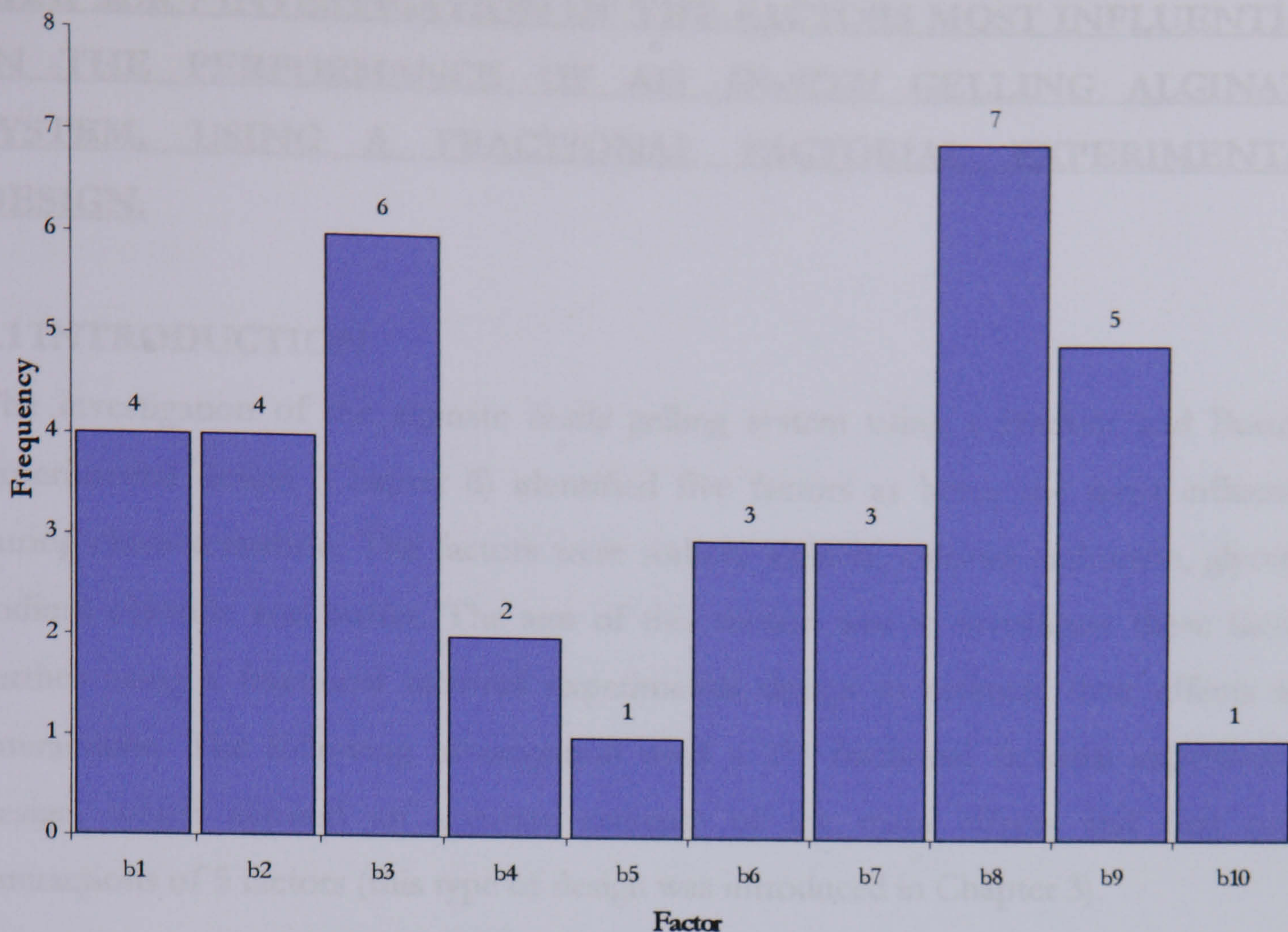


Figure 6.8 Frequency chart of the influential factors in the alginate gelling system.

#### 6.4 SUMMARY

The factors chosen for the next stage of the design process were Sodium alginate (b1), Calcium carbonate (b3), Glycerol (b6), Sodium chloride (b8) and Buffer (b9). The factors alginate type (b2) and Hyaluronic acid (b7) were not chosen for further investigation. The very rapid gelling time and viscosity effects exhibited by the low level alginate type (b2), suggested that further development with this factor level would not provide a gel of the required properties. Glycerol (b6) was chosen above Hyaluronic acid, because of its greater effect with water uptake and rate index.

The confidence in the results was high, because the advantage of the Plackett and Burman design was that the random error for a particular factor effect was spread over the whole experiment. By varying all the factors together the random error was distributed equally about zero<sup>[128]</sup> and the estimated precision of the results for the coded 0 formulations were used to determine the significance of any effects seen in the effects profile (introduced in Chapter 3). The screening procedure proved a very rapid way of characterising this system. However, interactions between factors could not be determined. The next chapter in this study, Chapter 7, investigates the factors chosen above for interactions to further refine the gelling system.



# **CHAPTER 7 INVESTIGATION OF THE FACTORS MOST INFLUENTIAL IN THE PERFORMANCE OF AN *IN-SITU* GELLING ALGINATE SYSTEM, USING A FRACTIONAL FACTORIAL EXPERIMENTAL DESIGN.**

## **7.1 INTRODUCTION**

The investigation of the alginate *in-situ* gelling system using a Plackett and Burman experimental design (Chapter 6) identified five factors as being the most influential during characterisation. The factors were sodium alginate, calcium carbonate, glycerol, sodium chloride and buffer. The aim of this section was to investigate these factors further using a fractional factorial experimental design to estimate their effects and interactions. The following investigation used a  $2^{5-1}$  fractional factorial experimental design, which allowed an accurate estimate of the main effects and first order interactions of 5 factors (this type of design was introduced in Chapter 3).

## **7.2 EXPERIMENTAL**

### **7.2.1 Materials**

Sodium alginate, LF 10/60, FMC Biopolymer, Drammen, Norway. Calcium carbonate, Sturcal f, Specialty Minerals, Birmingham, England. Glycerol, Spectrophotometric grade (99.5%), Sigma Aldrich, Gillingham, Dorset, England. Hyaluronic acid, MV, FMC Biopolymer, Drammen, Norway. Di-sodium hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset, England. Potassium di-hydrogen orthophosphate, AR, BDH Laboratory Supplies, Poole, Dorset, England. Sodium Chloride, AR, BDH Laboratory Supplies, Poole, Dorset, England. Hydrochloric acid, AR, BDH Laboratory Supplies, Poole, Dorset, England. Sodium carboxymethyl cellulose, low viscosity, Fluka Chimie AG, Buckinghamshire. England. Delta gluconolactone, Aldrich Chemical Company, Gillingham, Dorset, England.

### **7.2.2 Equipment**

Oertling Balance, 4 decimal places Oertling. Smethwick, West Midlands, England. Carrimed Rheometer, CSL<sup>2</sup> 500, TA Instruments Ltd., Surrey, England. Autoclave, Bench top, Dixons Surgical Instruments Ltd., Wickford, Essex, England. Glass Franz cells, Custom made by Mr. B. Chappel, Glass blower, Liverpool University, Liverpool, England. Overhead stirrer, Janke, BDH Laboratory Supplies, Poole, Dorset, England.



Bench pH meter, HI 8579 Hanna instruments, BDH Laboratory Supplies, Poole, Dorset, England. Water bath, Grant (model w38), BDH Laboratory Supplies, Poole, Dorset, England.

7.2.3 Methods

7.2.3.1 EXPERIMENTAL DESIGN

The  $2^{5-1}$  fractional factorial design used (Table 7.1), was based on a  $2^5$  factorial experiment partitioned at the highest order of interaction (introduced section 3.2.2). The resultant design enables the determination of the five main effects and the ten first order interactions of five factors without confounding<sup>[128]</sup>, the factors and their levels are found in Table 7.2. The five factors not investigated in this Chapter for effects and interactions were kept at the coded 0 levels from Table 6.1 (section 6.2.3), except for gelling temperature which was set at 25°C. The level of alginate was increased above that used in Chapter 6 to maximise the absorptive capacity without increasing the viscosity to an unmanageable magnitude.

Table 7.1 A  $2^{5-1}$  fractional factorial experimental design, allows the estimation of main effects and first order interactions without confounding.

Experiment	Effect	A	B	C	D	E
		Alginate	NaCl	CaCO <sub>3</sub>	Buffer	Glycerol
01	abcde	+1	+1	+1	+1	+1
02	a	+1	-1	-1	-1	-1
03	b	-1	+1	-1	-1	-1
04	c	-1	-1	+1	-1	-1
05	d	-1	-1	-1	+1	-1
06	e	-1	-1	-1	-1	+1
07	abc	+1	+1	+1	-1	-1
08	abd	+1	+1	-1	+1	-1
09	abe	+1	+1	-1	-1	+1
10	acd	+1	-1	+1	+1	-1
11	ace	+1	-1	+1	-1	+1
12	ade	+1	-1	-1	+1	+1
13	bcd	-1	+1	+1	+1	-1
14	bce	-1	+1	+1	-1	+1
15	bde	-1	+1	-1	+1	+1
16	cde	-1	-1	+1	+1	+1

7.2.3.2 CHARACTERISATION OF FORMULATIONS

The methods for preparing and characterising the sixteen formulations (experiments) of the experimental design (Table 7.1) can be found in sections 2.2 and 2.3.



Table 7.2 Factors and their levels for the 2<sup>5-1</sup> fractional factorial design.

	Factor	(-1)	(+1)	Unit
A	Alginate concentration	5	5.75	%w/w
B	NaCl concentration	0.25	0.5	%w/w
C	CaCO <sub>3</sub> concentration	0.20	0.26	%w/w
D	Buffer concentration	0.21	0.41	%w/w
E	Glycerol concentration	3.0	5.5	%w/w
	Factor	Constant level		
F	Alginate type	LF 10/60		-
G	NaCMC type	low viscosity		-
H	NaCMC concentration	0.62		%w/w
I	HA concentration	0.05		%w/w
J	Gelling temperature	25		°C

7.3 RESULTS AND DISCUSSION

The results, generated by the characterisation methods (Chapter 2), were used to estimate the effects and interactions of factors. These data are presented and discussed in the following three sections 7.3.1) Water uptake, 7.3.2) pH and 7.3.3) Rheological characterisation. For each characterisation method there are two graphs that display 1) the magnitude of factor effects and interactions and 2) the significance of any interaction. The graphs are presented together to avoid confusion.

7.3.1 Water Uptake

The results of the water uptake characterisation are found in Table 7.3, the main effects and interactions of factors are found in Table 7.4. The main effect profiles for both the 8 and 24h time points (Figures 7.1 and 7.3) retain a very similar form and will be discussed as one with allowance made for the effect of buffer. The positive effect of alginate on water absorption was seen in sections 2.3.4, 4.4, 5.2.5 and 6.3.2. Sodium chloride had a negative effect on absorptive capacity, as in section 5.2.5 and 6.3.2. The reasons for this are unclear. The discussions of Chapters 5 and 6 noted the effect of sodium chloride on the swelling and solubility of polyelectrolytes. A possibility not discussed so far is that mass is lost as both sodium and chloride ions diffuse across the semi permeable membrane (osmosis), to reach equilibrium, causing the negative effect.

However, Segeren *et al*<sup>[134]</sup> observed that it took an alginate gel three weeks to reach an equilibrium position. This suggested that the process was very slow and that the amount of sodium and chloride ions lost would be too small to induce the large negative effect seen in Figure 7.1.



Table 7.3 Water uptake method results for each formulation in the 2<sup>5-1</sup> fractional factorial experimental design ( $\bar{x}$ , n = 3 ( $\pm$  standard deviation)).

Experiment	Water Uptake g/g/cm <sup>2</sup>	
	24h	8h
01	0.021 ( $\pm$ 0.002)	0.009 ( $\pm$ 0.001)
02	0.028 ( $\pm$ 0.002)	0.012 ( $\pm$ 0.001)
03	0.020 ( $\pm$ 0.001)	0.008 ( $\pm$ 0.001)
04	0.021 ( $\pm$ 0.001)	0.009 ( $\pm$ 0.001)
05	0.024 ( $\pm$ 0.002)	0.010 ( $\pm$ 0.001)
06	0.025 ( $\pm$ 0.002)	0.011 ( $\pm$ 0.001)
07	0.020 ( $\pm$ 0.002)	0.008 ( $\pm$ 0.001)
08	0.022 ( $\pm$ 0.003)	0.010 ( $\pm$ 0.001)
09	0.023 ( $\pm$ 0.001)	0.011 ( $\pm$ 0.001)
10	0.024 ( $\pm$ 0.002)	0.011 ( $\pm$ 0.001)
11	0.026 ( $\pm$ 0.002)	0.011 ( $\pm$ 0.001)
12	0.027 ( $\pm$ 0.001)	0.012 ( $\pm$ 0.001)
13	0.016 ( $\pm$ 0.001)	0.007 ( $\pm$ 0.001)
14	0.017 ( $\pm$ 0.001)	0.008 ( $\pm$ 0.001)
15	0.022 ( $\pm$ 0.003)	0.010 ( $\pm$ 0.002)
16	0.021 ( $\pm$ 0.001)	0.010 ( $\pm$ 0.001)

Table 7.4 The main effects and interactions of factors for water uptake characterisation, in the fractional factorial experimental design.

Factor tests		Water uptake g/g/cm <sup>2</sup>	
		24h	8h
Main effects	Alginate	0.0033	0.0015
	NaCl	-0.0045	-0.0019
	CaCO <sub>3</sub>	-0.0030	-0.0014
	Buffer	-0.0004	0.0001
	Glycerol	0.0009	0.0009
Interactions	Alginate*NaCl	-0.0005	0.0001
	Alginate*CaCO <sub>3</sub>	0.0006	-0.0002
	Alginate*Buffer	-0.0004	-0.0003
	Alginate*Glycerol	-0.0002	-0.0003
	NaCl*CaCO <sub>3</sub>	-0.0004	-0.0004
	NaCl*Buffer	0.0004	0.0000
	NaCl*Glycerol	0.0004	0.0002
	CaCO <sub>3</sub> *Buffer	-0.0001	0.0002
	CaCO <sub>3</sub> *Glycerol	0.0001	-0.0002
	Buffer*Glycerol	0.0002	-0.0002



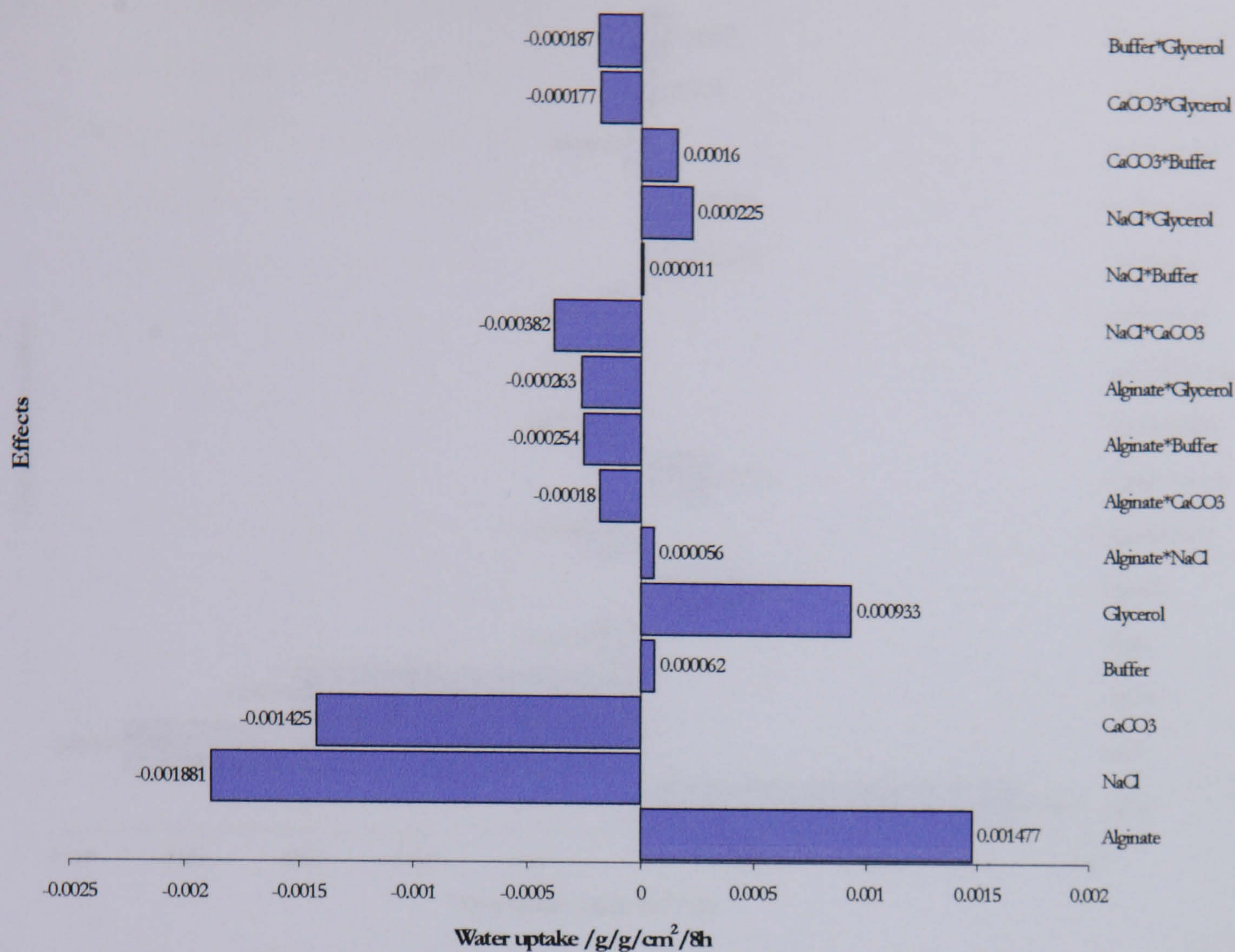


Figure 7.1 The profiles of the main effects and interactions of factors affecting water uptake (8h).

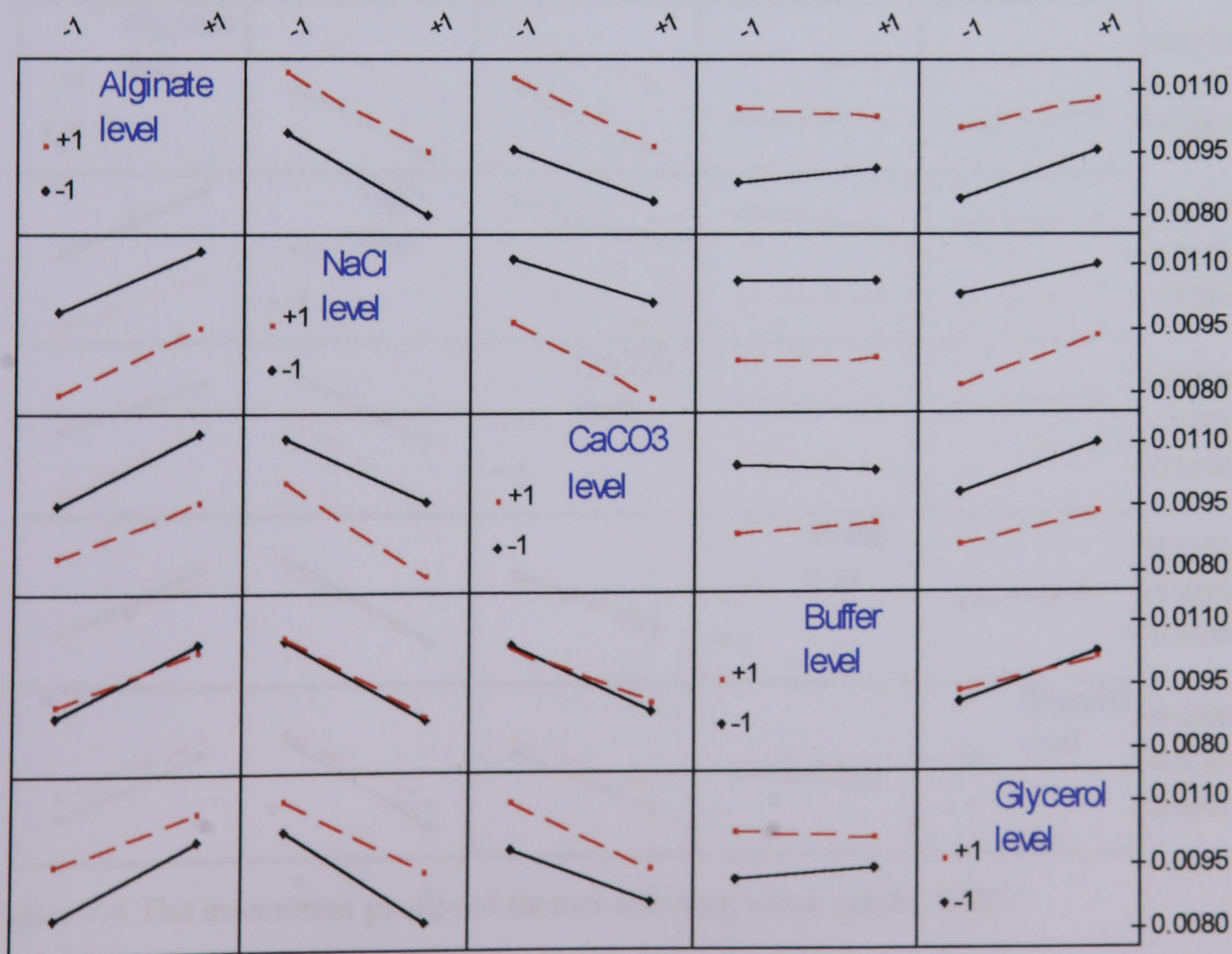


Figure 7.2 The interaction profile of factors affecting water uptake (8h).



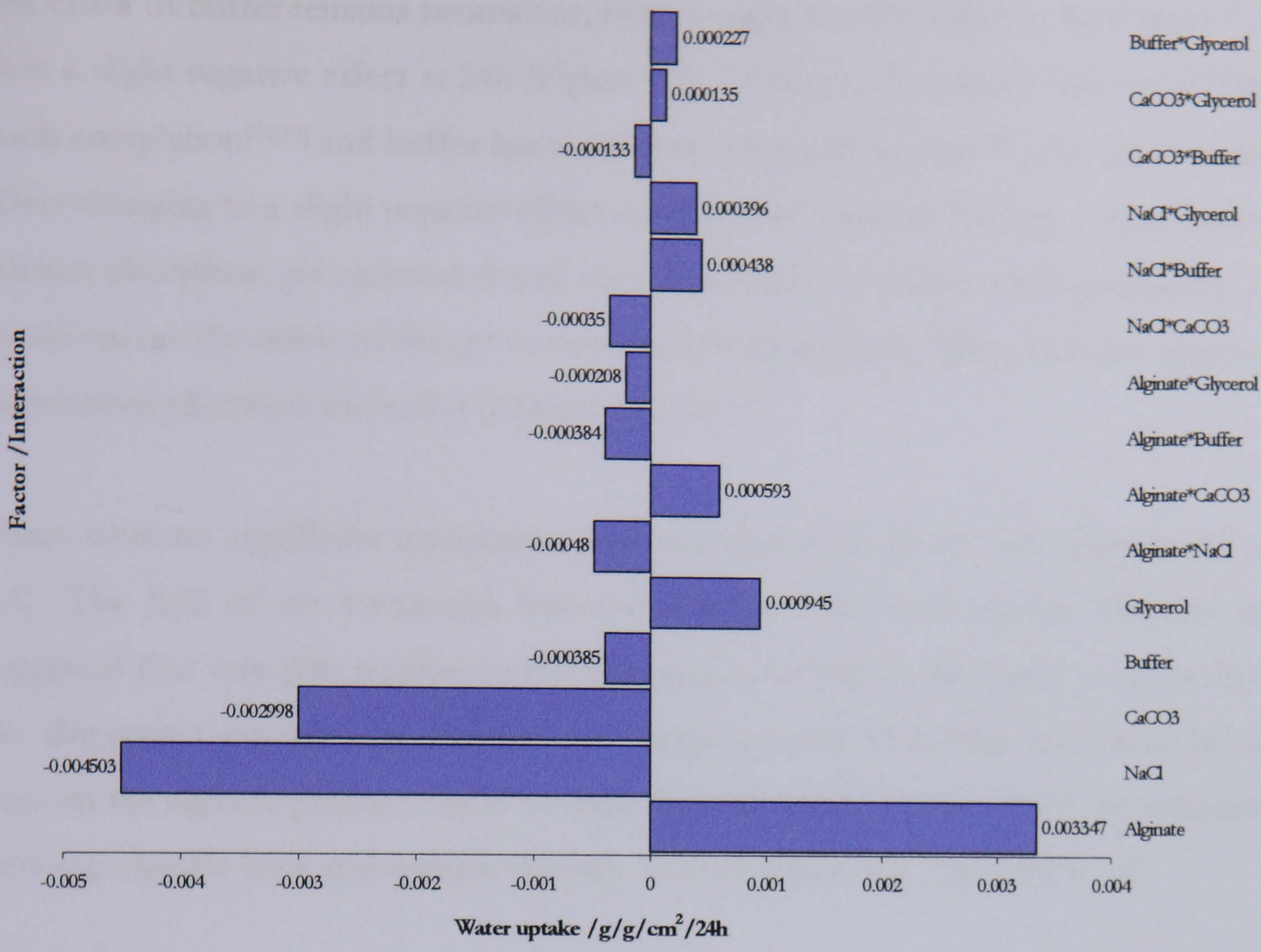


Figure 7.3 The profiles of the main effects and interactions of factors affecting water uptake (24h).

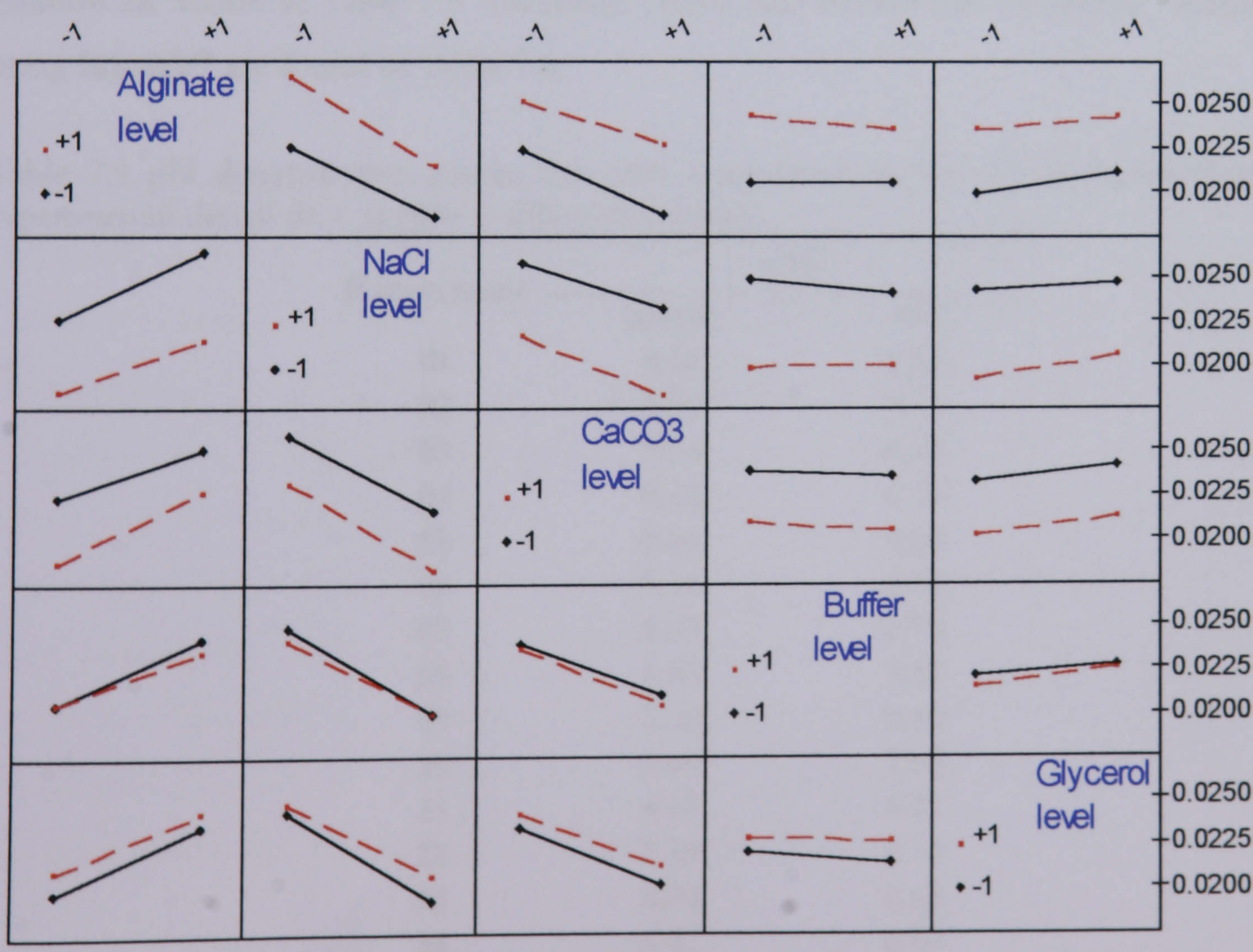


Figure 7.4 The interaction profile of factors affecting water uptake (24h).



The effect of buffer remains anomalous, from a slight positive effect at 8h (Figure 7.1) it gives a slight negative effect at 24h (Figure 7.3). Gelling is thought to take up to 24h to reach completion<sup>[74,99]</sup> and buffer has a role increasing gelling time<sup>[93]</sup>. The slight positive effect changing to a slight negative effect could be the result of calcium sequestered as a calcium phosphate, precipitated during the initial stages of gelation, being released. This would reduce the ability of the gel to swell as time progressed. There were no significant interactions identified for buffer (Figures 7.2 and 7.4)

There were no significant interactions between factors at 8h or 24h (Figures 7.2 and 7.4). The lack of an interaction between alginate level and sodium chloride level suggested that very little sodium or chloride ions were lost to the donor solution during the absorption experiments. The loss of a large amount of sodium ions from binding sites on the alginate polymer could increase the absorptive capacity<sup>[134,143]</sup>, an interaction between alginate level and sodium chloride level was therefore expected at 24h.

### 7.3.2 pH

The results of the determination of pH for each formulation when sterile and 48h after gelation are found in Table 7.5. The main effects and interactions of factors, calculated using Minitab® are found in Table 7.6.

Table 7.5 pH determination results for each formulation in the 2<sup>5-1</sup> fractional factorial experimental design (n = 1) (48h = 48h post gelation).

Experiment	pH	
	Sterile	48h
01	8.56	6.64
02	8.52	6.71
03	8.34	6.59
04	8.43	6.76
05	8.66	6.63
06	8.56	6.52
07	8.53	6.70
08	8.53	6.51
09	8.70	6.46
10	8.64	7.00
11	8.48	6.97
12	8.59	6.74
13	8.70	6.68
14	8.47	6.60
15	8.71	6.51
16	8.65	6.82



Table 7.6 The main effects and interactions of factors for pH determination, in the fractional factorial experimental design (48h = 48h post gelation).

Factor tests		pH	
		Sterile	48h
Main effects	Alginate	0.004	0.078
	NaCl	0.001	-0.183
	CaCO <sub>3</sub>	-0.019	0.188
	Buffer	0.126	0.028
	Glycerol	0.046	-0.040
Interactions	Alginate*NaCl	0.021	-0.095
	Alginate*CaCO <sub>3</sub>	-0.014	0.035
	Alginate*Buffer	-0.104	-0.015
	Alginate*Glycerol	-0.019	0.013
	NaCl*CaCO <sub>3</sub>	0.014	-0.050
	NaCl*Buffer	-0.011	-0.030
	NaCl*Glycerol	0.039	-0.028
	CaCO <sub>3</sub> *Buffer	0.034	0.000
	CaCO <sub>3</sub> *Glycerol	-0.081	0.013
	Buffer*Glycerol	-0.051	0.013

### 7.3.2.1 THE INFLUENCE OF FACTORS ON THE pH OF THE STERILE FORMULATIONS IN THE EXPERIMENTAL DESIGN

The factors with the greatest effect on pH were buffer and glycerol (Figure 7.5). The buffer (pH 7) was added to moderate the effect of calcium carbonate on system pH. The pKa of calcium carbonate is 9.5<sup>[99]</sup>. The results of the screening study show that pH was reduced by the presence of buffer (section 6.3.4). The effects profile for this investigation returned a positive effect for buffer, increasing pH. Calcium carbonate returned a negative effect on pH, reducing the pH of the system. The effects of alginate and sodium chloride are negligible, Smidsrød and Draget<sup>[136]</sup> noted that pH was dependant upon both ionic strength and alginate concentration. The large negative effect sodium chloride had on pH in section 6.3.4 (Figure 6.5) was not repeated here. The alginate\*buffer interaction was significant (Figure 7.6), a question arises, was the ionic nature of the buffer therefore affecting the pH more than the presence of calcium carbonate? The interaction between CaCO<sub>3</sub>\*glycerol was also significant. At high levels of calcium carbonate increasing the level of glycerol reduced pH, which was in contrast to the increase in pH at low levels of calcium carbonate.

The pH determination was based on the values taken post sterilisation, after the addition of glycerol. The stability of alginate during steam sterilisation is not completely



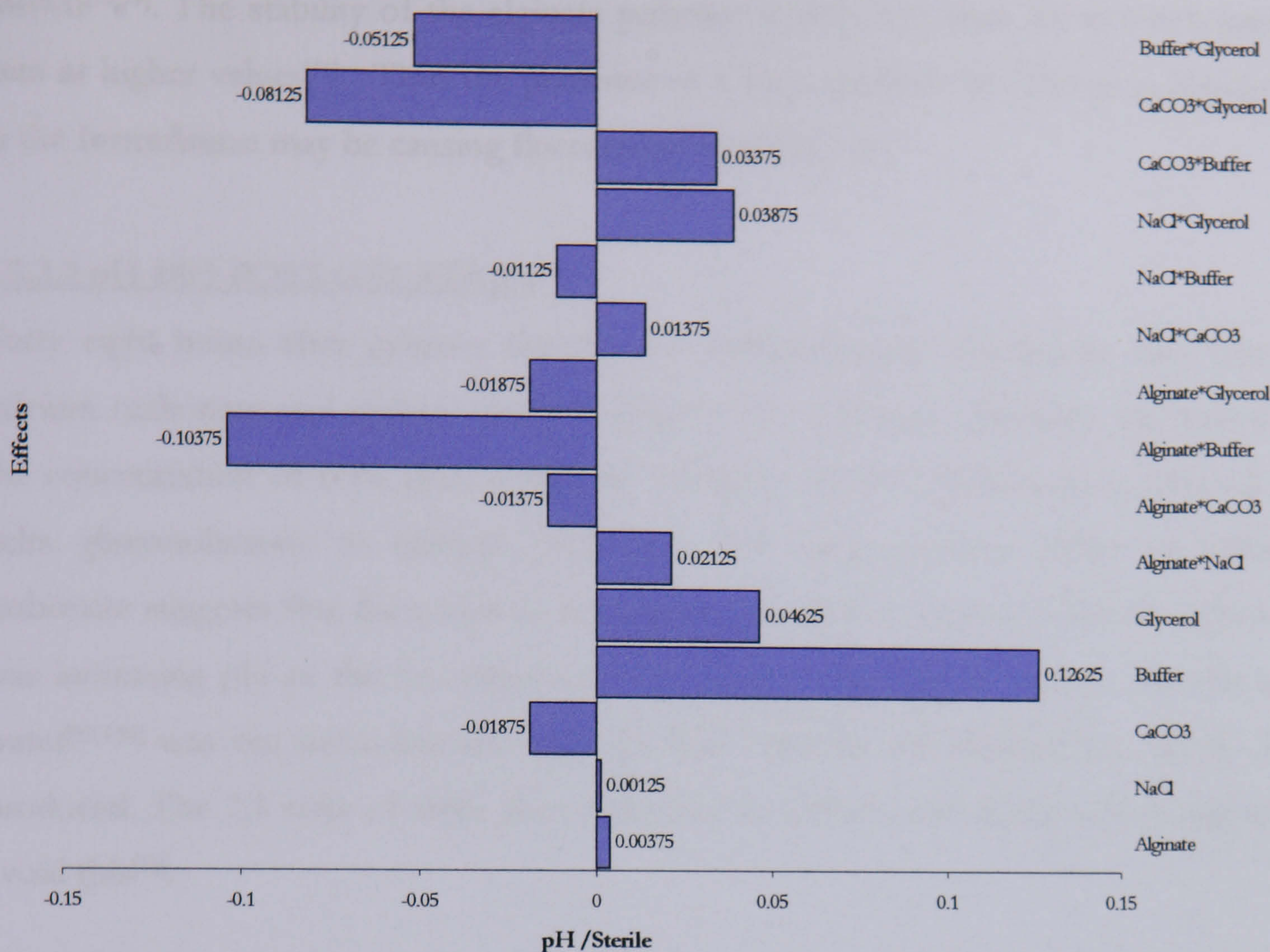


Figure 7.5 The profiles of the main effects and interactions of factors affecting pH of the sterile formulations.

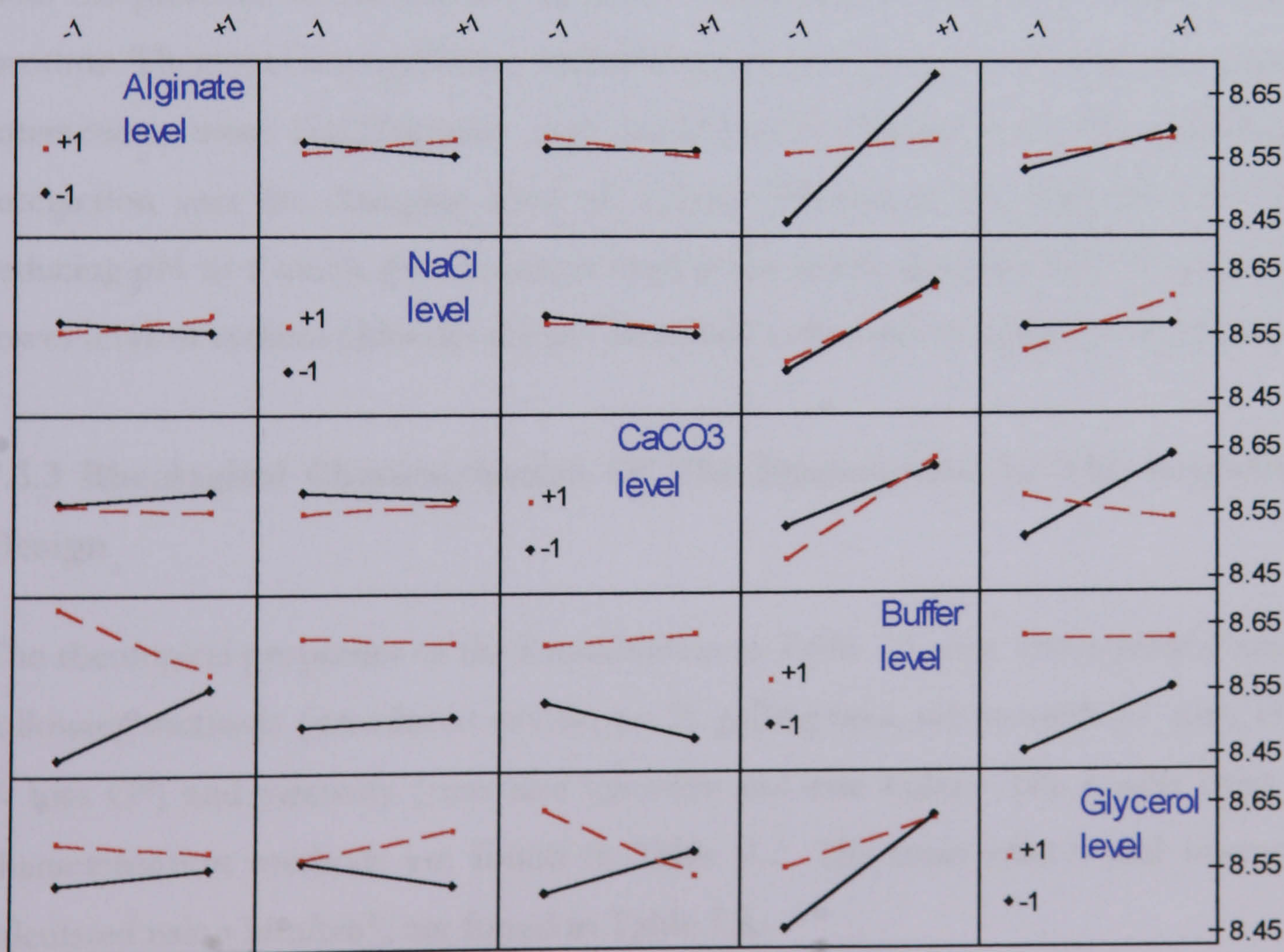


Figure 7.6 The interaction profiles of factors affecting pH of the sterile formulations.



known<sup>[74,99]</sup>. The stability of the alginate polymer at pHs less than 8.5 is much greater than at higher values<sup>[71]</sup>. Thus the presence of a large number of different constituents in the formulation may be causing fluctuations in the results.

#### 7.3.2.2 pH 48H POST GELATION

Forty eight hours after gelation the pH was predominantly affected by two factors: calcium carbonate and sodium chloride (Figure 7.7). Calcium carbonate was linked to the concentration of delta gluconolactone, added to induce gelation, by a ratio of 2:1 delta gluconolactone to calcium carbonate. The large positive effect of calcium carbonate suggests that there was an incomplete reaction. Unreacted calcium carbonate was increasing pH or the decomposition of calcium carbonate to carbon dioxide and water<sup>[62,136]</sup> was not occurring and only the half reaction was reached *i.e.*,  $\text{HCO}_3^-$  was produced. The 2:1 ratio of delta gluconolactone to calcium carbonate was designed to avoid this<sup>[74]</sup>.

The large negative effect of sodium chloride was a repeat from section 6.3.4 (Figure 6.6). AR grade sodium chloride was used to avoid impurities. However, the change in pH with the presence of sodium and its affect on binding with alginate could be releasing protons. There was no significant interaction between these two factors. The significant interactions were  $\text{NaCl} \times \text{alginate}$  and  $\text{NaCl} \times \text{CaCO}_3$  (Figure 7.8). The  $\text{NaCl} \times \text{alginate}$  interaction sees the changing level of sodium chloride at the high level of alginate reducing pH to a much greater extent than at the lower alginate level. However, at the lower level of sodium chloride, the pH increased with a rise in alginate concentration.

#### **7.3.3 Rheological Characterisation Of The Formulations In The Experimental Design**

The rheological properties of the formulations in Table 7.1 were characterised using the following methods (introduced in Chapter 2), gelling time, gel strength ( $G'$  max,  $G''$  and % loss  $G''$ ) and viscosity (zero rate viscosity and rate index). The results from these characterisation methods are found in Table 7.7. The main effects and interactions, calculated using Minitab<sup>®</sup>, are found in Table 7.8.



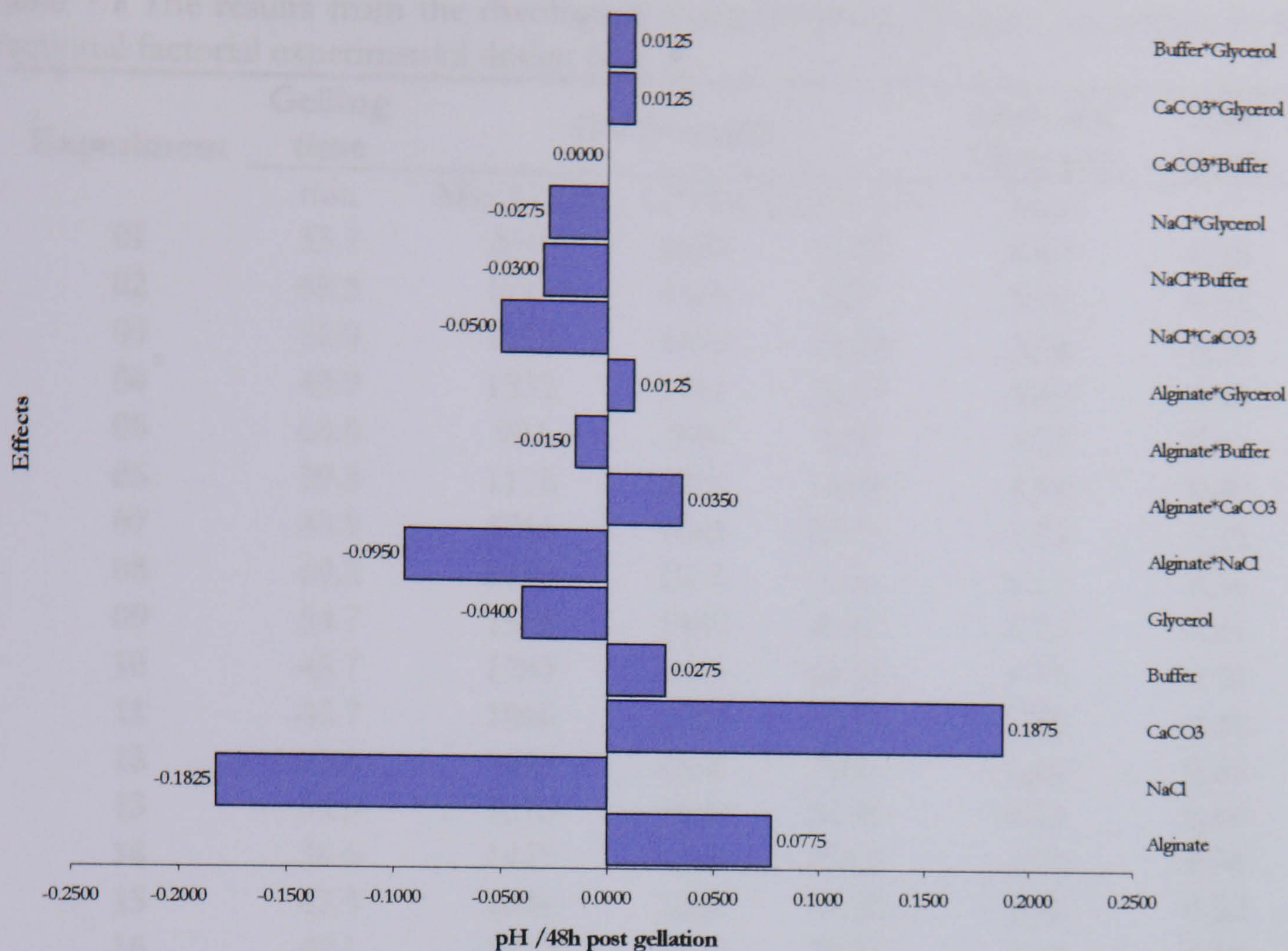


Figure 7.7 The profiles of the main effects and interactions of factors affecting pH 48h after gelation.

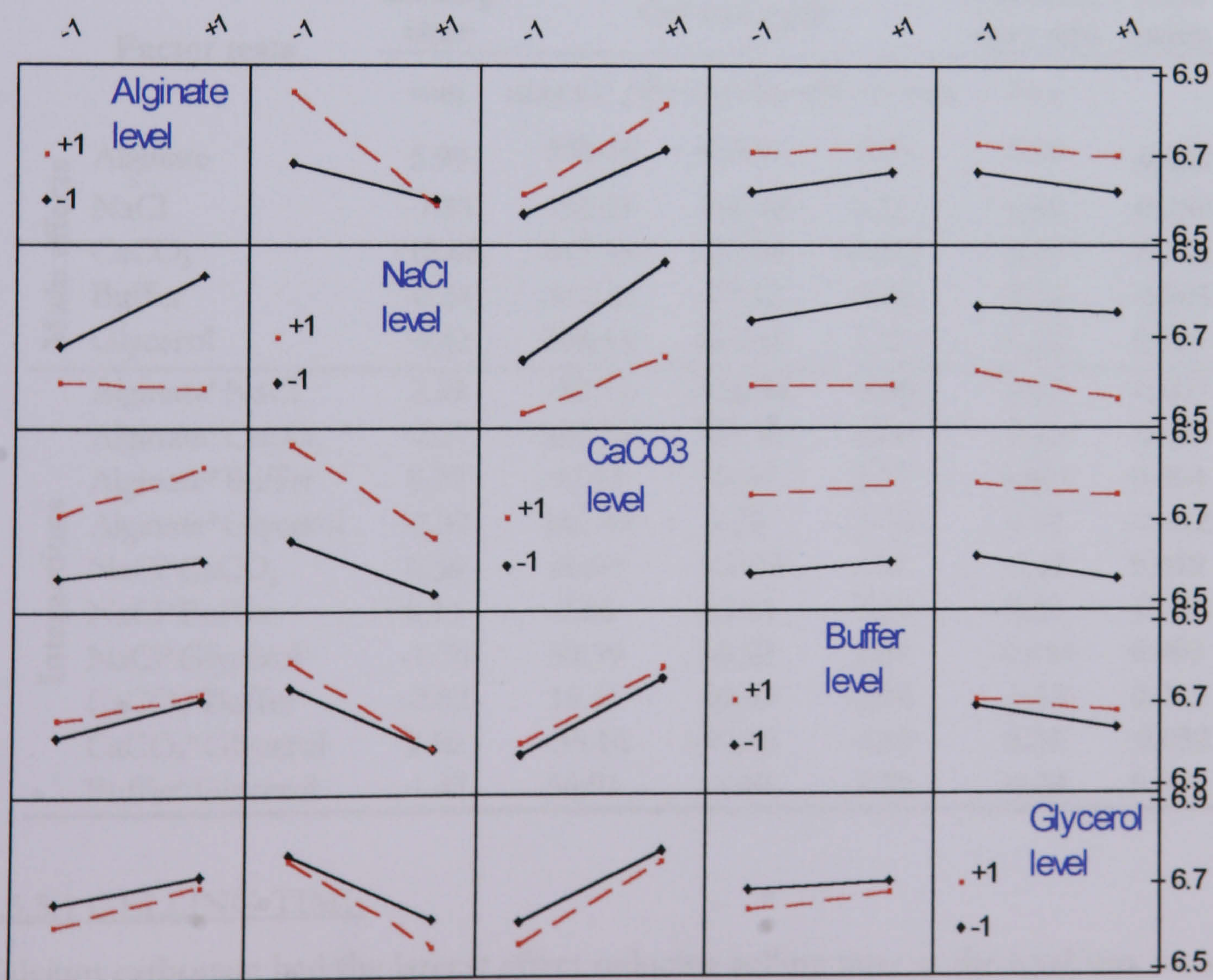


Figure 7.8 The interaction profiles of factors affecting pH 48h after gelation.



Table 7.7 The results from the rheological characterisation of each formulation in the 2<sup>5-1</sup> fractional factorial experimental design (n = 1).

Experiment	Gelling time	Gel Strength			Zero rate Viscosity	Rate index
	min	Max G' /Pa	G' /Pa	G' /% loss	Pa.s	
01	35.7	2049	1685	16.08	8.87	0.38
02	68.3	1215	1318	3.27	5.92	0.53
03	52.0	1214	1180	14.16	3.68	0.50
04	43.9	1532	1151	27.37	3.82	0.49
05	63.8	994	998	6.56	4.23	0.44
06	59.3	1178	1310	14.86	4.51	0.49
07	43.9	1765	1641	23.35	7.29	0.45
08	69.2	1000	1038	1.50	8.11	0.36
09	54.7	1378	1487	8.89	8.29	0.47
10	45.7	1785	2051	20.58	6.49	0.50
11	45.7	1966	2029	17.10	7.86	0.40
12	63.8	1432	1526	2.08	6.84	0.62
13	33.9	1270	928	24.86	4.14	0.49
14	36.6	1449	1160	45.62	4.70	0.50
15	47.5	1048	1262	15.78	4.63	0.52
16	42.1	1220	1170	20.32	4.54	0.51

Table 7.8 The main effects and interactions of factors from the rheological characterisation of formulations in the fractional factorial experimental design.

Factor tests		Gelling time	Gel strength			Viscosity zero rate	Rate index
		min	max G' /Pa	G' /Pa	G' /% loss	Pa.s	
Main effects	Alginate	5.99	335.66	452.10	-9.59	3.18	-0.030
	NaCl	-7.35	-18.59	-146.40	4.76	0.69	-0.039
	CaCO <sub>3</sub>	-18.88	447.16	212.04	16.02	0.19	-0.024
	Buffer	-0.34	-112.41	-77.42	-5.86	0.22	-0.003
	Glycerol	-4.41	118.16	165.44	2.39	0.82	0.018
Interactions	Alginate*NaCl	2.38	-32.91	-121.86	-3.06	0.67	-0.057
	Alginate*CaCO <sub>3</sub>	-2.37	187.84	297.39	-0.68	0.15	-0.037
	Alginate*Buffer	0.79	97.91	33.45	2.77	0.015	0.004
	Alginate*Glycerol	-2.37	146.84	4.25	-3.53	0.19	-0.012
	NaCl*CaCO <sub>3</sub>	0.56	26.09	-100.34	1.37	-0.12	0.019
	NaCl*Buffer	0.11	2.66	-61.61	-2.59	0.22	-0.043
	NaCl*Glycerol	-1.70	50.59	36.52	3.24	-0.002	0.003
	CaCO <sub>3</sub> *Buffer	-2.82	15.41	40.50	-2.04	-0.13	0.013
	CaCO <sub>3</sub> *Glycerol	2.60	-35.16	-97.35	-1.65	0.24	-0.052
	Buffer*Glycerol	-1.47	56.91	-8.40	-2.20	-0.34	0.041

7.3.3.1 GELLING TIME

Calcium carbonate had the largest effect reducing gelling time as the level was increased (Figure 7.9). This result was anticipated considering the nature of the alginate and calcium gelling mechanism<sup>[82,160,161,163]</sup>.



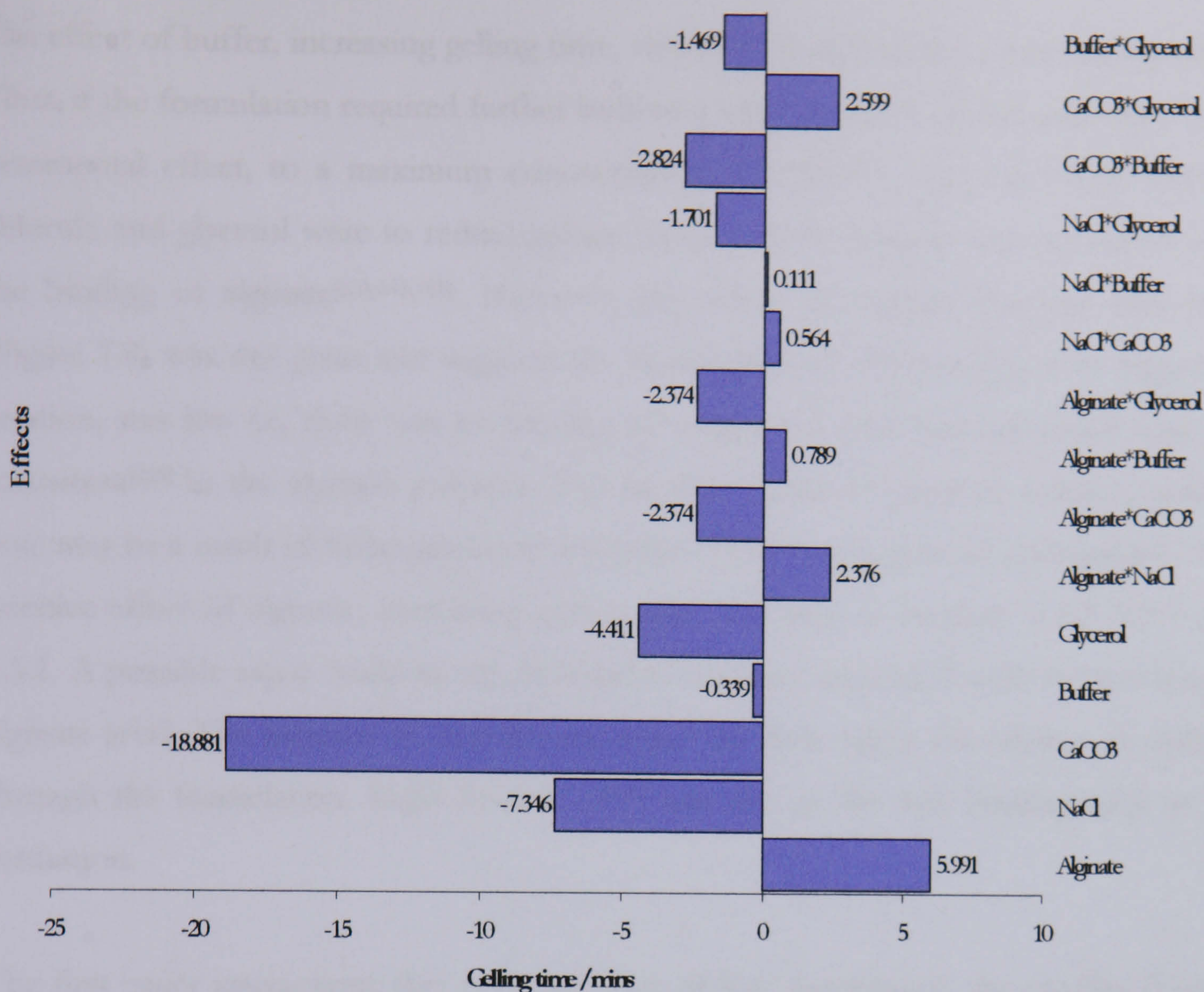


Figure 7.9 The profiles of the main effects and interactions of factors affecting gelling time.

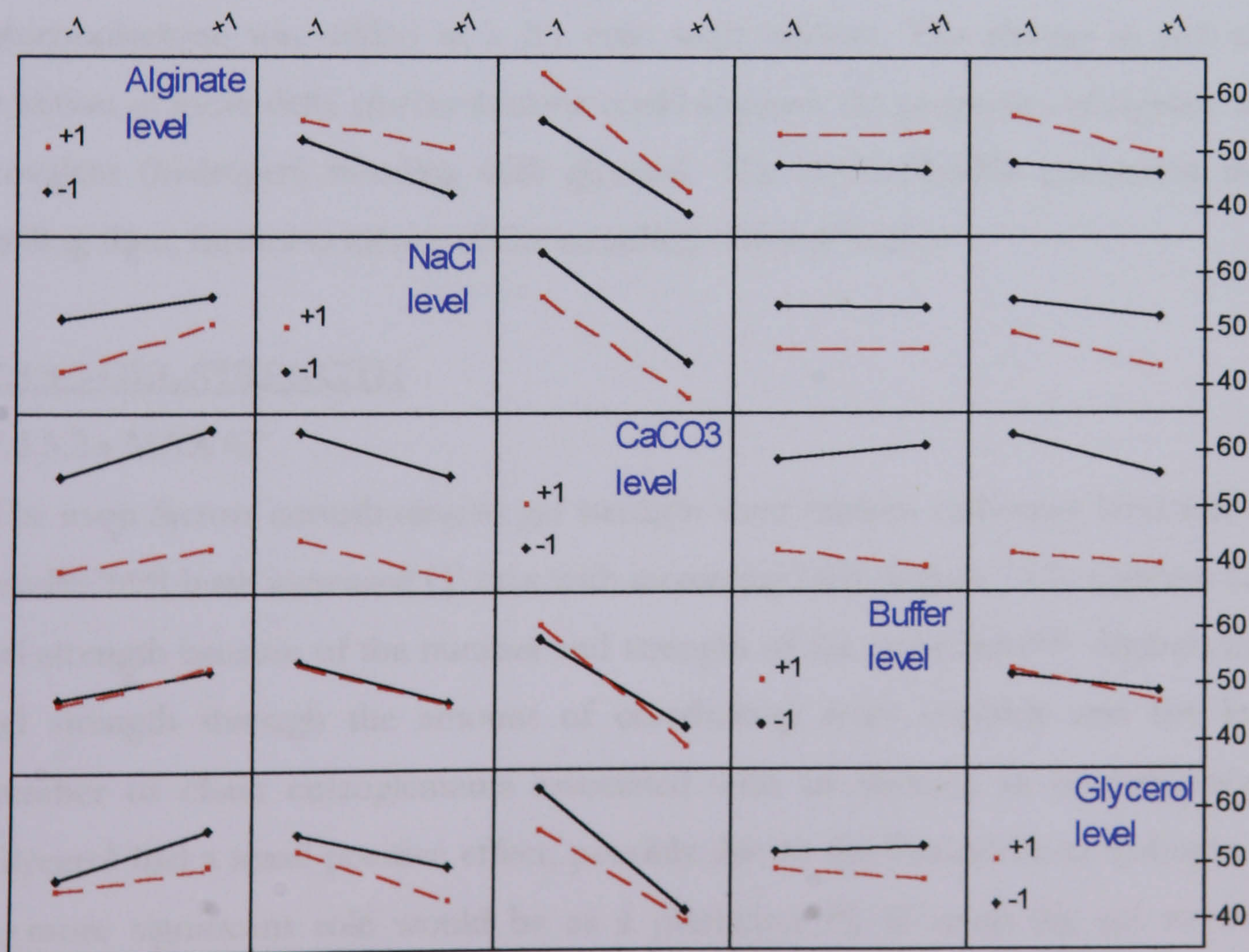


Figure 7.10 The interaction profiles of factors affecting gelling time.



The effect of buffer, increasing gelling time, exhibited in section 6.3.1 was not repeated. Thus, if the formulation required further buffering more could be added with out a large detrimental effect, to a maximum concentration of 0.1M<sup>[164]</sup>. The effects of sodium chloride and glycerol were to reduce gelling time, sodium chloride was associated with the binding of alginate<sup>[135,143,158]</sup>. However, the effect of sodium chloride seen here (Figure 7.9) was not great and suggests the significance of this binding, with regard to gelation, was low *i.e.*, there was no binding of sodium by areas not associated with gel formation<sup>[135]</sup> in the alginate polymer. The negative effect of glycerol, reducing gelling time may be a result of hydrogen bond formation<sup>[165]</sup>, between glycerol and alginate. The positive effect of alginate, increasing gelling time was seen in sections 5.2.4, 5.2.5 and 6.3.1. A possible cause could be the increase in viscosity associated with the increase in alginate level. The increase in viscosity increases the time taken for calcium to diffuse through the formulation. Skjåk-Bræk *et al*<sup>[135]</sup> saw this as the rate limiting step in gel formation.

The first order interactions that occurred were of low significance, the profiles (Figure 7.10) showed a reasonably parallel response to a change in the relevant factors. The largest of these interactions were between CaCO<sub>3</sub>\*buffer and CaCO<sub>3</sub>\*Glycerol. Delta gluconolactone was added in a 2:1 ratio with calcium. The change in pH with the addition of more delta gluconolactone could improve the properties of alginate for non-covalent (hydrogen) bonding with glycerol. The CaCO<sub>3</sub>\*buffer interaction increased gelling time, further evidence of the retarding effect of buffer.

### 7.3.3.2 GEL STRENGTH

#### 7.3.3.2.a MAX G'

The main factors contributing to gel strength were calcium carbonate level and alginate level<sup>[62,74,136]</sup>, both increased G' max with increasing level (Figure 7.11). Calcium increases gel strength because of the number and strength of the cross links<sup>[62]</sup>. Alginate increases gel strength through the amount of crosslinking areas available and the increased number of chain entanglements associated with an increase in concentration<sup>[166,167]</sup>. Glycerol had a small positive effect, possibly due to the formation of hydrogen bonds. A more significant role would be as a plasticiser<sup>[153]</sup>, allowing the gel to withstand applied stress<sup>[65]</sup> and thereby increase strength (see section 7.3.3.2.b). The negative effect of buffer and sodium chloride on G' max was a result of the retardation of gelation



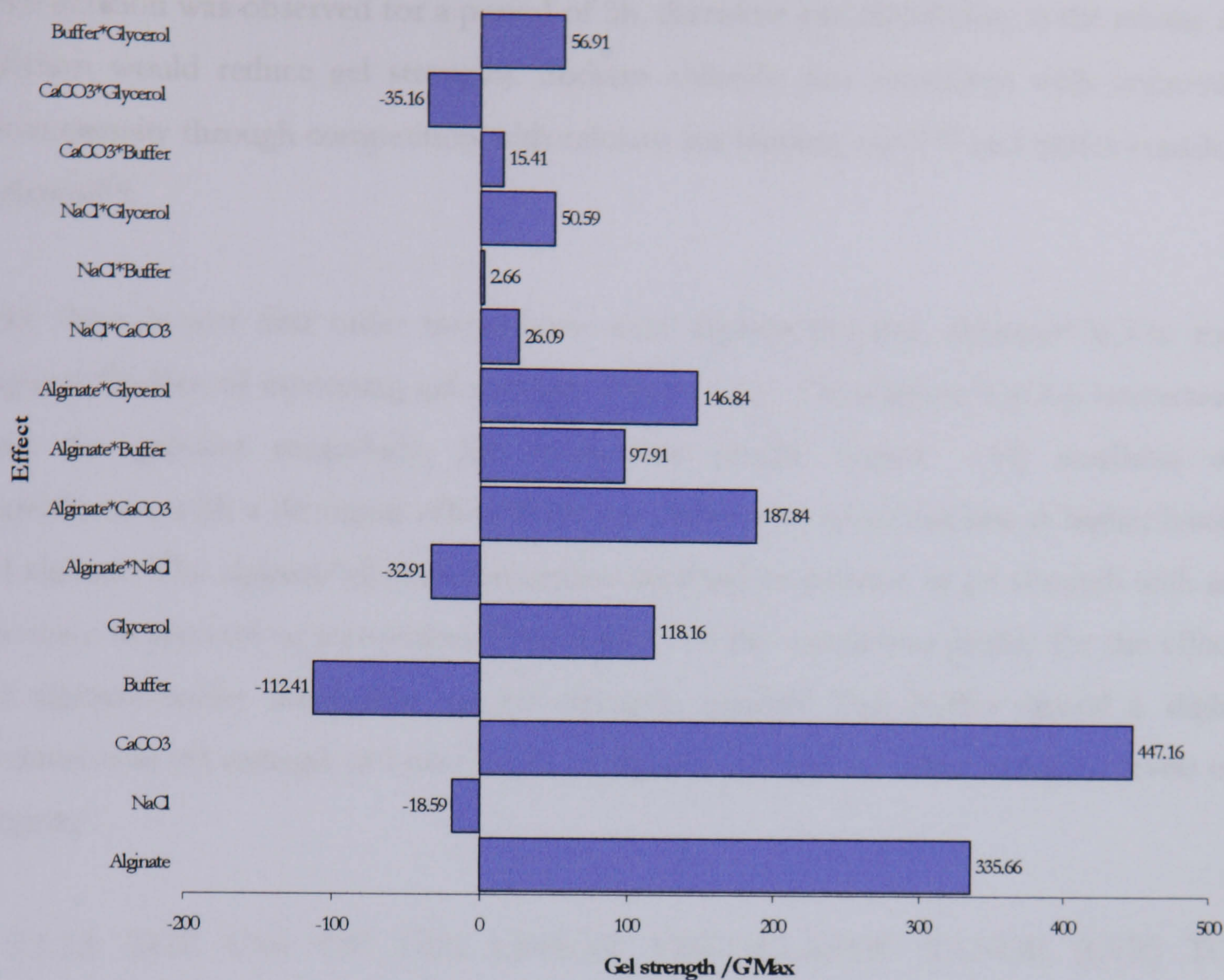


Figure 7.11 The profiles of the main effects and interactions of factors affecting gel strength (G' max).

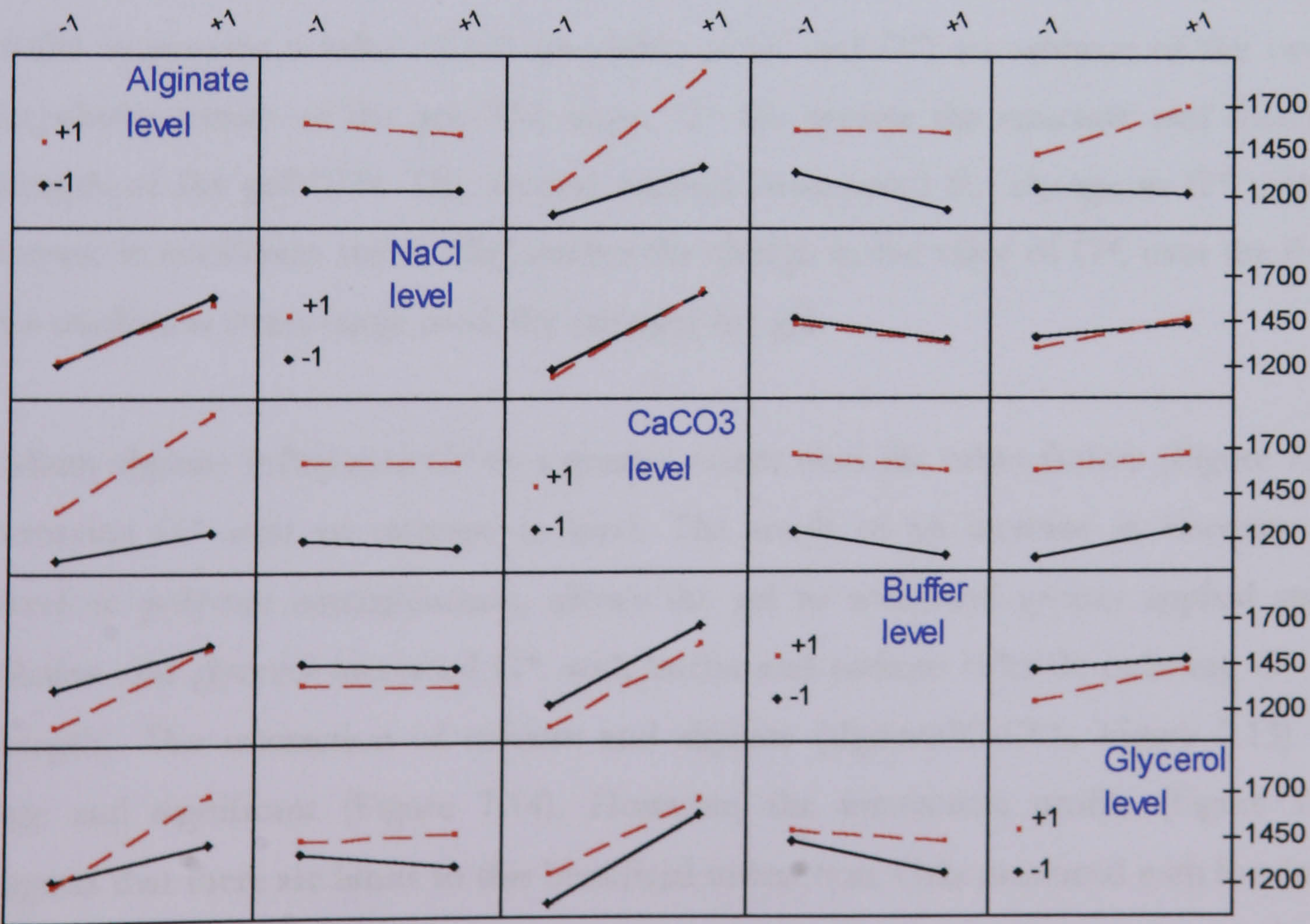


Figure 7.12 The interaction profiles of factors affecting gel strength (G' max).



(NB gelation was observed for a period of 3h, therefore any retardation in the release of calcium would reduce gel strength). Sodium chloride was associated with improved homogeneity through competition with calcium for binding sites<sup>[135]</sup> and buffer retarded gelation<sup>[93]</sup>.

The three largest first order interactions were alginate\*glycerol, alginate\*CaCO<sub>3</sub>, and alginate\*Buffer, all increasing gel strength (Figure 7.11). The alginate\*CaCO<sub>3</sub> interaction had the greatest magnitude, the interaction profile (Figure 7.12) confirms its significance, with a divergent effect from the addition of more calcium at higher levels of alginate. The alginate\*glycerol interaction involved an increase in gel strength with an increase in glycerol concentration. Examination of the interaction profile for the effect of alginate\*buffer interaction on gel strength, suggests that buffer caused a slight reduction in gel strength at lower levels of alginate but had no effect at higher levels of alginate.

#### 7.3.3.2.b THE USE OF THE LINEAR VISCOELASTIC RANGE (LVR) TO DETERMINE THE EFFECTS OF FACTORS ON GEL STRENGTH

The LVR was used in two ways to determine the effects of factors on gel strength (introduced, section 2.2.2.2). The first method used the y intercept from each LVR profile to provide a value of  $G^*$  (modulus of  $G'$  and  $G''$ ) an estimate of the overall viscoelastic nature of the gel. The larger  $G^*$  the greater the structure and therefore strength of the gel<sup>[115,116]</sup>. The second method determined the change in  $G^*$  with an increase in oscillation stress. The smaller the change in the value of  $G^*$ , over the 0-150 Pa.s oscillation stress range used, the stronger the gel.

Sodium alginate influenced  $G^*$  to a greater extent than the other factors (Figure 7.13), increasing  $G^*$  with an increase in level. The result of an increase in viscosity and therefore polymer entanglements, allows the gel to withstand greater applied stress. Calcium and glycerol increased  $G^*$ , with buffer and sodium chloride reducing  $G^*$  (gel strength). The interaction of calcium and alginate (alginate\*CaCO<sub>3</sub>, Figure 7.13) was large and significant (Figure 7.14). However, the interaction profile (Figure 7.14) suggests that there are limits to this beneficial interaction. Gels produced with low levels of alginate and high levels of calcium are known to be brittle<sup>[55]</sup>. Though the gels in this section were produced with a relatively high alginate level, the effect of calcium reducing



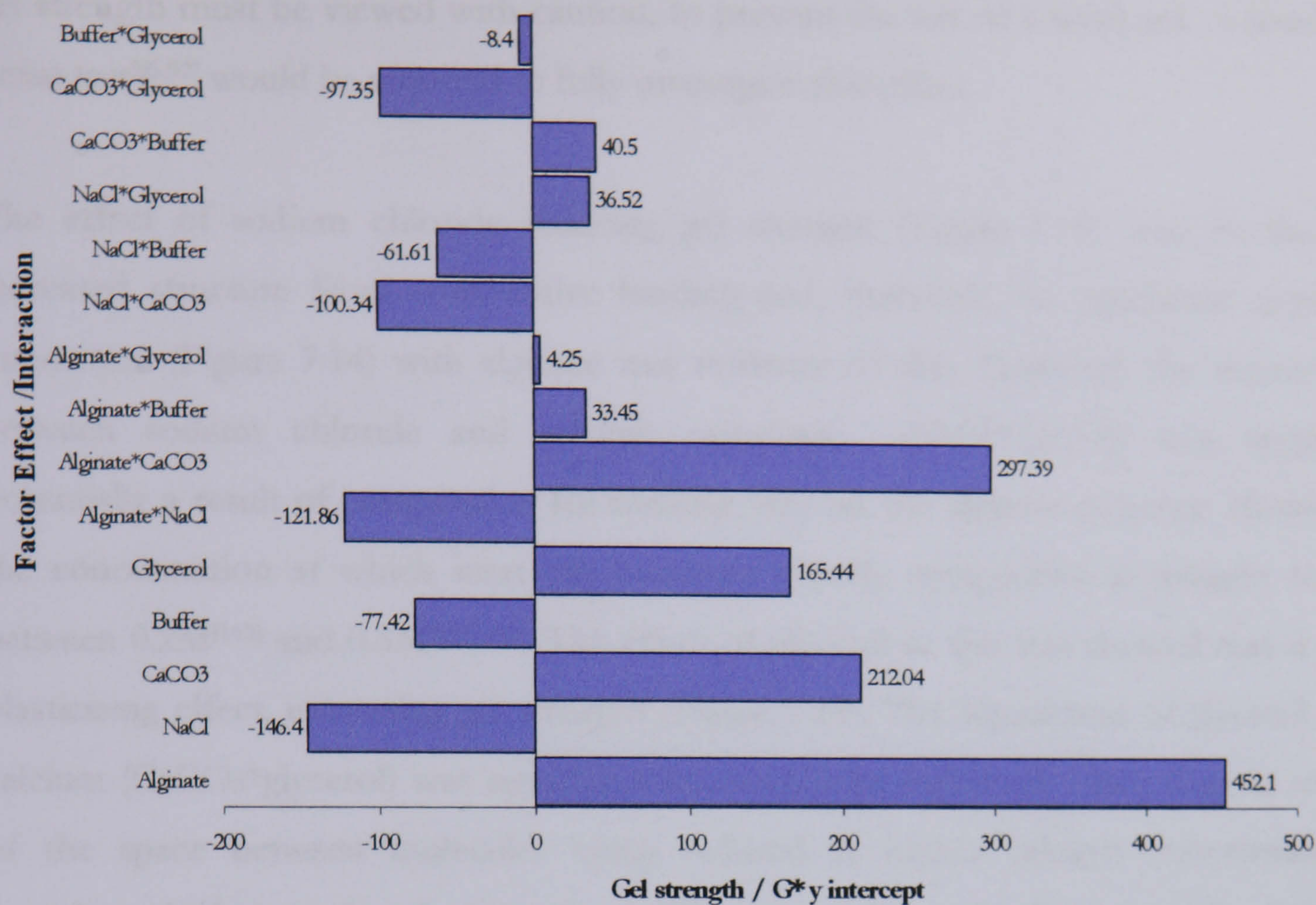


Figure 7.13 The profiles of the main effects and interactions of factors affecting gel strength ( $G^*$ ).

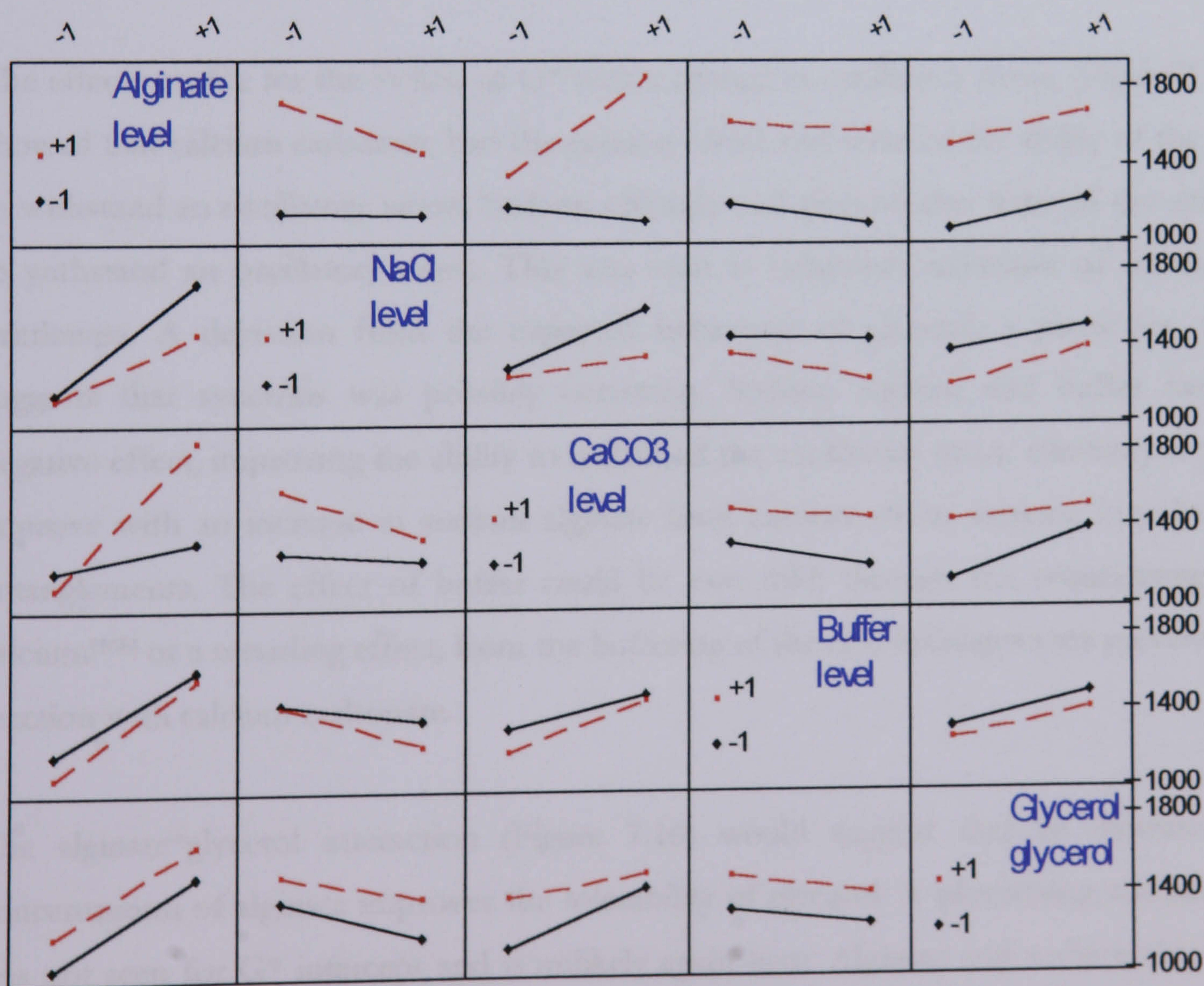


Figure 7.14 The interaction profiles of factors affecting gel strength ( $G^*$ ).



gel strength must be viewed with caution, to prevent the use of a weak gel. A breaking point test<sup>[85,92]</sup> would be required to fully investigate this effect.

The effect of sodium chloride reducing gel strength (Figure 7.13) may be due to increased structure from competitive binding and, therefore, its significant negative interaction (Figure 7.14) with alginate was evidence of this. However, the interaction between sodium chloride and calcium carbonate ( $\text{NaCl} \cdot \text{CaCO}_3$ ) was negative, potentially a result of competition for binding sites on the alginate polymer. However, the concentration at which inert salt becomes actively competitive is thought to be between 0.2M<sup>[143]</sup> and 0.5M<sup>[142,146]</sup>. The effect of glycerol in this test showed that it had plasticising effect, increasing gel strength (Figure 7.13). The interaction of glycerol and calcium ( $\text{CaCO}_3 \cdot \text{glycerol}$ ) was significant but cannot be explained, other than in terms of the space between molecules being reduced at higher calcium concentrations impeding hydrogen bond formation. The interaction of glycerol with alginate ( $\text{alginate} \cdot \text{glycerol}$ ) was not significant, which suggested that glycerol was potentially binding more strongly with other molecules.

The effects profile for the % loss of  $G^*$  with a change in oscillation stress, (Figure 7.15) showed that calcium carbonate had the greatest effect and reduced the ability of the gel to withstand an oscillatory stress. Sodium chloride and glycerol also reduced the ability to withstand an oscillatory stress. This was seen as behaviour indicative of increased brittleness. A deviation from the expected behaviour of glycerol, a plasticiser, and suggests that syneresis was possibly occurring. Sodium alginate and buffer had a negative effect, improving the ability to withstand the oscillatory stress. Elasticity would improve with an increase in sodium alginate level because of an increase in polymer entanglements. The effect of buffer could be two fold: through the sequestering of calcium<sup>[168]</sup> or a retarding effect, from the buffering of the free hydrogen ions preventing reaction with calcium carbonate.

The  $\text{alginate} \cdot \text{glycerol}$  interaction (Figure 7.16) would suggest that an increase in concentration of alginate improves the tolerability of glycerol. A plasticising interaction was not seen for  $G^*$  intercept and is unlikely again here. Alginate and sodium chloride ( $\text{alginate} \cdot \text{NaCl}$ , Figure 7.16) had a significant interaction, increasing the resistance of the



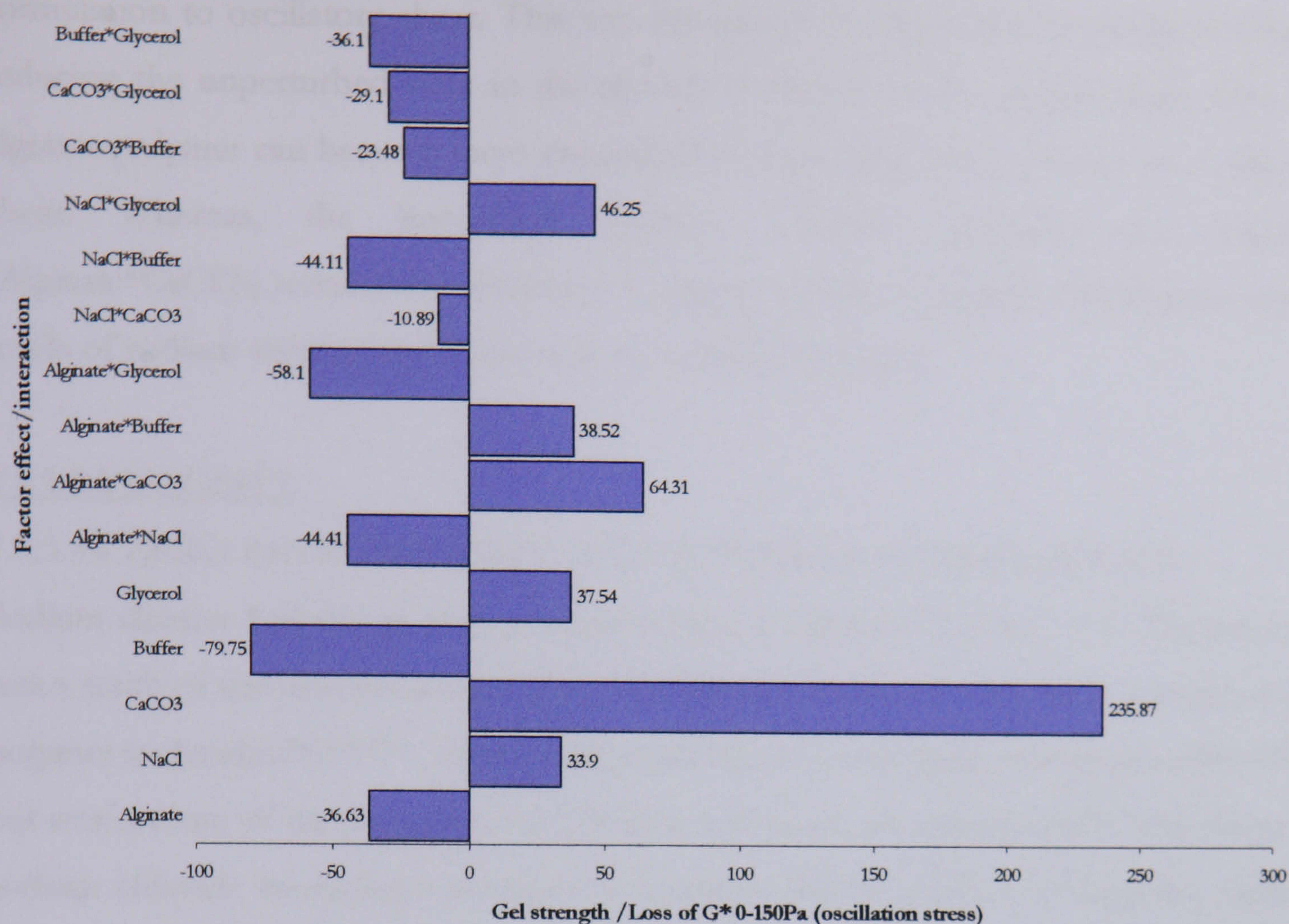


Figure 7.15 The profiles of the main effects and interactions of factors affecting gel strength (%G\* lost 0-150Pa).

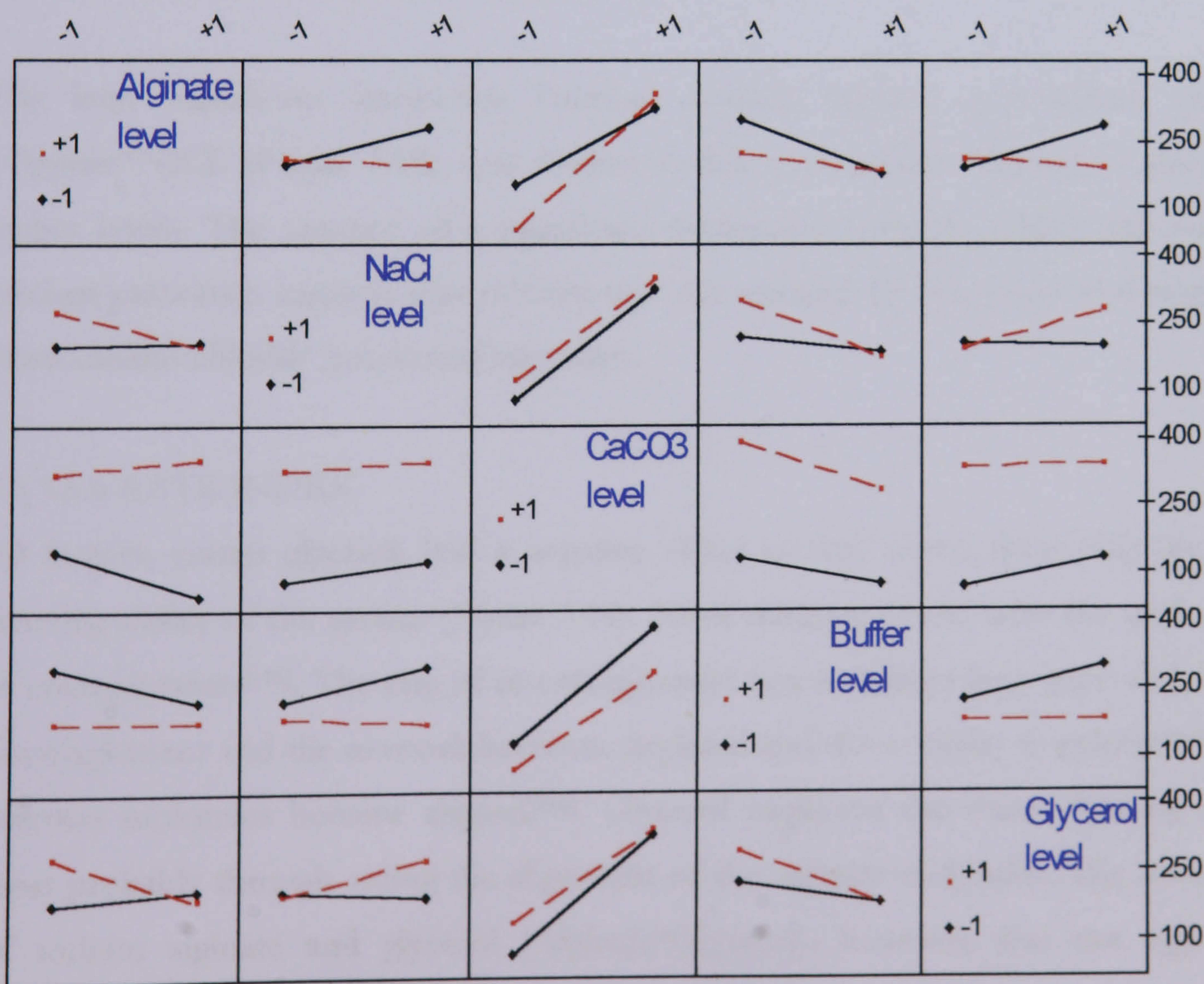


Figure 7.16 The interaction profiles of factors affecting gel strength (%G\* lost 0-150Pa).



formulation to oscillatory shear. This was thought to be the result of charge screening inducing the unperturbed state in the alginate molecule. In the unperturbed state the alginate polymer can become more entangled<sup>[116]</sup>, increasing the resistance to oscillatory shear. Whereas, the interaction between calcium carbonate and alginate (Alginate\*CaCO<sub>3</sub>) reduced the resistance to shear, a result of increased brittleness at low levels of sodium alginate and high levels of calcium carbonate.

### 7.3.3.3 VISCOSITY

#### 7.3.3.3.a ZERO RATE VISCOSITY OF THE STERILE FORMULATIONS

Sodium alginate had the greatest positive effect on viscosity (Figure 7.17). The increase was a result of the increase in level and a subsequent increase in the entanglement of the polymer molecules<sup>[146,169,170]</sup>. Alginate molecules have a very rigid conformation<sup>[140,154,155]</sup>, but retain some of the properties of a flexible coil in an extended form<sup>[66]</sup>. The effect of sodium chloride increasing viscosity was probably due to sodium binding the alginate molecules<sup>[171]</sup> rather than the encouragement of the unperturbed state<sup>[116,149]</sup>, which would decrease the viscosity<sup>[116]</sup>. Glycerol had a positive effect on viscosity possibly due to hydrogen bonding, mentioned in section 7.3.2.

The large significant interaction between sodium alginate and sodium chloride (Alginate\*NaCl) (Figure 7.18), was further evidence of sodium binding to alginate at higher levels. The absence of a significant interaction between sodium chloride and calcium carbonate suggests that calcium was not released by the reaction of impurities from sodium chloride (increasing viscosity).

#### 7.3.3.3.b RATE INDEX

All factors, except glycerol, had a negative effect on rate index, decreasing the shear thinning ability of the system (Figure 7.19). Shear thinning occurs with the gradual loss of entanglements<sup>[116]</sup>. The rate of re-entanglement can no longer keep pace with forced disentanglement and the network becomes depleted and the viscosity is reduced<sup>[172]</sup>. The polymer molecules become aligned<sup>[116]</sup>. Glycerol improves the shear thinning ability, most probably through aiding the alignment of the alginate molecules. The interaction of sodium alginate and glycerol (Alginate\*Glycerol), however, was not significant (Figure 7.20).



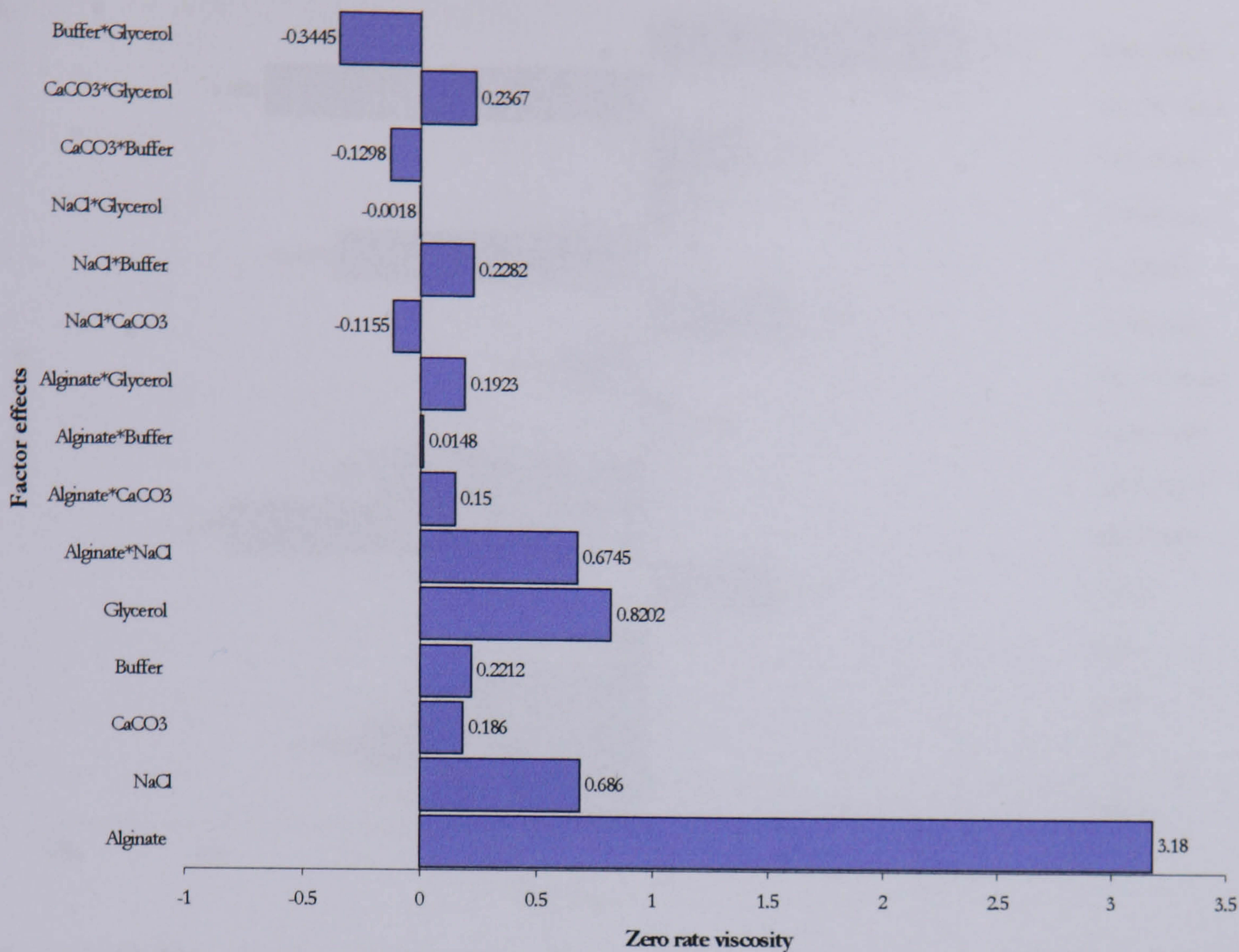


Figure 7.17 The profiles of the main effects and interactions of factors affecting zero rate viscosity of the sterile formulations.

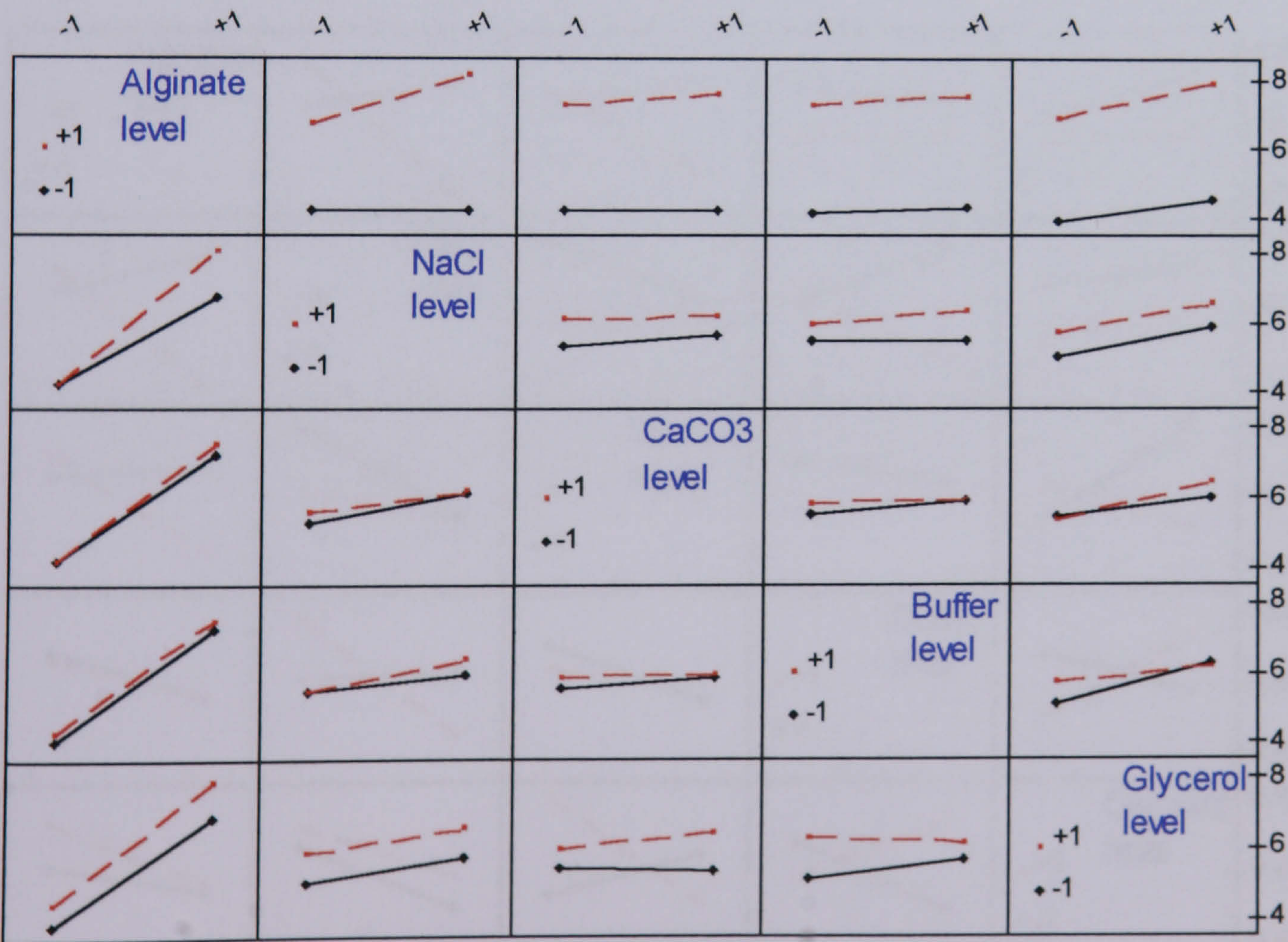


Figure 7.18 The interaction profiles of factors affecting zero rate viscosity of the sterile formulations.



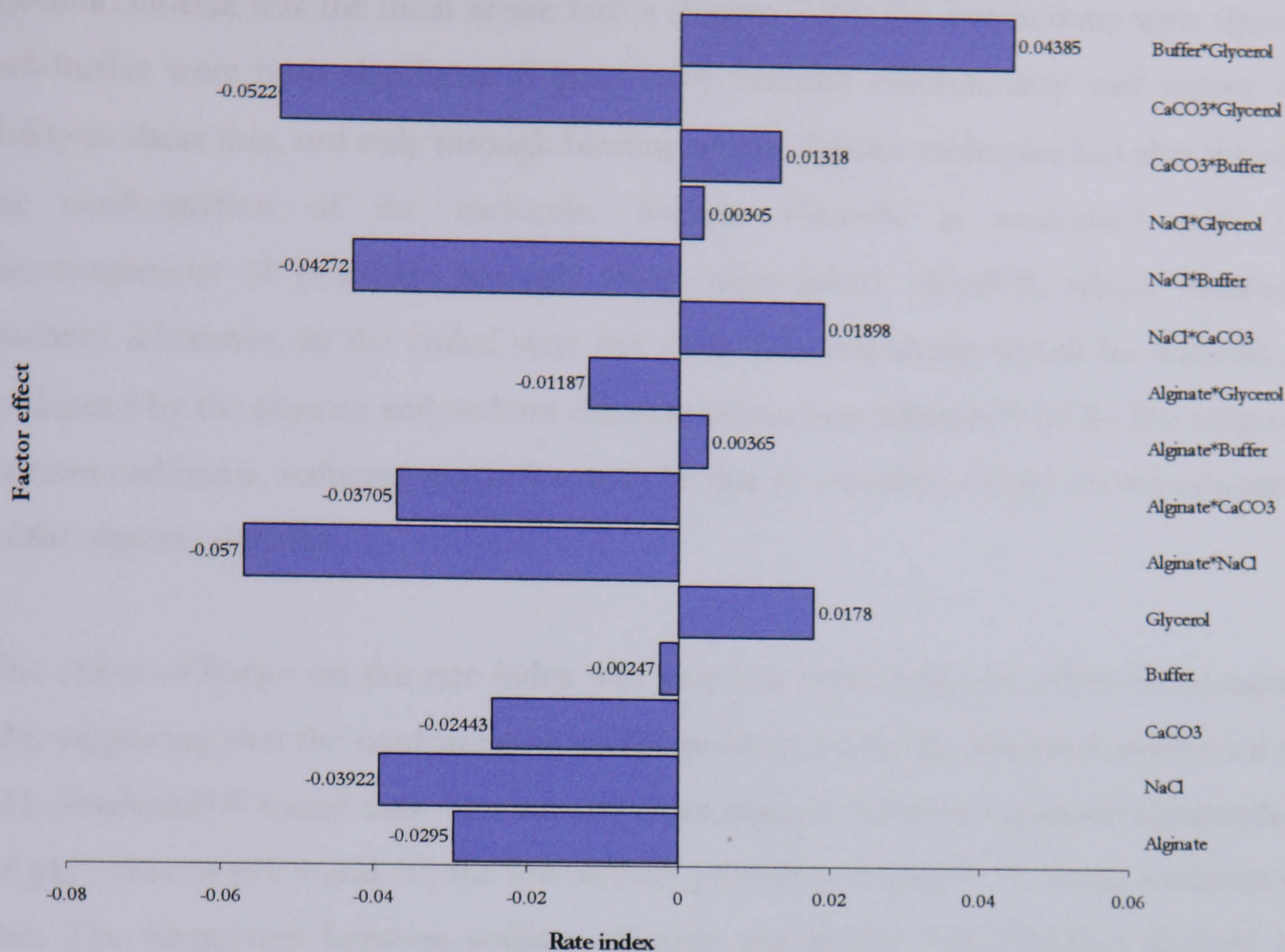


Figure 7.19 The profiles of the main effects and interactions of factors affecting the rate index of the sterile formulations

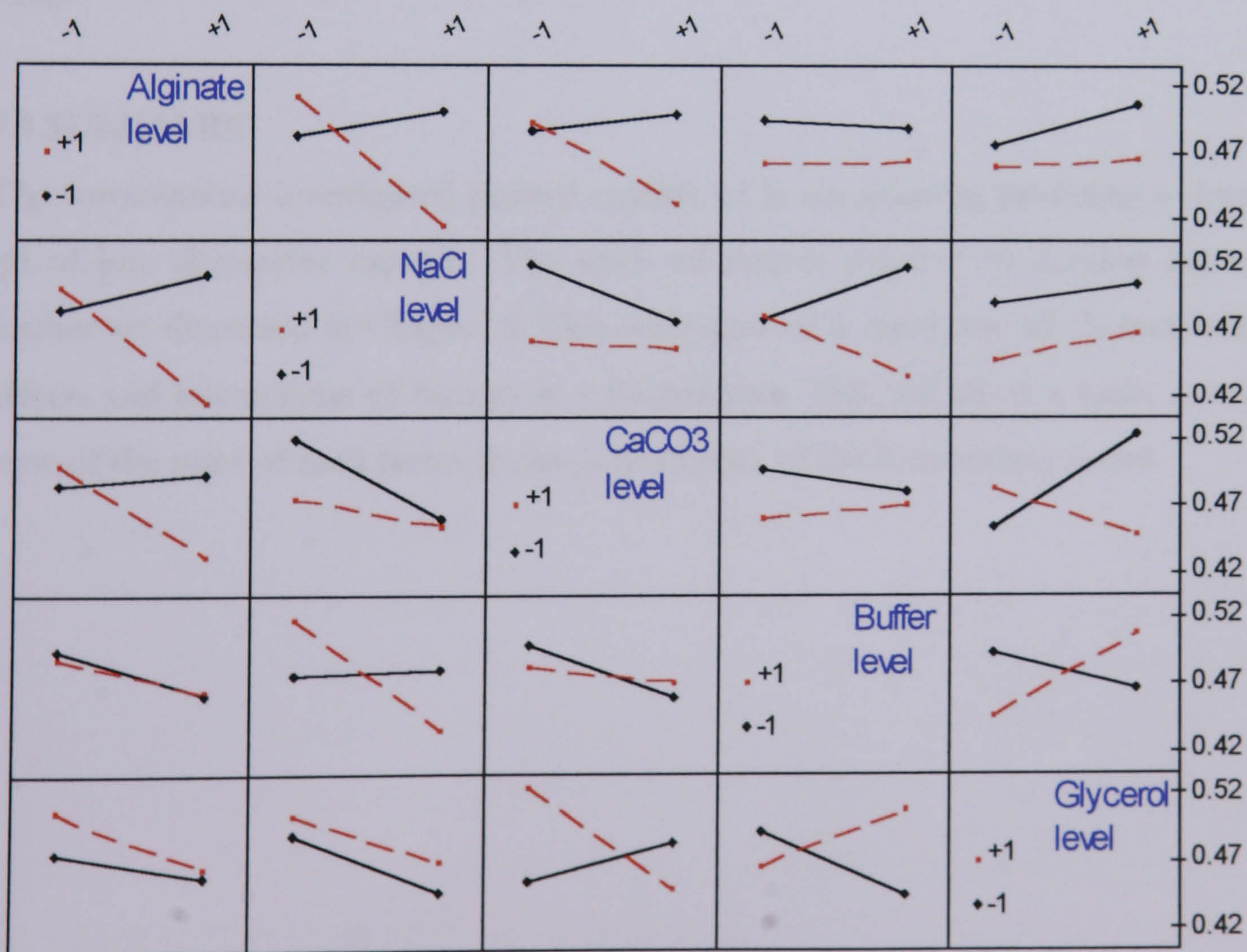


Figure 7.20 The interaction profiles factors affecting the rate index of the sterile formulations



Sodium chloride was the most active factor (Figure 7.19); the interactions with alginate and buffer were both significant (Figure 7.20). Sodium chloride may well reduce the ability to shear thin, not only through binding of the alginate molecules but also through the conformation of the molecule. Sodium chloride is associated with the encouragement of polymers towards their unperturbed states<sup>[116]</sup>, which decreases viscosity. However, in the coiled state the shear thinning ability would be reduced, as evidenced by the alginate and sodium chloride interaction (alginate\*NaCl). The effect of calcium carbonate, reducing rate index, may be due to the effect of pH on the ionisation of the alginate polymer.

The effect of buffer on the rate index was very low considering its effect in increasing pH, suggesting that the conformation of the polymer molecule remained unaffected by pH. Smidsrød<sup>[140]</sup> found that the viscosity of an alginate solution remained independent of pH between pH 4 and 10, the low activity of buffer (Figure 7.17) being evidence of this. The interaction between sodium chloride and buffer (NaCl\*buffer) showed no effect to a change in sodium chloride concentration at the lower level of buffer. At the higher level of buffer however, the change in rate index was markedly increased (Figure 7.20).

#### 7.4 SUMMARY

The formulations investigated proved capable of *in-situ* gelation, providing a structured gel of low absorptive capacity. The levels of factors required to develop the system further are discussed in Chapter 8. This study proved a rapid way of characterising the effects and interactions of factors in a formulation. This will allow a more considered view of the roles of each factor in the performance of the formulation *in-vivo*.



## **CHAPTER 8 GENERAL DISCUSSION**

### **8.1 OVERVIEW**

The available knowledge on the ability of sodium alginate to form gels dates back to the 1940's. The use of sodium alginate as a *in-situ* forming wound dressing was investigated by Blaine<sup>[173]</sup> and patented by McDowell<sup>[93]</sup>, but a commercially available dressing was not produced then, nor has one been reported since then. The versatility of sodium alginate is not in question. In the area of healthcare, alginate has found uses in many different areas such as dental moulds<sup>[174]</sup>, foams<sup>[52,97,98]</sup>, wound dressings<sup>[175,176]</sup>, haemostats<sup>[77,78,175]</sup>, for cell immobilisation<sup>[168,177,178,179,180,181,182,183]</sup>, tissue matrix materials<sup>[166,184]</sup> and pharmaceuticals<sup>[164,184,185,186,187,188]</sup>. In wound care fibre dressings are the mainstay of alginate use. Sorbsan<sup>®</sup> and Kaltostat<sup>®</sup> are two prominent examples; both have been exposed to numerous studies involving clinical trials<sup>[51,57,58,59,61]</sup>, dressing cytotoxicity tests<sup>[60,189, 190,191]</sup> and dental trials<sup>[192,193]</sup>. An amorphous hydrogel containing alginate is manufactured by Johnson and Johnson: Nugel<sup>®</sup>, which is a wound dressing claimed to benefit healing<sup>[194]</sup>. Nugel was characterised in Chapter 4 with two other amorphous hydrogels, Intrasisite<sup>®</sup> (Smith and Nephew Ltd.) and Sterigel<sup>®</sup> (SSL International, formerly Seton Healthcare).

The long history of the use of alginate and its biocompatibility suggests that its development as an *in-situ* dressing would provide a useful alternative to Cavi-Care<sup>®</sup> (Smith and Nephew Ltd.). An alginate system would also unlock the potential of including proteins and other sensitive additives, in the wound dressing, to promote healing at the wound site.

### **8.2 FORMULATION CHARACTERISATION**

The characterisation of the formulations in this project was based on rheological and water uptake investigations. The paucity of publications describing the absorptive capacity of hydrogels made this task difficult. Thomas and Loveless<sup>[195]</sup> used a 'nylon tea bag' to investigate the absorptive capacity of a number of dressings, including an Intrasisite<sup>®</sup> sheet dressing which had a semi-permeable backing. The base formulation was different from the one used in Chapter 4, hence a direct comparison could not be made. The water uptake characterisation was performed using a new method developed specifically for determining the absorptive capacity of amorphous and *in-situ* forming



hydrogels. Shigeyama *et al*<sup>[121]</sup> used a Franz cell in the conventional way *i.e.* solution in the lower compartment, to determine the absorptive capacity of a topical cream. The method developed and validated in section 2.3, used a Franz cell by filling the lower compartment with the gel and used the upper compartment to hold the donor solution. This allowed the uptake of water to be monitored by weight change over time.

The validation was successful and proved that the Franz cell method was reproducible, accurate, sensitive and easy to use. The robustness of the method was tested in three ways. Firstly, by testing the batch of formulation, prepared three times and the Franz cell used, no significance was attributed to either at the 95% confidence interval ( $P < 0.05$ ) (section 2.3.4.2.a). A second test was applied by using a second balance, to monitor the change in weight, during the linearity experiment: no significant difference was found between the balances (section 2.3.4.2.b). The final test of robustness was to change the glassware. The commercial samples were characterised using two sets of glassware, the two types of glassware were 1) bought Crown glass Franz cells and 2) custom made Franz cells by Mr Chappel (Glassblower). Water uptake results were compared using a one way ANOVA treating the type of glassware (section 2.3 Table 2.5). For both Intracel<sup>®</sup> and Nucel<sup>®</sup> there was a significant difference between glassware at the 95% confidence interval ( $P < 0.05$ ). However, for Sterigel<sup>®</sup> there was no significant difference at the 95% confidence interval ( $P > 0.05$ ). With this in mind, the only conclusion to be drawn was that a difference exists between the two sets of glassware. However, the reasons for this difference may not be solely due to glass type or dimensions. The physical properties of the gels may also have to be taken into account. Sterigel<sup>®</sup> was less viscous than either Intracel<sup>®</sup> or Nucel<sup>®</sup> (section 4.2 Figure 4.1). Lower viscosity could reduce gel susceptibility to the differences between glassware. The effects of temperature were not discussed because the laboratory was not under environmental control.

The accuracy and precision of the method was determined on the basis of the balance and its ability to provide a true measure of the change in gel weight over time. However, the accuracy of the method in terms of its ability to model the wound environment requires a comparison to *in-vivo* studies. The validity of this method as a model of the wound environment was based on the restriction the Franz cell provides, preventing complete swelling of the gel. *In-vivo*, amorphous hydrogels are used under a secondary



dressing and in combination with compression (for treating leg ulcers). Therefore during use the gel cannot fully swell with restriction.

Thomas and Loveless<sup>[195]</sup> characterised twelve commercially available hydrogels, the swelling characteristics of these gels did not match the performance of the gels *in-vivo*. Thomas and Loveless<sup>[195]</sup> concluded that the reason for this was that the swelling investigations did not mimic the constricted environment experienced *in-vivo*. The Franz cell method, developed in this thesis, would present a step forward in this area. The enclosed environment provided by the Franz cell, could mimic the constricted swelling under a secondary dressing.

The experimental designs in Chapters 5, 6 and 7 were used to efficiently investigate different factors and develop a formulation. The Plackett and Burman screening design used in Chapter 6 was an excellent way to investigate a large number of factors, to determine their significance in the formulation and therefore decide on further investigation. However, the disadvantage of the design was that no interactions could be determined and two formulations, within the experimental design, could not be compared directly. This was because of the large number changes in factor levels for each formulation. The use of a low resolution fractional factorial design could have helped in this, the disadvantage was that to be confident of the data quality a number of repeats would have to be performed. The advantage of the Plackett and Burman design was that the random error for a particular factor effect was spread over the whole experiment, because factor effects cannot be calculated without consideration of all results. The significance of an effect for a factor was determined by using the centre of the experimental design domain to find the random error for a particular test and then use this result to apply to the significance of an effect (a limiting value). If the effect of a factor was greater than the limiting value, an effect was considered significant.

The choice of factors for further investigation was determined using a tally of the order of significance for each physical characterisation test. The factors with the greatest effect in all characterisation tests were considered for further investigation. The amount of hyaluronic acid available for use restricted the number of factors to five. The experimental design used to investigate these factors was a fractional factorial design. Characterisation tests were performed only once, except for water uptake. This suggests



that the confidence in the data would be low without further repeats. However, the repeat formulations from the Plackett and Burman design indicate that the results of the tests were repeatable and accurate. Repeating the sixteen experiments three times to provide a concrete set of data was not feasible.

The full factorial designs used in Chapter 4 were time consuming, though informative, the single data points used for the rheological characterisation reduced the quality of the data. However, the water uptake data was based on the results from six Franz cells for each formulation (*i.e.*,  $n=6$ ).

### 8.3 *IN-SITU* GELLING WOUND DRESSING DEVELOPMENT

The investigations from Chapter 4 were not successful with regard to developing an *in-situ* gelling wound dressing, the gels produced were amorphous hydrogels. The method used to prepare the gels was potentially flawed. Sterilising the gels after mixing, rather than mixing sterile formulations to form a gel, was not a true test of how the formulations were required to perform. In terms of the absorptive capacity of the gels compared to a blank alginate solution, the only improvement seen was with Aquasorb<sup>®</sup> based gels. Celquat<sup>®</sup> reduced and Natrosol<sup>®</sup> had no effect on the absorptive capacity.

The charge of added polymer could go some way in describing the properties of the alginate polymer systems produced. The Aquasorb<sup>®</sup> polymer (sodium carboxymethyl cellulose) had a negative charge available for hydrogen bonding water from the pendant group. Celquat<sup>®</sup>, by contrast, had a large, positively charged, ammonium group as the pendant group. Natrosol<sup>®</sup> had a neutral overall charge, the hydroxyethyl groups possibly masking the absorptive nature of the polymer backbone.

The Natrosol<sup>®</sup> system (section 4.4.5) can be described using the 3%w/w Natrosol<sup>®</sup> and 8%w/w Alginate formulation (summarised Table 8.1). The relatively low viscosity compared to other formulations, was a potential benefit. However, this was tempered by the low creep recovery results. The formulation would be easy to syringe but would not regain enough structure to remain in the wound without secondary bandaging. Natrosol<sup>®</sup> was chosen for its shear thinning ability, so that higher concentrations could be used and still remain easy to syringe into the wound. However, the rate index (result 0.6) was much lower than expected for a system designed to have good shear thinning



properties. The effect profiles for the effects of factors on rate index (Figure 4.26) show that Natrosol® increased shear thinning with an increase in level. The water uptake result was lower than blank alginate but higher than Celquat®. This suggests that discounting the Natrosol® formulation out of hand may be premature.

Table 8.1 A summary of results from the characterisation of amorphous hydrogels from Chapter 4 (Section 4.2.2 and Tables 4.5, 4.6, 4.9, 4.10, 4.13, 4.14, 4.17 and 4.18).

Formulation	Viscosity	Rate Index	Creep Recovery	Water Uptake
%w/w	Pa.s	-	%	g/g/cm <sup>2</sup> /24h
8% Alginate	123	0.4	36.3	0.042
+3% Aquasorb®	18850	0.8	70.0	0.043
+3% Celquat®	4187	0.5	48.5	0.030
+3% Natrosol®	5186	0.6	33.0	0.040
Nugel®	40630	0.6	44.3	0.041
Intrasite®	62510	0.7	59.3	0.039
Sterigel®	6312	0.7	84.3	0.023

Celquat®, by contrast (section 4.4.4), was less viscous and had a much lower rate index, creep recovery and water uptake capacity. The reason for this could be explained by there being a positive attraction between the quaternary ammonium group on the Celquat® polymer and the co-ordinating G block regions of the alginate polymer, resulting in a reduced capacity to shear thin. This type of attraction is a possibility according to Smidsrød<sup>[136]</sup>. An alternative to this physical interaction is a chemical interaction. Taguchi and Tanaka<sup>[196]</sup> investigated the crosslinking of hyaluronic acid and collagen, negatively and positively charged respectively. They found that acidic and basic groups on the respective polymers were prone to react. Such a situation could explain the negative effect Celquat® had on the water uptake capacity (Figure 4.12). Steam sterilisation would provide conditions for a reaction to progress.

The final system studied was composed of sodium alginate and Aquasorb® (section 4.4.6). The viscosity of the formulation was the highest of the four systems developed (Table 8.1) but remained approximately half the viscosity of Nugel®. This relatively large viscosity was tempered by a high rate index, which showed that the formulation was potentially able to shear thin, providing a much easier formulation to syringe than Nugel® or Intrasite®. The improved shear thinning compared to the Natrosol® formulation was explained by the charge of the polymer, the negative charge prevents extensive polymer entanglements and also ensures that under stress the polymers flow



past each other with much greater ease. The presence of sodium chloride in the formulation could prevent this from occurring, through charge shielding.

The presence of Aquasorb® in the formulation had a greater effect on the water uptake capacity of the formulation compared to either Natrosol® or Celquat®. Aquasorb® increased absorptive capacity to a greater extent than that of sodium alginate alone (Table 8.1). The charged carboxy group and counter ion were thought to provide a much greater potential for binding water. The method of determining the absorptive capacity could also affect the results. The linearity experiment, carried out during the Franz cell method validation (section 2.3.4), suggested that as concentration increased the ability of the Franz cell to allow proportional swelling was diminished (Figure 2.13). The Franz cell environment was physically challenging the formulations, as a result their ability to swell was being diminished. Increasing the final time point from 24h to 48h or 72h, would provide a method to reach full capacity of the gels and determine the time for which the gels could be kept in the wound.

Calcium was present in the alginate polymer systems to induce gelation. This was based on the principle of a delivery system consisting of two syringes. To expel the contents, the syringes would be emptied together through a mixing nozzle: a double barrel syringe<sup>[97,98]</sup>. Mixing the solutions together; syringe 1 containing polymer and calcium solution and syringe 2 containing sodium alginate solution, would lead to gel formation. However, considering the speed at which calcium can induce alginate to gel<sup>[197]</sup> the scenario where the gel formed during mixing, blocking the nozzle, was far more likely to occur. The beneficial properties of Aquasorb® on the alginate polymer system suggest that calcium would not be required to develop the formulation as an amorphous hydrogel. The formulation was a dirty green colour, which looked rather disgusting and would result in poor patient efficacy. Improving formulation colour by mixing the alginate solution with activated charcoal then filtering, to produce a much lighter almost transparent solution<sup>[198]</sup>, was considered a necessary step prior to manufacture.

The gelation of an alginate formulation was achieved in Chapter 5 using the methods described by Draget *et al*<sup>[74,91]</sup> to develop a microbiological culture media. Draget *et al*<sup>[74,91]</sup> used calcium carbonate as an insoluble sequestering agent and the slow addition of an acidic proton, dissolved calcium carbonate to release calcium ions, carbon dioxide



and water. Hydrolysis degraded delta-gluconolactone to gluconic acid, releasing an acidic proton. The binding of calcium occurs in seconds<sup>[197]</sup>, a problem which causes gel heterogeneity. This arises when the alginate forms a gel before calcium ions can diffuse evenly throughout the system. Even mixing of the system with an insoluble salt, of very small particle size, avoids this problem. The presence of gluconic acid may restrict the use of such a dressing in diabetic patients, though the body is able to tolerate its presence<sup>[199,200]</sup>.

The gelling system investigated in Chapter 5 was developed further in Chapter 6, with the addition of excipients. A Plackett and Burman screening experimental design was used to investigate the effects of ten factors on the performance of the formulation. These factors were 1) Sodium alginate, 2) Sodium alginate type, 3) Calcium carbonate, 4) Sodium carboxymethyl cellulose viscosity, 5) Sodium carboxymethyl cellulose concentration, 6) Glycerol, 7) Hyaluronic acid, 8) Sodium chloride, 9) Buffer and 10) Gelling temperature. The screening procedure was effective in determining the influence of these factors on the alginate gelling system. The most active factors were chosen for further investigation, these factors were alginate concentration (b1), calcium concentration (b3), glycerol concentration (b6), sodium chloride concentration (b8) and buffer concentration (b9). These factors were investigated in Chapter 7 using a fractional factorial experimental design that estimated the main effects and first order interactions of factors at two levels.

The effects of sodium chloride on sodium alginate are well documented<sup>[81,135,140,142,143,146,158,170,171,201]</sup>. The presence of sodium chloride in the formulation reduced absorptive capacity. This could simply be an osmotic effect. However, sodium ions were associated with binding sodium alginate in a number of studies<sup>[135,142,143,145,146,158]</sup>. The pI of the formulation was such that alginate would be fully ionised<sup>[143,157]</sup>. Thus, the potential for free sodium ions to be bound was high.

The effects of sodium chloride cannot be fully delineated from the experiments carried out. The effects of sodium chloride on polyelectrolytes include conformational<sup>[137,202,141,203,204]</sup> and physical effects<sup>[152,169,205]</sup>. The conformational effects of sodium chloride are thought to be surpassed at an alginate concentration of 3%<sup>[206]</sup>. The beneficial effects of sodium chloride were i) reducing gelling time, ii) improved



strength, iii) reducing pH of the formulation and iv) increasing shear thinning (section 5.2.5). The gel strength profile in section 5.2.5, shows sodium chloride had a greater effect increasing gel strength at lower levels of sodium alginate and calcium carbonate, which was also noted by Zheng *et al*<sup>[142]</sup>. The effect of sodium chloride reducing pH, could be explained by the Henderson Hasselbalch equation<sup>[207]</sup>; ionic strength, accounting for activities, reduces pH. The increase in shear thinning imparted by the sodium chloride seen in sections 5.2.5.2.f and 6.3.3 would be beneficial when syringing or pouring the formulation into the wound. The reason for this increase may be that the binding of sodium was such that the viscosity increased, but the low strength of the binding left the attraction susceptible to shear dependant behaviour<sup>[116,172]</sup>, an effect observed by Herraiez-Dominguez *et al*<sup>[208]</sup> when investigating sodium carboxymethyl cellulose.

Sodium alginate was the dominating factor, with the largest effects in most areas of performance. The level of alginate was constrained by the viscosity required: liquid enough to pour or syringe easily (plastic 5ml syringe; no needle), but capable of absorbing water when in the gel state. The level of calcium carbonate controlled the gelling time. However, the more alginate used the greater the amount of calcium required to induce gelation<sup>[74]</sup>. The strength of the gel was determined by the G:M block ratio<sup>[62,99,136,145,209]</sup> and the level of calcium carbonate.

The type of alginate used in the factor influence study (Chapter 7) was Protanal LF10/60 which had a guluronic acid content of 69%<sup>[99]</sup> and a molecular weight of 190-210 kDa <sup>[99,156]</sup>, the gels from this alginate are strong<sup>[99,209,210]</sup>. The high level of G residues gives mechanically stronger gels with enhanced stability to anti-gelling ions and calcium sequestrants<sup>[178]</sup>. The MG block regions in alginate polymers have been associated with water absorption<sup>[145]</sup>, the effect of sodium chloride on the absorptive capacity could involve these regions.

The ability of alginate to absorb water was reduced by the presence of calcium, so much so that in the wound environment serum could prevent the formulation from absorbing any fluid, the result of the effect of salt on a polyelectrolyte<sup>[68,152,205]</sup>. Simply increasing the level of alginate was not practical because of the associated increase in viscosity. The incorporation of alginates of a different type was an alternative, but the type used in



Chapter 6 (Protanal LF 10/60D) had associated problems with viscosity and gel strength. The use of alginates with lower levels of glucuronic acid would require higher levels of calcium to form a strong gel. The associated effect of calcium carbonate, on pH and therefore stability, would be detrimental and a larger amount of delta gluconolactone would be required to release calcium ions. There are reports that high concentrations of calcium in the wound are detrimental to wound healing<sup>[60,176]</sup>.

The stability of sodium alginate during sterilisation was considered acceptable<sup>[62,74]</sup>, but the storage of the molecule at a pH where a degradation pathway exists<sup>[71,211]</sup> required accurate control of the formulation pH. Smidsørd and Haug<sup>[71,212]</sup> investigated the pH stability of alginate and found that with small changes in pH, the stability was considerably improved. The closer to pH 7 the more stable the alginate molecule would be<sup>[71,212]</sup>. The tests were performed at elevated temperatures. At lower temperatures, the stability would be greater, but during sterilisation the pH must be as close to 7 as possible. The interaction of alginate and sodium chloride had a number of benefits. It improved the shear thinning and reduced the pH (section 5.2.5). Therefore higher levels of alginate could be used, but the negative effect of sodium chloride on water uptake (Chapters 5, 6 and 7) suggests that sodium chloride level must be kept low.

The formulations investigated in Chapter 4 contained similar levels of calcium to the formulations in Chapter 6, but remained amorphous. The result of the method of calcium introduction completely altered the state of the formulation. The pH induced by calcium carbonate had a number of disadvantages, i) the stability of the alginate molecule was compromised by high pH<sup>[71,212]</sup> and ii) the wound will not tolerate high pH<sup>[11]</sup>. However, the rapid change in pH (Appendix 2) suggests that after 15 minutes, a pH acceptable to the wound was achieved.

The reaction of calcium carbonate with the acid proton, released by delta gluconolactone, should proceed to completion *i.e.*, carbon dioxide and water. However, bubble formation in the formulations was low, which was related more to the viscosity of the formulation allowing carbon dioxide to escape prior to gelation. However, the rising pH (Appendix 2) suggested that reaction of calcium carbonate with the acid proton did not go to completion and the bicarbonate ion remained. The role of the



buffer must not be ruled out, not only in terms of its ability to absorb a proton but also in its ability to sequester calcium.

Sodium alginate level affected both gelling time and gel strength. As the level of alginate was increased gelling time increased (sections 5.2.4, 5.2.5, 6.3.5 and 7.3.3.1), possibly a result of the associated increase in polymer entanglement with an increase in polymer concentration. Though the proximity of the polymer chains increases, the time taken to form stable junction zones increases. The minimum number of glucuronic acid monomers required to form a stable junction zone is thought to be between 14<sup>[63]</sup> and 20<sup>[133,134]</sup>. The high glucuronic acid content alginate Protanal LF10/60 would ensure a high number of G block sequences with a sufficient number of glucuronic acid monomers. The ability of the polymer chains to align would be reduced by the increase in entanglement.

Awad *et al*<sup>[213]</sup> found that gel strength was dependant upon sodium alginate concentration. Protanal LF10/60 used in this study had a high glucuronic acid content alginate, associated with forming strong gels. However, the effects of increasing alginate level were contradictory in Chapter 5. Results section 5.2.4 suggested that increasing alginate level increased gel strength whereas those in section 5.2.5 suggested that increasing alginate level reduced gel strength. An interaction with sodium chloride must be taken into account for this difference. The effects of increasing alginate level in section 6.3.5 were to reduce gel strength. However, the effect of alginate type was demonstrated when considering the effect of alginate type. Increasing the level of alginate type from a low to high glucuronic acid alginate (LF10/60D to LF 10/60) increased gel strength. The level of calcium carbonate also affected gel strength. The effects were dependent upon the type of alginate used, because the G/M ratio dictates the properties of a gel (section 1.4). A high level of calcium in a high G content gel could lead to syneresis<sup>[62]</sup>. Gels formed without syneresis would tend to be hard and brittle, whereas, a low level of calcium with a high G content alginate solution a gel may not form.

In Chapter 7 different methods for determining gel strength were used in addition to the G' max method used in previous sections. Though the increase in the level of alginate using % loss G\* suggested that gel strength was decreased by an increase in alginate



level, the determination of  $G'$  Max and  $G^*$  intercept both indicated that increasing alginate level increased gel strength. The disadvantage of the gel strength tests, used in this study, was that they were performed 3h post gelation, gelation using sequestered salts is thought by some authors to take up to 24h.

The presence of a buffer in the formulation did not prevent gelation occurring. This was the main worry associated with its inclusion. McDowell<sup>[93]</sup>, Monshipouri and Price<sup>[168]</sup>, and Kuo and Ma<sup>[166]</sup> have actively used phosphates as sequestering agents. The dissolved calcium was sequestered by phosphate, before binding, thus retarding gelation. The role of buffer was discussed in section 7.3.2. The pH profiles in Appendix 2 show that the pH drops dramatically from pH 8 to pH 6 over approximately 3h and then rises slowly afterwards over 24h. A pH 6.5-7 was seen in most formulations after 48h, which was acceptable for a wound dressing.

Water uptake, gelling time and shear thinning were all improved by the presence of glycerol. Glycerol was included primarily as a plasticiser and improved gel strength (sections 6.3.5 and 7.3.3). When the gel dried the glycerol imparted soft and malleable properties, ensuring that the gel would not harden and disrupt the wound environment. Glycerol was not steam sterilised because it is an organic liquid, the sterilisation method used was 150°C for two hours<sup>[214]</sup>. The inclusion of this factor in the formulation could therefore complicate its manufacture. This was the only drawback associated with the use of glycerol. An alternative method of sterilisation would be gamma ( $\gamma$ )-irradiation, but this is known to damage the alginate molecule<sup>[73]</sup>. Alternative plasticisers are sorbitol and propylene glycol, both having humectant properties<sup>[153]</sup>.

The choice of five factors for further investigation left five factors at a constant level. The levels chosen were based on the screening procedure (Chapter 6). The gelling temperature was set at 25°C standard temperature, because temperature had very little effect on the formulation. The choice of LF10/60 as the type of alginate was based on the LF 10/60 having a lower viscosity which ruled out the use of LF10/60D, though the use of LF10/60LF in future formulations was not ruled out. Sodium carboxymethyl cellulose (low viscosity) was kept at a level to ensure that advantage could be taken of its shear thinning ability without increasing the viscosity of the formulation to unmanageable levels. The studies of Chapter 4 showed that a high concentration of



Aquasorb<sup>®</sup> (sodium carboxymethyl cellulose) was required in a formulation to increase the absorptive capacity above that of blank alginate. The concentration of sodium carboxymethyl cellulose must be kept below 5% because of a toxic effect toward cells at that level<sup>[215]</sup>, but, such a concentration would be impractical to use as a gel because of a very high viscosity. The level of hyaluronic acid was dictated more by the availability of stock, though the screening procedure showed hyaluronic acid to have a neutral influence on the formulation. At higher concentrations, its effects on water uptake and viscosity may become prohibitive. Again the stability of the hyaluronic acid during sterilisation was a consideration<sup>[216]</sup>.

The biocompatibility of alginate as mentioned earlier (section 1.4.6), has been brought into question<sup>[58,59,60]</sup>. High M block alginate was thought to elicit a foreign body reaction, which can impede healing. Klöck *et al*<sup>[67]</sup> found that low molecular weight fragments which leaked from a dressing, lead to a foreign body reaction. Other investigations have found alginate contains phenolic compounds<sup>[217]</sup>, an artifact from manufacture and many immunogenic factors<sup>[67]</sup>. Methods have been developed to remove these compounds from alginate improving stability and biocompatibility<sup>[67]</sup>. The calcium carbonate method of internal gelation is quoted as being mild and non toxic<sup>[166]</sup>, but before further development could be undertaken the formulations would have to be tested for toxicity either by the British standard method<sup>[189,218]</sup> or the L929 mouse fibroblast method developed by Spyratou<sup>[23]</sup> and used on numerous occasions as a sensitive test of toxicity<sup>[60,219,220]</sup>.

Advances in the area of scarless healing of secondary wounds<sup>[108]</sup> prompted the inclusion of hyaluronan, which plays an active role in the reduction of scar tissue<sup>[110]</sup> and has a wider role in wound healing<sup>[107,108,221,222]</sup>. Foetal wound healing studies have demonstrated scarless healing<sup>[223,111]</sup> with the general acceptance that foetal wounds heal by regeneration<sup>[111]</sup>. Amniotic fluid<sup>[104]</sup> and foetal skin<sup>[223]</sup> are rich in hyaluronan, but, adult skin still heals with a scar when bathed in amniotic fluid<sup>[112]</sup>. In non-foetal models hyaluronan facilitates healing<sup>[100,215]</sup>. The role of hyaluronan in wound healing still remains to be properly elucidated<sup>[107]</sup> but there is no doubt that it has a beneficial effect upon healing<sup>[224,225]</sup>. The use of growth factors to promote scarless healing is also an area of current research<sup>[226,227,228,229,230,231]</sup>. Alginate is seen as an ideal method for presenting agents to the wound in a controlled manner<sup>[224]</sup>, but, the role of individual growth



factors is a matter of debate. Transforming growth factor  $\beta$ -1 was shown to promote healing<sup>[226,230]</sup> and reduced scar formation<sup>[231]</sup>.

The promotion of healing by the inclusion of factors beneficial to the healing wound was investigated by Viljanto and Raekallio<sup>[232]</sup> and more recently by Lindenbaum *et al*<sup>[233]</sup>. The major fear would be that such a method provides an environment ideal for bacterial growth. Neither investigation<sup>[232,233]</sup> mentioned the presence or promotion of infection. Lindenbaum *et al*<sup>[233]</sup> used frequent dressing changes and washes to prevent infection, antibiotics were not used. In fact topical antibiotics are actively discouraged<sup>[22,234]</sup> and they are even thought to retard healing. Their systemic use has no affect in the topical environment<sup>[16]</sup>.

## 8.4 CONCLUSION

In conclusion, this study introduced a new method for the characterisation of the absorptive capacity of wound gels (amorphous and *in-situ* gelling). The method was validated as accurate, reproducible and sensitive. The investigations carried out in Chapter 4 produced an amorphous hydrogel that could compete with Intrasisite<sup>®</sup> (Smith and Nephew Ltd.) and Nugel<sup>®</sup> (Johnson and Johnson), based on the characterisation methods that were used. The formulation was 8%w/w Sodium alginate (LF10/60, FMC Biopolymer) and 3%w/w Aquasorb<sup>®</sup> A500 (Hercules). However, the formulation did not fulfil the aims of this thesis; to develop an *in-situ* gelling wound dressing.

Chapters 5, 6 and 7 covered the development of an *in-situ* gelling wound dressing that fulfilled the aims of this thesis. The properties of the dressing (Chapter 7) were also capable of fulfilling the ideal properties of a wound dressing (section 1.3 Table 1.5). The formulation that would be beneficial as a wound dressing is detailed in Table 8.2.



Table 8.2 A formulation for an *in-situ* gelling wound dressing.

Constituent	Amount	Unit
Sodium alginate	5.5	%w/w
Sodium chloride	0.25	%w/w
Calcium carbonate	0.22	%w/w
Phosphate buffer	0.25	%w/w
Glycerol	5	%w/w
Sodium alginate type	LF 10/60	-
Sodium carboxymethyl cellulose type	low viscosity	-
Sodium carboxymethyl cellulose	0.62	%w/w
Hyaluronic acid	0.05	%w/w

A gel formulated as above would gel within approximately 1h. A secondary dressing would be required to ensure efficacy. The gelling formulation could be put to use in the treatment of burns and leg ulcers. In the treatment of burns it would be used by covering the wound with the formulation as it gels. The loss of water to the atmosphere, from a wound covered in the gel, would cool the wound and regulate exudate absorption. Any secondary dressing employed would have to be selected to allow vapour transmission. The use of the gel in conjunction with compression would help in treating leg ulcers.



## **CHAPTER 9 RECOMMENDATIONS FOR FURTHER WORK**

The following comprises a summary of recommendations for further work.

The Franz cell method for determining the absorptive capacity of hydrogels, was validated in section 2.3. This method requires further investigation before it can be accepted as an *in-vitro* model of the wound environment. Investigating the effects of cell dimension on the absorptive capacity and the internal pressure exerted by swelling gels would allow a comparison to the *in-vivo* wound environment. The validation of the method using a serum mimic would also be required.

The physical hydrogels investigated in Chapter 4 exhibited interesting properties. The formulation 8%w/w Sodium alginate (LF10/60, FMC Biopolymer) and 3%w/w Aquasorb® A500 (Hercules) was seen as a potentially viable wound dressing.

The *in-situ* dressing developed and characterised in Chapters 5, 6 and 7 would require further development to produce a commercially viable product. Initially the method of delivery, cytotoxicity work and the absorptive capacity in the presence of a serum mimic were seen as important areas of investigation. Integrating the cytotoxicity work with an experimental design would allow the investigation of formulation performance and its efficacy at different stages prior to and during application to the wound. This would also indicate why a dressing of a similar type has not been developed commercially.

The type and source of alginate was seen as a further area of investigation to maximise absorptive capacity, gel strength and gelling time. Haug *et al*<sup>[64]</sup> used bacteria as a source of alginates and investigated genetic modification to produce specific types of alginate. It is also possible to tailor an alginate polymer by *in-vitro* enzymatic modification<sup>[235]</sup>.



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**APPENDIX 1 OERTLING BALANCE CALIBRATION**

Table A1.1 Balance calibration data recorded for 10 repeat weighing for each calibration point (weights not traceable).

Oertling balance		Balance reading /g					
Weight /g		0.05	0.5	5	50	100	200
1		0.052	0.4989	5.0009	50.0112	99.96	200.0096
2		0.0518	0.4985	5.0012	50.0111	99.9601	200.009
3		0.0519	0.4986	5.001	50.0111	99.9605	200.0101
4		0.0517	0.4988	5.0012	50.011	99.96	200.0094
5		0.0517	0.4988	5.0012	50.0112	99.9601	200.0095
6		0.0515	0.4988	5.0012	50.0111	99.9604	200.0096
7		0.0518	0.4987	5.0014	50.0112	99.9609	200.0094
8		0.052	0.4987	5.0011	50.011	99.9604	200.0094
9		0.0517	0.4987	5.0013	50.0111	99.9601	200.0097
10		0.0516	0.4987	5.0015	50.0114	99.9605	200.0091
Mean		0.0518	0.4987	5.0012	50.0111	99.9603	200.0095
STDEV		0.0002	0.0001	0.0002	0.0001	0.0003	0.0003
RSD		0.3161	0.0228	0.0035	0.0002	0.0003	0.0002

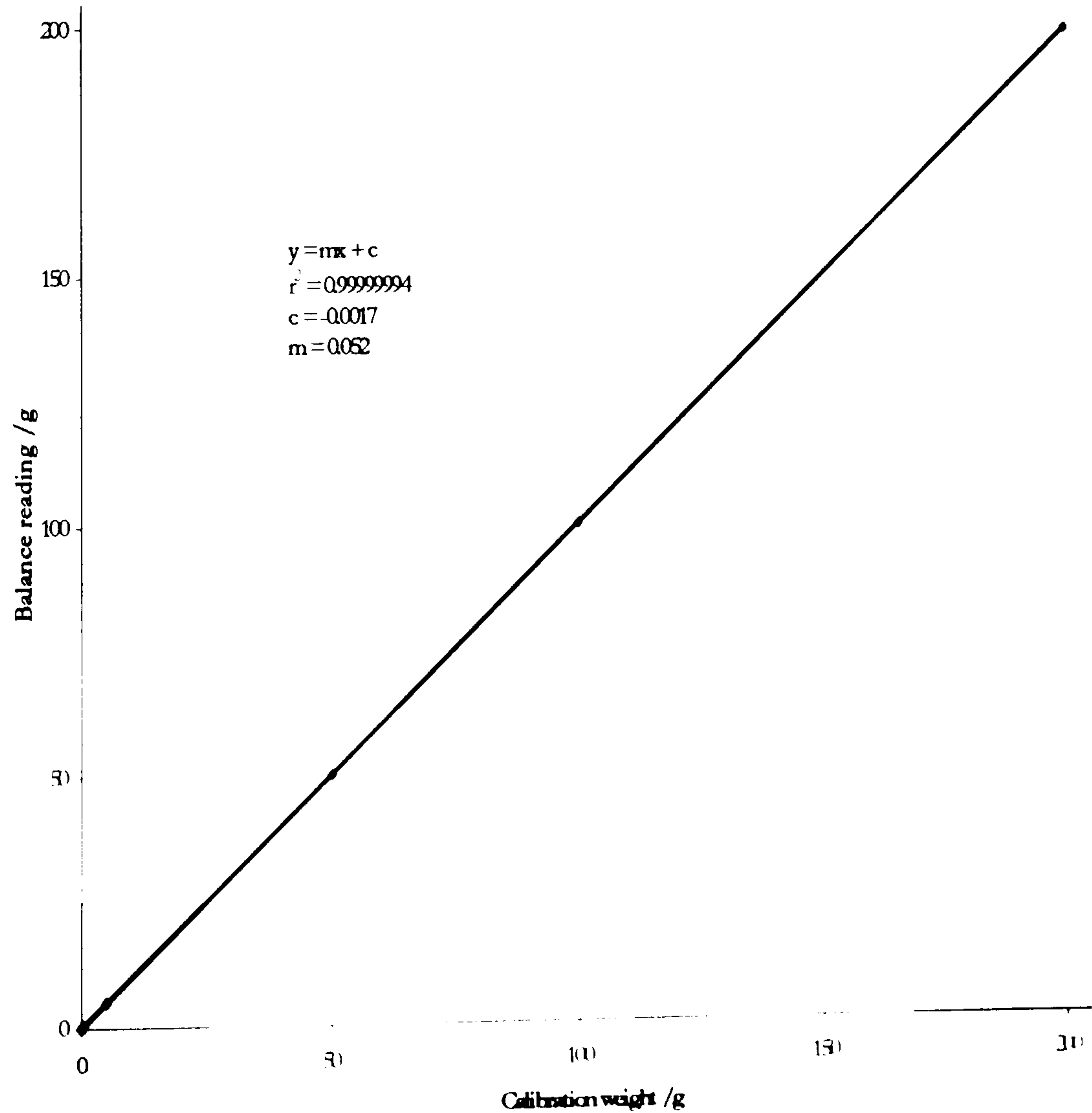


Figure A1.1 Linear regression analysis of balance calibration data; r<sup>2</sup> value of 0.9999.



**APPENDIX 2 INVESTIGATING THE pH PROFILE OF THE CALCIUM CARBONATE/SODIUM ALGINATE SYSTEM DURING GELATION**

**A2.1 AIM**

The aim of the experiment was to observe the change of formulation pH with time during gelation. The factors, their levels and the Plackett and Burman experimental design are outlined in Tables A2.1 and A2.2 respectively.

Table A2.1 Factors and levels for the Plackett and Burman screening design.

Factors to be screened	levels	+	0	-
Sodium alginate (%w/w)	X <sub>1</sub>	5	3.5	2
Alginate type	X <sub>2</sub>	lf 10/60	lf10/60ls	lf10/60d
Calcium carbonate (%w/w)	X <sub>3</sub>	0.32	0.24	0.16
Carboxymethyl cellulose (η)	X <sub>4</sub>	High	Medium	Low
Carboxymethyl cellulose (%w/w)	X <sub>5</sub>	1	0.75	0.5
Glycerol (%w/w)	X <sub>6</sub>	5	2.5	0
Buffer (%w/w)	X <sub>7</sub>	1	0.5	0

Table A2.2 Experimental design for Plackett and Burman screening procedure.

Factor Experiment	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>
1	+	+	+	-	+	-	-
2	-	+	+	+	-	+	-
3	-	-	+	+	+	-	+
4	+	-	-	+	+	+	-
5	-	+	-	+	+	+	+
6	+	-	+	-	-	+	+
7	+	+	-	-	-	-	+
8	-	-	-	-	-	-	-
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0

**A2.2 METHOD**

The materials were equivalent to those in section 6.2.1. The methods for gel manufacture can be found in section 2.1.

**A2.3 RESULTS**

The results are represented graphically below Figures A2.1- A2.3



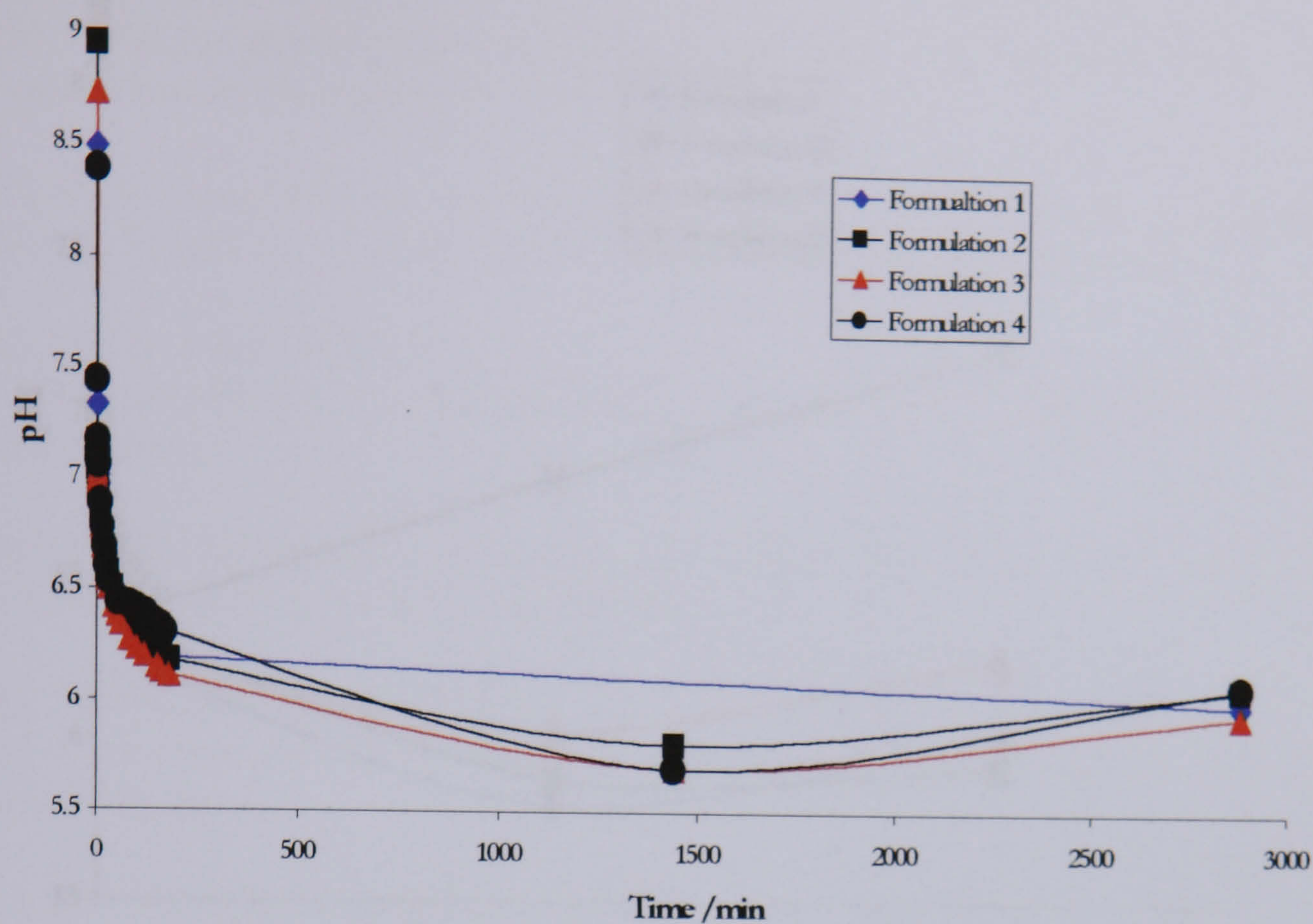


Figure A2.1 The pH profiles of formulation 1, 2, 3 and 4.

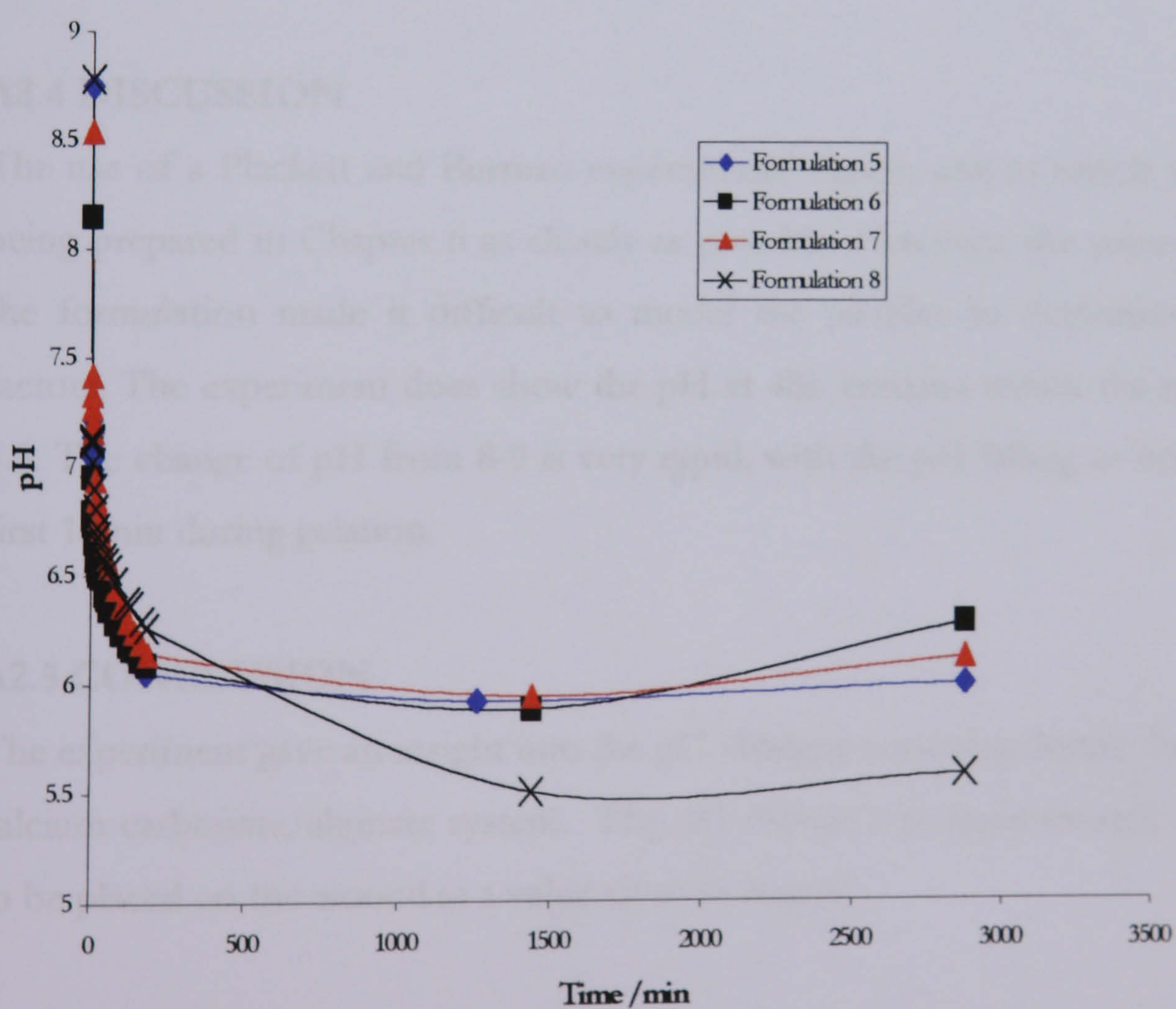


Figure A2.2 pH profiles of formulations 5, 6, 7 and 8.



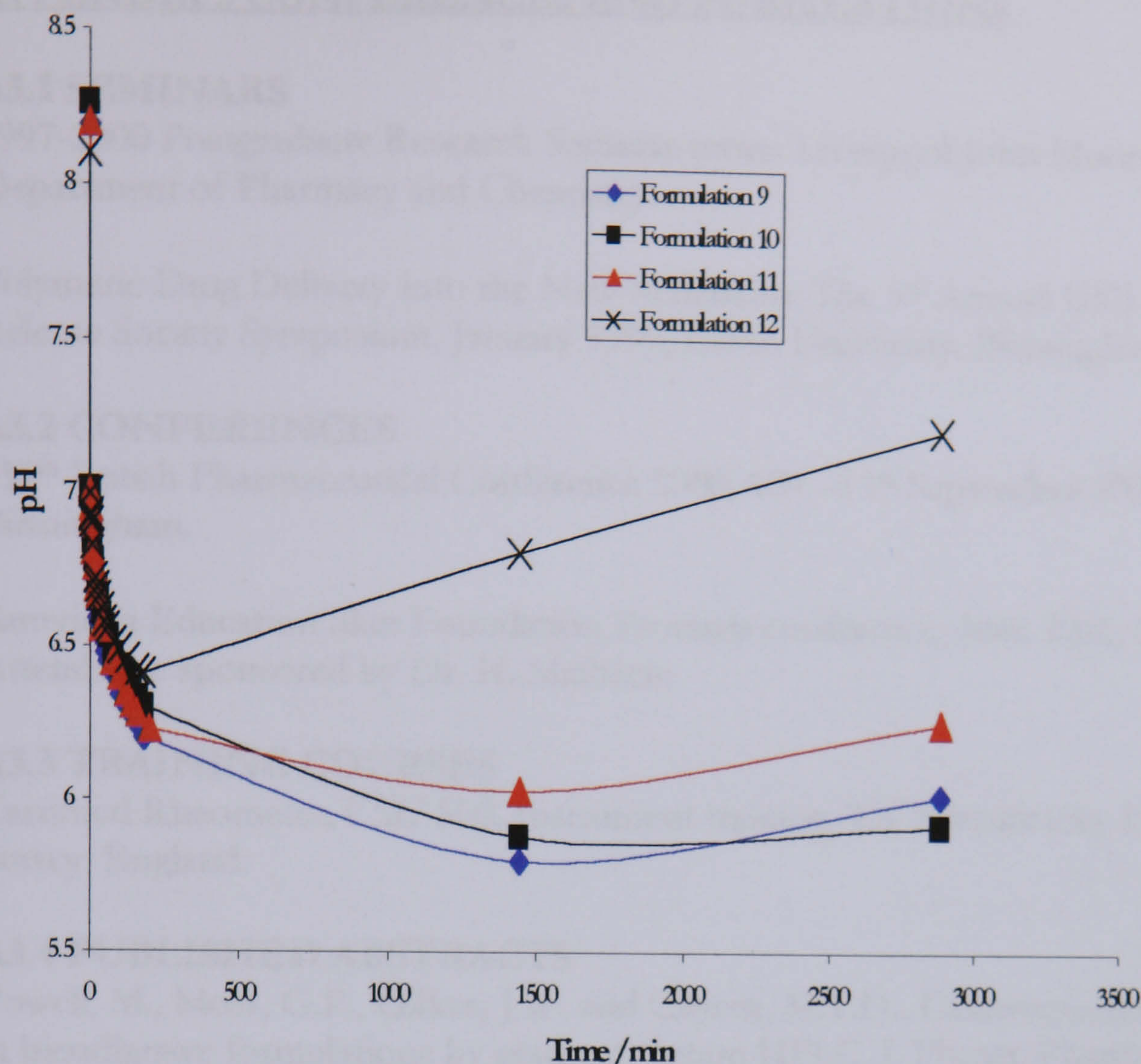


Figure A2.3 pH profiles of formulations 9, 10, 11 and 12.

## A2.4 DISCUSSION

The use of a Plackett and Burman experimental design was to match the formulations being prepared in Chapter 6 as closely as possible. However, the presence of buffer in the formulation made it difficult to model the profiles to determine the effects of factors. The experiment does show the pH at 48h remains within the range of pH 7.5-5.5. The change of pH from 8-9 is very rapid, with the pH falling to below pH 7 in the first 10min during gelation.

## A2.5 CONCLUSION

The experiment gave an insight into the pH changes occurring during the gelation of the calcium carbonate/alginate system. The pH change was rapid enough for the dressing to be placed on the wound at a value close to neutral.



## **APPENDIX 3 CONFERENCES AND PUBLICATIONS**

### **A3.1 SEMINARS**

1997-2000 Postgraduate Research Seminar series. Liverpool John Moores University, Department of Pharmacy and Chemistry.

Polymeric Drug Delivery into the New Millenium. The 5<sup>th</sup> Annual UKI Controlled Release Society Symposium, January 1999, Aston University, Birmingham.

### **A3.2 CONFERENCES**

137<sup>th</sup> British Pharmaceutical Conference 2000, 10<sup>th</sup> –13<sup>th</sup> September 2000, ICC Birmingham.

American Education Skin Foundation Psoriasis conference, June 2001, San Francisco. Attendance sponsored by Dr. H. Maibach.

### **A3.3 TRAINING COURSES**

Carrimed Rheometer, CSL<sup>2</sup> 500, Instrument training. TA Instruments, Leatherhead, Surrey. England.

### **A3.4 PUBLISHED ABSTRACTS**

Powell, M., Moss, G.P., Gilkes, J.W. and Cronin, M.T.D., Determination of bleomycin in bioadhesive formulations by gradient elution HPLC. J. Pharm. Pharmacol. 50 (supplement): 91 (1998). [Attached page 189].

Gilkes, J.W., Ford, J.L., Rowe, P.H., and Malhi, J. Water absorption by wound gels: validation of a wound model. J. Pharm. Pharmacol. 52 (supplement): 89 (2000). [attached page 190].



Determination of bleomycin in bioadhesive formulations by gradient-elution HPLC

M. POWELL, G. P. MOSS, J. W. GILKES AND M. T. D. CRONIN

School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF

Bleomycin is a mixture of antineoplastic antibiotics employed in the treatment of various squamous cell carcinomas, including those of the cervix and the skin, and in the treatment of Hodgkin’s disease and other lymphomas. It has been formulated for localised administration to the cervical canal as double-layered sticks (Iwata et al 1987) or as a compressed disk (Machida et al 1979). The aim of the present study was to develop a rapid HPLC assay for the determination of bleomycin in bioadhesive dosage forms based on those reported previously (Woolfson et al 1995).

Formulations were prepared using methods discussed previously (Woolfson et al 1998). Analysis was performed by reverse-phase high performance liquid chromatography (HPLC) on a Waters Alliance system with photodiode array detection. The column used was a Waters NovaPak (3.9mm x 150mm; 5µm d.p) at 30°C. The mobile phase consisted of HPLC water and acetonitrile, each containing trifluoroacetic acid (TFA) (0.1%). Gradient elution was performed at 1.0 ml min<sup>-1</sup> (Table 1).

Time (mins)	0	5	10	11	12	15
%A (Acetonitrile/TFA)	10	20	50	50	10	10
%B (H <sub>2</sub> O/TFA)	90	80	50	50	90	90

Table 1. Experimental conditions for gradient elution HPLC analysis of bleomycin.

Wet formulations were spiked at two levels (1.5 and 0.15 units g<sup>-1</sup>) with bleomycin sulphate USP. The purpose of the lower level sample was to characterise the performance of the method close to the expected limit of detection. A calibration curve was constructed by spiking HPLC water with bleomycin covering the range 0-3.0 units g<sup>-1</sup>. Samples (n = 4) were prepared for analysis by 100-fold dilution with HPLC water. 5µL of the resulting solutions were injected and a chromatogram extracted at 290nm from the diode array data. Areas

from the two major bleomycin peaks (USP, 1985) were compared to standards and mean recoveries calculated (Table 2). Limits of detection were determined from signal to noise ratios (6:1) on the sample spiked at 0.15 units g<sup>-1</sup> (Table 2).

	Peak 1	Peak 2
Retention times (min)	3.28	3.89
r <sup>2</sup>	0.994	0.999
% recovery from gels (1.5 units g <sup>-1</sup> )	91 ± 6.5	86 ± 6.4
% recovery from gels (0.15 units g <sup>-1</sup> )	162 ± 21	148 ± 39
Limits of detection (units/g)	0.3	0.45

Table 2. Recoveries and limits of detection for bleomycin USP in bioadhesive formulations

Determination of peak areas corresponded to the requirements for bleomycin analysis (USP, 1985). The results obtained indicate that the method employed may be used to determine reproducibly therapeutically relevant levels of bleomycin with a limit of detection of 0.3 units g<sup>-1</sup>. Chromatograms obtained from standard samples and formulations are readily comparable, indicating both the full and efficient extraction of bleomycin from the dosage form. Further, the use of a gradient elution method ensures that all the relevant peaks may be determined within 10 minutes, compared to the 30 minutes of the established method of analysis (USP, 1985).

Iwata, M. et al (1987) *Drug Des. Del.* 1: 253-260  
Machida, Y. et al (1979) *Chem. Pharm. Bull.* 27: 93-100  
U.S. Pharmacopeia XXI (1985) 126-127  
Woolfson, A.D. et al (1995) *J. Cont. Rel.* 35: 49-58  
Woolfson, A.D. et al (1998) *Int. J. Pharm.* In press



Water absorption by wound gels:  
validation of a wound model.

JONATHAN W. GILKES, JAMES L. FORD, PHILIP H. ROWE  
and JASPAL S. MALHI.

*School of Pharmacy and Chemistry, Liverpool John Moores  
University, Byrom Street, Liverpool. L3 3AF.*

Hydrogels are utilised for their ability to absorb wound exudate and facilitate the successful healing of a wound (Thomas 1990). There is a paucity of methods describing the capacity of wound gels to absorb water. The aim of this study is to validate a method for determining the absorptive capacity of a wound gel, using Franz cells. These are normally used to determine the absorption of substances across a semi-permeable membrane (Brinon et al 1999). The cells were adapted to model the wound environment under a secondary dressing, providing a realistic and efficient method for determining the absorptive capacity of a gel.

(Table 1: A, B & C); and the results duplicated (Table 1: X<sub>1</sub>, X<sub>2</sub> etc). Samples were poured into the lower compartment (A) of a Franz cell (Fig 1). The Visking tubing (C) and upper compartment (B) were then secured in place, using a horseshoe clamp (E). To ensure no sample loss due to swelling or drying during the experiment, an extra piece of plastic tubing with a cover (D) was attached to the side arm. Water absorption was observed by recording the change in weight of the Franz cell with time. Compartment (B) was emptied of water, dried with tissue paper and the weight recorded at hourly intervals for 12 h, then at 24 h.

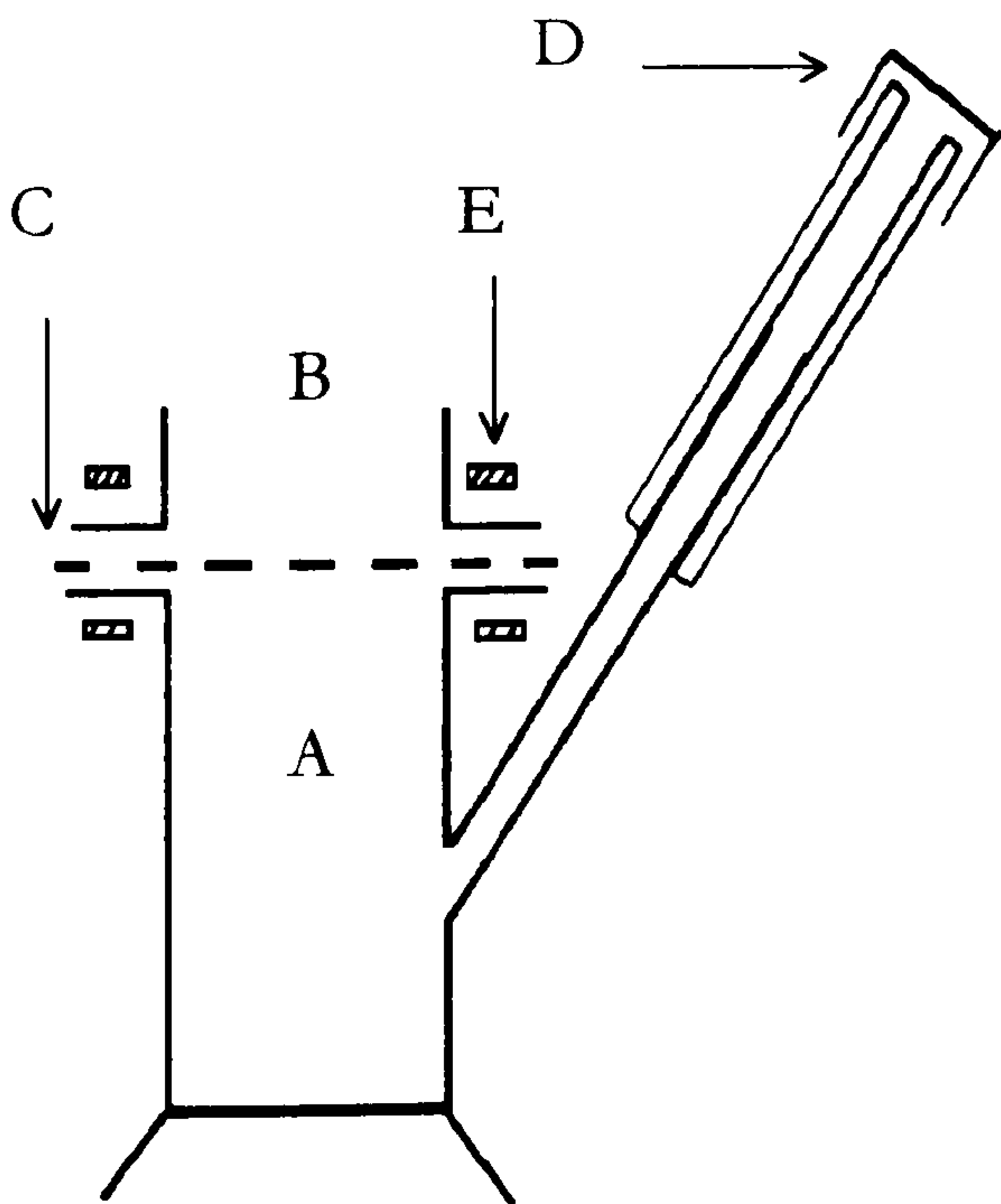


Figure 1. Schematic diagram of a Franz Cell.

Three batches of 8% w/w sodium alginate were prepared by dissolving sodium alginate (Protanal LF 10/60, Pronova Biopolymer) in isotonic calcium chloride solution. The beneficial properties of sodium alginate products in wound management have been well documented (McMullen 1991). For each batch, three separate Franz cells were used

Table 1. Water absorption results at 24 h  
(g water / g alginate gel).

Batch	Franz Cell		
	A	B	C
X <sub>1</sub>	0.049	0.055	0.058
X <sub>2</sub>	0.057	0.053	0.058
Y <sub>1</sub>	0.054	0.050	0.056
Y <sub>2</sub>	0.056	0.056	0.056
Z <sub>1</sub>	0.052	0.047	0.053
Z <sub>2</sub>	0.055	0.056	0.053

Representative data (24 h) are seen in Table 1. The mean absorption for 3 batches was 0.054 g water/ g alginate gel (n = 18, SD = 0.003 g water/ g alginate gel and RSD = 5.7%). A two way ANOVA was performed treating both batch (X, Y & Z) and cell (A, B & C) as factors; no significant effect of either factor was demonstrated (P>0.05). The method has proved to be efficient and repeatable. It is suggested that this is a useful method for determining water absorption by gels used in wound management.

Brinon, L. et al (1999) *Journal of Controlled Release* 60: 67-76.  
McMullen, D. (1991) *Dermatology and Nursing* 3: 216-217  
Thomas, S. (1990) *Hydrogel Dressings*. In: Thomas, S. *Wound Management and Dressings*. The Pharmaceutical Press, London, pp. 50-54.