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***AN INVESTIGATION INTO THE USE OF ALGINATES AS  
BIOADHESIVE DELIVERY SYSTEMS***

***By***

***Douglas Banning***



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in  
The Faculty of Medicine  
of  
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**The School of Pharmacy  
University of London  
Brunswick Square  
London WC1N 1AX**

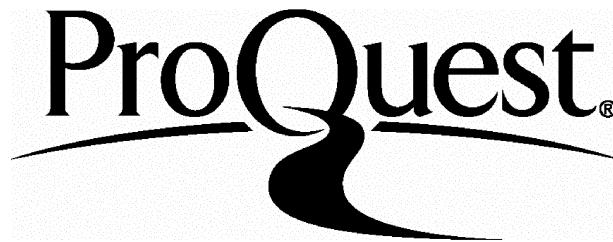
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## ABSTRACT

Sodium alginates are naturally occurring polymers consisting of two monomers, mannuronate and guluronate. Their gel-forming properties and their potential use as bioadhesives have attracted particular attention within the pharmaceutical industry. The aim of this study is to characterise the gelling behaviour of several batches of sodium alginates of differing chemical characteristics and to relate this behaviour to their bioadhesive properties.

Three methods based on the internal bulking gelation were used to assess factors that may affect the basic characteristics of alginate gels, particularly gel strength. Isothermal oscillatory rheology and thermorheology, as well as texture analysis<sup>15</sup> were used to assess the behaviour of these gels. It was possible from these analyses to demonstrate that the level of calcium, the method of preparing these gels, the fraction of guluronate, and the amount of sodium alginate all affect the gel behaviour.

In order to relate these findings to bioadhesion, two formulations, solid compacts and solutions and their interactions with various mucin types were examined. It was found that sodium alginate interaction with all types of mucin was more pronounced when the alginate consisted of predominantly mannuronate monomer. Further examinations revealed that there was also a temperature-dependent thermogelation process when alginate/mucin mixes were subjected to heat/cool cycles.

Finally, the bioadhesive potential of solutions of sodium alginate to porcine oesophageal tissues was assessed using a purpose-designed bioadhesion test system within a humidity chamber, based on flow channel technique. Fluorescently labelled sodium alginate solutions were applied to oesophageal tissues, continuously perfused with de-ionised water, phosphate buffer pH 7.4, or artificial saliva over 30 minutes. The total amount left after 30 minutes was calculated and expressed as a bioadhesive index. Mannuronate-rich alginates were found to exhibit better bioadhesive properties than their guluronate-rich alginates.

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*In the ever loving memory of Grandpa*

*To believe in something not yet proved.....  
is the only way we can leave the future open.  
Man surrounded by facts, permitting himself no surmise,  
no intuitive flash, no great hypothesis, no risk, is a locked cell.  
Ignorance cannot seal the mind and imagination more surely.*

Lillian Smith (1897-1966)

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## LIST OF SYMBOLS

$\delta$	Phase angle
$\tan \delta$	Loss tangent
$\Delta l/l$	Relative deformation
$\varepsilon$	Fracture energy
$\gamma$	Shear stress
$\dot{\gamma}$	Shear rate
$\gamma_0$	Strain amplitude
$\gamma(t)$	Deformation with time
$\dot{\gamma}(t)$	Strain rate
$\eta$	Intrinsic viscosity
$\eta^*$	Viscosity of 1% solution
$\mu\text{m}$	Micrometre
$\mu\text{J}$	Micro Joules (units of work of adhesion)
$\theta$	Angle
$\rho$	Density
$\sigma_0$	Generated stress
$\sigma(t)$	Resultant stress
$\tau$	Stress
$\omega$	Angular frequency
$A$	Surface area ( $\text{m}^2$ )
<b>Ba</b>	Barium
<b>Ca<sup>2+</sup></b>	Calcium ions
<b>Cd</b>	Cadmium
<b>Co</b>	Cobalt
<b>Cu</b>	Copper
<b>D<sub>b</sub></b>	Mucoadhesive material diffusion coefficient
<b>F</b>	Force (N)
<b>Fe</b>	Ferrous (iron)
<b>G'</b>	Storage (elastic) modulus



<b><math>G''</math></b>	Loss (viscous) modulus
<b>L</b>	Critical crack length upon separation of 2 surfaces
<b>Me</b>	Molecular weight between entanglements
<b>Mg</b>	Magnesium
<b>Mn</b>	Manganese
<b>n</b>	Number of observations
<b>Ni</b>	Nickel
<b>p</b>	Probability value
<b>Pb</b>	Lead
<b>r</b>	Radius
<b><math>r^2</math></b>	Linear regression coefficient
<b>S</b>	Spreading coefficient
<b>Sr</b>	Strontium
<b>t</b>	Time
<b><math>W_A</math></b>	Work of fracture of adhesion
<b>Zn</b>	Zinc

## LISTS OF ABBREVIATIONS

<b>AlgCOOH</b>	Alginate acid
<b>Anova</b>	Analysis of variance
<b>AUC</b>	Area under the curve
<b>BNF</b>	British National Formulary
<b>BP</b>	British Pharmacopoeia
<b>Ca(Alg)<sub>2</sub></b>	Calcium alginate
<b>CD</b>	Circular dichroism
<b>CM</b>	Crude mucin (isolated from freshly sacrificed porcine gastric)
<b><sup>13</sup>C-NMR</b>	Carbon 13 nuclear magnetic resonance
<b>DP</b>	Degree of polymerisation
<b>DPGM</b>	Dialysed porcine gastric mucin (type III from Sigma)
<b>DTG</b>	Derivative thermogravimetric
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>Eqn.</b>	Equation
<b>F<sub>G</sub></b>	Fraction of guluronic acid residues
<b>F<sub>GM.MG</sub></b>	Fraction of alternating sequences
<b>G</b>	$\alpha$ -L-guluronic acid
<b>GDL</b>	G-gluconic acid lactone
<b>GG-Blocks</b>	Homopolymeric blocks of guluronic acid
<b>GI</b>	Gastrointestinal
<b>GIT</b>	Gastrointestinal tract
<b><sup>1</sup>H-NMR</b>	Proton nuclear magnetic resonance
<b>HM</b>	Homogenised mucin (isolated from crude mucin)
<b>kDa</b>	KiloDalton
<b>M</b>	$\beta$ -D-mannuronic acid

<b>MM-Blocks</b>	Homopolymeric blocks of mannuronic acid
<b>MG-Blocks</b>	Alternating sequence of mannuronic and guluronic acids
<b>M:G ratio</b>	Ratio of mannuronic acid to guluronic acid content
<b>Mw</b>	Molecular weight
<b>NaCMC</b>	Sodium carboxymethyl cellulose
<b>NaN<sub>3</sub></b>	Sodium azide
<b>N<sub>G&gt;1</sub></b>	Typical average length of guluronic acid blocks larger than 1
<b>NMR</b>	Nuclear magnetic resonance
<b>NSAIDs</b>	Non steroidal anti-inflammatory drugs
<b>PGA</b>	Propylene glycol alginate
<b>PGM</b>	Porcine gastric mucin (type III from Sigma)
<b>PHEMA</b>	Poly(hydroxymethyl methacrylate)
<b>Phosph.</b>	Phosphate
<b>PMA</b>	Polymethacrylic acid
<b>PMSF</b>	Phenylmethylsulfonyl fluoride
<b>RH</b>	Relative humidity
<b>SGM</b>	Stimulated gastric mucus
<b>SM</b>	Mucosa surface
<b>T<sub>g</sub></b>	Glass transition
<b>TG</b>	Thermogravimetry
<b>TGA</b>	Thermogravimetric Analyser
<b>USP</b>	United States Pharmacopoeia
<b>UV</b>	Ultraviolet
<b>WOA</b>	Work of adhesion

**CHAPTER 1: INTRODUCTION**

## 1.1 General introduction

Alginates are naturally occurring co-polymers obtained from marine brown algae (*Phaeophyceae*) and as capsular polysaccharides in soil bacteria (Painter, 1983). Alginates consist of L-guluronic acid (G) and D-mannuronic acid (M), joined by (1, 4) glycosidic bonds (Figure 1.1). In the salt form, the terms guluronate and mannuronate are used respectively.

**Figure 1.1: Structural data and monomer sequencing of alginates (a) the monomers in alginate; (b) the alginate chain; (c) alginate chain sequence.**

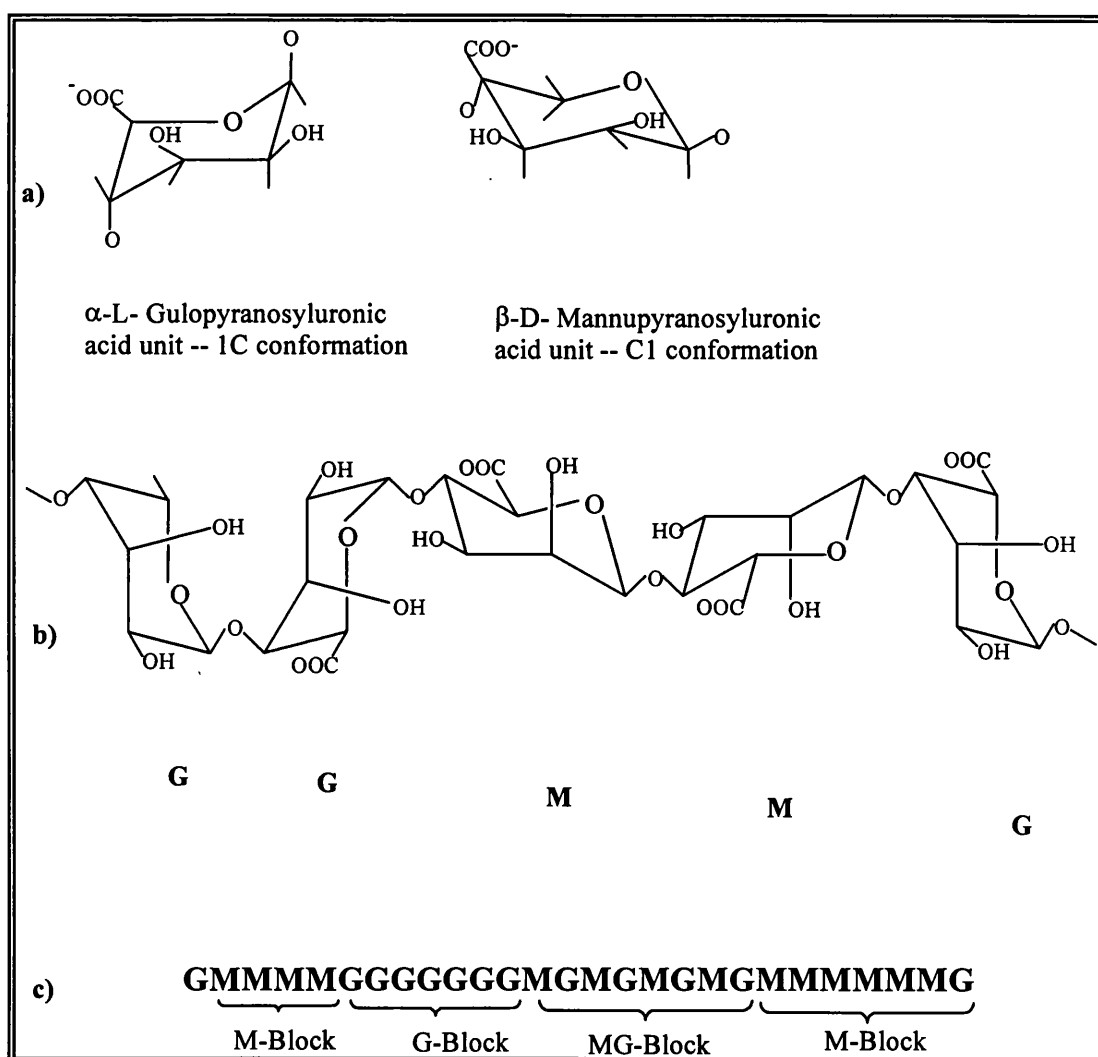


Figure 1.1a and 1.1b adapted from Smidsrød and Draget, (1996).

In addition to the homopolymeric sequences of both monomers (Haug *et al.*, 1966; 1967a), alginates also contain heteropolymeric regions that can vary from almost statistically random to regularly alternating (Grasdalen *et al.*, 1977). This arrangement depends on botanical source and the state of maturation of the plant. The function of alginate has been suggested to give strength and flexibility to the algal tissue (Smidsrød and Draget, 1996). This has been attributed to their ability to accumulate divalent metal ions and form gels of the required mechanical strength with these ions (Smidsrød, 1974).

The structure of bacterial alginates is essentially the same as the algal material although there are some minor but important differences. The bacterial alginate is invariably O-acetylated and this substituent appears to be associated exclusively with D-mannuronate residues (Skjåk-Bræk *et al.*, 1985). The distribution of the various block structures within bacterial alginates may be atypical in some cases. *Azotobacter vinelandi* produces alginates with a range of block structures that are very similar to those found in some species of seaweed. However, detailed analysis of alginates derived from various species of *Pseudomonas* have shown that the polysaccharide is derived polyguluronate blocks and therefore very different from the algal material (Hacking *et al.*, 1983).

### ***1.1.1 Sources of Alginates***

Although many species of brown seaweed that have been examined contain alginates, only a few are sufficiently abundant to be suitable for commercial processing. The most widely used are species of *Laminaria* (British Isles, Norway, France, North America, Japan), *Macrocystis* (USA) and *Ascophyllum* (British Isles) (McDowell, 1986). The *phaeophyceae*, all of which contain algin, are varied family of the plants. They grow on rocky shores or in ocean areas at high tide line. Others exist in a belt along the shore wherever the depth is less than 40 metres (the limit of sunlight penetration). In areas of minimal wave actions, plants may have a 15 year life span (*Laminaria hyperborea*), whilst others which grow in areas of yearly storm cycles are annuals (*Neveocystis leutkenaria*). The geographical location of the brown seaweed is quite diverse. Whilst the giant kelp, *Macrocystis pyrifera*, grows

in abundance along the coast of North and South America, New Zealand, Australia and Africa, the *Laminaria* species are mostly found along the coast of North America, Europe and Asia.

### 1.1.2 Chemical Composition of Alginates

Although the chemical composition of alginates was partially analysed by Fisher and Dörfel (1955), the first information regarding the sequential structure of alginates was not available until 1964 (Haug, 1964; Haug *et al.*, 1966; Haug and Smidsrød, 1965). Using the method of partial acid hydrolysis and subsequent fractionation, Haug and co-workers (1966) were able to separate alginate into three fractions, which varied widely in their composition. Further studies by the same workers established that a part of alginate was resistant to hydrolysis in mineral acid, and that this part could be divided into two fractions, each enriched to more than 90% in one of the two acids, and both having degrees of polymerisation above 20 (Haug *et al.*, 1966). A non-resistant material of intermediate composition could be prepared with approximately the same degree of polymerisation and was found to contain a large fraction of the two monomers arranged in a strictly alternating sequence (Larsen *et al.*, 1969; 1970). This work led to the realisation that alginate is a block copolymer containing two kinds of homopolymeric blocks (MM-blocks and GG-blocks) together with blocks of an alternating sequence (MG-blocks).

### 1.1.3 Analysis of composition

As with most biopolymers, the chemical composition ultimately determines the physical nature and, to large extent, the functionality of the polymer. The principal parameter usually determined with respect to alginates is the ratio of the mannuronate to guluronate (M:G ratio). As the solution properties of alginates are dependent on the chemical composition it is clearly desirable to be able to analyse the M:G ratio and, ideally, the individual blocks structure of the polymer. The contribution of individual blocks to the overall structure of alginate may be quantified by chemical or physical methods. The M:G ratios and properties of the polymannuronic and polyguluronic acid isolated from brown algae are shown in

Tables 1.1a and 1.1b. The differences in composition and fine structure as indicated in these Tables account for the differences in properties and functionality of alginates isolated from different sources of brown algae.

**Table 1.1a** *Mannuronic acid (M) and Guluronic acid (G) composition of alginic acid obtained from commercial brown algae.*

<i>Species</i>	<i>Mannuronic Acid Content (%M)</i>	<i>Guluronic Acid content (%G)</i>	<i>M/G Ratio</i>	<i>M/G Ratio range</i>
Macrocystis pyrifera	61	39	1.56*	-
Ascophyllum nodosum	65	35	1.85 (1.1)*	1.40-1.95**
Laminaria digitata	59	41	1.45*	1.40-1.60**
Laminaria hyperborea (stipes)	31	69	0.45*	0.40-1.00**
Eclonia cava and eisenia bicyclis	62	38	1.60*	-

\* Haug (1964) and Haug and Larsen (1962) for commercial algin samples of the two ratios shown for *Ascophyllum nodosum*. The algin samples manufactured in Canada have the higher M/G ratio value; the lower value corresponds to an European sample.

\*\* Data of Haug (1964) showing the range in composition of mature algae collected at different times of each of several locations.

Haug *et al.* (1967a) used partial hydrolysis and fractional acid precipitation, coupled with reaction with phenol sulphuric total carbohydrate assay to determine the 3 types of block structures of alginates. In spite of the reliability and wide acceptance of this method, the various blocks should not be considered as the ideal structure as, for example, the poly-L-guluronate fractions will routinely contain 10-15% D-mannuronate. The application of nuclear magnetic resonance (NMR) and circular dichroism (CD) techniques has led to the development of several non-destructive methods for the analysis of block structure.



**Table 1.1b: Properties of polymannuronic acid, polyguluronic acid, and alternating segments in alginic acid isolated from brown algae.**

<i>Source</i>	<i>Polymannuronic acid segment (%)</i>	<i>Polyguluronic acid segment (%)</i>	<i>Alternating segment (%)</i>
Macrocystis pyrifera	40.6	17.7	41.7
Ascophyllum nodosum	38.4	20.7	41.0
Laminaria hyperborea	12.7	60.5	26.8

Data from Penman and Sanderson, 1972.

The pioneering work by Morris *et al.* (1975) revealed that CD spectrum of alginates depends on the M:G ratio and on the arrangement of the block structure within the molecule. However, the distinguishing features of the spectrum are very sensitive to minor variations in calcium ion concentration, particularly for samples containing poly G blocks (Morris *et al.*, 1978). Penman and Sanderson (1972) developed the use of <sup>1</sup>H-NMR for the analysis of block structures in alginate. The original method, which included partial degradation and fractionation of the alginate into its block structures before analysing each of them separately, has since been improved upon (Grasdalen *et al.*, 1979). This improved method allows the use of high temperatures (90°C) coupled with solvent suppression techniques so that the intact or minimally depolymerised samples of alginate may be analysed. Grasdalen *et al.* (1979, 1981) have also used <sup>13</sup>C-NMR spectroscopy to study the monomer sequences in intact and slightly depolymerised alginates. This technique not only allowed the evaluation of the monomeric composition (M:G ratio) but also the monomeric sequence in terms of a complete set of four diad and eight triad frequencies and composition of the end units and of the units adjacent to M-residues at the non-reducing end.

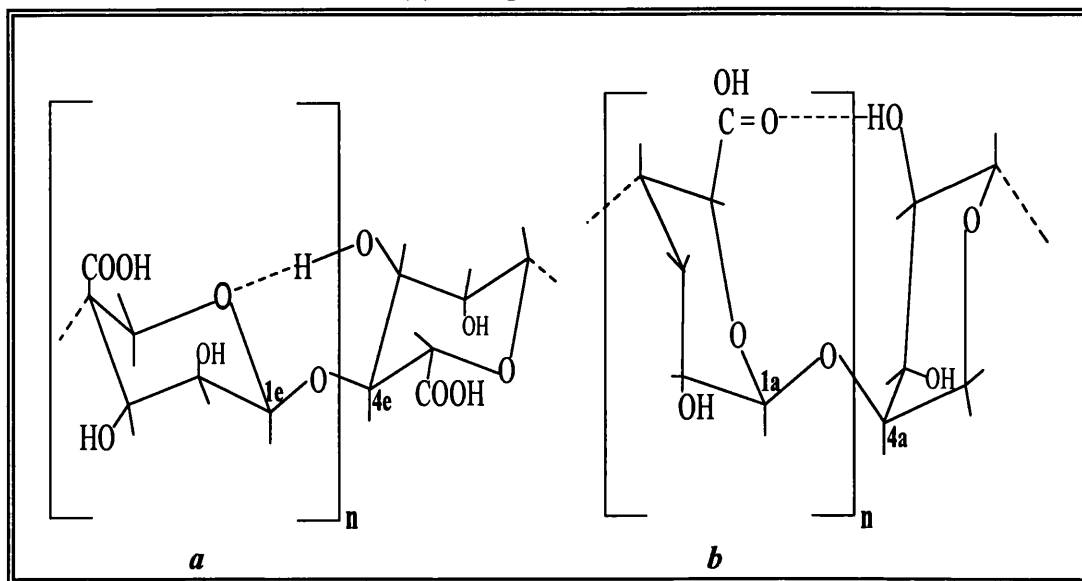
### 1.1.3.1 *The effects of composition*

It has been established (Haug, 1961) that there is a close correlation between the chemical composition of alginates and their physical properties. There is a close link between the mannuronic acid to guluronic acid ratio of the alginates and their ion exchange properties and acid dissociation (Haug, 1961) and gel forming ability (Smidsrød and Haug, 1965). The solubility of alginates, especially in acidic solutions, has been shown to depend on the monomer composition of the alginate. Furthermore, alginates containing high guluronic acid tend to yield strong, brittle gels with divalent cations (except magnesium); such gels usually appear transparent or glassy. In contrast, gels formed from alginate of high mannuronic acid content yield rather weak, but more elastic gels with translucent appearance. These properties are discussed in more detail in section 1.2.3

## 1.2 *Physical properties of alginates*

X-ray diffraction studies in fibres of alginic acid and polarised infrared spectroscopy of orientated films have resulted in information on the crystalline structure of polymannuronic acid and polyguluronic acid (Atkins *et al.*, 1971). The shape of the polyguluronic acid chain is quite different from that of polymannuronic acid. Polyguluronic acid is a buckled, ribbon-like molecule in which the guluronic acid is in the  $1C$  conformation and, therefore, diaxially linked. The conformation is stabilised by an intramolecular hydrogen bond between the hydroxyl group on carbon 2 and the hydrogen atom of the carboxyl group in adjacent units. Polymannuronic acid, on the other hand, is flat, ribbon-like molecule, the mannuronic acid being in  $C1$  conformation and therefore, di-equatorial linked. The molecule is stabilised by the formation of intra-molecular hydrogen bond between the hydroxyl group on carbon 3 of one unit and the ring oxygen atom O(5) of the next sugar unit in the chain (Figure 1.2). The interchain bonds in polymannuronic acid involve a water molecule, which is situated such that it functions twice as a hydrogen bond donor and twice as an acceptor, the hydrogen bonds so formed being in a range of 2.7-2.9Å (Atkins *et al.*, 1971)

**Figure 1.2 Glycosidic linkage in polymannuronic acid (a) and polyguluronic acid (b) in alginate chains**



### 1.2.1 Solubility of alginates

Alginates are essentially hydrophilic and simple alginates are insoluble in non-aqueous solvents. Alginic acid is insoluble in cold water and only slightly soluble in hot water. The dissociation constant for alginic acid depends on the ratio of mannuronic acid to guluronic acid. Haug (1964) reported pKa values of 3.38 and 3.65 for mannuronic acid and guluronic acid, respectively. The pH required to precipitate alginic acid from a solution of sodium alginate depends on the degree of polymerisation, the populations of mannuronic and guluronic acid residues, and perhaps on their distribution through the chain. Undegraded alginates are precipitated over the pH range 3.5 to 2.5, those with higher guluronic acid content and higher degree of polymerisation being the most readily precipitated.

The calcium salt also has only very limited solubility in water. However, the sodium, potassium, and ammonium salts of alginic acid and the propylene glycol ester are readily soluble in hot or cold water (McDowell, 1986). The salts of most of the di- and polyvalent metals are insoluble in water. The insolubility of these alginates is a consequence of their polymeric nature and the way in which carboxyl and hydroxyl groups are arranged. Köhn and Larsen (1972) have obtained in

solution calcium salts of polymannuronic acid with degree of polymerisation (DP) up to 4000 and have measured the calcium activities. They concluded that while in solutions of the calcium uronate monomers the proportion of free calcium dropped with increasing size of polymer, it reaches a much lower level in the guluronates than in mannuronates. This indicated a strong bonding of calcium to the guluronate polymers even while these remained in solution.

### 1.2.2 *Viscosity of alginate solutions*

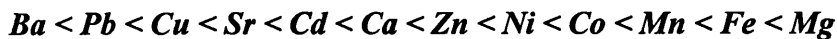
The viscosity of an alginate solution in water depends on temperature, the concentration of the alginate, the degree of polymerisation of the alginate and the nature of ions and other substances in the solution. As viscosity is determined largely by the length of the molecules in solution, those which are stiffer produce a higher viscosity than more flexible ones of the same molecular weight.

The viscosity of sodium alginate solutions is depressed by the addition of monovalent salts (McDowell, 1986). As is typical of polyelectrolytes, the algin polymer contracts as the ionic strength of the solution is increased. The viscosity of very dilute solutions of alginate should give some indication of their molecular size and shape, at least in theory. Alginate molecules are much less flexible than those of many polymers and it has been found that G blocks are stiffer (Smidsrød *et al.*, 1973) than M blocks which are in turn stiffer than the MG regions. This is the reverse trend of the order of solubility of alginates. At very low concentrations of calcium ions, alginate solutions tend to be more viscous or turbid. However, at high concentrations, the  $\text{Ca}^{2+}$  ions cause gelation of the alginate solutions. This effect of divalent cations on alginate solution is discussed at length in the next section.

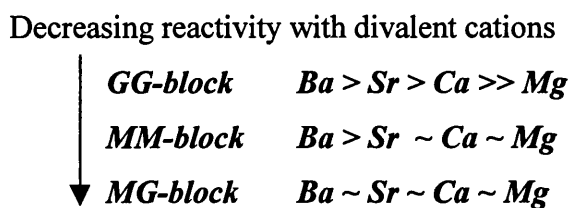
### 1.2.3 *Gelation of Alginates*

Perhaps one of the most well known properties of alginates is their ability to form gels with divalent cations, through precipitation when added to alginate solutions. Different divalent metals show different affinities for (and level of interaction with) alginate (Haug and Smidsrød, 1965; Smidsrød, 1974). The formation of gels from

alginate solution with divalent cations has been shown (Haug and Smidsrød, 1965) to be in the following order:



Smidsrød (1974) has also shown that there is a clear correlation between gel-formation and ability for a strong inter-chain binding to calcium ions. Furthermore, he showed the following order of alkaline earth metal ability to form gels with alginates:



Single chain segments of GG-blocks may bind divalent metal ions selectively by a binding site composed of COO<sup>-</sup>, O(5) and O(4) in one unit and O(2) and O(3) in the preceding unit. The GG-blocks therefore yield gels that are easily formed and generally strong, in the presence of divalent cations. Such binding of divalent cations to GG-blocks has been shown to be a strong autocoooperative, and indicates the heterogeneity with respect to binding sites. In contrast, the MM-blocks and the alternating MG-blocks have low calcium ion selectivity and little or no signs of autocoooperativity (Smidsrød, 1974).

Grant *et al.* (1973) originally proposed the most well known theory on alginate gelation which has since been referred to as the ‘egg-box’ model for the cooperative mechanism of binding involving two or more chains. According to this model, chains of alginate are viewed as corrugated egg-box with interstices in which the cations may pack and be coordinated. The strength and selectivity of cooperative binding is determined by the comfort with which “eggs” of the particular size may pack in the “box”, and with which the layers of the box pack with each other around the eggs. Although this model has stood the test of time, it has recently come under intense scrutiny and other theories are currently being considered for the mechanism(s) involved in the gelation of alginate gels by divalent cations.

In both food and pharmaceutical industries, the choice of divalent cations required for the gelation of alginates is based not only on their reactivity with alginates but more importantly, on their toxicity profiles. Calcium has remained the most preferred divalent cation as barium, lead, copper and cadmium are all heavy metals of reasonably high toxicity. Besides the choice of divalent cation, consideration must be given to the following factors when making alginate gels:

*(i) Type of alginate used*

Penman and Sanderson (1972) have shown that different species of the brown seaweed yield alginate that differ in monomer composition and block structure and a given alginate has its own characteristic calcium reactivity and gelation properties. Alginates rich in guluronic acid content tend to produce strong brittle gels that are heat stable. In contrast, alginates rich in mannuronic acid content provide weaker more elastic gels that are less heat stable but possess greater freeze/thaw stability. The explanation for this is that at low calcium levels there is sufficient calcium to stabilise a greater proportion of the G blocks in aggregates in high M than in high G alginates. Gels formed from the reaction between divalent cations and guluronic acid-rich alginates have been shown to consist of very stiff open pore structure with high modulus (Skjåk-Bræk *et al.*, 1986), whilst those from their mannuronic acid-rich counterparts are much more compact but of low modulus. Furthermore, Smidsrød and Haug (1972) have reported that the molecular weight of the alginate above a degree of polymerisation of 40 ( $M_w \sim 9\ 000$ ) does not affect the rigidity of the gel. This suggests that the number of junctions per molecule now becomes so high that each macromolecule loses its individuality in the network. However, lower molecular weight alginates generally give brittle gels and high molecular weights a texture that is more elastic (Smidsrød and Haug, 1972).

*(ii) The degree of conversion to calcium alginate*

The degree of conversion of monovalent salts of alginates (e.g. sodium alginate) to calcium alginate (i.e. the exchange of sodium ions for calcium ions) is perhaps the most important criterion for desirable gelation. The reaction between sodium

alginate and calcium salts, such as, calcium chloride, may be represented by the following equation:



This implies that at calcium to alginate molar ratio of 0.5, there is theoretically sufficient calcium to completely replace the sodium. This is expressed as calcium conversion of 100 per cent. Several studies, including those of Papageorgious *et al.* (1994) have investigated the optimum ratio of calcium to sodium for ideal homogeneous alginate gels. Others have predicted that maximum gel strength occurs when the calcium conversion is about 30% (i.e. 1%w/w sodium alginate solution being equivalent to 15mM of calcium ions), provided that the molecular weight of an alginate monomer is about 200 (Moe *et al.*, 1992). Above this percentage of calcium conversion, problems such as syneresis and aggregation begin to occur.

*(iii) Source of calcium ions and method of preparation.*

The source of calcium ions required to bring about gelation will very much depend on the method of preparation and the pH of the final gel. There are essentially two main methods of preparing alginate gels, namely the dialysis (or diffusion) method and the internal setting (or bulk) method.

In the dialysis method, allowing calcium ions to diffuse into an alginate solution forms alginate gels. One way of achieving this by placing alginate solution in perspex cylinders sealed at both ends with cellulose membranes, and placing them in solution of calcium salts, most commonly calcium chloride (Johnson *et al.*, 1997). Simply increasing the calcium concentration may increase the diffusion rate. This technique has been utilised for the production of alginate gels both in the food industry for making artificial berries or other restructured food. It has also been employed in biotechnology for making gel beads for immobilisation purposes (Skjåk-Bræk and Martissen, 1991). The main drawbacks of this method are inhomogeneity of the gels formed and the process being slow. The concentration of alginate in such gels tends to be considerably lower in the centre of the gel than at the

edges. This is because during diffusion of the metal ions into an alginate solution, the rapid formation of a network produces an inwardly moving gelling zone. The diffusion of alginate from the centre of the gel towards the gelling zone leads to a depletion of alginate in the centre.

In the internal or bulk setting method, which is normally carried out at ambient temperature, the calcium is released under controlled conditions from within the system. Several calcium salts may be employed in this method, including insoluble calcium carbonate (Papagiorgious *et al.*, 1994; Draget *et al.*, 1989) or dicalcium hydrogen orthophosphate (Nussinovitich and Peleg, 1990). The rate at which the calcium is made available to the alginate molecules depends primarily on pH, the amount and (with the exception of calcium chloride) the particle size and the solubility characteristics of the calcium salts. Calcium carbonate with large particle size, for example, often gives inhomogeneous gels when used with alginates with a low viscosity grades (Draget *et al.*, 1989).

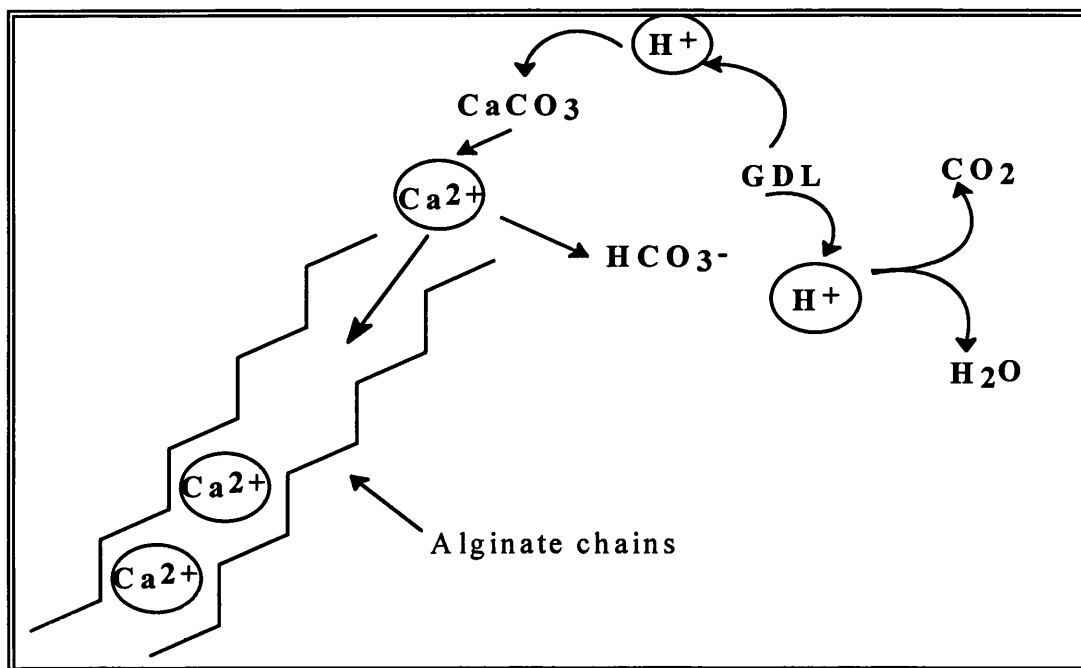
Sequestrants (or calcium chelators) are an essential component in almost all alginate gels made by the internal setting method in order to control the rapid release of calcium ions. These compounds, which are usually in excess, ensure that the calcium ions are not rapidly released in an uncontrolled manner by binding all the available calcium ions. Trisodium citrate (Papagiorgious *et al.*, 1994), EDTA (Pronova Biopolymer A.S., undated) and hexametaphosphate (Nusinovitich and Peleg, 1980) have all been used to control the release of calcium ions. Other strategies include the use of insoluble or poorly soluble calcium salts such as calcium carbonate in combination with a releasing agent such as D-gluconic acid lactone (GDL), which breaks down calcium carbonate (Draget *et al.*, 1989) releasing carbon dioxide in the process (Figure 1.3). The CO<sub>2</sub> may be removed from the gel by gently heating the preparation to near boiling point or by vacuum.

Temperature is another factor that affects the rate of gel formation in internal setting. It has been suggested (Sime, 1984; Papagiorgious *et al.*, 1994) that although the calcium ions required for the setting reaction are already in solution with the alginate, setting does not occur at elevated temperatures because the alginate chains



have too much thermal energy to permit interaction. It is only when the solution is cooled that the calcium-induced interchain can occur. This is the basis of hot-made alginate gels. Some of these criteria for the formation of ideal alginate gels are investigated in Chapter 2.

**Figure 1.3: Schematic representation of main reactions during the formation of an alginate gel using GDL and  $\text{CaCO}_3$**



Adapted from Smidsrød and Draget, (1996).

### 1.3 Uses and applications of alginates

Alginates have wide ranging uses and applications in the food and pharmaceutical industries, textile and printing, in the fields of dentistry, toxicology and medicine. This is because of the ability of alginates to form gels of various strengths, chelation with divalent cations, function as excipients and as the main components in medicinal preparations.

### ***1.3.1 Pharmaceutical uses of alginates***

#### ***1.3.1.1 Solid dosage forms — Controlled release preparations***

Alginate (in the form of alginic acid) is an excellent tablet disintegrant due to its wicking and rapid swelling properties. It performs equally well in direct compression or wet granulation processes. Alginic acid is effective at concentrations of 2% or higher, and levels as high as 10% are used when formulating an effervescent tablet. In controlled release preparations, alginate salts can be used alone or in combination with other polymers, such as xanthan gum, to control drug release from a hydrophilic matrix tablet (Onsøyen, 1996). Due to the swelling properties of sodium alginate, if this system is combined with a water-soluble bioactive agent, the agent will dissolve and diffuse throughout the swollen matrix. In gastric fluid, the hydrated sodium alginate is converted into a porous, insoluble alginic acid skin. Once passed into the higher pH of the intestinal tract, the alginic acid skin is converted into a soluble viscous layer. This pH dependent behaviour of alginate alone can be exploited to customise release profiles. To achieve optimum profiles, alginate alone is typically used between 20 and 50%. At these levels, a hydrocolloidal matrix forms with sustained release of the active ingredients as a result (Onsøyen, 1996). In combination with other polymers, alginate can be used at concentrations below 20%.

#### ***1.3.1.2 Encapsulation and film forming***

In addition, sodium alginate has been used in the encapsulation of enzymes and live cells. The encapsulation process normally involves the use of 1-4% sodium alginate solution containing the encapsulant. This solution is added dropwise to a 2-5% solution of calcium chloride or other divalent cation (Espevik and Skjåk-Bræk, 1996). The calcium cross-links the alginate molecules to form a three-dimensional network (gel). Diffusion through the gel is dependent on the pore size of the gel as well as the solubility and molecular weight of the diffusant. The cross-linking can be reversed by ion exchange (Espevik and Skjåk-Bræk, 1996).

Alginate films can be prepared by drying a thin layer of a soluble alginate solution, by treating a soluble film with a divalent cation or with an acidic solution, or by extruding a solution of alginate into a precipitating bath which produces an insoluble alginate (Onsøyen, 1996). In film coatings, alginates have been found to produce uniform coats and have been used commercially in tablet coating systems. A 5-10% solution of alginate will produce a clear, glossy, water-soluble film, which then will function as a simple enteric barrier in the stomach (where the sodium alginate is converted into insoluble alginic acid). Films with greater integrity are produced if a plasticiser such as glycerine or propylene glycol are incorporated (Glicksman, 1969).

### *1.3.2 Liquid dosage forms*

Hydrated, linear, alginate polymers increase solution viscosity rapidly with increasing alginate concentration. Optimum results for thickening and suspending with alginates is normally achieved with concentrations in the range of 0.3-2%w/w. Low concentrations of calcium ions may be used to increase the viscosity of a given alginate solution. Care must be taken, however, because gelation can occur if excess calcium ions are introduced, especially at higher alginate concentrations, as outlined in section 1.2.3. Algin derivatives, propylene glycol alginates (PGAs), are stabilisers and secondary emulsifiers designed for lower pH products than other alginates. Concentrations of 0.25-2%w/w are typically used to produce stable emulsions and suspensions. The esterification of carboxyl groups with the more lipophilic hydroxyl propyl groups reduces the overall hydrophilic character of the molecules and imparts surface-active properties. The resulting ability of PGAs to reduce the surface tension of water phase enables them to efficiently stabilise emulsions. Substitution of 50% or more of the carboxyl groups also produces greater acid tolerance than other alginates because fewer carboxyl groups are available to convert to insoluble, free-acid form. Further uses and applications of alginates are summarised in Table 1.2.

*Table 1.2: Some uses and applications of alginates*

<b>Main Property Used</b>	<b>Use</b>	<b>Reason for Use</b>
Thickening Powder	Controlling the viscosity of food products, e.g. sauces and syrups	Alginates are edible
	Thickening cosmetic creams, liquid detergents and shampoos.	Harmless on skin
	Thickening textile printing pastes	Easy washed out; special Advantages with certain Dyes
General colloidal Properties	Stabilising ice cream	Checks phase separation
	Stabilising foam on beer and water ices	Checks dripping
	Stabilising imitation ice cream	Gives quick whipping
	Preventing liquid separation in toothpaste	
Gel formation	Milk desserts, confectionery jellies	Jellies can be made in the cold and are heat stable
	Dental impression materials	Can be used in the cold
	Semi-solid pharmaceutical and cosmetic preparation	
Formation of films on surfaces	Binding pharmaceutical tablets	Disintegrating on wetting
	Oil permeable barrier creams for protecting the skin	
	Surface sizes and coating additive for paper	Controls penetration for oily substance
Base exchange and chemical reactions	Analytical separation	
	Purification of bases Salts of physiologically active bases	Medicinal uses

Data from McDowell, (1986)

#### 1.4 Bioadhesion

Adhesion is an interfacial phenomenon influenced by surface energies. When an adhesive bond is established between two materials the total surface energy of the system is diminished, recreating a new surface. When the adhesion involves biological surfaces, the phenomenon is referred to as bioadhesion (Peppas and Buri, 1985). Bioadhesion is therefore defined as the state in which two bodies (one or both of the adherents, are of a biological nature) are held together for extended periods of time by interfacial forces. These forces may range from valence forces to mechanical interactions, or some combination of chemical and physical interactions (Park and Robinson, 1985). A bioadhesive can therefore be defined as a substance which has the ability to interact with biological materials and is capable of being retained on the biological substrate for a period of time (Park and Robinson, 1985). If the biological surface is mucus, the term mucoadhesion is used. Since adhesion to biological surfaces almost always involves the mucus layer or some components of mucus, the terms bioadhesion and mucoadhesion have been used interchangeably.

The field of bioadhesion/mucoadhesion is not new, given that cells attach to each other with great strength, and bacterial attachment to many surfaces of the human body has been known for a long time. Moreover, the attachment of synthetic polymers to tissues is equally well established, as illustrated by retention of dentures in the mouth. A great deal of increased attention has been given to the possibility of using bioadhesive/mucoadhesive polymers for drug delivery purposes and the attendant benefits to be derived therefrom (Gurny *et al.*, 1984; Nagai 1985; Ch'ng *et al.*, 1985; Longer *et al.*, 1985; Hui and Robinson 1985; Yukimatsu *et al.*, 1994). Bio(muco)adhesive dosage forms for controlled drug delivery may be an important means of locally administering drugs at various sites of application, thus improving bioavailability characteristics and avoiding some side effects exhibited by conventional dosage forms. Leung *et al.* (1991) postulated that the reason for the interest in bio(muco)adhesive polymers and/or copolymers is that they fulfil the following desirable features of controlled release system:

- Localisation of the dosage form in specific region to improve and enhance bioavailability of drugs.

- Promotion of intimate contact of the formulation with the underlying absorbing surface to allow modification of tissue permeability as might be required for absorption of macromolecules, e.g. peptides and ionised species or to inhibit protease activity.
- Prolonged residence time to permit once-a-day dosing, such that patient compliance can be improved
- Improve the viability of non-parenteral, non-oral routes of drug administration.

#### ***1.4.1 Elements of mucoadhesion***

Two different components are involved in mucoadhesive drug delivery (Leung *et al.*, 1991); firstly, the mucoadhesive drug delivery system itself, which acts as a platform and reservoir for the drug, and secondly, the underlying substrate, which may be the mucin layer or the mucosal epithelium or perhaps some of each. The entire gastrointestinal tract (from the mouth to the anus) is lined with mucus and this represents the potential site of attachment for any bioadhesive delivery system (Marriot and Gregory, 1990). This is presented as a continuous unstirred gel layer over the mucosal epithelium that performs a protective and lubricative function and is the first layer that interacts with foreign materials, e.g. foods, drugs, bacterial organisms and chemical entities (Clamp *et al.*, 1978). The nature and thickness of this layer of mucus may alter significantly during disease, which might lead to a change in the behaviour of a delivery system (Marriot and Gregory, 1990; Allen, 1983). One would expect the physical and chemical properties of the gel mucus to differ from species and from site to site if only because the environment into which it is secreted varies. In the gastrointestinal tract, for example, the gel is mixed with micro-organisms, secreted and leached protein, and is at least an underlying uniformity of structure of mucus from different sources based on a large molecular weight glycoprotein (Marriot and Gregory, 1990).

### **1.4.2 The mucus layer**

Mucus is a unique secretion which is composed of viscous, sticky, water-insoluble glycoproteins (mucins), enzymes, water, electrolytes, sloughed epithelial cells, bacteria and bacterial products and various other materials depending on the source and location of the mucus (Clamp *et al.*, 1978; Allen, 1983; Leung *et al.*, 1991). The principal molecular component of mucus responsible for the viscous and gel-forming properties of mucus is glycoproteins. The mucin glycoproteins have strong attachment properties, not only to each other but also to other bioadhesive molecules and firmly bind to the epithelial cell surface presenting as a continuous unstirred gel layer over the mucosa

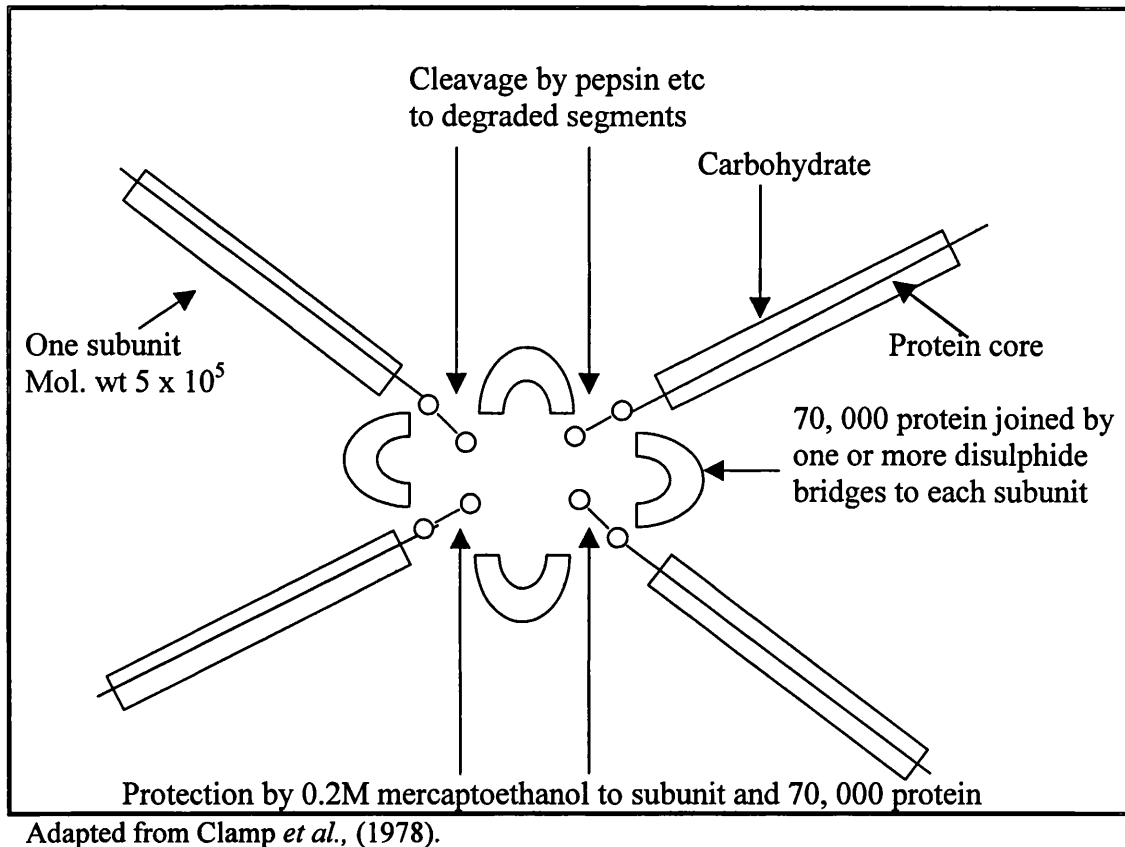
#### **1.4.2.1 Chemical composition and structural function of mucus**

Typically, mucus consists of about 1% by weight of salts and other dialysable components, 0.5-1% of free protein, and a similar proportion of the carbohydrate-rich mucus glycoproteins (referred to as mucins), and 95% or more of water (Allen, 1978). Mucus glycoproteins are characterised by a high carbohydrate to protein ratio, the carbohydrate constituting usually more than 65% of the dry weight (Clamp *et al.*, 1978). These carbohydrate side-chains form a dense, fairly uniform hydrophilic cover for different glycoproteins, although considerable microheterogeneity in length and complexity exists among the carbohydrate side chains (Gibbons, 1978; Phelps, 1978). Glycoproteins are biopolymers, which contain one or more carbohydrate chains covalently linked to a polypeptide backbone (Schachter and Brockhausen, 1989). The principal unit is a relatively high molecular weight glycopeptide, often called subunit which is covalently bound to other subunits through disulphide bonds and probably interacts intermolecularly with other subunits through ionic bonds and entanglements (Figure 1.4).

Native pig gastric mucus glycoprotein is the most studied of all the sources of mucus glycoproteins. It has a molecular weight of  $2 \times 10^6$  and is a polymer of four glycoprotein subunits (molecular weight  $5 \times 10^5$ ) joined by disulphide bridges (Allen *et al.*, 1982). Both proteolysis and reduction cleave the glycoprotein into lower

molecular weight glycoprotein subunits with the loss of their viscous and gel forming properties (Figure 1.4).

**Figure 1.4:** A diagrammatic representation of the structure of the pig mucus glycoprotein (Mol. wt  $2 \times 10^6$ )



### 1.4.3 Physicochemical Properties of Mucus

#### 1.4.3.1 Thickness of mucus layer

The thickness of the mucus layer has been proposed as the distance between the solution interface and the mucus-mucosa interface (Leung *et al.*, 1991). *In-vivo*, mucus is presented as a thin but continuous translucent gel cover, heterogeneous in thickness, over the mucosal epithelium surface. Thickness of the mucus layer varies considerably with organ and species (Table 1.3). In rats, for example, the thickness has been quoted as being between 5 and 200  $\mu\text{m}$ , and occasionally up to 500  $\mu\text{m}$ ,



with a mean thickness of around 80  $\mu\text{m}$  (McQueen *et al.*, 1984). The thickness of the mucus layer can be observed directly on thick, unfixed mucosal sections (Allen *et al.*, 1984).

The continuity and thickness of the mucus layer is important to its protective function against physical and chemical insults. The depth of the gel layer will depend on the difference between the rate of secretion and removal of mucus (Allen *et al.*, 1982). Certain chemicals (e.g. luminal pepsin) rapidly dissolve the mucus but the secretion of fresh mucus maintains its continuity (Allen *et al.*, 1984). Others such as carbenoxolone, prostaglandins and carbachol increase mucus thickness (Williams and Turnberg, 1982).

**Table 1.3: Species and organs variation of mucus thickness**

<i>Organ</i>	<i>Mean Thickness (<math>\mu\text{m}</math>)</i>
Human Stomach	192 <sup>(1)</sup>
Rat Stomach	77 <sup>(1)</sup>
Rat Duodenum	81 <sup>(1)</sup>
Human eye conjunctiva	1.4 <sup>(2)</sup>

(1) Allen *et al.* (1984) (2) Nichols *et al.* (1985)

#### **1.4.3.2 Mucus as a gel**

Mucus is a cross-linked network of disulphide bonds and physical entanglements of linear, flexible and random coil glycoprotein molecules that form a viscoelastic gel (Silberberg, 1989). It can be studied rheologically by conventional techniques, and it has been demonstrated that mucus is an incipient gel that has optimal properties in so far as mucocilliary clearance in respiratory tract is concerned (Gelman and Meyer, 1979). The

glycoprotein can be isolated from mucus and when redissolved at the physiological concentration it makes a gel, which possesses rheological properties similar to those of fresh mucus (Allen *et al.*, 1976; Mantle and Allen, 1981).

#### ***1.4.3.3 Mucus glycoprotein charge***

The oligosaccharide side chains at the terminal end of the mucin network contain either sialic acid, which is negatively charged. In addition, sulphate may be linked to either galactose or N-acetylglucosamine. The sialic acid (pKa 2.6) is completely ionised at physiological pH of 7.4. This implies that the mucin network carries a substantial negative charge at physiological pH of 7.4 (Johnson and Rainsford, 1972). Quinton and Philpott (1973) have suggested that fixed anionic sites cross-linked with membrane-fixed cationic sites, coordinated with metallic cations, and form hydrogen bonds with other electronegative centres. Thus, epithelial anionic sites might be partly responsible for holding the mucus layer close to the underlying mucosa. Similarly, anionic sites on the mucin network might play an important role in mucoadhesion.

#### ***1.4.3.4 Rheological and gel-forming properties of mucus***

Rheological studies show that mucus is a water-insoluble, viscoelastic gel created by the cross-linked polymer structure (Litt *et al.*, 1977; Allen, 1978). At physiological pH and ionic strength, the glycoprotein organises water in its vicinity efficiently and is capable of gel formation and a wide range of rheological behaviour (Litt, 1984). Mucus shows a number rheological properties including viscosity, elasticity, flow elasticity or retraction (tendency of mucus to assume its original shape following deformation produced by stress or pressure), plasticity (property of the mucus which permits it to be deformed without rupture) and tack (stickiness of the mucus) (Elstein, 1978).

There are two structural features of mucus glycoprotein that influence the viscous and gel-forming properties of mucus (Allen, 1978). Firstly, the degree of expansion of the isolated glycoprotein molecule in solution, which determines the concentration

at which gel formation occurs. Secondly, the polymerisation of the glycoprotein subunits into the native glycoprotein. Litt *et al.* (1976) investigated the rheological differences accompanied by variations in sample pH, macromolecular concentration and ionic strength and found that these three parameters influenced the viscoelastic properties of tracheal secretions. For example, a higher viscosity for gastric glycoproteins has been observed at lower ionic strength (below 10 mM) (Allen *et al.*, 1982). Since glycoproteins and other proteins in the mucus are polyions, mucus is capable of swelling, the extent of which depends on the pH and ionic strength of the hydrating medium (Verdugo, 1984). Thus, the rheological properties of mucus may be regulated via control of the transepithelial movement of water, ions and soluble protein (Verdugo, 1984).

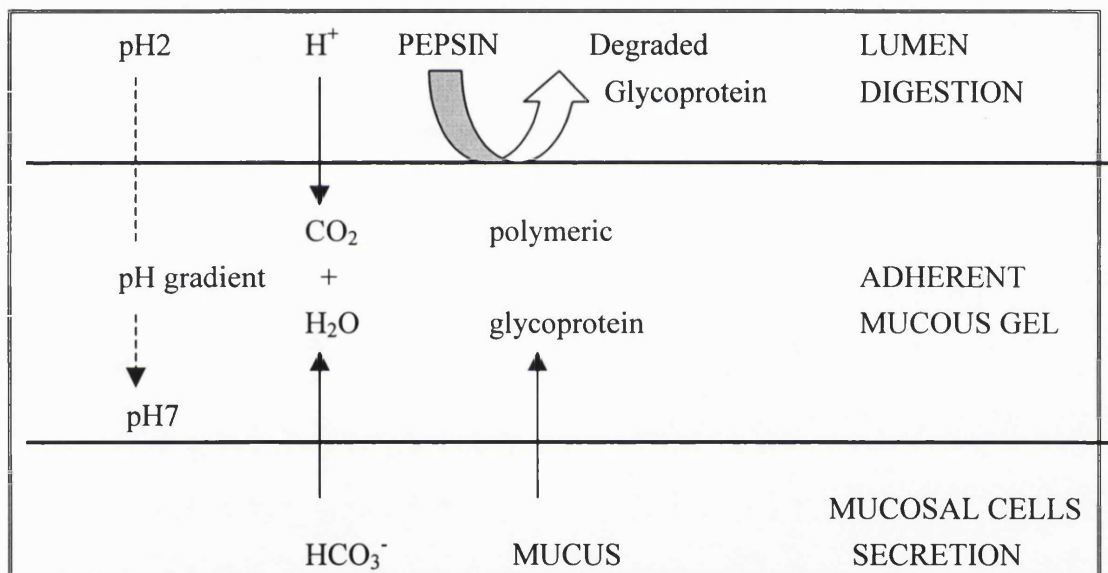
The conformational model for the mucus polymer network predicts that variation in the rheological properties of the mucus should depend on one or more of the following (Verdugo, 1984): the degree of entanglement between glycoprotein chains, variation in the stillness of the glycoprotein chains and the amount of the interaction between the glycoproteins network and the water medium. Mucus is unstable and its gel-like structure declines with time.

#### ***1.4.3.5 pH gradient of mucus***

Almost forty years ago, Heatley (1959) proposed that gastric mucus may act as an unstirred water layer in which hydrogen ions diffusing from the lumen are neutralised by bicarbonate secreted from the surface epithelium. This created a pH gradient across the mucus layer adhering to the gastric mucosa. Heatley's proposal was later supported by work of Ross *et al.* (1981), Williams *et al.* (1981) and Flemstrom and Kivilaakso (1983), using antimony microelectrodes to measure the pH. When the luminal pH was 2.0, the maximum pH reached on the traversing the mucus layer was  $6.68 \pm 0.71$  (n=30) and a stable gradient could be maintained across the mucus for over 100 minutes. The demonstration of a pH gradient confirms the presence of a "barrier" against acid on the gastric mucosa (Figure 1.5).

The glycoprotein matrix in the mucus effectively prevents pepsin and other large molecule from passing through. Hydrogen and hydrogen ions are found to diffuse 3-4 times slower than that through a similar thickness of unstirred layer (Turnberg and Ross, 1984; Turnberg, 1985). The rate of alkaline secretion in humans is about 10-20% of the basal acid secretion (Turnberg, 1985) which is not enough to neutralise all the acid. It therefore appears that the mucus may act by preventing mixing of bicarbonate with hydrogen ions (Takeuchi *et al.*, 1983), rather than by inhibiting diffusion of these ions.

**Figure 1.5: Possible protective mechanism of the gastro-intestinal mucosa against gastric acid and pepsin**



#### 1.4.4 Functions of mucus

Protection is commonly the primary role of mucus (Allen and Garner, 1980), although mucus in different body locations has unique functions (Litt, 1984). For example, the stomach and duodenum are protected against the gastric juices by mucus, by providing a permanent unstirred layer over the mucosal surface which supports surface neutralisation and prevents the small quantity of bicarbonate from mixing with the bulk of the secreted acid in the lumen (Litt 1984). In Table 1.4, various types and functions of normal mammalian epithelial mucus is shown. The effectiveness of the protective capacity of mucus gel layer very much depends on the

thickness of adherent mucus covering the surface and the structure of this mucus gel. The thickness of the mucus gel layer depends on the mucus turnover of the organ in consideration. Mechanical damage is prevented by lubrication of the surface of epithelial cells and, in this regard, the terminal sialic acid is suggested to play an important role (Lee and Ogilvie, 1982). Agents which increase the thickness of the adherent mucus include anti-ulcer drugs and prostaglandins by stimulating its rapid secretion. Prostaglandins and non-steroidal anti-inflammatory drugs (NSAIDs) also markedly stimulate and inhibit, respectively, mucosal bicarbonate secretions (Flemström and Garner, 1982). On the other hand, ulcerogenic, non-steroidal, anti-inflammatory drugs (e.g. diclofenac sodium and aspirin) have long been known to inhibit mucus biosynthesis. Furthermore, due to lipid binding, the hydrophobic mucosal lining of the stomach may contribute to the ability of the mucus gel to resist back diffusion of the hydrochloric acid from the lumen to the epithelial surface.

The polymeric structure of the glycoprotein component is critical for formation of the mucus gel. Although the mucus gel layer presents a barrier to acid and pepsin, this enzyme will degrade the secretion at its luminal surface. Degraded mucus glycoprotein is therefore lost into the lumen and must be replenished by the secretion of new material at the mucosa. In contrast, however, the glycoprotein from the mucous gel adherent is predominantly undegraded and of higher molecular weight. There is an overall weaker mucus gel structure (less polymer) which offers a poorer protective cover in ulcer patients (Younan *et al.*, 1982). The luminal pepsin activity is not noticeably higher in ulcer patients and it is thought that the increased molecular weight glycoprotein in their mucus may arise at the mucosal surface, possibly due to increased lysosomal protease activity associated with cell shedding.

**Table 1.4: Types and suggested functions of normal mammalian epithelial mucus**

<i>Types of secretion</i>	<i>Functions</i>
Respiratory	Clearance of mucosal insults Maintenance of water balance in the airway Maintenance of water balance in mucosa Ion transport and regulation
Middle ear	Clearance of cellular debris? Water balance
Salivary	Lubrication Water balance Anti-microbial Immunochemical complex
Gastrointestinal	Mechanical protection Lubrication Mixing and diffusion barrier Water balance
Cervical	Regulation of sperm transport Sperm reservoir Anti-microbial Sperm energy source Sperm selectivity Water balance

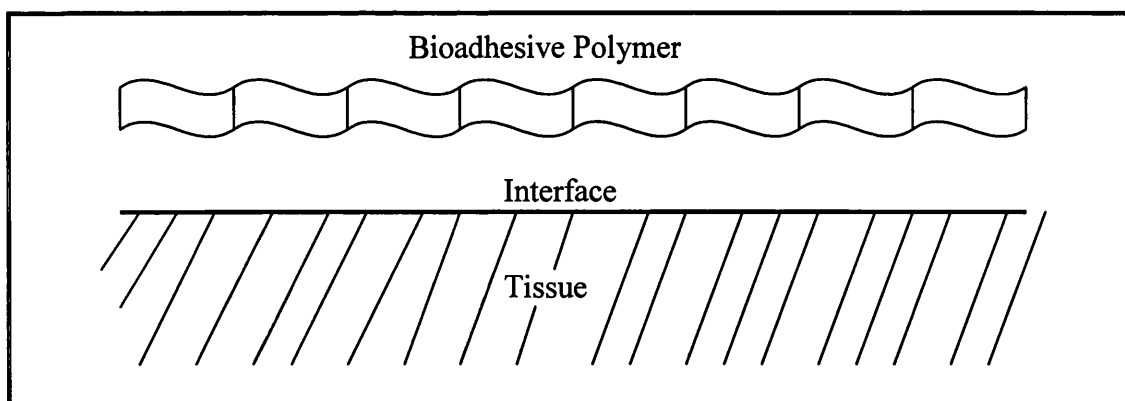
### ***1.5 Some proposed theories of mucoadhesion or structure-adhesion relationships.***

Mucoadhesion is a very complex phenomenon and, therefore it is not surprising that various theories have been proposed by various workers to (or at least in part) explain the concept behind this phenomenon. However, no single theory is sufficient enough account for every case of mucoadhesion (Park and Robinson, 1985; Park and Park, 1990)

### 1.5.1 Mechanisms of mucoadhesion

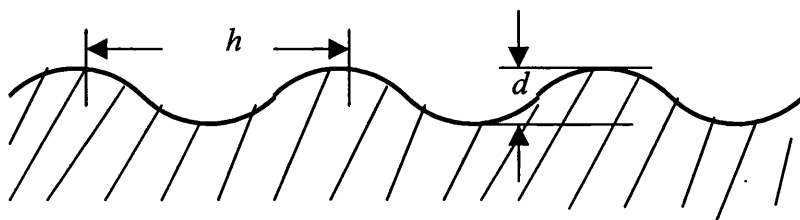
In order for a satisfactory adhesive bond between a mucoadhesive system and the substrate to occur, consideration must be given to the surface of the mucoadhesive material, the first layer of the mucosa and the interfacial layer between the mucoadhesive and mucosa (Peppas and Buri, 1985). This is illustrated in Figure 1.6. The process of mucoadhesion can be divided into two stages: (1) establishment of intimate contact (physical or mechanical bonds) and (2) formation of chemical bonds. In the first stage, the establishment of contact by viscoelastic deformation between the mucoadhesive and substrate is obtained by deposition and inclusion of the adhesive material in the fissures of the tissue (Peppas and Buri, 1985). Thus, the surface roughness of the substrate (Kinloch, 1980), the composition of the mucoadhesive and mucin and the applied force or pressure are important factors in mucoadhesion.

**Figure 1.6: Proposed regions involved in the formation of mucoadhesive bond**



For a surface of maximum depth  $d$ , and maximum width of  $h$ , its roughness is defined as the ratio of the depth to width (Figure 1.7). Supposing that this surface determines which materials are potential mucoadhesive, it would imply that only highly fluid materials that can be incorporated within these anomalies of the tissue can be considered successful adhesive systems. Therefore, the viscosity of liquid mucoadhesive controlled-release formulations would be very important in the development of satisfactory mucoadhesive stability (Davis and Khanderia, 1980).

**Figure 1.7: Schematic representation of the surface roughness of tissue**



In the second stage of mucoadhesion, after contact, interfacial bond formation is established. This interfacial bonding occurs through secondary bonding e.g. electrostatic and hydrophobic interactions, hydrogen bonding, and van der Waals intermolecular interactions (Peppas and Buri, 1985). For mucoadhesives with charged groups, electrostatic interactions and hydrogen bonding appear to be of primary importance. Electrostatic bonds are caused by Coulombic attraction between ions of opposite net charges. However, two surfaces, although negatively charged, may attract each other by long-range forces created by atomic and molecular vibrations that produce fluctuating dipoles of similar frequencies on each surface.

Recognising that mucin contains both anionic and cationic sites, as well as regions of hydrophobicity, several mechanisms of attachment appear possible. In addition, given that bridging cations such as calcium can also serve as an adhesive mechanism between the polyanionic polymer and mucin, it is clear that a single mechanism to explain the attachment is unlikely (Park and Robinson, 1985). In any case, all mechanisms require highly intimate contact between the polymer and the mucin and an expanded network in both substances would favour strong adhesion.

## **1.5.2 Theories of Mucoadhesion**

### **1.5.2.1 Fracture theory of mucoadhesion**

One method of analysis and understanding of polymer adhesive failure is through the fracture characteristics. The fracture theory of mucoadhesion attempts to relate the difficulty of separating the two surfaces after adhesion, to the adhesive bond strength. Kammer (1983) offers a thorough review of this theoretical framework.



The fracture strength,  $\sigma$ , which is equivalent to the mucoadhesive strength can be calculated by the equation:

$$\sigma = (E\varepsilon/L)^{1/2} \quad \text{Eqn. 1.2}$$

where  $E$  is the Young's modulus of elasticity,  $\varepsilon$  is the fracture energy and  $L$  is the critical crack length upon separation of the two surfaces.

The work of fracture or adhesion,  $W_A$ , of an elastomeric network increases with molecular weight,  $M_C$ , of the network strands (Ahagon and Gent, 1975) and are related by the equation:

$$W_A = K (M_C)^{1/2} \quad \text{Eqn. 1.3}$$

where  $K$  is a constant relating to the density of the polymer, the effective mass, length and flexibility of a single main chain bond, and the bond dissociation energy. One of the important parameters that affect the polymer fracture energy and strength is the molecular weight. This energy and strength can be used to describe the permanence of the mucoadhesive bond. The fracture energy of brittle polymers increases with the polymer molecular weight,  $M$ , up to the critical value,  $2M_e$  corresponding to the onset of chain entanglement. The parameter  $M_e$  is the molecular weight between entanglements. For values of  $M$  larger than  $2M_e$ , the fracture energy increases abruptly with  $M$  and eventually reaches a plateau value. Polymers of molecular weights smaller than  $2M_e$  are characterised by virtual lack of chain entanglements.

According to the fracture theory the adhesion can be quantified by breaking strength ( $F_{\max}$ ) of a mucoadhesive from a tissue, or by the work of adhesion ( $W_A$ ) of the mucoadhesive joint which may be calculated as the area under the typical force elongation curves. The reproducibility of the  $F_{\max}$  requires a tissue and an adhesive with constant elongation characteristics, while the analytical principle of the  $W_A$  does not require constant elongation during detachment as a prerequisite (Ponchel *et*

*al.*, 1987; Jacques and Buri, 1992). This theory assumes that in a separation experiment, the failure of the mucoadhesive bond occurs exactly at the interface. However, Ponchel *et al.* (1987) has shown that the adhesive failure almost never occurs at the interface but close to it.

### ***1.5.2.2 Electrostatic theory of mucoadhesion***

This theory states that an electrical double layer is produced at any interface and the consequent Coulombic attraction largely accounts for adhesion and resistance to separation. Deryaguin *et al.* (1977) suggested that the bioadhesive polymer and the glycoprotein network have different electronic structures. Ultimately when the two surfaces come into contact, electron transfer is likely to occur, which will in turn lead to the formation of a double layer of electrical charge at the bioadhesive interface. Adhesion occurs due to attraction forces across the electrical double layer. Thus, the adhesive/substrate system is treated as a capacitor, which is charged when two different surfaces come in contact and discharged when they are separated.

### ***1.5.2.3 Diffusion theory of mucoadhesion***

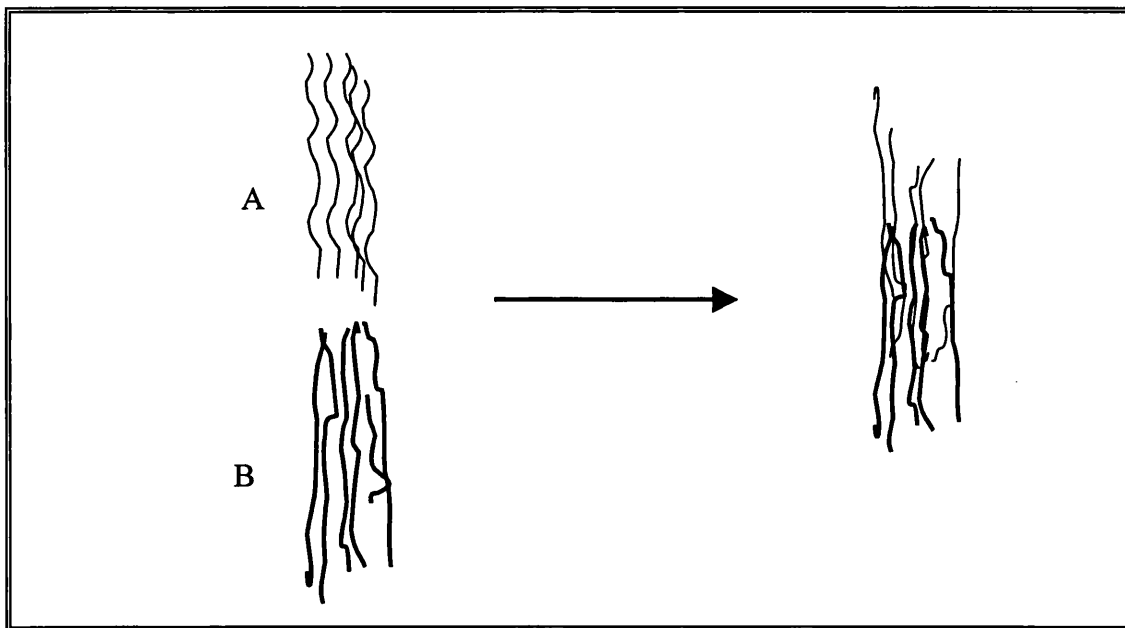
The diffusion theory of adhesion is a simple concept originally described by Voyutskii (1963), who suggested that if two polymer surfaces were in sufficiently close contact, parts of the long-chain molecules would diffuse across the interface. The two polymers will interpenetrate and eventually the interface will disappear and the two parts will become one (Figure 1.8). For interpenetration to be effective, the molecules involved must be relatively mobile. When the adhesive polymer and mucus interpenetrate each other to a sufficient depth, it creates a semi-permanent adhesive-bond. During chain interpenetration the molecules of the mucoadhesive and glycoprotein network are brought into an intimate contact, and due to concentration gradient, the mucoadhesive polymer chains penetrate at rates which are dependent on the diffusion coefficient of a macromolecule through a cross-linked network and the chemical potential gradient (Mikos and Peppas, 1990). This phenomenon can be observed with liquid and uncross-linked solid (or swollen) bioadhesive controlled release systems (Peppas and Buri, 1985).

With cross-linked polymers, interpenetration of large chains is more difficult to occur. In any case, small chains and chain ends may still contribute to interpenetration. Although the exact interpenetration depth needed to achieve mucoadhesion is not known, it is hypothesised to be the order of 0.2-0.5  $\mu\text{m}$  (Peppas and Buri, 1985). The representative mean diffusional path,  $S$ , for macromolecules can be estimated as (Campion, 1974):

$$S = (2tD)^{1/2} \quad \text{Eqn. 1.4}$$

where  $D$  is diffusion coefficient.

**Figure 1.8: Molecular model of chain interpenetration during mucoadhesion of a polymer (A) with mucus (B)**



Adapted from Peppas and Buri (1985).

The diffusion coefficient has been found to depend on the molecular weight of the polymer strand, and to decrease significantly with increasing cross-linking density, indicating that flexibility and chain segment mobility of the mucoadhesive polymer and mucus glycoprotein molecules are important parameters that control interdiffusion (Gupta *et al.*, 1994). Typical values of the polymer diffusion

coefficient through the glycoprotein network of the mucus may be in the range of  $10^{-10}$ -  $10^{-16}$  cm /sec (Peppas and Buri, 1985).

It is possible to determine as well the characteristic time for mucoadhesion,  $t$ , by the equation:

$$t = (l)^2 / D_b \quad \text{Eqn. 1.5}$$

where  $l$  is the interpenetration depth and  $D_b$  is the mucoadhesive material diffusion coefficient.

#### 1.5.2.4 Wetting theory of mucoadhesion

This theory analyses adhesive and contact behaviour in terms of the ability of a liquid or a paste to spread over a biological surface (Peppas and Buri, 1985). The ability of the adhesive to spread spontaneously on mucin influences establishment of intimate contact between the mucoadhesive and mucin, and consequently influence mucoadhesive strength. The thermodynamic work of adhesion is a function of the surface tensions of the surfaces in contact, as well as the interfacial tension. A small value of the interfacial tension would mean a more intimate contact between the two surfaces (Gupta *et al.*, 1990).

The Young's equation (Eqn. 1.6)

$$\gamma_{SF} = \gamma_{SL} + \gamma_{LF} \cos \theta \quad \text{Eqn. 1.6}$$

considers a liquid drop rest on ideal solid surface and in particular the triple line of contact where solid S, liquid L and surrounding fluid, F, meet. A free interfacial tension is attributed to each interface ( $\gamma_{SL}$ ,  $\gamma_{SF}$ ,  $\gamma_{LF}$ ). A force acts along each interface.  $\theta$  is the contact angle measured between the solid-liquid interface and the tangent to the liquid-fluid interface at the triple line (Shanahan, 1992). Equation 1.6 can be extended (Eqn. 1.7) to explain the relation between the thermodynamic work

of adhesion to the surface and interfacial tension of the fluid bioadhesive formulation (*b*) with the tissue substrate (*t*) in the gastric contents (*g*) (Peppas and Buri, 1985).

$$\gamma_{tg} = \gamma_{bt} + \gamma_{bg} \cos \theta \quad \text{Eqn. 1.7}$$

Mucus and tissue from the buccal area are systems which have low surface free energies. Characterisation of the interfacial tension of liquid bioadhesion systems with the tissue,  $\gamma_{bt}$ , could be done *in vitro* or *in situ* using excised sections of tissue from animals and applying the classical Zisman analysis (Fox and Zisman, 1950; Shafrin and Zisman, 1960)

The free energies of wetting, adhesion and spreading are expressed in terms of the free energies of each interface,  $\gamma$  (Paddy, 1992). The wetting energy is:

$$W_e = \gamma_{bt} - \gamma_{tg} \quad \text{Eqn. 1.8}$$

The spreading energy of liquid *b* (gel) on solid *t* (tissue) in the presence of fluid *g* (gastric contents) is often termed the “spreading coefficient” and is defined as:

$$S = \gamma_{tg} - \gamma_{bt} - \gamma_{bg} \quad \text{Eqn.1.9}$$

which is interpreted as the net force provoking radial spreading of the liquid on the solid for a hypothetical contact angle of zero. Each  $\gamma$  term and *s* are both temperature and composition dependent. For the bioadhesive material to displace the gastric contents and adhere spontaneously on the mucus (tissue), the spreading coefficient  $S_b$  must be positive.

Dupre’s thermodynamic work of adhesion  $W_a$  for the adhesive and the substrate in the presence of fluid is given by:

$$W_a = \gamma_{tg} + \gamma_{bg} - \gamma_{bt} \quad \text{Eqn. 1.10}$$

where  $\gamma_{tg}$  and  $\gamma_{bg}$  are the surface tension and  $\gamma_{bt}$  is the interfacial tension. Therefore, the interfacial energies are responsible for the contact between the two surfaces and for the adhesive strength (Kammer, 1983).

The work of cohesion  $W_c$  of the liquid is:

$$W_c = 2\gamma_{bg} \quad \text{Eqn. 1.11}$$

therefore:

$$S = W_a - W_c \quad \text{Eqn. 1.12}$$

Thus, the higher the value of S the greater is the thermodynamic work of adhesion compared to the cohesive energy of the adhesive (Shanahan, 1992).

The interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities have been studied, and the best adhesion was found with moderately wettable polymers (Van Wachem *et al.*, 1985). The adhesion of cellulose polymers-cell increases with increasing contact angle of the polymeric surfaces. Thus a proper surface treatment is important to establish a desirable strength of bioadhesion.

#### ***1.5.2.5 Adsorption theory of mucoadhesion***

The most widely accepted theory of adhesion is the adsorption theory (Kinloch, 1980), which proposes that materials will adhere because of interatomic and intermolecular forces which are established between the atoms and molecules in the surfaces of the adhesive and the substrate. Accordingly, an adhesive polymer adheres to tissues because of van der Waals forces (dipole interactions and London dispersion), often referred to as secondary bonds. In addition, primary bonds (ionic or covalent) form across the interface. (Good, 1977; Tabor, 1977). Thus the forces involved are those which act between the atoms and molecules (valence forces) (Allen, 1992), although London dispersion forces, dipole interactions and hydrogen bonds are universally present since they only depend upon the presence of nuclei and electrons. Other types of forces can only act when appropriate chemical groupings occur. Because these forces are all of very short range, adhesives have the necessity

to achieve close intimate contact and interaction (wetting and spreading) (Allen, 1992). The formation of adsorption bonds at the interface depends greatly on the properties of macromolecules, molecular weight, chemical structure, flexibility of the chain-segment and charge density (Eirich, 1977).

### 1.6 *Bioadhesive Polymers/Copolymers*

Bioadhesives used or tested to date are all polymers of high molecular weight that are able to form hydrogels. The term hydrogel is typically reserved for polymeric materials that can absorb a significant amount of water (>20% of its dry weight) while maintaining a distinct three-dimensional structure. This definition is normally taken to include dry polymers that will swell when placed in water, as well as the already swollen elastic, transparent, semisolid materials called gels. Gels contain a high ratio of solvent to gelling agent (Gehrke and Lee, 1990).

Bioadhesive polymers have been the subject of considerable attention over a number of years (Robinson and Park, 1984; Peppas and Buri, 1985; Caramella *et al.*, 1994). Much of this attention has been focused on the possibility of formulating drugs in bioadhesive dosage forms which adhere to the mucosal surface of the GI tract, thereby increasing residence time with a concomitant increase in drug absorption. There is still debate regarding the prerequisite physical and chemical characteristics of putative bioadhesive polymers. Robinson and Park, (1984) using the fluorescence technique, concluded that :

- Cationic and anionic polymers bind more effectively than neutral polymers.
- Polyanions are better than polycations in terms of binding/potential toxicity, and that water-insoluble polymers give greater flexibility in dosage form design compared to rapidly or slowly dissolving water-soluble polymers.
- Anionic polymers with sulphate groups bind more effectively than those with carboxylic groups.
- Degree of binding is proportional to the charge density on the polymer.

Although an anionic nature is preferred for a good mucoadhesive, a range of nonionic molecules (e.g. cellulose derivatives) and some cationic (e.g. chitosan) have been successfully used.

Peppas and Buri (1985) proposed that a good bio(muco)adhesive must have strong hydrogen bonding groups (-OH, -COOH), strong anionic charges, high molecular weight and sufficient flexibility to penetrate the mucus network or tissue crevices, amongst other characteristics. Sodium alginates fulfil all of the above criteria, yet there still remain questions regarding their mucoadhesive nature. For example, while Charreau *et al.* (1993) have stated that all alginates are 'poor' bioadhesives, Smart *et al.* (1984) and Chen and Cyr (1970) ranked sodium alginates as 'excellent' bioadhesive. These discrepancies may be in part be due to the lack of a universally standardised method of bioadhesion, as many methods are available which may not necessarily yield equivalent results (Park and Park, 1990). It is therefore of interest to study the bioadhesion process using more than one technique in order to allow comparison between data sets. In addition, very little information is available on the bioadhesive properties of solutions, hence there have been difficulties associated with differentiating between the intrinsic molecular interaction between the polymer and mucin from effects caused by the viscoelastic properties of the bioadhesive polymers. In the next section, some of the techniques for measuring bioadhesion are reviewed.

### ***1.6.1 Methods of mucoadhesion assessment and evaluation***

A number of methods have been employed in an attempt to measure the bio(muco)adhesion exhibited by polymers, with some technique designed specifically for the measurement for mucoadhesion. The first step in the selection of a bioadhesive for a particular application is to determine if its properties are suitable for the intended application. Owing to the large number of bioadhesives and potential bioadhesives in different physical forms and biological substrates of different nature, evaluation of bioadhesive properties is complex and diverse. Therefore, evaluation and comparison of the properties of various bioadhesives can be obtained only if the entire test conditions and experimental procedures are kept constant.

It is not easy to extrapolate the behaviour of a bioadhesive from a test to its performance *in vivo*, since testing is generally made under a controlled environment



that is different from in-use conditions (Park and Park, 1990). In addition, each test measures a particular aspect of bioadhesion and a particular property of a bioadhesive while the actual *in vivo* performance of a bioadhesive depends on various interdependent properties. Although certain test methods represent the actual *in vivo* performance of a bioadhesive better than others, it is not clear what parameter is most suitable for evaluating the *in vivo* bioadhesive performance. Therefore, various properties of a bioadhesive should be measured, and the parameters obtained compared with actual *in vivo* performance of the bioadhesive.

### 1.6.1.1 *In vitro* methods

Many techniques are available for measuring bioadhesion (Park and Park, 1990), each is different in design and each has limited areas of application. These adhesion testers are usually adaptations of the existing laboratory apparatus such as tensiometers, microbalances and motor driven pulley systems incorporating spring balances as measuring devices.

Ishida *et al.* (1983) investigated the shearing stickiness of carbopol ointments by changing the content of Carbopol® in the bases at 25°C. A sample of ointment of thickness 0.3-0.4 mm was placed between two glass plates. A string was wound by means of a motor at a constant speed of 140 cm/min and the reading on the spring balance represented the value of shearing stickiness when the two glasses separated. The adhesion of polyethylene gels containing various amounts of sodium carboxymethyl cellulose (NaCMC) were also analysed (Gurny *et al.*, 1984) Upon hydration, adhesion studies were performed with a tensile tester (Instron, model 1114) equipped with a custom made cell for measurement of the adhesive strength. The bioadhesive preparations were hydrated with equal amounts of artificial saliva for 120 minutes. The stress-strain curves were recorded.

Further bioadhesive tests based on *force of detachment* measurements include that of Smart *et al.* (1984), who developed an *in vitro* test method to investigate the adhesiveness of various materials (including sodium alginate) to mucus. The force required to detach a glass plate, coated with the test material, from an isolated mucus

gel was measured, as the surface of the mucous membranes are covered with a continuous layer of mucus, and adhesion to this layer is a requirement for mucosa-adhesion. This technique was based on a Wilhelmy plate method used for surface tension measurements, which consists of a glass plate suspended from a microbalance. A 5ml glass vial containing the mucus samples, was placed into water bath at 20°C. This was then placed on a platform that could be mechanically lowered. The platform was raised until the plate had penetrated the mucus gel or model gel to touch the base of the container. The maximum force when the plate detached from the gel was noted.

A technique (Park and Robinson, 1984) using a fluorescent probe was developed to measure the bioadhesion of various soluble polymers to a cell membrane. In this technique, the adhesiveness of test polymer molecule to the cell surface was quantified by the change in membrane viscosity. An alteration in membrane viscosity after polymer binding was measured from the change in fluorescent spectra of pyrene, which was incorporated, into the lipid bilayer of the cell membrane. A number of charged and neutral polymers were tested and compared in a quantitative manner. Following on from this technique Park and Robinson, (1985) related charge density and hydrophobicity to bioadhesion of the polymer to a mucosal surface. The force required separating a polymer from freshly excised rabbit stomach tissue was measured using a precision balance adapted for bioadhesion measurements.

Later, Leung and Robinson (1988) studied the vertical tensile stress and the shear stress of mucoadhesives to rabbit stomach using a modified tensiometer method. The dual tensiometer has two parts, one of which is a regular modified tensiometer to adjust the position for mucoadhesion and counterbalance the weight of the stopper, and the other is a second tensiometer to measure the strength of shear stress. Improvements on this technique continued to be made into the 1990s. Jacques and Buri (1992) described a method for determination of bioadhesion between a polymer of acrylic acid and a piece of bovine sublingual mucosa. The tests were performed using an adapted tensile tester, fitted with force and displacement transducers driven by a control unit. Mucoadhesion was evaluated by the work of elongation of the mucosal sample during the detachment of the tablet. The reproducibility of the

results obtained depended upon the thickness of the tissue and the hydration conditions.

A novel approach (based on the forces of detachment) which has become the benchmark for *in vitro* mucoadhesion studies was described by Tobbyn *et al.* (1993), when they studied the mucoadhesion of Carbopol<sup>®</sup> 974 discs to pig gastric tissue using a TA.XT2 Texture Analyser (Stable Micro Systems). Carbopol<sup>®</sup> discs were affixed to the instrument probe. USP Simulated Gastric Mucus (SGM) was maintained at 37°C by the use of a thermostatically controlled magnetic heater/stirrer. A receptacle was constructed holding the tissue fast during the withdrawal phase of the test. The withdrawal rate of the instrument probe was set at 0.1 mm/sec and the probe was withdrawn for 15 mm. The effect of contact force and contact time were studied, as well as the reproducibility of the method. The force/time curve was collected during the first and second phases of the mucoadhesive test. Data was collected which allowed the calculation of the work of adhesion (area under the force-distance curve).

The use of rheology in the mucoadhesion assessments has also been reported. Mortazavi and Smart (1995) used a modified rheometer for tensile and shear studies, capable of measuring the maximum force required, as well as the total work necessary, to detach a mucoadhesive containing disc from a model mucosal surface. Similarly, Tamburic and Craig (1996) determined the *in vitro* mucoadhesive properties of polyacrylic acid, using porcine gastric mucin in the form of 30%w/w gel in water as a mucus substitute.

#### **1.6.1.2 *In vivo* methods**

There are comparatively few published data on the *in vivo* measurements of bio(muco)adhesion, due to the numerous difficulties involved. However, different authors have described *in vivo* or *in-situ* methods to measure bioadhesion of different polymers (Anders and Markle, 1989; Ch'ng *et al.*, 1985; Chen and Cyr, 1970). Ch'ng *et al.* (1985) and Longer *et al.* (1985) have both investigated bioadhesion of capsule formulations containing a proposed bioadhesive,

polycarbophil, in the rats. They demonstrated that these formulations showed delayed GI transit and gave improved availability of poorly absorbed drug, chlorothiazide.

Other notable *in vivo* mucoadhesion work includes those of Harris *et al.*, (1990a, b). In their work, Harris and co-workers assessed the GI transit of potential bioadhesive systems, both in rats and in man, using gamma scintigraphy and have also studied the GI transit of putative bioadhesive formulation in man.

Park and Robinson (1984) examined a broad range of polymers as to their binding affinity to mucin-epithelial surface. Interpretation of the results from this study suggested that a polyanionic polymer is preferred over a neutral drug delivery system or polycationic polymer and that water-insoluble polymer would offer advantages in design over water-soluble polymer. Moreover, within the polyanionic polymer category, it was decided that carboxylic acid polymers would be a useful starting point in elucidating structural features as they relate to bioadhesion. It was light of this that sodium alginate, a water-soluble, polyanionic polymer was chosen for this study.

In this work various techniques (some, adaptations of those listed above, and some totally innovative) are used to assess and quantify the mucoadhesiveness of sodium alginate preparations, with oesophagus as the target tissue. While the aim and objectives of the study are described in detail later, the physiology and disease states of the oesophagus are outlined here.

### ***1.7 Anatomy and physiology of the oesophagus***

Previous bioadhesion studies of the GI tract have paid very little attention to the oesophagus, despite the fact that this organ may be host to a number of disease states. This therefore makes the oesophagus a potential organ to target for the purpose of bioadhesion and for drug delivery.

The oesophagus begins in the neck where it connects with the pharynx; it runs slightly to the left of the midline through the thorax and enters the abdomen where it joins the stomach (Bowman and Rand, 1990). The oesophagus is a muscular tube lined mostly by squamous epithelium and is approximately 40 cm long in man. At the upper end there is cricopharyngeal sphincter, which contains striated muscle fibres enabling voluntary control over the initiation of swallowing. The remainder of the muscular tube is composed of smooth muscle, which propels food boli by peristalsis and is under autonomic control.

The oesophageal wall also has several distinct layers. They are an outer adventitial layer of connective tissue (the serosa), a muscular layer containing circularly and longitudinally arranged fibres, a submucosa of connective tissue containing large blood vessels and mucous glands, and a mucosa consisting of (1) smooth muscle fibres (the muscularis mucosa), (2) a lamina propria of loose connective tissue and (3) an outer epithelial layer of stratified squamous cells.

In the upper part of the oesophagus most of the muscle fibres are striated, in the middle portion both striated and smooth fibres are present, while in the lower part smooth muscle predominates. The circularly arranged rings of the smooth muscle of the upper and lower ends of the oesophagus are thicker and more powerful than elsewhere along its length; except during swallowing, they remain in a state of tonic contraction. These regions are described as the pharyngeal and cardiac sphincters, respectively. The principal innervation of the oesophagus is by the vagus nerves, which supply both motor neurons to the striated muscle and parasympathetic fibres to the smooth muscle. Sensory fibres from the oesophagus also run in the vagi, they are activated by distension of the lumen. The entry of food into the stomach is facilitated by relaxation of the distal sphincter. The rate of passage of materials through the oesophagus depends on their consistency. Liquids are conveyed slowly and a solid mass formed in a bolus moves more slowly still. Contractions of the oesophagus which are ineffective in moving a swallowed mass give rise to a painful sensation; this may occur when swallowed mass becomes lodged, or when the circular muscle in advance of the mass fails to relax.

The mucosa of the pig's oesophagus (which is used as a model in the present study) is slightly keratinized. The lamina propria is made of a feltwork of fine collagen fibres with an abundance of evenly distributed elastic fibres (Stinson and Calhoun, 1993). The muscular layer in the pig's oesophagus contains only longitudinally arranged smooth muscle bundles, which are absent in the cranial end of the oesophagus. The muscular layer is especially well developed in the caudal end, where it is as thick as the outer layer of the tunica muscularis. Unlike in man, the submucosa in the pig is loose connective tissue containing large, longitudinally orientated arteries, veins, large lymph vessels and nerve trunks. While the layer contains mucous glands in man, seromucous glands containing mucous acini with serous demilunes characterise this layer in the pig. Furthermore, in the pig, the glands are abundant in the cranial half but do not extend to the caudal half. Density of the glands may be as much as four times greater near the stomach (caudally) than at the beginning of the organ (cranially). The loose nature of the relaxed oesophagus allows it to form longitudinal folds.

### ***1.7.1 Normal physiology of the oesophagus in man***

The body of the oesophagus lies within the negative pressure thoracic cavity, while the abdominal cavity has a positive pressure gradient. Without natural defence mechanisms the pressure gradients would favour continual reflux of gastric material into the oesophagus. The lower oesophageal sphincter is manometrically defined as a high-resting-pressure zone (15-35 mmHg above gastric baseline pressure). This sphincter is normally in a tonic state preventing reflux of gastric material from the stomach, but relaxes upon swallowing to permit the free passage of food into the stomach.

The lower oesophageal sphincter forms a muscular ring, which by increasing its tonus is able to prevent reflux of the stomach content. There is also hiatus mechanism, which may be compared to what happens when a sack is tightened. When the stomach is pressed upwards against the diaphragm, it is squeezed up so that leakage of the gastric content to the oesophagus is only possible through the very

narrow mucosa folds then formed. Another important protection for the oesophageal mucosa is the continuous rinsing by swallowed saliva.

In spite of these protecting functions together with several others, it very often happens that the acid stomach content reaches the oesophageal mucosa, which in contrast to the stomach mucosa is not adapted to the acid and enzymes present in the gastric juices. The mucosa will heal itself, but if the contact with gastric juice continues for longer periods of time, it may result in conditions such as oesophagitis, oesophageal stricture, hyperplasia of the oesophageal epithelium lining (hyperplastic reflux), oesophageal ulcers and Barrett's oesophagus.

### ***1.7.2 Diseases of the oesophagus***

The symptoms and/or severity of damage produced by gastroesophageal reflux are partially dependent upon the duration of contact between the gastric contents and the oesophageal mucosa (Stanciu and Bennett, 1974). This contact time is dependent on the rate at which the oesophagus clears the noxious material and the frequency of reflux. The oesophagus is cleared by primary peristalsis in response to swallowing, secondary peristalsis in response to oesophageal distension and gravitational effects. Swallowing may also contribute to oesophageal clearance by increasing salivary flow. Saliva may buffer the residual gastric material on the surface of the oesophageal mucosa.

#### ***1.7.2.1 Reflux Oesophagitis***

The hallmark symptom of oesophagitis is heartburn, which is classically described as a substernal sensation of warmth, or burning that may radiate to the neck. Other symptoms that may occur secondary to oesophagitis include regurgitation, water brash and haemorrhage (Bowman and Rand, 1990). Chronic blood loss may occur in oesophagitis, however, acute blood loss is rare. Dietary factors such as spicy foods, orange juice, tomato juice, and coffee may precipitate symptomatic reflux by direct mucosal irritation (DiPiro *et al.*, 1989). Furthermore, pregnancy, chaliasia and scleroderma are conditions in which reflux is common.

### **1.7.2.2 Histopathology of Oesophagitis**

Reflux oesophagitis is a condition in which the oesophageal epithelium is damaged by gastroesophageal reflux of predominantly acid and pepsin. High-grade changes of gastroesophageal reflux are characterised by severe epithelial injury or destruction commonly accompanied by mucosal infiltration neutrophils and eosinophils or both. Epithelial erosion, ulceration, and accompanying inflammatory infiltrate are confined mostly to the oesophageal mucosa, lamina propria, and muscularis mucosa; however, perfusion through the wall of the oesophagus can occur in unusual circumstances. Exposure of the squamous mucosa to refluxed acid (together with bile in patients with entero-gastric reflux) leads to cell injury and accelerated desquamation. In response to this injury the proliferation of the germinative cells of the epithelium is increased (basal cell hyperplasia), resulting in less mature cells occupying most of the epithelial thickness and is accompanied by elongation of the connective tissue papillae (Underwood, 1996)

Where reflux is severe, cell proliferation cannot keep pace with cell desquamation, which can be the source of haemorrhage and may even proliferate in most severe cases. Healing is achieved by the fibrosis and epithelial regeneration; subsequent shrinkage of fibrous tissue can produce a segmental narrowing (stricture) in the areas of healed ulceration. Restoration of epithelial continuity is usually achieved by proliferation of squamous cells, but in some patients the lost squamous epithelium is replaced by columnar epithelium giving rise to a condition known as 'Barrett's oesophagus'

### **1.7.3 Treatments of Reflux Oesophagitis**

Therapeutic modalities in the treatment of reflux oesophagitis are targeted at reversing the various pathophysiologic abnormalities (DiPiro *et al.*, 1989). In general therapy is directed at boosting the normal defence mechanisms that may prevent reflux and/or decrease the aggressive factors that potentiate reflux or mucosal damage. The stepwise treatment of reflux oesophagitis may be categorised into non-invasive lifestyle modification, drug treatment, and in rare cases, anti-reflux surgery.



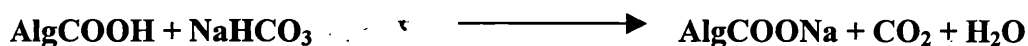
Simple, non pharmaceutical steps such as the elevation of the head of the bed 15-20 cm, avoiding spicy foods, abrasive food (burn toast, crisps), and large meals at least 2 hours before retiring to bed have been found to be very useful in patients with reflux oesophagitis. In addition, weight reduction and smoking cessation (if necessary), are also known to be effective. As mucosal damage correlates with the extent of acid exposure, drug treatment of reflux oesophagitis has been primarily aimed at reducing this exposure either by neutralisation of the acid (antacids) or by suppressing gastric acid secretion, histamine H<sub>2</sub>-antagonists). Other drug treatments include those that provide mucosal protection (alginic acid, sucralfate), increase lower oesophageal sphincter pressure (bethanechol, metoclopramide) or promote gastric emptying (metoclopramide, cisapride). Antacids or histamine H<sub>2</sub>-antagonists (e.g. ranitidine) are usually the first drugs to be tried. Antacids frequently give symptomatic relief, and are widely used for self-medication and can often relieve symptoms in both ulcer and non-ulcer dyspepsia, and in reflux oesophagitis. They are best given when symptoms occur or are expected, usually between meals and at bedtime, four or more times a day. Alginates added as mucosal protectants against reflux oesophagitis may be useful. However, the use of H<sub>2</sub>-antagonists can mask the symptoms of gastric carcinoma, and in older patients who are at greater risk, early investigation may be desirable (Martindale 1996).

### ***1.7.3.1 The anti-reflux mechanism and effect of alginates***

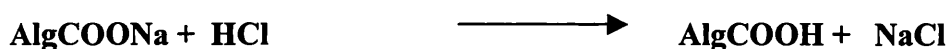
Alginate-containing antacids such as Algicon<sup>®</sup> (Rhône-Poulenc Rorer), Gastrocote<sup>®</sup> (Seton), Gaviscon<sup>®</sup>, Gaviscon<sup>®</sup> Advance, and Gaviscon<sup>®</sup> Infant (Reckitt & Colman) contain varying amounts alginate the form of alginic acid, magnesium alginate or sodium alginate; and are all available as 'over the counter' medication. A previous study (Washington *et al.*, 1987) has demonstrated that not only does the form of alginate important but also the presence of other ions may contribute to the overall protective properties of these antacids. Perhaps, the floating alginate foam or "raft" provide suppression of reflux. The results from this study indicated that Gaviscon<sup>®</sup> liquid formed stronger rafts than Algicon<sup>®</sup>. The authors argued that reason for this observation was that the Algicon<sup>®</sup> formulation demonstrated problems

of competition for available acid local to the raft, between the bicarbonate required to elevate the raft and entrapped antacid component.

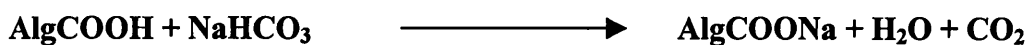
The mechanism for raft formation of alginate-containing antacids may be explained as follows: When alginic powder is stirred in water in the presence of bicarbonate, it reacts in the following manner:



In the acid environment of the stomach, further reaction takes place. Thus,



There is precipitation of a colloidal alginic acid gel around the carbon dioxide bubbles. The gel is further stabilised through the action of *in situ* calcium ions at the same time as it expands in the body temperature. The light gel will form a cover floating on the stomach contents. The acid gastric juice can not enter the oesophagus, because the light alginic acid raft will first reach the hiatal opening and seal the mucosa folds, which otherwise would let the gastric juice pass and irritate the sensitive mucosa of the oesophagus (Martindale 1996; BNF 1998). The patient is protected for several hours from gastric reflux, heartburn and retrosternal pain. In time, as the stomach contents empty into the duodenum, the alginic acid gel will also disappear. Finally, the alginic acid gel is neutralised by sodium bicarbonate and the following reaction occurs.



Sodium alginate will therefore pass through the enteric tract and be excreted unchanged in the faeces.

#### 1.7.4 Barrett's Oesophagus

The distal oesophagus is lined by squamous epithelium, which, when exposed to chronic reflux of gastroduodenal contents, is sometimes replaced by the glandular epithelium of Barrett's oesophagus. Barrett's oesophagus is a premalignant

condition, which exhibits three kinds of columnar mucosa, namely, *junctional type* (resembling normal gastric cardia), *atrophic fundal type* (containing scant specialised gastric secretory cells) and '*specialised*' mucosa (in which the epithelium undergoing a further metaplastic changes towards an intestinal type and has acquired goblet cells).

Although cancer is not common in absolute terms, the risk of malignancy is approximately 100 times higher among patients with Barrett's oesophagus than in the general population (Kumar and Clark, 1990). Once the condition has been diagnosed, it is advisable to put patients on regular endoscopic surveillance and regular biopsies are taken for the detection of dysplasia.

### **1.7.5 Tumours of the oesophagus**

#### **1.7.5.1 Benign Tumours**

A variety of benign tumours occur in the oesophagus. These are usually small, rarely over 3 cm in diameter, and occur mostly as intramural, solid, grey submucosal masses. Benign tumours are uncommon and comprise about 5% of all neoplasms of the oesophagus; the type most frequently encountered is a leiomyoma, but fibromas, lipomas, haemangiomas, neurofibromas, lymphangioma, and squamous papillomas may also arise in this location. These tumours are rarely large enough to cause symptoms and are usually chance findings at post-mortem (Underwood, 1996).

#### **1.7.5.2 Carcinomas**

Carcinoma of the oesophagus accounts for 2-2.5% of all forms of malignant disease in the United Kingdom (Kumar and Clark, 1990; Underwood, 1996). The majority of malignant tumours occur in the middle (50%) and lower third (25%) of the oesophagus. The incidence of carcinoma is higher among those with oesophagitis, achalasia, strictures, oesophageal diverticula and oesophageal web than in a control group (Robbins *et al.*, 1984).

Dysphagia is the most common clinical manifestation of oesophageal carcinoma and is progressive. The initial difficulty in swallowing solids may be extended to liquids

as the condition deteriorates. The lesion is usually ulcerative in nature, extending around the wall of the oesophagus to produce a stricture (Underwood, 1996). Direct invasion of the surrounding structures rather than widespread metastases occur. Weight loss, as a result of dysphagia as well as of anorexia, frequently occurs.

The prognosis and the survival rate of oesophageal carcinomas are poor (2%, 5-year survival) and only symptomatic and palliative treatment is a realistic possibility. Dilatation of the stricture and placing a tube to keep the oesophagus open is the usual therapy and can be performed via an endoscope. Surgery carries a high morbidity and mortality and little chance of anything but palliation. Radiotherapy and chemotherapy can be used for squamous carcinoma with limited success (Underwood, 1996).

## **1.8 Aims and objectives of study**

### **1.8.1 Aim**

The aim of this work was to study the bio- and mucoadhesive properties of sodium alginate, with particular emphasis on oesophageal delivery

### **1.8.2 Objectives**

- To characterise alginate gels with particular view to develop texture analysis as method of measuring Young's modulus. In addition the effects of different preparation methods, the level of calcium conversion, and the chemical composition characteristics of the alginate batches were examined using both texture analysis and rheology. The thermal effects on the gels were also assessed using thermorheology.
- The interactions between sodium alginates and mucins were examined in order to understand and assess the mucoadhesive properties of these alginate batches. Both the source and quality of mucin and their concomitant interactions with alginates were investigated using texture analyser and also measuring the synergy between mucin and alginates with the aid of rheology.

- Alginate adherence to the porcine oesophagus was quantified using an in-house built apparatus with the view to understanding bioadhesion to this organ. The relationship between the chemical composition of alginates and their functionality was evaluated using this technique.

**CHAPTER 2: CHARACTERISATION OF ALGINATE GEL  
SYSTEMS**

## 2.1 Some basic concepts of oscillatory rheology

Rheology is the science concerned with the deformation of matter under the influence of stresses, which may be applied perpendicularly to the surface of a body (a tensile stress), tangentially to the surface (a shearing stress), or at any other angle to the surface. The deformation that results from the application of a stress may be divided into two types:

1. spontaneously reversible deformations or elastic deformations, and
2. permanent or irreversible deformations that are referred to as flow and are exhibited by viscous bodies.

The work used in producing an elastic deformation is recoverable when the body returns to its original shape after removal of the applied stresses. However, in irreversible deformations the work used in maintaining deformation is dissipated as heat and is not recoverable mechanically when the stress is removed.

The description of an ideal viscous fluid behaviour is Newton's Law which is

$$\textit{Shear stress} = \textit{Viscosity} \times \textit{Shear rate} \quad (\tau = \eta\dot{\gamma}) \quad \text{Eqn. 2.1}$$

where shear stress is the intensity of shear force per unit area of cross section.

Similarly, the description of a perfect elastic solid behaviour is Hooke's Law:

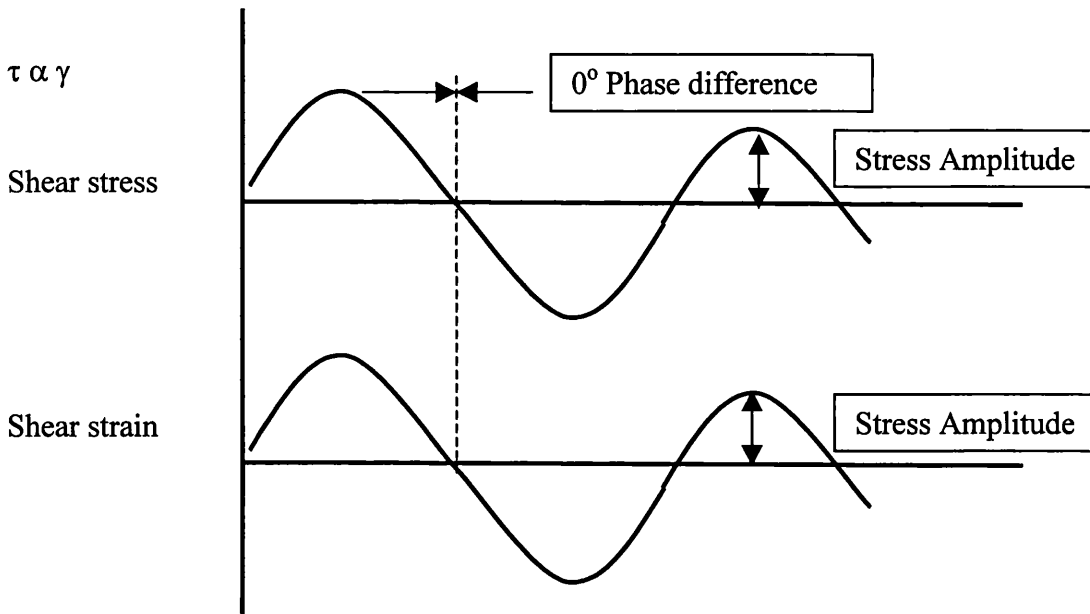
$$\textit{Shear stress} = \textit{Elasticity} \times \textit{Strain} \quad (\tau = G\gamma) \quad \text{Eqn. 2.2}$$

where strain is defined as the relative deformation of a solid body in response to a stress. If the stress in each case is applied in a sinusoidal fashion then, in accordance with Hooke's Law expression, a sinusoidal stress wave applied to ideal elastic will generate a sinusoidal strain wave completely in phase (Figure 2.1a).

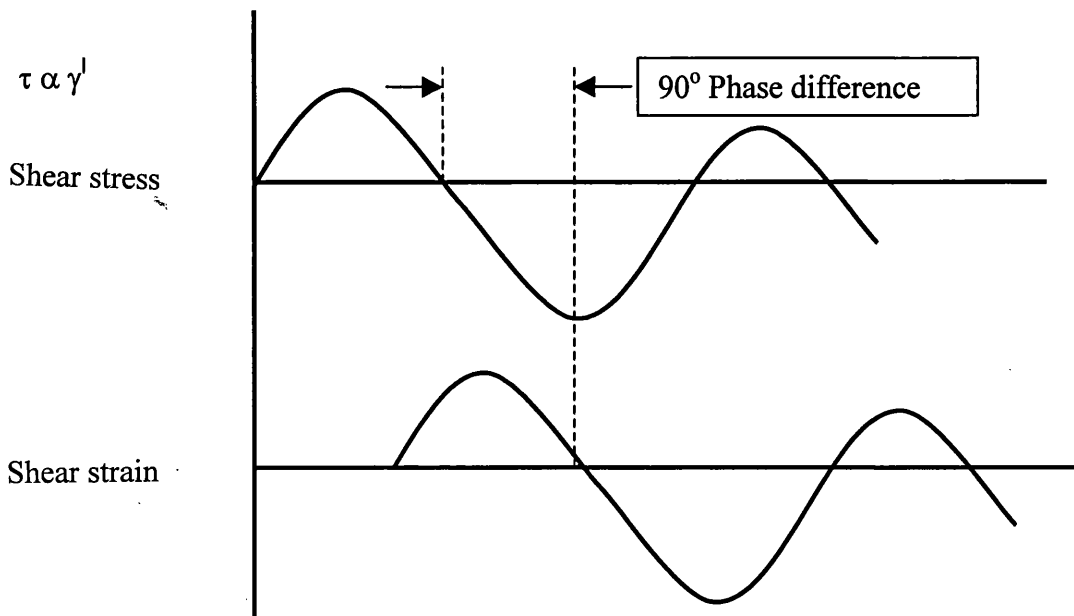
For a Newtonian fluid, an applied sinusoidal stress wave will generate stress and strain waves, which are out of phase by  $90^\circ$ . This is because the slope of a sine wave (derivative with respect to time) is at its maximum when the sine wave goes through zero. Hence when stress is at a maximum then Newton's Law predicts that shear rate

or strain rate is maximum and therefore, the stress maximum will coincide with the zero crossing point of the strain wave (Figure 2.1b). Hence, the stress and strain maxima are  $90^\circ$  apart.

**Figure 2.1a: Hookean Solid**



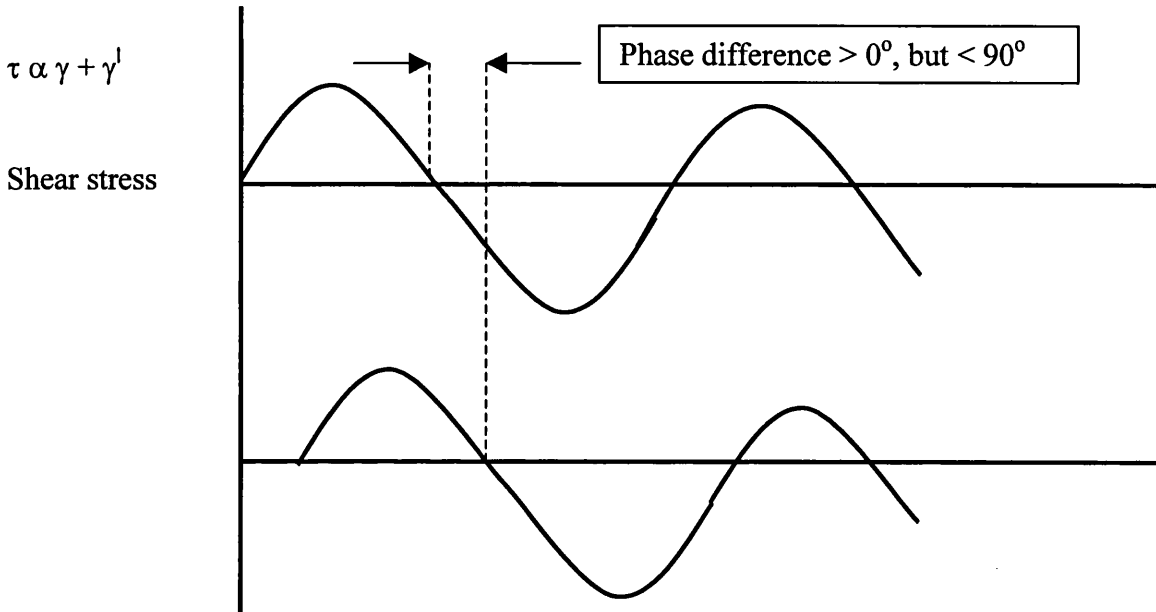
**Figure 2.1b: Newtonian Fluid**





It follows that for a viscoelastic material, the phase difference is more than zero, but less than  $90^\circ$  (Figure 2.1c).

*Figure 2.1c: Viscoelastic material*



Pharmaceutical materials which are viscoelastic conform to the last description of sine waves. It implies that in such systems, there are components of both elasticity and viscosity. In oscillatory (dynamic) measurements, a small, sinusoidal oscillatory strain, or deformation,  $\gamma(t)$  at time  $t$  according to equation 2.3, is applied to the sample.

$$\gamma(t) = \gamma_0 \sin \omega t \quad \text{Eqn.2.3}$$

where  $\gamma_0$  is the strain amplitude and  $\omega$  is the angular frequency.

The applied strain generates two stress components in the viscoelastic material: an elastic component in-phase with the strain and out-of-phase viscous component. Differentiation of Eqn. (2.3) yields Eqn. (2.4), which shows the strain rate  $\dot{\gamma}(t)$  for evaluating the viscous component to be  $\pi/2$  radians out of phase with the strain:

$$\gamma^I(t) = \gamma_0 \omega \cos \omega t \quad \text{Eqn. 2.4}$$

Oscillatory measurements need to be carried out in the linear visco-elastic region. This region, where the range of stresses (torques) and/or strains (displacements) over which the modulus and viscosity are constant, needs to be found before further measurements such as frequency, temperature or time sweeps can be carried out.

For deformation within the linear viscoelastic range, Eqn. (2.5) expresses the generated stress ( $\sigma_0$ ) in terms of an elastic, or storage, modulus  $G^I$  and a viscous, or loss, modulus  $G^{II}$ . In this region, the range of stresses (torques) and/or strains (displacements) over which the modulus and viscosity are constant.

$$\sigma_0 = G^I \gamma_0 \sin \omega t + G^{II} \gamma_0 \cos \omega t \quad \text{Eqn. 2.5}$$

For a viscoelastic material, the resultant stress is also sinusoidal but shows a phase lag of  $\delta$  radians when compared with the strain. (Figure 2.1c). The phase angle  $\delta$  covers the range  $0 - \pi/2$  as the viscous component increases. Equation (2.6) also expresses the sinusoidal variation of the resultant stress:

$$\sigma(t) = \sigma_0 \sin (\omega t + \delta) \quad \text{Eqn. 2.6}$$

Combining Eqns.(2.5) and (2.6), the following viscometric expressions emerge that define viscoelastic behaviour:

$$G^I = [\sigma_0 / \gamma_0] \cos \delta \quad \text{Eqn. 2.7}$$

$$G^{II} = [\sigma_0 / \gamma_0] \sin \delta \quad \text{Eqn. 2.8}$$

$$\tan \delta = G^{II} / G^I \quad \text{Eqn. 2.9}$$

where  $G^I$  is the storage modulus,  $G^{II}$  is the loss modulus, and  $\tan \delta$  is the loss tangent.

The modulus  $G'$  (storage or elastic modulus) is defined as the stress in phase with the strain in a sinusoidal shear deformation divided by the strain. It is a measure of the energy stored in the material (or recoverable per cycle of deformation) when different systems are compared at the same strain amplitude.  $G''$  (the loss or viscous modulus) is defined as the stress at  $90^\circ$  out of phase with the strain divided by the strain. It is a measure of the energy dissipated or lost as heat per cycle of sinusoidal deformation, when different systems are compared at the same amplitude. The loss tangent, therefore, is the ratio of the energy dissipated to that stored per cycle of deformation. These viscoelastic functions have important roles in the rheology of structured polysaccharides.

One characteristic of viscoelastic substances is that in measurements with increasing frequency, the slope of the elastic modulus with respect to frequency is steeper than the slope of the viscous modulus. A useful parameter which is dimensionless and conveys no physical magnitude but a measure of the ratio of energy lost to energy stored in a cycle deformation is the loss tangent,  $\tan \delta = G''/G'$ . Almdal *et al.*, (1993) have proposed that 'true gels' of semisolid nature (such as alginate gels used here) are characterised by the absence of an equilibrium modulus, a storage modulus  $G'(\omega)$  which exhibits a pronounced plateau extending to times of the order of seconds. In addition, such gels are characterised by a loss modulus,  $G''(\omega)$  which is considerably smaller than the storage modulus in the plateau region. This implies that in such gels  $\tan \delta$  values are much less than 1.

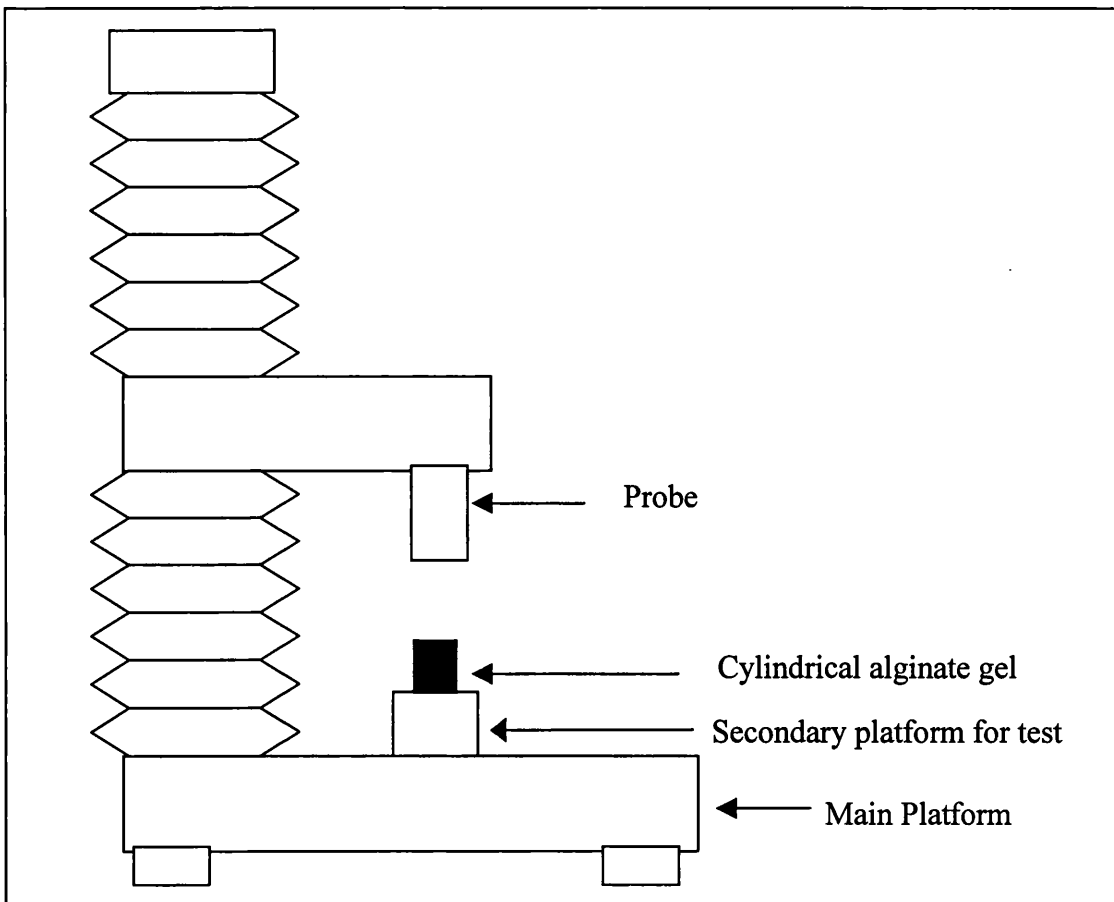
## 2.2 Texture analysis of alginate gels

Gels are commonly used as vehicles in pharmaceutical and cosmetic preparations for many types of applications (dermatological, ophthalmic and intramuscular injectable). Gel formation is also involved in drug release mechanisms from hydrophilic swellable matrices, which are widely used in prolonged medication (Colombo *et al.*, 1987; Conte *et al.*, 1988). In such systems, polymer hydration results in the formation of an outer gel layer that controls drug diffusion and that eventually undergoes erosion. Therefore, the drug release also depends on the

physical and mechanical properties of the gel layer and it has been suggested that the strength rather than the viscosity of the gel layer that plays a major role in the drug release process (Van Aerde and Ramon, 1988; Herman *et al.*, 1989; Herman and Remon, 1989).

The use of texture analyser (Figure 2.2) to measure properties of gels has gained (and continues to gain) more attention in both pharmaceutical and food industries. Amongst the various properties of gels measured with this apparatus is the gel strength, or the Young's modulus. The experimental details of this apparatus are discussed in section 2.4.3. For gels in containers, the gel strength has been defined as the ratio between the force (penetrating force) at a given time and the displacement covered by the probe inside the sample (Ferrari *et al.*, 1994).

**Figure 2.2 Diagrammatic representation of SMS Texture Analyser TA.XT2**



Other ways of expressing the strength of gels include measuring the ‘work of fracture’ which is the work required to crack a unit area of the gel (Oates *et al.*, 1993). In the case of ‘isolated’ gels (those made from plastic moulds), the strength is expressed as the Young’s modulus of the gel (Draget *et al.*, 1994), section 2.4.3. The combination of rheology and textural analysis offers a better understanding of gel behaviour both at the physical and molecular level.

### 2.3 *Scope and rationale for study*

Various methods of preparing desirable alginate gels have been discussed in Chapter 1. The method of preparation of such gels ultimately determine the rheological and other physical properties of these systems, although there has not been a systematic comparison of alginate gels prepared using different protocols. It was therefore the aim of this study to explore some of the methods of preparing alginate gels with divalent cations, using different preparation conditions and concentrations of sodium alginate. The information obtained with oscillatory rheology should shed some light on the molecular arrangements within the gel systems. In addition, the use of texture analyser was employed to determine the Young’s moduli of these gels.

### 2.4 *Materials and Methods*

#### 2.4.1 *Materials:*

Chemical	Supplier & Batch number
Calcium carbonate	BDH (K22397699)
D-gluconic acid lactone	Sigma (74H0888)
Citric acid	BDH (K22028738)
EDTA	BDH (K16983264)
Sodium hydroxide	BDH (4X500GMS)
Calcium chloride	BDH (K21330898)

In addition, five medium molecular weight sodium alginates, three of which were of high guluronic acid content (SF 120, SF/LF 40, and SF 200) and two low guluronic

acid (LF 120L and SF 60L) were supplied by Pronova Biopolymer a.s., Drammen, Norway. The data sheet for these alginate samples are shown in Table 2.1.

**Table 2.1: Chemical composition, intrinsic viscosity and molecular weight of the alginate sample used**

Sample	$\eta$ (dl/g)	$\eta^*$ (mPa.s)	Molecular weight (kDa)	FG	FGM.MG	Dry matter content (%)	NG>1
SF 120	9.1	110	225 (220)	0.694	0.141	91.00	14.5
SF/LF 40	12.5	410	315 (n.k)	0.630	0.050	86.50	20.3
SF 200	15.0	990	380 (n.k)	0.690	0.110	91.50	20.3
LF 120L	9.91	121	245 (240)	0.444	0.196	90.60	6.9
SF 60L	n.k	950	n.k (350)	0.438	0.184	86.00	7.4

$\eta$  = intrinsic viscosity,  $\eta^*$  = viscosity of 1% solution, ( ) = molecular weight average, FG = fraction of guluronic acid residues, FGM.MG = fraction of alternating sequences, NG>1 = typical average length of guluronic acid blocks larger than 1, n.k = not known. Data supplied by Pronova Biopolymer A.S, Drammen, Norway.

### Equipment

For all rheological work, a Carri-med CSL 500 rheometer (TA instruments, Leatherhead, England) with Tempette Junior temperature controlled water bath, model TE-8J (Techne Inc., USA) was used. For the determination of gel strength, the SMS TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, UK) interfaced with an IBM computer (Opus personal computer, Opus Technology, Surrey, UK, model CM 1438T) for automatic collection and processing of the experimental data. Hi Resolution Thermogravimetric Analyser (TGA 2950, TA Instruments, UK) was used for all thermogravimetric analysis, using open aluminium pans.

## 2.4.2 Methods:

### 2.4.2.1 Thermogravimetric analysis

Thermogravimetry (TG) is a technique in which the mass of the sample is monitored while the temperature of the sample, in a specified atmosphere, is programmed. In order to avoid confusion with the glass transition,  $T_g$ , most manufacturers prefer to call this technique thermogravimetric analysis (TGA). In addition to the normal mass change with temperature of the thermogravimetric curve, the rise of mass change with time,  $dm/dt$  is often plotted. This is known as the derivative thermogravimetric (DTG) curve.

Sodium alginate powder is known to contain a certain amount of water even when 'dry'. This water which adds to the overall weight has to be taken into consideration when weighing out (or calculating the %w/w of) the sample. The amount of sodium alginate in solutions and gels is based on 'absolute' dry material (ADM), as given by Pronova Biopolymer a.s., The following equation shows the relation between alginate as absolute dry material, the weight required and the dry matter content (%):

$$\text{ADM} = [\text{weight required} \times 100] / [\text{dry matter content (\%)}] \quad \text{Eqn. 2.10}$$

where the value of the dry matter content is as quoted by the manufacturer (Table 2.1).

The manufacturers have used a method based on prolonged drying of the powder at 105°C until there is no further weight change, to quantify the water (or moisture) content. The use of thermogravimetric analyser (TGA) offers a more accurate and rapid determination of the water content.

Approximately 15 mg of sodium alginate samples were carefully loaded into the aluminium pan and heated at a rate of 10°C per minute and a temperature range of 10°C to 160°C. This temperature was chosen to incorporate the browning temperature of 130°C of sodium alginate, but to avoid the decomposition of the sample. The weight change within the temperature range was taken as the percentage

'water loss', assuming that there were no other volatile substances in the sample. Furthermore, the temperature at which the water loss was at its maximum was also determined. Each alginate type was run at least four times (using a fresh sample each time) for reproducibility of data.

#### ***2.4.2.2 Alginate gel preparations:***

Following an extensive literature review and some initial screening of various methods and techniques of making homogeneous alginate gels, three internal setting methods (Chapter 1, section 1.1.2.3) were chosen for comparative studies.

In the first method, described as 'Pronova's standard method 2204 for gel strength' (Pronova Biopolymer a.s., undated and unpublished), the alginate gels were prepared as follows:

Ethylenediaminetetraacetic acid (EDTA) (18.6g) was dissolved in 500g de-ionised water by gently heating to approximately 40°C in a 1000ml beaker (solution 1). In a 2000ml beaker, 7.35g of calcium chloride were dissolved in 500g de-ionised water (solution 2). The two solutions were mixed together and the pH adjusted to 7-7.5 by adding 5M sodium hydroxide (solution 3). A 1000ml-glass beaker was weighed and the weight recorded. Into this beaker, 135g of solution 3 and 315g of de-ionised water were placed (solution 4). Sodium alginate powder (6.0 g absolute dry weight) was dissolved in solution 4 while stirring with a mechanical stirrer (Heidolph Instruments, Germany) (solution 5). Complete mixing was achieved by initially stirring at about 1400 rpm while introducing the alginate powder during 3 seconds. Stirring continued at about 2000 rpm for 20 minutes, reducing the speed if necessary to avoid loss of solution. In a 100ml beaker, 4.82 g of glucono- $\delta$ -lactone (GDL) was dissolved in 45g of de-ionised water. This solution was freshly prepared and added to solution 5, stirring with a glass rod. The total weight of the solution was adjusted to 600g by adding de-ionised water. For clarity, this method of preparing alginate gels is designated 'method 1'.



For rheological measurements, aliquots of the solution were placed into flat-bottomed, 8-welled tissue culture plates to form gels of 6 cm diameter and 1 mm thickness. In the case of gel strength measurement in the form of Young's modulus, the preparation was poured into flat-bottomed, 24-welled tissue culture plates of 18 mm height and 16 mm internal diameter and covered with lids in both cases. After 16 to 18 hours of storage at room temperature, de-mouldable gels were obtained, which contained 1%w/w sodium alginate, 15 mM calcium ions and 45 mM GDL. The final pH of thoroughly homogenised gel sample was found to be  $4.3 \pm 0.1$ . Depending on the amount of gel required, the above regimen was adjusted accordingly.

Draget *et al.* (1991) have argued that the problems associated with the use of complexed  $\text{Ca}^{2+}$  are the relatively low pH required in order to release calcium and the potent toxicity of the complexing agent itself. The second method (designated method 2) was an adaptation of that described by Draget *et al.* (1989). Calcium carbonate powder was firstly passed through sieves of mesh size of  $40\mu\text{m}$  to obtain fine and uniform particulate of  $\text{CaCO}_3$  powder. This was necessary to eliminate the non-dispersing particles of  $\text{CaCO}_3$  with large pore sizes. In a flat-bottomed 500ml wide-necked autoclave bottles, calcium carbonate (0.32g) was dispersed in 190g de-ionised water with some gentle stirring. Sodium alginate (2g dry weight) was also dispersed into the above and the dispersion autoclaved in a bench-top autoclaves for  $115^\circ\text{C}$  at a pressure of 10 psi, for 25 minutes. The autoclaving of the preparation not only ensured that the  $\text{CaCO}_3$  and the sodium alginate were properly dispersed but also yielded a sterile preparation. After cooling down to room temperature, freshly prepared solution of GDL made by dissolving 1.14g of GDL powder in 10g of de-ionised water was added to the alginate- $\text{CaCO}_3$  mix. The mixture was then thoroughly stirred before it was poured into the containers described above, covered, and left to form homogeneous gels between 16 and 18 hours. The final gel contained 1% w/w sodium alginate, 32 mM GDL and 15 mM  $\text{Ca}^{2+}$  ions, and a final pH of 7.2. This method is henceforth designated 'method 2'.

Method 3 is based on suggestions by Toft (1980) and Sime (1984), validated by Papageorgiou *et al.*, (1994), that when sodium alginate, calcium salts and sequestrants are dissolved in hot water and the solution allowed to set by cooling,

homogeneous gels free of precipitates are formed. Homogeneous alginate gels were prepared by dry blending 1-3%w/w absolute weight with 0.15% trisodium citrate (TSC) and adding them to calcium chloride solutions (30 to 80% of the stoichiometric equivalent, equation 1.1) at 90°C. This allowed the examination of the effects of calcium ions and sodium alginate content on the physical properties of the gels formed. Samples were then mixed thoroughly for approximately 5 minutes before they were poured into either 24-welled tissue culture plates (for gel strength measurements) or into 8-welled tissue culture plates (for rheological measurements). The plates were then covered with lids and allowed to cool to room temperature, before storing at 5°C for 72 hours. The final pH of the formed gels was measured at  $6.8 \pm 0.1$ .

#### 2.4.3 Estimation of the (apparent) Young's modulus of alginate gels:

The (apparent) Young's moduli of all the alginate gels prepared using the three different methods were measured using texture analyser in the "return to start mode" and "force versus distance" mode as follows. Cylindrical gels (16 mm diameter and 18 mm height) were carefully removed from the culture plates (usually by inverting the plates with the lids still on) and placed on the platform of the instrument, the surface of the gels carefully blotted off with paper tissues. A lightly greased cylindrical probe (20 mm diameter) made of plexiglass was lowered at a constant speed of 5 mm/s before contact was made with the gel (pre-test speed) and at 0.2 mm/s after contact has been established (test speed). It was necessary to grease the probe surface with low viscosity silicone oil in order to eliminate forces due to friction. Once in contact with the gel, the probe was lowered for a total distance of 5 mm before it was returned at a constant speed of 0.2 mm/s until the gel has recovered from the stress of the applied force. The probe's withdrawal was continued at 5 mm/s (post test speed). The magnitude of the contact force was as low as possible (0.049N) and at an acquisition rate of 50 points per minute. Parallel measurements of at least  $n = 10$  were taken for each set of alginate gels. The initial part of the force versus distance curve was linear and the Young's modulus (E) was defined by the equation:

$$E = [F/A] / [\Delta l/l] \quad \text{Eqn. 2.11}$$

where  $F$  is the force (in Newton) related to the relative deformation ( $\Delta l/l$ ) of the initial slope of the force/distance curve and  $A$  ( $\text{m}^2$ ), the surface area of the gel cylinder.

#### 2.4.3.1 Derivation of Eqn. 2.11

Consider a cylindrical gel of radius  $r$  and height  $l$  is compressed with a force,  $F$  to a new height  $l_0$ . The stress on the gel,  $\sigma$ , is given by the relationship,  $\sigma = F/A$ , where  $A$  is the cross sectional area of the gel (Figure 2.3a and 2.3b). The strain  $\varepsilon$ , (dimensionless) of such gel will be given by,

$$\varepsilon = (l - l_0)/l = \Delta l / l$$

where  $l_0$  = the height of gel at final compression point

$l$  = the original height of gel before compression

$\Delta l$  = the change in height

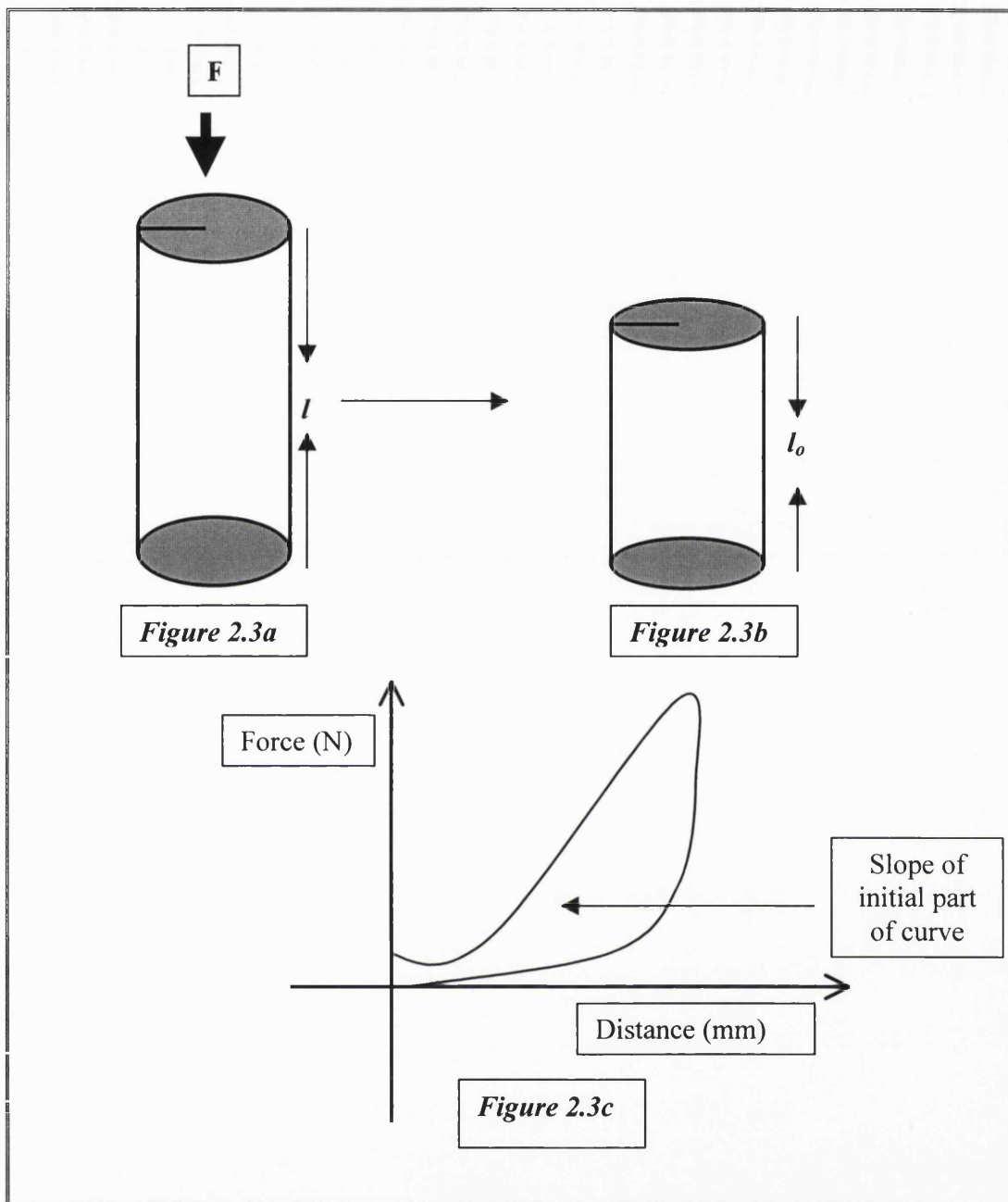
Hence, the Young's (or apparent Young's) modulus of the gel,  $E$ , is the ratio of the stress to strain, and is given by

$$E = \text{stress/strain} = \sigma / \varepsilon \text{ or } [F/A] / [\Delta l/l]$$

This is represented by the Force vs. Distance curve (Figure 2.3c), with the slope of the initial part of the curve used in the calculations.

*Assumption:*

*There was no change in the diameter of the gels during compression, or the change in diameter was equal in all samples.*



#### 2.4.4 Rheology of alginate gels:

The rheological properties of alginate gels should be of particular interest for two reasons. Firstly, because it is possible by varying the concentration of the cation to alter the density of crosslinks independently of the polysaccharide concentration, thus making the results more amenable to interpretation. Secondly, as alginate gels

do not melt on heating (Papageorgiou *et al.*, 1994), it might be expected that they would show different rheological properties to thermoreversible gels.

Both the elastic (or storage) and viscous (or loss) moduli of alginate gels prepared as detailed above were determined using Carri-med CSL 500 rheometer equipped with 60 mm diameter parallel plate with solvent traps and transparent acrylic covers. The routine procedure included carefully removing the gel (6 cm diameter x 1 mm thickness) from the 6 cm diameter tissue culture plates and placing it on the rheometer. A torque sweep was then performed on the gel to establish the linear viscoelastic region, where the range of stresses (torques) and/or strains (displacements) over which the modulus and viscosity are constant. Both frequency (0.1-10 Hz) and temperature scans (10°C – 90°C, 2°C/min) were performed using a strain of 0.025 which was found to be within the linear viscoelastic region of these gels. The gels were covered with low viscosity ( $\approx 5$  mPa.s) silicone oil and the solvent trap filled with de-ionised water to prevent evaporation of the gel during the temperature scans. The gel was then allowed to equilibrate at 10°C before monitoring structural changes during heating to 90°C, which was immediately followed by cooling at the same rate to 10°C. All temperature scans were performed at 1 Hz. Frequency scans were also performed using the same plate and gel geometry and at 25°C, at least four times.

## 2.5 Results

### 2.5.1 Thermogravimetric analysis

Table 2.2, summarises the moisture content of 'dry' alginate powders used in this work, as determined with TGA. A close inspection of Table 2.2 shows that when the values of the 'dry matter content (%)' are added to those of '% weight loss', the resultant value is approximately 100%.

**Table 2.2: Temperature dependence loss of water (moisture) from alginate powder**

<i>Sample</i>	<i>*Dry matter content (%)</i>	<i>**% Water Loss</i>	<i>**Temperature at peak water loss (°C)</i>
SF 120	91.00	9.52 ± 0.16	70.00 ± 1.50
SF/LF 40	86.50	12.97 ± 0.45	78.00 ± 0.67
SF 200	91.50	10.57 ± 0.15	70.00 ± 1.00
LF 120L	90.60	9.45 ± 0.31	65.00 ± 1.67
SF 60L	86.00	14.26 ± 0.28	80.00 ± 0.50

\* Data supplied by Pronova Biopolymer A.S., Drammen, Norway

\*\* Determined on Thermoanalyser, values are the means and standard deviations of four measurements

The thermogravimetric analysis has therefore proved to be a quick, reproducible and a very efficient method of quantifying the moisture (water) content of alginate powders. In addition, the analyses have validated the manufacturer's data sheet, and may be used as method for monitoring changes in the moisture content of sample during storage.

## 2.5.2 Young's modulus determination

### 2.5.2.1 Effect of calcium conversion on the Young's moduli of alginate gels

The amount of calcium ions (in the form of level of % calcium conversion) has been reported (Papageorgiou *et al.*, 1994) to have an overall effect on the structure and characteristics of alginate gels. In Table 2.3, such effect is reported, using method 3 (hot-made method) of preparing alginate gels. Preparations containing either 20% or 80% calcium conversion failed to yield cylindrical gels after 72 hours storage at 5°C.

**Table 2.3: The effect of % calcium conversion on the Young's moduli of alginate gels**

<i>% Calcium conversion</i>	<i>Young's modulus (kPa), n=10</i>
20	Gels too weak to measure
30	$67.42 \pm 1.78$
40	$47.84 \pm 0.68$
50	$28.44 \pm 1.05$
60	$21.73 \pm 0.96$
80	Gels too weak to measure

The alginate gels were made with 2%w/w SF 120 and 0.15% trisodium citrate, using method 3.

Further storage at this temperature showed no further improvement in the gel formation and was therefore discarded. The failure of 20% calcium conversion to form a reasonable homogeneous gels may be explained by the fact the amount of calcium ions present were not sufficient to create the necessary bridges between the monomers of alginate in order to form a rigid gels network.

Whilst Papageorgiou *et al.* (1994) have reported that the modulus of rigidity (or the gel strength) increased with increasing % calcium conversion up to and including 40%, the current work found that 30% calcium conversion was the optimum calcium concentration for similar modulus of rigidity. The discrepancy could be attributed to the differences in the guluronate content in the samples used. Whereas Papageorgiou *et al.* (1994) have used alginate samples containing 58% guluronate, the sample used in this study (SF 120), contains 69.4% guluronate. In the case of the lower guluronate content, more calcium ions are required to form bridges between the monomers of the alginate chains, leading to gel formation. The same level of calcium conversion

exceeds the requirements for alginate sample containing a higher proportion of guluronate, as outline in Chapter 1.

On the other hand, the failure of excessive quantities of calcium salt to meet expectations for higher gel strength (in the form of Young's modulus) may be attributed to the fact that the amount of free  $\text{Ca}^{2+}$  exceeded the optimal alginate concentration correlated to their content of guluronic acid residues. This in turn leads to an irreversible syneresis and weakening of the gel (Martinsen *et al.*, 1989; Skjåk-Bræk *et al.*, 1989). Furthermore, the formation of less homogeneous gel systems with higher salt concentration (visible to the eye) may be an indication of structural weakening of alginate gels at higher levels of salt, which is accompanied by complex textural changes in polymeric assemblage, even at elevated temperatures. This is typified by the lower values of Young's modulus obtained with calcium level at and above 60% of the theoretical requirement for alginate than those obtained with 40% or 30% calcium conversion. As a result, at a  $\text{Ca}^{2+}$  level of 60% of the theoretical equivalent for alginate, the ultimate modulus of the system remains below the corresponding gel strength at 40% conversion and the 80% stoichiometric equivalent forces the network to fall short of the final modulus attained at 30% conversion.

#### ***2.5.2.2 Effects of both concentration and type of alginate on the Young's modulus of their corresponding gels.***

In preliminary studies, it was observed that both the alginate type and the polymer concentration independently affected the Young's modulus of the resulting gels. In addition, maximum gel strength within a series of sodium alginate systems may be correlated to the G-content in the alginate sample. Draget *et al.* (1991) have reported that there is virtually no difference in rate of gelling and, indeed, the gel strength between a low-G and high-G alginates up to 1.5 hours. After 1.5 hours, however, high-G alginates enter a secondary gelling phase with increasing gel strength, whereas the low-G sample seem to enter a very pronounced plateau phase where, the gel strength remains almost independent of the gelling time. Given that all gels for this study were allowed to form over 16 –18 hours, it is reasonable to assume that the final strength of these gels would not be dependent on the gelling time, but rather on



the proportions of guluronate or mannuronate within each sample. It was therefore necessary to explore these influences further.

In order to examine the influence of guluronate content and the concentration of alginates on their gel strengths, five alginate samples of varying proportions of guluronate were used. A careful examination of Table 2.4, reveals three important observations regarding the effect of type and concentration of alginate used on the gel strength. Firstly, the gels that yielded relatively high Young's modulus values (SF 200, SF 120, and SF/LF 40) are all rich in guluronic acid content. Furthermore, the three samples have high  $NG>1$  (typical average length of guluronic acid blocks larger than 1) values. Within a group of sodium alginates of similar guluronic acid content, increasing molecular weight leads to increasing gel strength. For example SF 200 (mw 380 kDa, %G = 69%) yielded higher gel strength at all concentrations of alginate examined than SF 120, which has similar G content (69.4%) but of lower molecular weight (225 kDa). This finding is consistent with the findings from the work of Draget *et al.* (1996) that the gel strength has been shown to increase over a broad range of molecular weight, especially for alginates rich in guluronic acid. Secondly, the gels which yielded low Young's modulus (LF 120 and SF 60L) were of relatively low G content and have comparatively low  $NG>1$  values. However, it must be borne in mind that the magnitude of the Young's modulus of alginate gels does not only depend on the guluronic acid content, but also on the sequencing of the monomers (Draget *et al.*, 1994). This is exemplified by higher Young's modulus values for SF 60L gels (44.4% G;  $NG>1 = 6.9$ ) than LF 120L (43.8%G;  $NG>1 = 7.4$ ). Thirdly, within a group of alginate samples of similar guluronic acid content and  $NG>1$  values, molecular weight of the sample plays an important role in the magnitude of the Young's moduli of their gels. It has been proposed (Draget *et al.*, 1991) that the reason why mannuronic and guluronic acid blocks are not equivalent in the context of their effects on the Young's modulus may be due to factors such as spatial arrangements of the monomers along the polymer chain which is of importance to the formation and the stability of intermolecular bonds. Furthermore, the fraction of the alternating sequences (FGM.MG) does not seem to be compatible with the high gel strength (Table 2.1). In fact, it has been shown (Draget *et al.*, 1991)

that in some cases, a high fraction of alternating sequences may even lead to a lower Young's modulus.

**Table 2.4 Effect of both type and concentration of alginate on Young's moduli of alginate gels**

Conc. of alginate (%w/w)	Young's modulus (kPa), mean $\pm$ s.d., n = 10				
	SF 200 (69.0% G)	SF 120 (69.4% G)	SF/LF 40 (63.0% G)	LF 120L (44.3% G)	SF 60L (43.8% G)
0.50	37.86 $\pm$ 2.45	33.92 $\pm$ 3.21	38.75 $\pm$ 2.4	24.23 $\pm$ 5.30	24.23 $\pm$ 4.60
1.00	66.60 $\pm$ 5.32	63.80 $\pm$ 5.60	65.53 $\pm$ 4.20	29.39 $\pm$ 6.90	38.83 $\pm$ 2.60
1.50	93.83 $\pm$ 4.78	82.83 $\pm$ 6.20	94.40 $\pm$ 4.70	59.49 $\pm$ 7.80	50.56 $\pm$ 3.50
2.00	120.34 $\pm$ 5.98	109.83 $\pm$ 4.40	108.10 $\pm$ 3.90	67.79 $\pm$ 9.40	59.79 $\pm$ 4.80

All gels were prepared according to Pronova's standard method 2204 (Method 1) and contained 30% calcium conversion.

This has been partially attributed to the fact that in such sequences, there is the lack of homopolymeric sequences, which in turn makes it difficult to create stable, intermolecular bonds.

### 2.5.2.3 Effects of methods of preparation on the Young's moduli of alginate gels

As discussed in Chapter 1, the method of preparing alginate gels has a profound effect on the physical characteristic of these gels. In Table 2.5, the effects of three methods of preparing alginate gels (detailed in section 2.4.2.2) at varying concentrations of sodium alginate on the Young's moduli of such gels is shown. In this study, up to and including concentrations of 1% w/w of sodium alginate, there was no significant difference ( $p < 0.05$ ) between the three methods employed. However, significant differences ( $p < 0.01$ ) were observed between the three methods as the concentration of sodium alginate was increased to 1.5% w/w. The

magnitude of the Young's modulus of the 2% w/w gels made by method 2, for example, was found to be more than twice that of 1.5%w/w.

**Table 2.5: Young's modulus dependence on method of preparation of alginate gels**

Method	Young's modulus (kPa), mean $\pm$ s.d., n = 10			
	0.5% w/w	1.0% w/w	1.5% w/w	2.0% w/w
1	37.86 $\pm$ 2.45	66.60 $\pm$ 5.32	93.83 $\pm$ 4.78	120.34 $\pm$ 5.98
2	33.14 $\pm$ 3.60	63.90 $\pm$ 6.90	109.62 $\pm$ 4.60	280.28 $\pm$ 7.50
3	30.12 $\pm$ 2.40	58.72 $\pm$ 7.40	78.42 $\pm$ 4.90	98.69 $\pm$ 6.40

Method 1 = Pronova's standard method 2204 for gel strength, method 2 = CaCO<sub>3</sub> as source of calcium ions, and method 3 = Hot-made method. Sodium alginate SF 200 was used in all three methods.

The increase in the apparent Young's modulus with increasing concentrations of sodium alginate was not surprising as, Mitchell and Blanhard, (1976) have previously reported that in some cases the stiffness (a measure of gel strength) of alginate gels could be as high as the square of the concentration. In addition, the stiffness is independent of the degree of polymerisation (DP) above a weight average DP of about 400 ( $M_w \approx 90\ 000$ ) and decreases with increasing temperature.

Oates *et al.* (1993) have reported that the work of fracture (the work performed in creating a unit area of crack within a gel) of alginate gels decreased as the concentration of alginate was increased, with this effect being more apparent for high G content alginate gels. Unfortunately the authors reported neither the concentrations nor indeed, the type of alginates used for the above observations. The authors, however, partly explained this observed decrease in 'gel strength' (or brittleness) that at high concentrations of alginates there is a problem with dispersion of the samples leading to inhomogeneity within the gel. Such problems of inhomogeneity were considered when alginate gels were made during this study by using a chelator

(EDTA) and a sequestrant (GDL), to avoid the uncontrolled binding of divalent cations to alginate molecules.

A reason for the high Young's modulus values obtained using method 2 is probably due to the superior diffusion of calcium ions leading to a more even gel. This superior diffusion is in turn due to the fact that the insoluble calcium carbonate is only made soluble by the addition of GDL to the previously heated and cooled alginate/CaCO<sub>3</sub> mixture. With both method 1 (Pronova's method) and method 3 (hot-made gels), the calcium source is the soluble calcium chloride. Although in both cases a chelating agent (EDTA and trisodium citrate, respectively) was used to ensure a controlled binding between alginate and calcium ions, this may not be as effective as using the insoluble calcium salt.

### 2.5.3 Rheology of sodium alginate gels

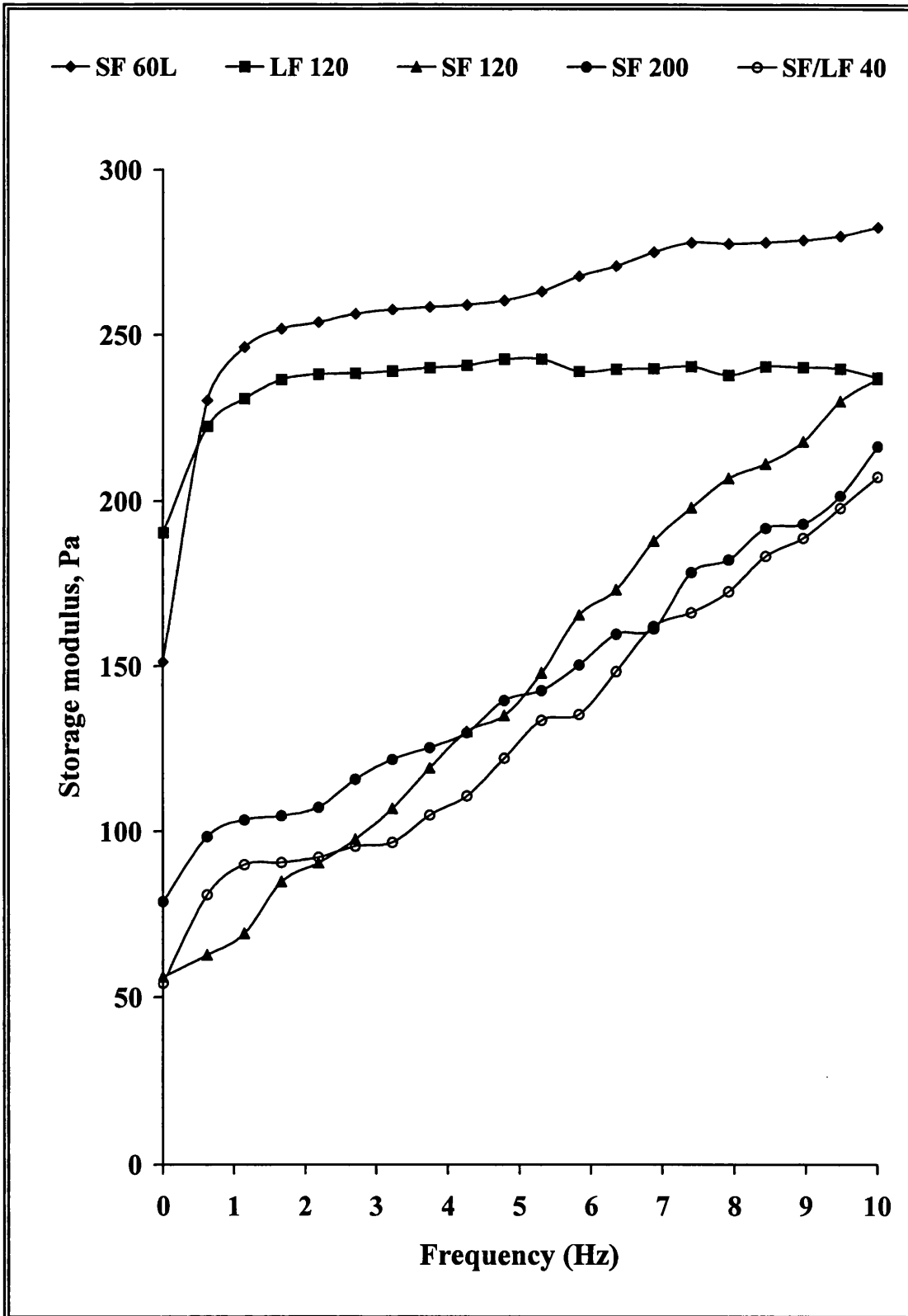
Many biopolymer gels are similar to rubbers in that they consist of crosslinked networks of long polymer molecules. Similarities in behaviour are therefore to be expected. They do contain, generally, a significant sol fraction, but such effects also occur in rubber systems and can be treated. It has been suggested that in biopolymer gels the junction zones are continually dissociating and reforming (Ferry, 1980) although the kinetics of this process appear to be such that they do not affect the mechanical properties under normal experimental time scales.

The effect of increasing frequency on the storage (elastic) response of alginate gels has been documented by several workers (Papageorgiou *et al.*, 1994, Draget *et al.*, 1989) who used different methods of preparing such gels. In Figure 2.3, the elastic responses of alginate gels prepared according to Pronova's standard method 2204 are shown. All the gels contained 1% w/w sodium alginate and 30% stoichiometric equivalent of Ca<sup>2+</sup> ions. These alginates not only vary in their molecular weight and M:G ratio, but also differ in terms of monomer sequencing. The elastic response of both LF 120L and SF 60L showed the typical plateau effect following the initial rise in elastic response with frequency. These two alginates are identical in terms of their guluronic acid content and sequencing ( $N_{G>1} = 6.9$  and  $7.4$ , respectively). The

higher elastic response showed by SF 60L (in comparison with LF 120L) further demonstrates that in series of alginate with similar guluronic acid content, the elastic modulus of the gels is very much affected by the molecular weights of the individual alginate. In contrast, however, the elastic responses of SF 120L, SF/LF 40, and SF 200 (high G content) displayed almost a linear relationship with frequency. These observations are consistent with the chemical compositions of sodium alginate samples used. The assertion that sodium alginates poor in guluronic acid content tend to form gels that are weak but more elastic than those from the guluronic acid-rich sample (Haug and Smidsrød, 1965; Draget *et al.*, 1989; Mikkelsen and Elgsaeter, 1995) is confirmed by the results displayed in Figures 2.4a-c. The magnitudes of the storage moduli of gels made from alginates of low guluronate content (SF 60L and LF 120L) were found to be higher than those of guluronate-rich alginates, (Figure 2.4a and 2.4b). In addition, the corresponding loss tangent (Figure 2.4c) of these gels indicates that whilst the guluronate-rich alginates exhibited more viscous component the mannuronate-rich alginate showed properties of elastic gels, with loss tangent values remaining well below unity, throughout the frequency range.

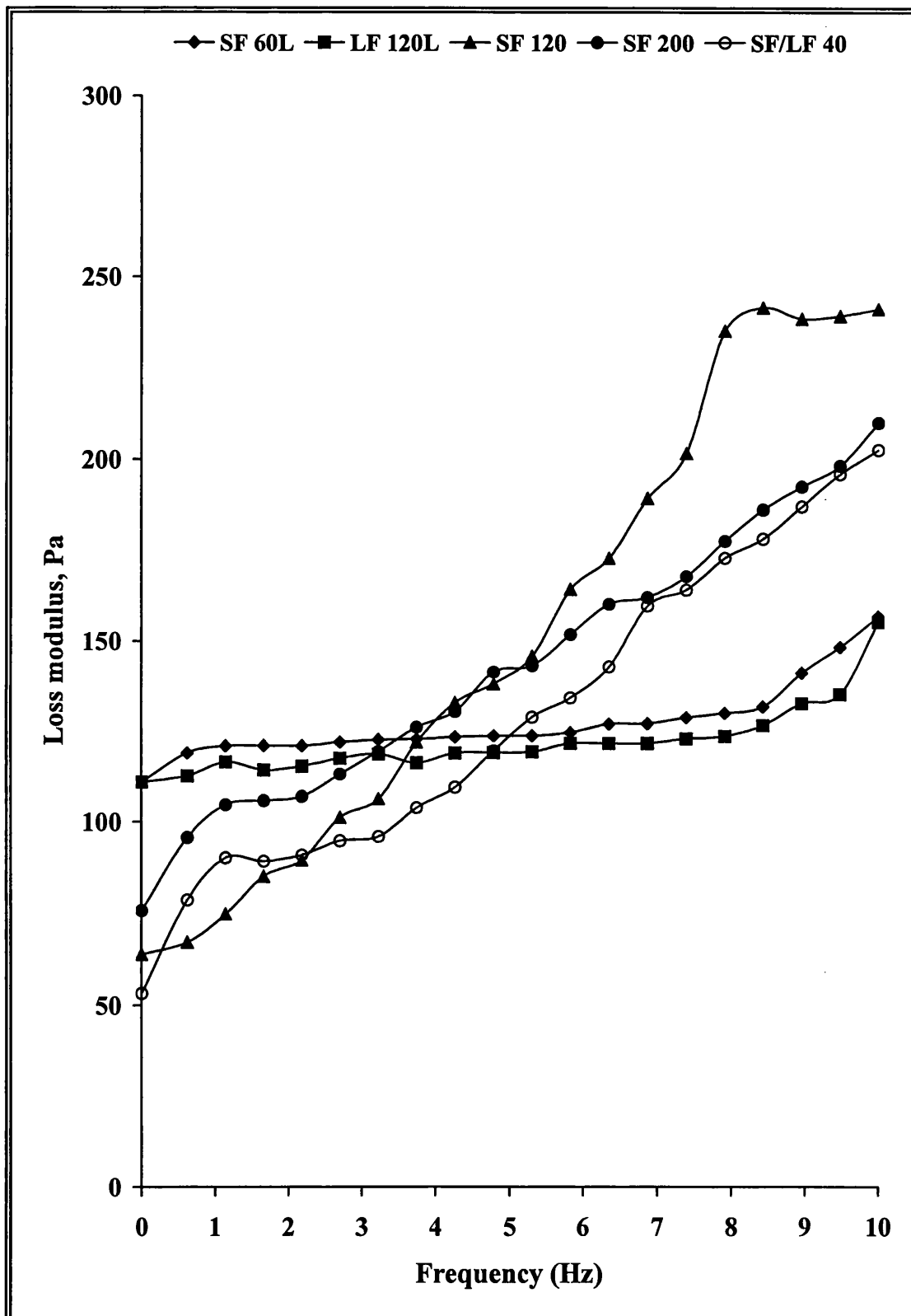
Papageorgiou *et al.* (1994), using alginate gels have validated the hypothesis proposed by Almdal *et al.* (1993), a 'true' gel (which is solid-like) is one whose elastic moduli are considerably greater than its loss moduli within the frequency range examined. Figure 2.5 depicts a typical viscous and elastic profile of alginate gels, in which the elastic modulus is considerably greater than the loss modulus throughout the entire frequency range examined (0.01 – 10 Hz). In addition, the viscous component of both samples is very much identical, an indication of the alginate gels behaving as 'true' gels. The two alginate samples were chosen because in Figure 2.4, they displayed the typical rheological profiles of 'true' gels.

Figure 2.4a: Frequency dependence of the storage (elastic) moduli of alginate gels

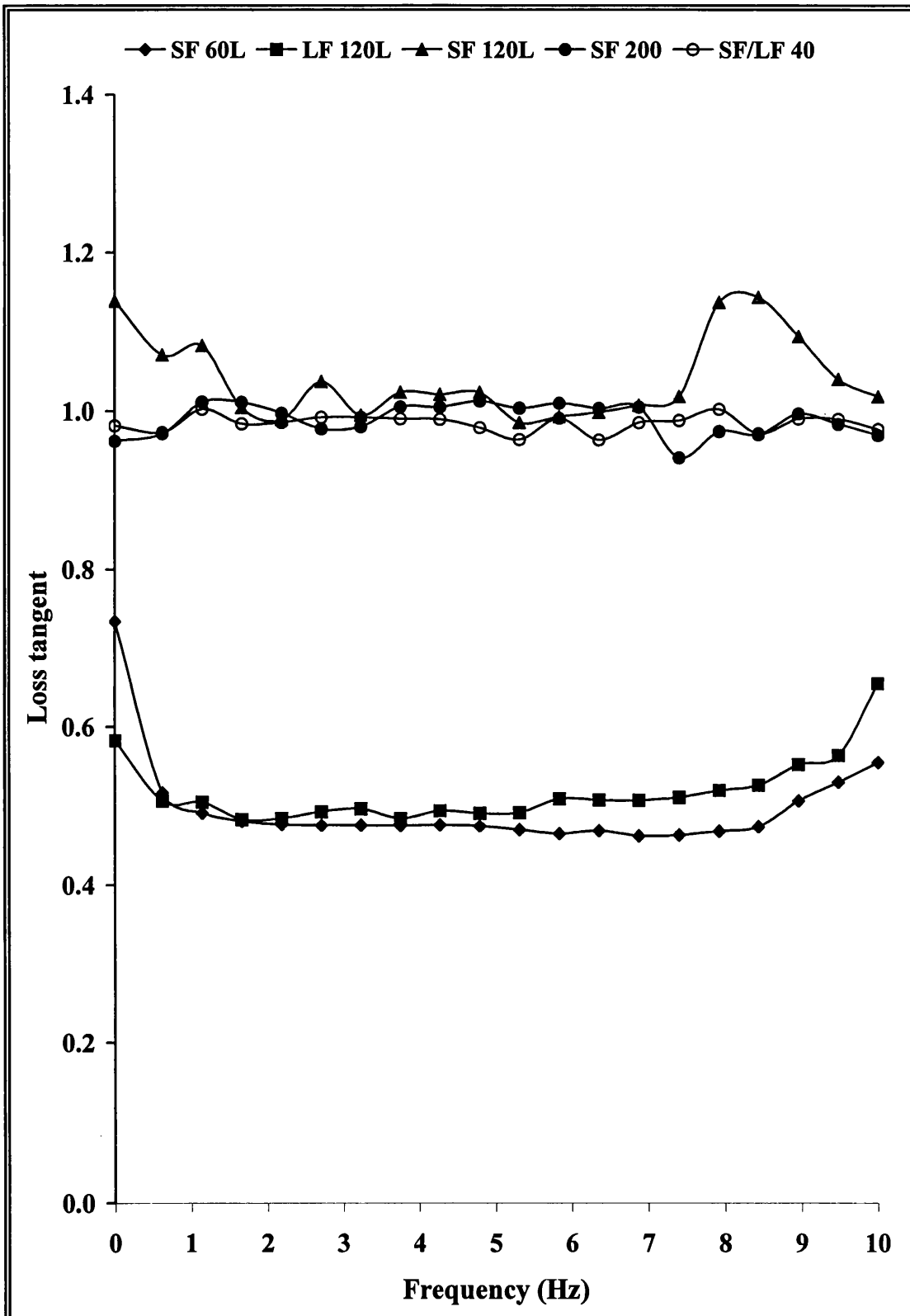


All gels were prepared by Pronova's method (method 1), using 1%w/w sodium alginate and 30% calcium conversion | Median data of n = 5 plotted.

Figure 2.4b: Frequency dependence of the loss (viscous) moduli of alginate gels



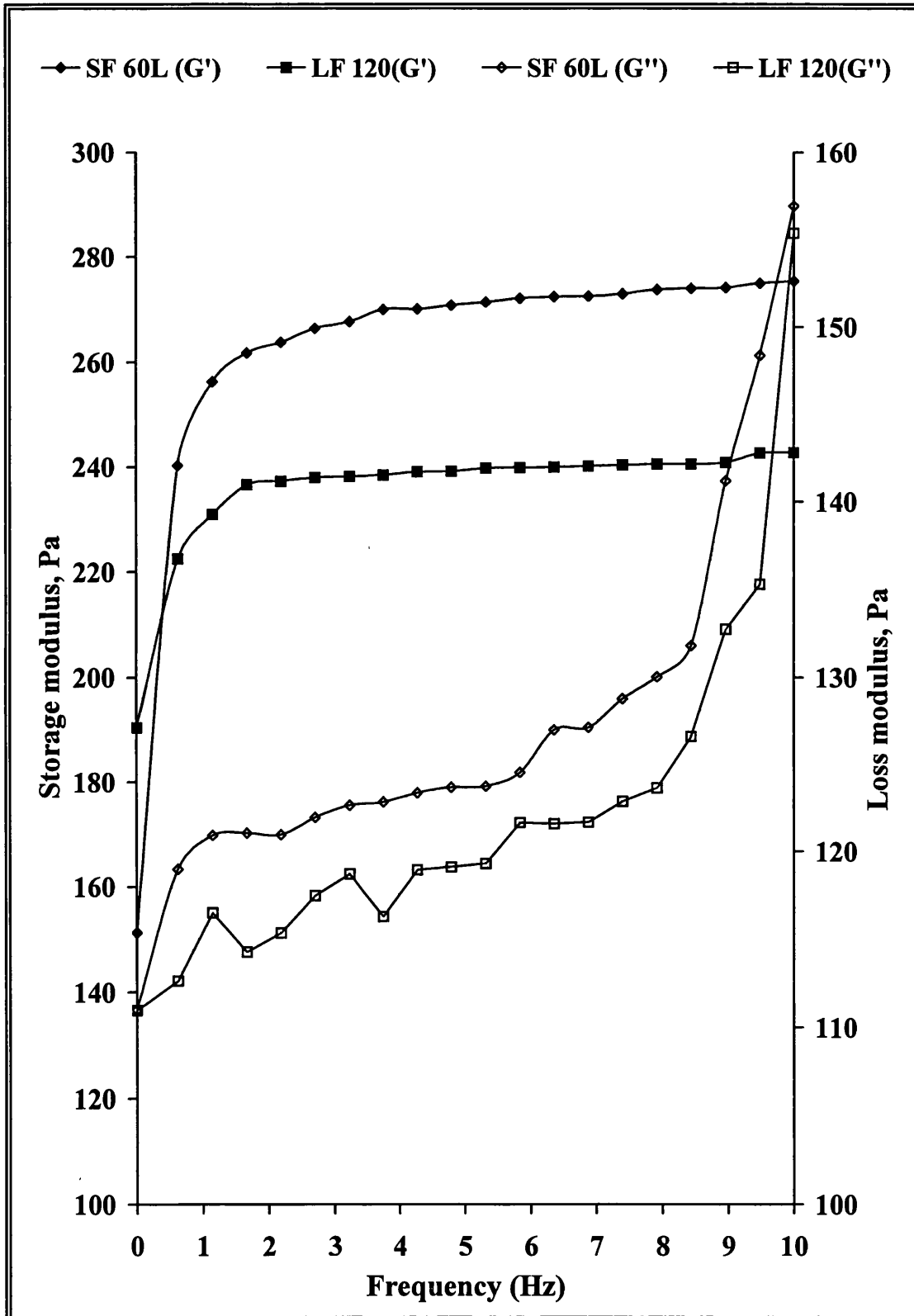
All gels were prepared by Pronova's method (method 1), using 1%w/w sodium alginate and 30% calcium conversion. Median data of n = 5 plotted.

*Figure 2.4c: Loss tangent dependence of frequency of alginate gels*

Median data of n = 5



**Figure 2.5: The influence of frequency on both the storage and loss moduli of alginate gels**



All gels were prepared by method 1, using 1% w/w sodium alginate.

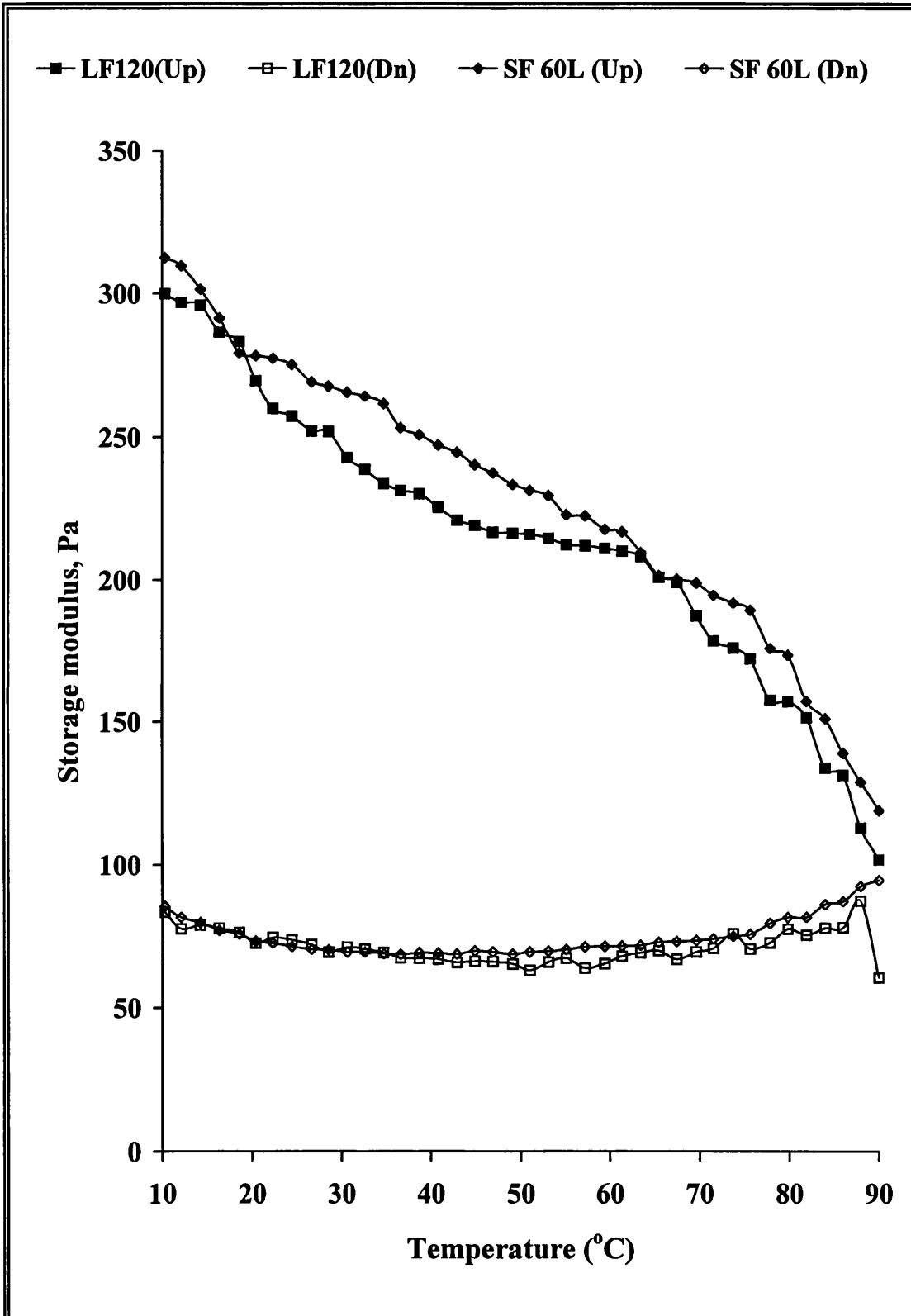
Median data of  $n = 5$  plotted.

Evaluation of  $G'$  and  $G''$  as a function of frequency, time, and temperature provide useful information on the phenomenon of gelation and the melting characteristics of thermoreversible gels. In thermally irreversible gels, the moduli are generally observed to decrease with an increase in temperature, apparently as a result of a reduction in network linkages (Clark, 1992). The thermo-irreversible nature of alginate gels is shown in Figure 2.6. During the heating cycle (up curves) the storage moduli of the alginate gels gradually and continuously decreased with the increasing temperature, as expected. A stiff coil can, upon heating, increase its flexibility because of the increased number of allowed torsion angles in the chain with increasing temperature. Stiff chain network can thus be expected to show a decrease in storage modulus with increasing temperature. In addition the melted material augments the viscous component of the gel, causing a progressive decrease in the elastic modulus. Richardson and Ross-Murphy (1981) have showed that the storage moduli for gels made from globular protein decrease with increasing temperature. This has been interpreted as (enthalpic elasticity) for this network (Clark and Ross-Murphy, 1987). On cooling, however, the previously broken network within the gel is not fully reformed, leading to a loss in the storage modulus.

The effect of temperature on the elastic moduli of alginate gels is very well documented (Papageorgiou *et al.*, 1994; Moe *et al.*, 1992; Andersen and Smidsrød, 1977). Using gels prepared by dialysing alginate solutions extensively against  $\text{CaCl}_2$  solutions, Andersen and Smidsrød (1977), found that the elastic moduli of gels decreased with increasing temperature. The decrease in elastic moduli was attributed to the behaviour of the non-permanent crosslinks of the gel rather than to the behaviour of the alginate chains themselves. Such decrease has been interpreted as a breaking and rearrangement of junction zones when force was applied to the dense network of stiff chains.

In Figure 2.6, the thermorheograms of two alginate samples, which showed 'true' gel characteristics in isothermal conditions, are depicted. During the heating cycle the elastic moduli of these gels continued to decrease with increasing temperature. This decrease in elastic moduli with temperature is due to the breaking and rearrangement of the junction zones between the guluronate-calcium ions, with some contribution

**Figure 2.6: Temperature dependence of the storage moduli of alginate gels**



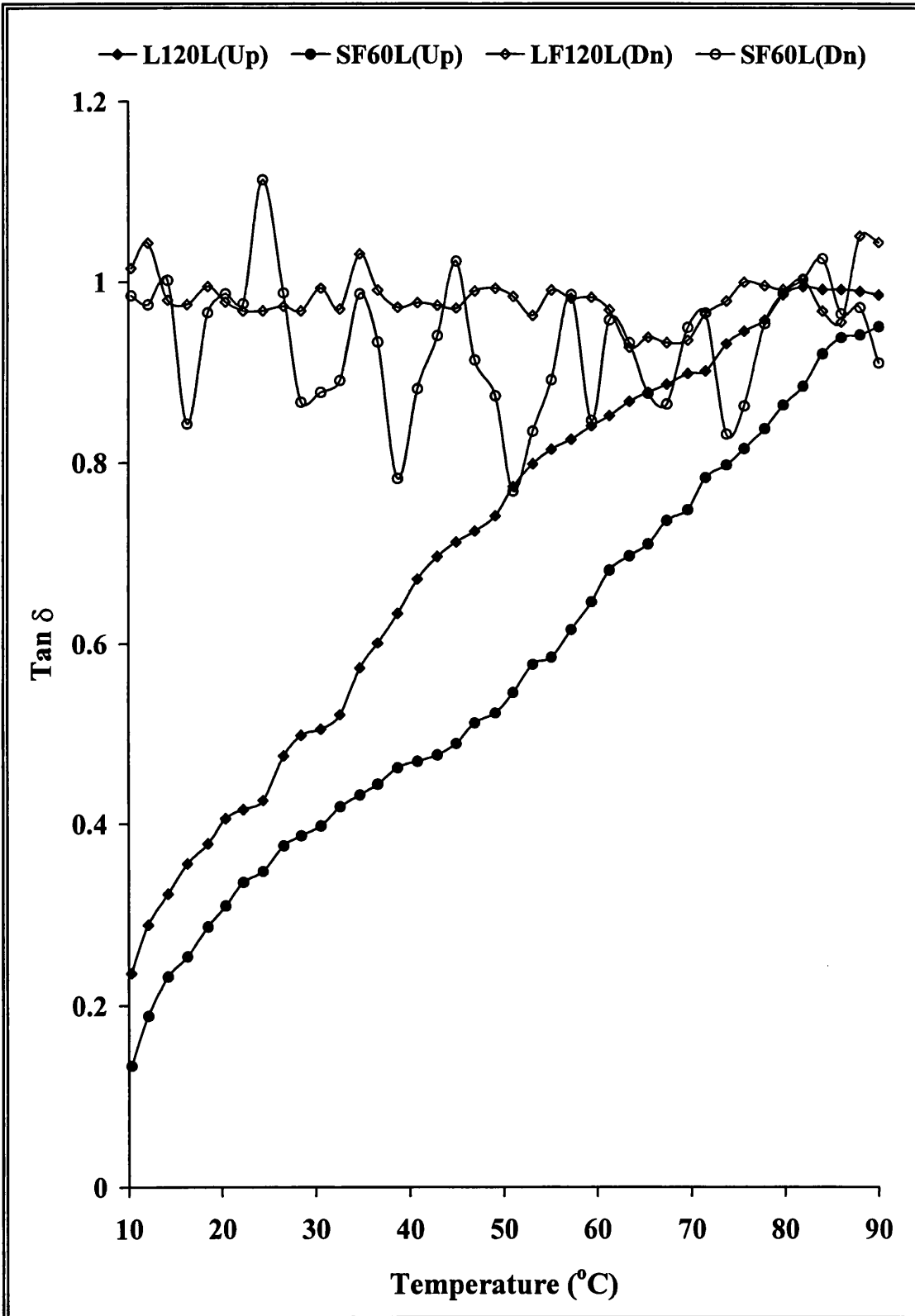
All the gels were prepared by method 1 and contained 1%w/w sodium alginate and 30% calcium conversion. Median data of n = 5 plotted.

from the mannuronate monomers. On cooling, however, the loss in the elastic moduli is not fully compensated and the elastic moduli are of much less magnitude during this cycle (Figure 2.6). Further confirmation of the breakdown of the alginate gel system during this heating/cooling cycle is manifested in the loss tangent values ( $\tan \delta$ ) (Figure 2.7). Whilst the heating of these gels was accompanied by a steady rise in  $\tan \delta$  values, the cooling of the same gels showed very little dependence on temperature. The increase in the loss tangent values with increasing temperature indicates that the gels were undergoing structural changes, as they lose some of their elastic properties and assuming more viscous properties. However, the loss tangent values for both gel systems during the heating phase did not reach unity, an indication of the gels maintained some of their elastic properties. The loss tangent values for these gels during the cooling cycles gave the indication that the gel systems have been destroyed during the heating cycle, as they remained relatively constant and yielded high loss tangent values.

## 2.6 Discussion

The understanding of parameters which affect the physical characteristics of alginate and their relationship with gel forming properties, is of paramount importance in any field where these biopolymers are applied. Some of these parameters have been investigated in this work. The use of thermogravimetric analysis to confirm the water (moisture) content of the 'dry' sodium alginate powder as quoted by the manufacturer and supplier Pronova Biopolymer a.s., proved to be very efficient with a high degree of precision and accuracy. This method offers a quick way of assessing moisture content in 'dry' or wet materials, requiring only small quantity of the sample (in mg).

Alginates are known to form gels at pH higher than the pKa of the individual monomers (guluronic and mannuronic acids). The final pH of the gels is therefore important in food science, as too acidic or too basic gels may not be acceptable to the consumer. Moreover, the properties of the gels, such as the strength may be affected by the final pH of the gel. The final pH of the gels prepared via the Pronova's method was found to be 4.3. The pH of the other two methods were almost neutral

*Figure 2.7 Temperature dependence of the loss tangent of alginate gels*

Median data of  $n = 5$  plotted.

(7.2 and 6.8 for the  $\text{CaCO}_3$  and hot-made gels, respectively). Direct comparison of the effect of pH on the gel strength was impossible since the last method differs from the other two, in that the gels were made at elevated temperatures.

The rheological behaviour of the alginate gels examined was quite different from the textural analysis of the same gels. Whilst rheological measurements have indicated that gels made from alginates rich in mannuronate (SF 60L and LF 120L) were more elastic than their guluronate-rich counterparts (SF 120, SF 200 and SF/LF 40), observations from textural analysis did not confirm such findings, as mannuronate-rich alginates were shown to have lower Young's moduli than guluronate-rich alginates. Although there are no published data on the concomitant use of rheology and texture analysis in characterising alginate gel systems, the two sets of results do not contradict each other. Rheology measures (in oscillatory mode) the molecular arrangements within the gel systems; using sinusoidal stresses within the linear viscoelastic regions of the samples. This implies that samples are allowed enough time for recovery after each cycle of imposing stresses upon them. Elastic samples therefore yield higher elastic modulus values ( $G'$ ) and low loss modulus values ( $G''$ ). Furthermore, it is known that gels containing a small number of crosslinks or junction zones for a given polymer concentration are more elastic than gels with a high crosslinks density. This is not only because the former have a lower rigidity modulus but also in the sense that a greater part of an induced strain is immediately recoverable on removal of an applied stress (Mitchell, 1976). Alginates rich in mannuronate content tend to have lower degree of junction zones for calcium binding than their guluronate-rich counterparts.

The texture analysis, on the other hand, imposes a direct force (compression) on cylindrical gels to either a set depth of the gels or for a set time, without any considerations for the elastic limits of the systems. It is therefore conceivable that the measurement does not differentiate between the viscous and the elastic components of the gel systems. True Young's modulus is restricted to totally elastic systems, and as the gel systems used here were of viscoelastic and the semisolid nature, it is conceivable that apparent rather than true Young's modulus was measured. The apparent Young's modulus could be related to the complex modulus of the system (a

combination of both the elastic and viscous components). Owing to their strength and brittleness, guluronate-rich alginates resist the compression forces much better than their mannuronate-rich counterparts, hence yielding higher apparent Young's modulus values.

## **2.7 Conclusions**

The method used to prepare alginate gels has been demonstrated to have profound effects on the rheological and textural properties of these gels. In addition, the final calcium salt concentration in the gels was also shown to affect the gel strength, with both low and excessive salt concentration producing weaker gels. The overall picture is one in which the textural studies complements those of rheological studies, the most fundamentally important parameters assessed for acceptance of alginate gels, in both pharmaceutical and non-pharmaceutical applications.

**CHAPTER 3: ALGINATE-MUCIN INTERACTIONS**



### 3.0 Introduction

In sections 1.1 and 1.2, various chemical and physical characteristics of alginates and mucin have been discussed. For every non-parenteral route of administration there is the benefit of prolonging the duration of residence of the dosage form in that route to achieve a once or twice daily dosing regimen of patient compliance. This alone is justification for the use bioadhesive/mucoadhesive systems, whereby the dosage form, by virtue of containing a bioadhesive polymer, adheres to the mucus or tissue surface until the polymer dissolves or until the mucin replaces itself.

It is not surprising therefore, that mucoadhesive polymers have attracted considerable attention for a number of years (Park and Robinson, 1984; Smart *et al.*, 1984; Peppas and Buri, 1985, Caramella *et al.*, 1994; Madsen *et al.*, 1998). Much of this attention has been focused on the possibility of formulating drugs in bioadhesive dosage forms which adhere to the mucosal surface of the gastrointestinal tract, thereby increasing residence time with a concomitant increase in drug absorption (Ch'ng *et al.*, 1984; Harris *et al.*, 1990a,b). Amongst various ways of assessing the *in vitro* mucoadhesive properties of polymers is oscillatory rheology.

To date, the tests used to assess bioadhesion may be broadly described as those examining the behaviour of bioadhesive dosage forms, particularly force of detachment methods, and those examining the molecular interaction between the bioadhesive and mucin. In particular, the use of rheological testing on bioadhesive/mucin mixes has proved to be a useful approach in this respect (Caramella *et al.*, 1994; Rossi *et al.*, 1992; Mortazavi *et al.*, 1992; Madsen *et al.*, 1998). The method follows work by Hassan and Gallo (1990) who suggested that any molecular interaction taking place between the bioadhesive and mucin should be reflected by the elastic moduli of those mixes. Several other workers including Mortazavi and Smart (1994) and Rossi *et al.* (1994) have suggested that rheological synergy of mucin-polymer mixtures can be related to the strength of mucoadhesion. Thus, associative interactions between mucin and polymer and the related rheological behaviour are of interest with regard to potential mucoadhesive behaviour.

Another method extensively used in vitro technique of bioadhesion is the texture analysis. This technique, based on the well-established method of penetrometry has recently emerged as a useful technique in the assessments of mucin-polymer interaction (Park and Robinson, 1985; Ponchel *et al.*, 1987; Caramella *et al.*, 1994). More recent studies carried out by Tobyn *et al.*, (1992, 1995, 1996a,b, 1997) have popularised the use of this technique in the field of bio (muco) adhesion. A detailed description of this method has been given in Chapter 2.

### **3.1 Scope and Rationale of study**

The aims of this chapter were to examine alginate-mucin interactions using the techniques of rheology and texture analysis, with the view to understanding parameters which may influence such interactions. In addition, the rheological synergy and the work of adhesion between various sodium alginate batches and mucin would be used as indices of the mucoadhesive potential of these alginates.

### **3.2 Materials and Methods**

#### **3.2.1 Materials**

In addition to the five batches of sodium alginate used in the preceding chapter, two more alginate samples (also supplied by Pronova Biopolymer a.s., Drammen, Norway) were also used in this chapter. The data sheets for the additional alginates are summarised in Table 3.1

The following chemicals and reagents were obtained from Sigma (Poole, Dorset) Chemicals Co. Ltd.

Chemical/Reagent	Batch Number
Mucin type III (Porcine stomach)	104H7177
Potassium thiocyanate	115H0760
Phenylmethylsulfonyl Fluoride	37H1340

**Table 3.1: Chemical composition and other characteristics of the alginate used.**

<i>Grade</i>	<i>Batch number</i>	$\eta^*$ <i>mPa.s</i>	<i>Mw</i> <i>(kDa)</i>	<i>FG</i>	$N_{G>1}$	$F_{GM,MG}$	<i>Botanical Source</i>
H 120L	645209	950	350	0.460	7.0	0.190	<i>Lessonia nigrescens</i>
LFR 5/60	599345	5.7	40	0.640	15.7	0.127	<i>Laminaria hyperborea</i>

$\eta^*$  = viscosity of 1% solution,  $M_w$  = molecular weight calculated from intrinsic viscosity, \* estimated molecular weight,  $F_G$  = fraction of guluronic acid residues,  $F_{GM,MG}$  = fraction of alternating sequences,  $N_{G>1}$  = typical average length of guluronic acid blocks larger than 1.

From Avocado Research Chemicals Limited, Heysham, Lancs., UK, the following were obtained:

Chemical	Batch number
Fluorescein disodium salt	A2850A
Sodium azide	B7938A
Potassium thiocyanate	A4327A

The following chemicals were obtained from BDH, Poole, Dorset, UK:

Chemical	Batch number
Disodium edetate	668235
Sodium chloride	476297
Dialysing tubing visk (0.28 mm diam.)	527200
Ethylenediaminetetraacetic acid (EDTA)	462478

### 3.2.2 *Methods*

### 3.2.3 *Isolation, preparation and characterisation of mucin from porcine stomach*

#### 3.2.3.1 *Preparation of crude mucin*

Crude mucin was obtained by carefully scraping cleaned batches of 25 porcine stomachs obtained fresh from the abattoir and was homogenised by gentle mixing. The pooling together of the scrapings ensured the elimination of inter batch variations resulting from the different mucin sources. The percentage w/w of 'solids' was determined by leaving approximately 0.5g on a watch glass at 50°C for 48 hours (Mortazavi *et al.*, 1993). The mechanical properties of native mucus gels stored at -20°C have been shown to be identical to those obtained from fresh samples (Bell *et al.*, 1984), provided the gels were left to recover for 1.5 hours after thawing. Therefore, whenever the mucus was not used straightaway, aliquot batches were sealed in plastic vials and kept at -20°C until required.

#### 3.2.3.2 *Preparation of homogenised mucus gel*

Homogenised mucin gel was prepared by a method originally described by Mortazavi *et al.* (1992), with a slight modification. Briefly, batches of crude mucus were obtained by scraping the surfaces of freshly obtained porcine stomachs (15-20). The entire preparation was performed at 4°C and the samples were kept on ice throughout to minimise the possible denaturation of the mucus. The crude mucus was then homogenised with an equal amount of an isotonic solution containing PMSF (0.0175% w/v), sodium azide (0.02% w/v), sodium edetate (0.186% w/v) and NaCl (0.9% w/v). Potassium thiocyanate (4.276% w/v) was added to the solution to aid solubilisation of the mucus. Following centrifugation at 1°C for 1 hour at 2 500 xg, the gel layers were removed from each tube, pooled, and exhaustively dialysed for 24 hours at 4°C and finally homogenised by blending. In order to produce a high concentration of mucus gel, the preparation was dialysed (12–14 kDa molecular weight 'cut-off') and then centrifuged at 20 000 xg (Beckman L8-M Ultracentrifuge, Beckman Instruments, Inc., USA) for 1 hour. The supernatant was discarded and re-

centrifuged at 120 000 xg for 1 hour and the gel layer taken. The dry weight of each batch was determined by placing a small quantity (0.5g) in an open glass vial at 50°C for 48 hours.

### ***3.2.3.3 Preparation of mucin gels from porcine gastric mucus (PGM)***

Gels from the commercially available partially purified mucin from the porcine stomach (Sigma porcine gastric mucin type III, PGM) were prepared as follows. Batches of 10g of mucin gels (30% w/w) were prepared by accurately weighing 3g of the dry mucin powder and carefully adding 7g of de-ionised water. The container was covered with parafilm and the preparation allowed to hydrate for at least an hour before the gel was then stirred with a glass rod, re-covered with parafilm, and kept at 4°C for 16-18 hours to promote complete hydration of the gel. All mucin gels prepared this way were used within 48 hours of preparation.

### ***3.2.3.4 Preparation of dialysed partially purified Sigma mucin***

As a means of comparing the effect of mucin type on the rheological synergy with sodium alginate preparations, PGM was dialysed and used according to a method described by Rossi *et al.* (1995) with some modifications. Batches of 30g of 60%w/w mucin gel were prepared by slowly adding 12 ml of de-ionised water to carefully weighed 18g of mucin powder, allowing it to hydrate before stirring with a glass rod. The samples were covered with parafilm and were left to equilibrate for at least 12 hours at 4°C. They were then dialysed in batches with a 12 000–14 000 molecular weight 'cut-off' membrane, previously boiled for 15 minutes in 10 mM ethylenediaminetetraacetic acid (EDTA) solution and washed in de-ionised water. It was necessary to boil the dialysing membrane in EDTA in order in to ensure that there were no calcium ions available to react with the mucin preparation during dialysis. The dialysis was carried out against de-ionised water, at 4°C and was continued until the weights of the mucin gels were approximately half of the initial weight (30%w/w).

### **3.2.4 Preparation of mucin-alginate mixes**

In all mucin-alginate mixes, the mucin gel was prepared as detailed above. The alginate solutions were made by dissolving the powder in de-ionised water under vortex created by the stirring action of a mechanical stirrer (Heidolph, Germany) at 1400 rpm for 20 minutes or until all the powder has gone into solution with no lumps. The resulting solution was allowed to stand at room temperature for 24 hours to equilibrate and also to ensure that the viscosity remained constant. Batches of 30g total weight of mucin-alginate mixes were made by the addition of 15g of accurately weighed 4%w/w sodium alginate preparation to 15g of previously hydrated 30%w/w mucin gel. The mucin-alginate mix was stirred with a glass rod, and kept at 4°C for further 12 hours prior to rheological measurements. The final mucin-alginate mixes contained 15%w/w mucin and 2%w/w sodium alginate, unless otherwise specified. For the control runs, 15%w/w and 2%w/w of mucin and sodium alginate respectively were used alone.

### **3.3 Rheological examination of mucin, alginate and mucin-alginate mixes**

The basic concepts of oscillatory rheology have been given in Chapter 2 and only experimental conditions are discussed here. Both frequency and temperature scans were performed on the aforementioned systems. Frequency scans were performed using the cone and plate geometry, with a 6 cm cone diameter and an angle of 1.59°. Unless otherwise specified, all frequency scans were carried out by carefully loading the sample onto the rheometer and allowing it to equilibrate (2 minutes) before scanning within the frequency range of 10-0.01 Hz and at 25°C. The amplitude was set at  $7.4 \times 10^{-4}$  rads., which was found to be within the linear viscoelastic region of all the systems described above.

The cone and plate geometry has the advantage that, provided the cone angle is small enough (less than 0.1 rad.) the nominal strain across the sample may be considered constant. However, it does have the disadvantage that the cone truncation can be extremely small (ca. 56  $\mu$ m). The percentage expansion and contraction of the plate,

following heating and cooling respectively, ultimately leads to errors in the rheological measurements. Consequently, this geometry cannot be used if the temperature is altered, either by heating or cooling depending on the particular system, to induce gel formation. Hence, all temperature scans were performed using the parallel plate geometry, with a 6 cm diameter plate and a gap of 500  $\mu\text{m}$ . The usual procedure included carefully loading the sample onto the rheometer, covering it with low viscosity silicone oil ( $\approx 5 \text{ mPa}\cdot\text{s}$ ) and scanning from 10°C to 90°C and back to 10°C, at a rate of 4°C/min., a frequency of 1 Hz and a set displacement of  $7.4 \times 10^{-4}$  rads, unless otherwise indicated. In addition, during all temperature scans, a solvent trap (filled with de-ionised water) was used in order to minimise the potential loss of water from these systems, especially during the heating process. In both frequency and temperature scans, each run was repeated at least four times for reproducibility of data.

### **3.4 Estimation of work of adhesion between alginate preparations and mucin**

In this set of experiments, mucoadhesion was measured using the *work of adhesion* as the indicator. For the purpose of this study, four different batches of sodium alginate (Protanal LFR 5/60, LF 120L, SF 60L and SF 200) were chosen from a total set of nine, to cover the entire spectrum of the characteristics of sodium alginates (M:G ratio, molecular weight, and viscosity). Two formulations (directly compressed tablets or compacts and solutions) of sodium alginate and 3 different mucin sources. These mucin types were Sigma mucin type III (PGM), intact mucus on the surface of porcine stomach (SM), and crude mucin (CM). The operational details of the texture analyser have been discussed in Chapter 2, and only the details pertaining to this study will be given here.

#### **3.4.1 Preparation of sodium alginate formulations**

Batches of sodium alginate compacts were made by compressing accurately weighed sodium alginate powder (250 mg), using an IR press to form compacts of 1.3 cm diameter and 1.5 mm thickness. Various compression forces and times were examined until compacts of desirable firmness and thickness were obtained. On the

other hand, solutions of sodium alginate (2% w/w) were made by simply weighing out the appropriate amount of the powder and dissolving in de-ionised water using a mechanical stirrer (Heidolph, Germany) at 1 500 rpm. The solutions were allowed to reach their respective maximum intrinsic viscosities and also to be rid of any air bubbles by keeping covered with parafilm for 24 hours at room temperature before they were used.

### ***3.4.2 Preparation of mucin (the biological substrate)***

For the purpose of this study, crude mucin as described above and two other forms of mucin were prepared. In the first instance, two concentrations of PGM, 15% w/w and 30% w/w were prepared. Batches of 15% w/w (10g) of mucin gels were made by adding 8.5g of de-ionised water to 1.5g of accurately and carefully weighed mucin powder and allowed to hydrate as described in section 3.2.3.3. In the case of the 30% w/w preparation, 7g of de-ionised water was added to 3g of mucin powder and treated as in the case of 15% w/w preparation. In addition, 'innate mucin' as the biological substrate was prepared by carefully washing the stomach with cold 0.9% sodium chloride solution to remove the stomach contents, degraded mucus and mucus degrading enzymes. Sections, 3 cm diameter were then cut from the pyloric gland region of porcine stomachs. This region of the stomach is believed to closely resemble the human gastric mucosa. The stomach pieces were quickly frozen in liquid nitrogen before they were stored at -20°C until required. Prior to each experiment, the tissues were removed from the freezer and stored at 4°C (in the refrigerator) to thaw overnight.

### ***3.4.3 Measurement of the work of adhesion***

#### ***3.4.3.1 Liquid sodium alginate formulation and mucin***

With the liquid alginate formulation, approximately 50mg of the mucin preparation (15% w/w or 30% w/w) was layered onto a filter paper disc (20 mm diameter). The paper disc (Whatman benchkote, polyethylene backed absorbent paper), which has an impermeable side to avoid the penetration of the mucin preparation, was affixed



to the lower platform of the texture analyser using a double-sided adhesive tape (3M, UK). After 2 minutes rest, 50mg of the alginate of interest (2% w/w) were applied to another paper disc and affixed to a 20 mm diameter upper probe made of plexiglas, using a double-sided adhesive tape. In both cases, the preparation was layered to absorbing side of the paper discs and the non-absorbing side attached to either the platform or the probe. The work of adhesion between the two preparations was measured in the *Return to Start Test Mode*, measuring the forces of adhesion in *Tension*. The two preparations were brought into contact with each other by steadily lowering the probe at a pre-set speed of 5 mm/s. Once in contact, the probe speed was reduced to 0.2 mm/s (test speed) to a total distance of 20 mm before the probe was withdrawn at a speed of 0.2 mm/s to a distance of 20 mm. From initial experiments this distance was found to be well beyond the point where the two surfaces separated during the probe withdrawal. The force used throughout was 0.5N (unless in the case of examining the effect of force on adhesion whereby the force was varied). Blank measurements were taken by replacing the mucin with 0.1 ml of de-ionised water. The experimental set-up is shown in Figure 3.1.

#### ***3.4.3.2 Solid alginate formulation and mucin***

The test protocol was similar to that described for the liquid formulation, with slight modifications. The 1.3 cm diameter compact was affixed to the centre of a 2 cm diameter probe (using a double-sided adhesive tape) and the mucin applied as in the liquid formulation. In the case of the porcine stomach tissue, the piece was affixed to the platform of the instrument. Ponchel *et al.* (1987) have demonstrated that with the solid preparations, factors such as the rate of probe withdrawal following contact between the bioadhesive polymer and mucin has been established may affect the work of adhesion. In this work, the effect of the rate probe withdrawal was examined by using withdrawal rates of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mm/s, keeping all other parameters constant. Another parameter examined that may have an effect on the work of adhesion between the alginate compacts and mucin preparations was the contact force, at five different forces of 0.5, 1.0, 2.0, 5.0, and 10.0N.

In order to study the effect of contact time on the work of adhesion, the compact was allowed to remain in contact with the mucin preparation(s) for 10, 30, 60, 120, 300, 450, and 600 seconds before the probe was withdrawn. In a parallel experiment the compacts were allowed to hydrate for the above times by wetting the centre of one side of a compact with 0.1 ml of de-ionised water (instilled by a syringe onto the centre of the compact and instantly spread over the whole surface) before being placed in contact with the mucin preparation for one minute. This enabled the effect of hydration on work of adhesion to be assessed. Finally, the dependence (if any) of the work of adhesion on the physical characteristics of the compacts themselves was investigated by keeping the compaction time constant (60 seconds) and varying the force from 1 ton to 15 ton.

### **3.5 Results**

#### **3.5.1 Characterisation of 'crude' and homogenised mucus**

Following the preparation and drying of both the crude and the homogenised mucus the weights of the individual aliquots were recorded and the means and the standard deviations were recorded (Table 3.2). The dry weights of the 'crude' mucus were generally higher than those obtained from the homogenised mucus. This is accounted for by the fact that in the 'crude' mucus, there was much more unidentified 'solid' debris. Although nature of the debris is unknown, it is believed that fat, some mucosal tissues, and perhaps undigested food might have contributed to the overall weight. This may in part affect the rheological profile of the mucus, as the glycoprotein component may be still bound to the debris.

#### **3.5.2 Oscillatory rheological characterisation of mucin, sodium alginates, and mucin-alginate mixes.**

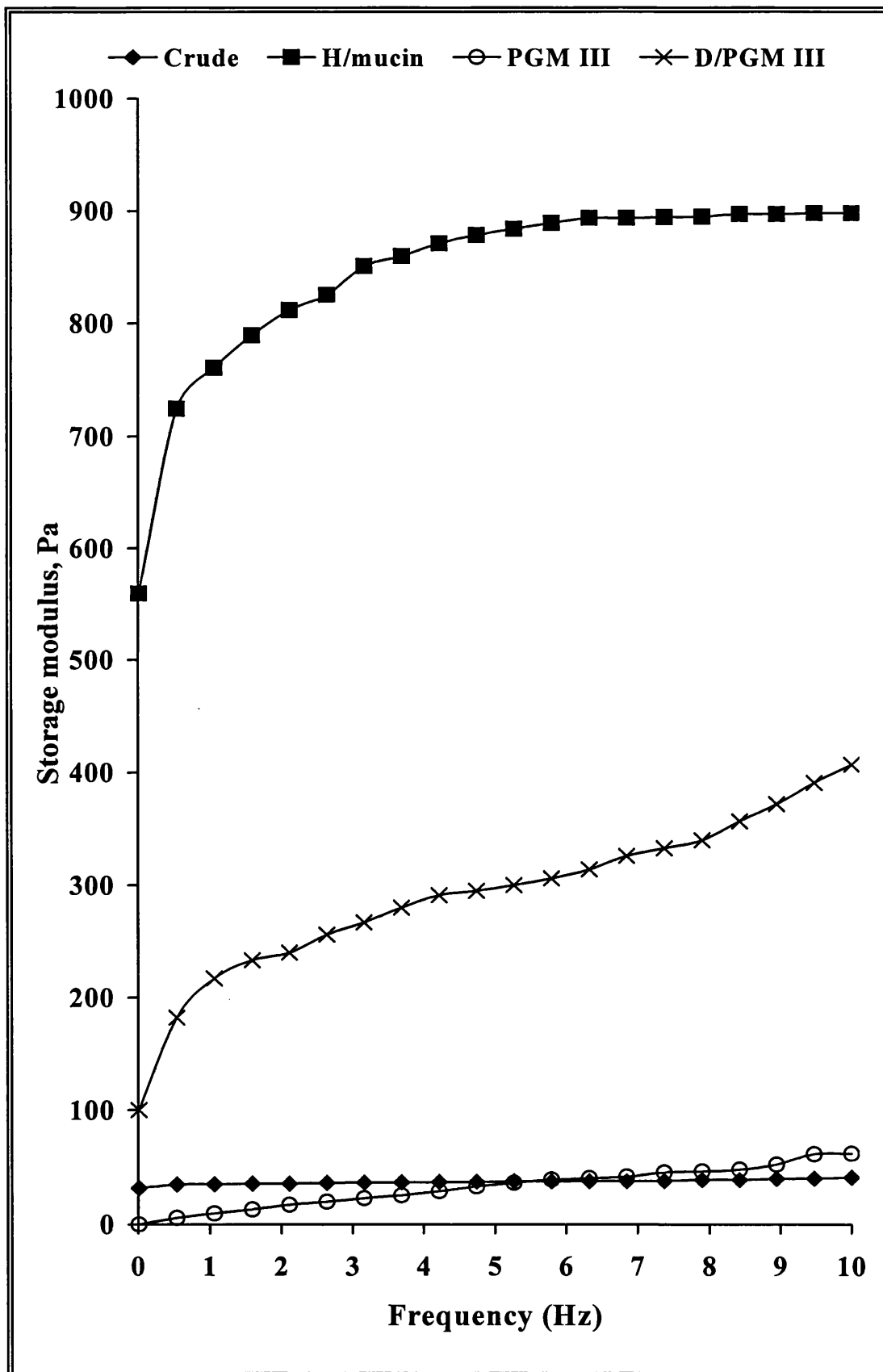
While there has been extensive work in the area of isothermal rheological studies on various mucin types (Foster *et al.*, 1994; Caramella *et al.*, 1994; Marriott *et al.*, 1982), considerably less is known about their interactions with the putative bioadhesive sodium alginate. However, some light has been shed on the extent of interaction

**Table 3.2: Dry weights of crude and homogenised mucus**

<i>Mucus type</i>	<i>% Dry weight (n= 20)</i>
'Crude' Mucus	14.48 ± 2.03
Homogenised Mucus	8.96 ± 3.67

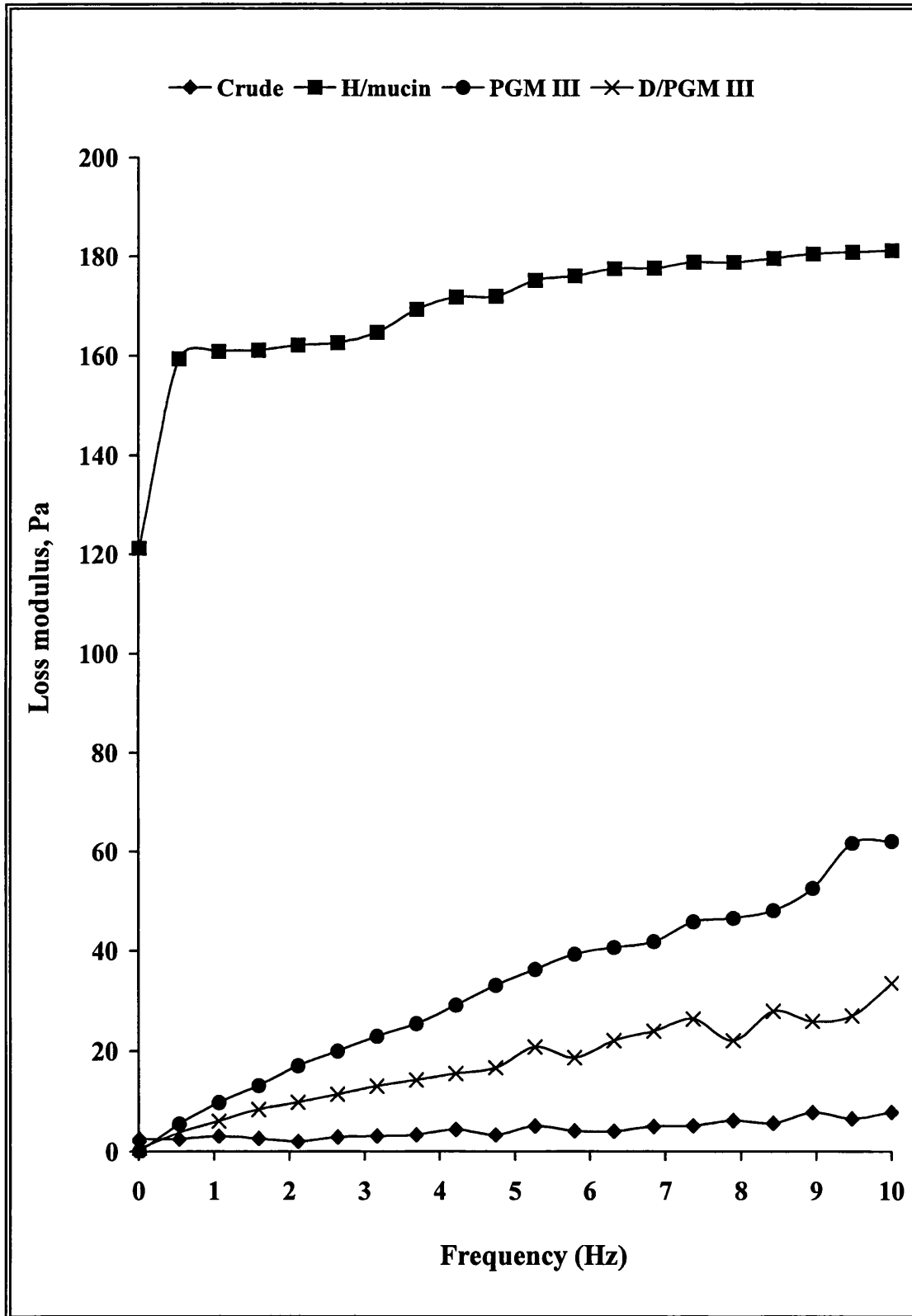
between sodium alginate and mucin by Fuongfuchat *et al.*(1996). In their work, Fuongfuchat *et al.* (1996) reported that mucin-alginate interactions lead to the formation of smaller aggregates of the individual mucin and alginate species. In addition, they pointed out that the interaction of alginate with mucin is more effectively analysed using mixtures containing excess mucin, as results from viscometry and light scattering of dilute solutions indicated the hydrodynamic sizes of the mixed solutes are smaller than those of the pure polymers.

The effects of frequency on the storage modulus (Figure 3.1a) and the loss modulus (Figure 3.1b) of 15%w/w 'gels' or preparations of various sources of mucin grades are shown. While the frequency scans of the crude mucin obtained from the porcine stomach and that of the commercially obtained mucin (PGM) showed a very similar pattern and low storage moduli magnitude, those of the homogenised mucin (HM) and dialysed PGM (DPGM) yielded a different and much greater magnitude of storage moduli. In addition, the latter two mucin preparations showed more gel-like characteristics, whereby the storage moduli remained much greater than the loss moduli throughout the frequency range employed. The rather higher storage modulus of HM and DPGM reflect the quality of the two preparations. In the case of DPGM, impurities were removed from the original PGM via an exhaustive dialysing process. The high values of the storage modulus for HM reflect the 'quality' and perhaps, the form in which the glycoprotein component exists in this mucus preparation.

*Figure 3.1a: Comparison of the storage moduli of the mucin types used*

Median data of  $n = 5$  plotted.

Figure 3.1b: Comparison of the loss moduli of the mucin types used



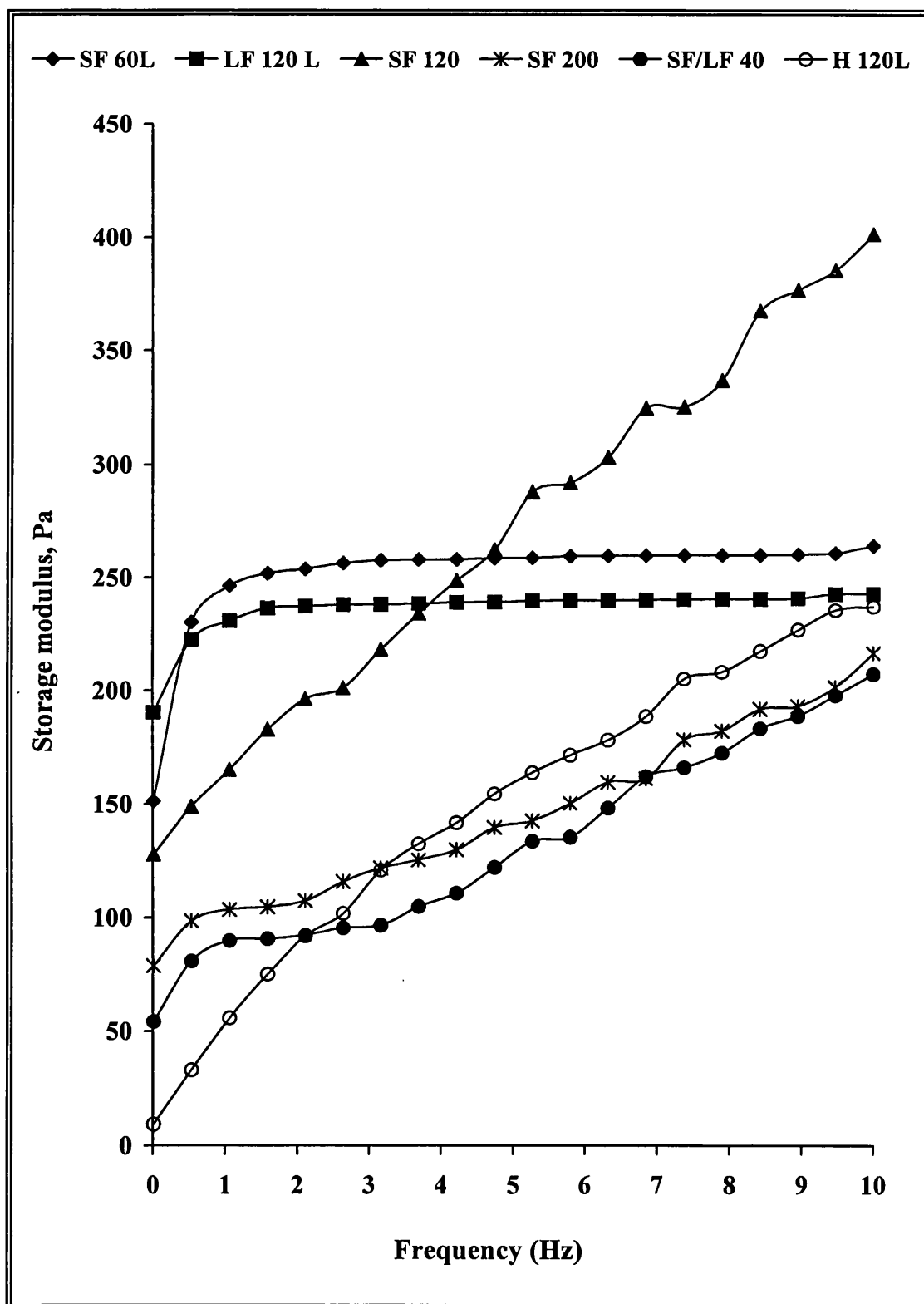
Median data of  $n = 5$  plotted.

On the other hand the low magnitude of storage moduli obtained for the porcine gastric mucin from Sigma (PGM) and that of the crude mucin (CM) could be

accounted for by the presence of impurities, in particular, the CM and/or the method of treatment (DPGM). Another interesting observation from the frequency scans was that whilst HM, DPGM and CM all showed true gel properties ( $G' > G''$  throughout the frequency range), that of PGM exhibited properties of viscous liquid ( $G' < G''$ ) in most cases, at similar concentrations. The corresponding loss moduli (Figure 3.1b) are of similar magnitude and pattern to those of the storage moduli. The ability of CM, PGM, and DPGM to exhibit gel properties may have rheological implications in as far as their interactions with sodium alginates are concerned. The results displayed in Figure 3.1a suggest that dialysing of PGM not only changed the gelling behaviour of the mucin but also led to substantial increase in the magnitude of its elastic response. The rather low elastic response values for PGM has the advantage of any subsequent synergy with sodium alginate being easily quantified.

The oscillatory frequency scans of sodium alginate preparations are depicted in Figure 3.2a. At 2%w/w, preparations of sodium alginate SF 60L and LF 120L showed properties of gels as the frequency dependence of the storage modulus shows a plateau after some initial rise with frequency. These results are in accordance with the definition of true gels as proposed by Almdal *et al.* (1993) who gave a phenomenological definition which has been discussed at some length in Chapter 2. The corresponding loss moduli of these alginate preparations are shown in Figure 3.2b. Although 2%w/w preparations of SF 200, SF/LF 40, SF 120, and H 120L showed patterns characteristic of viscous liquids with their storage moduli increasing with frequency throughout the frequency range used, the loss tangent values (Figure 3.2c) of SF 200 and SF 120 do not, however, reflect these observations. The loss tangent figures of SF 200 and SF 120 remained below 1 during the frequency scans. This implies that in these preparations, the elastic moduli exceeded the loss moduli within the frequency range, a characteristic of gels, though these preparations physically differ from the gels encountered in Chapter 2. In such preparations, factors such as the individual alginate viscosity, molecular weight and the M:G ratio may all play a role in determining the pattern and the magnitude of the storage modulus.

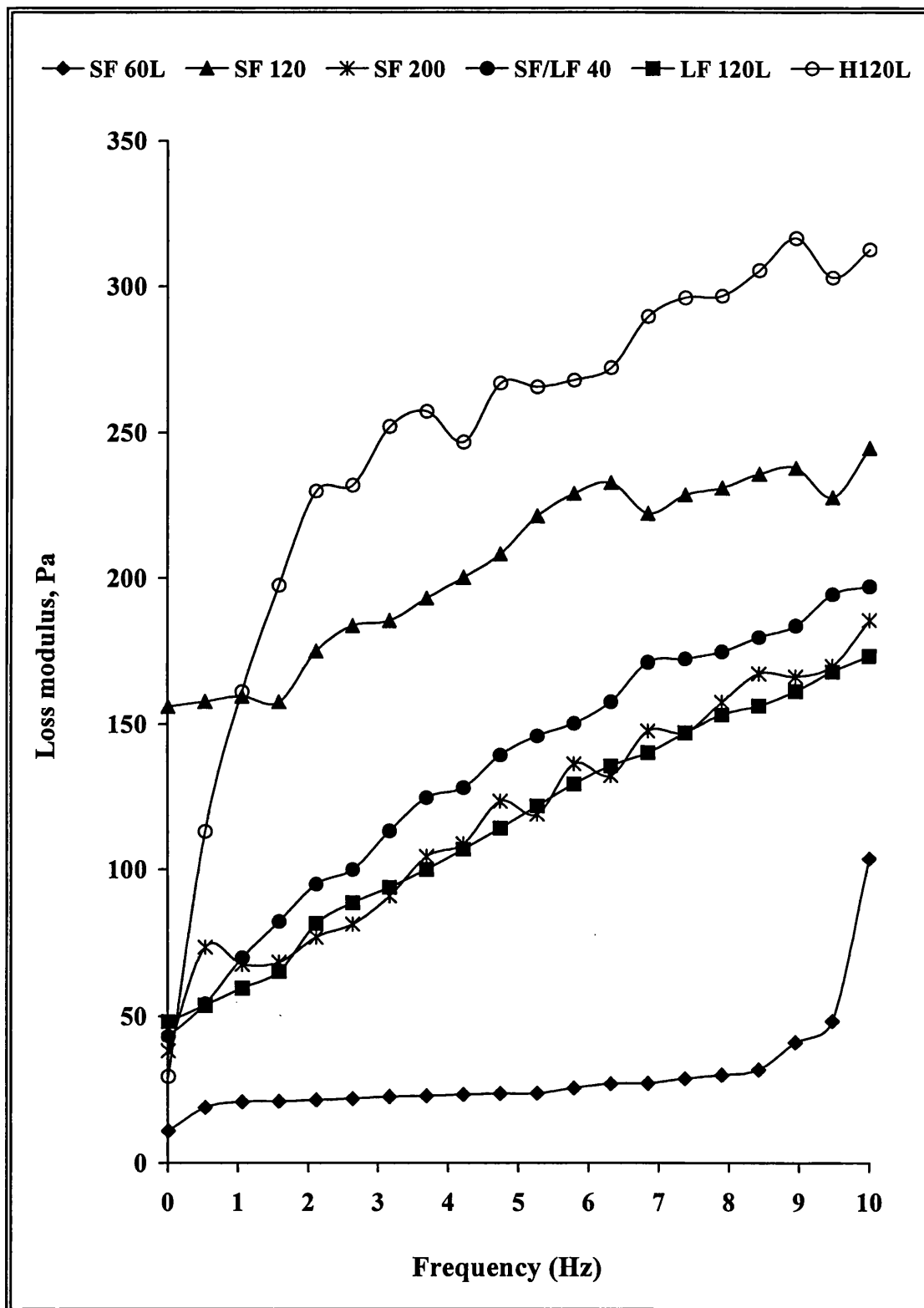
**Figure 3.2a: Storage modulus dependence of frequency of 2%w/w alginate preparations**



Median data of  $n = 5$  plotted.

Nonetheless, the trend observed in Figure 3.2b does not reflect to the quoted individual viscosities of the sodium alginate batches used (Table 3.1).

**Figure 3.2b: Loss modulus dependence of frequency of 2%w/w sodium alginate preparations**

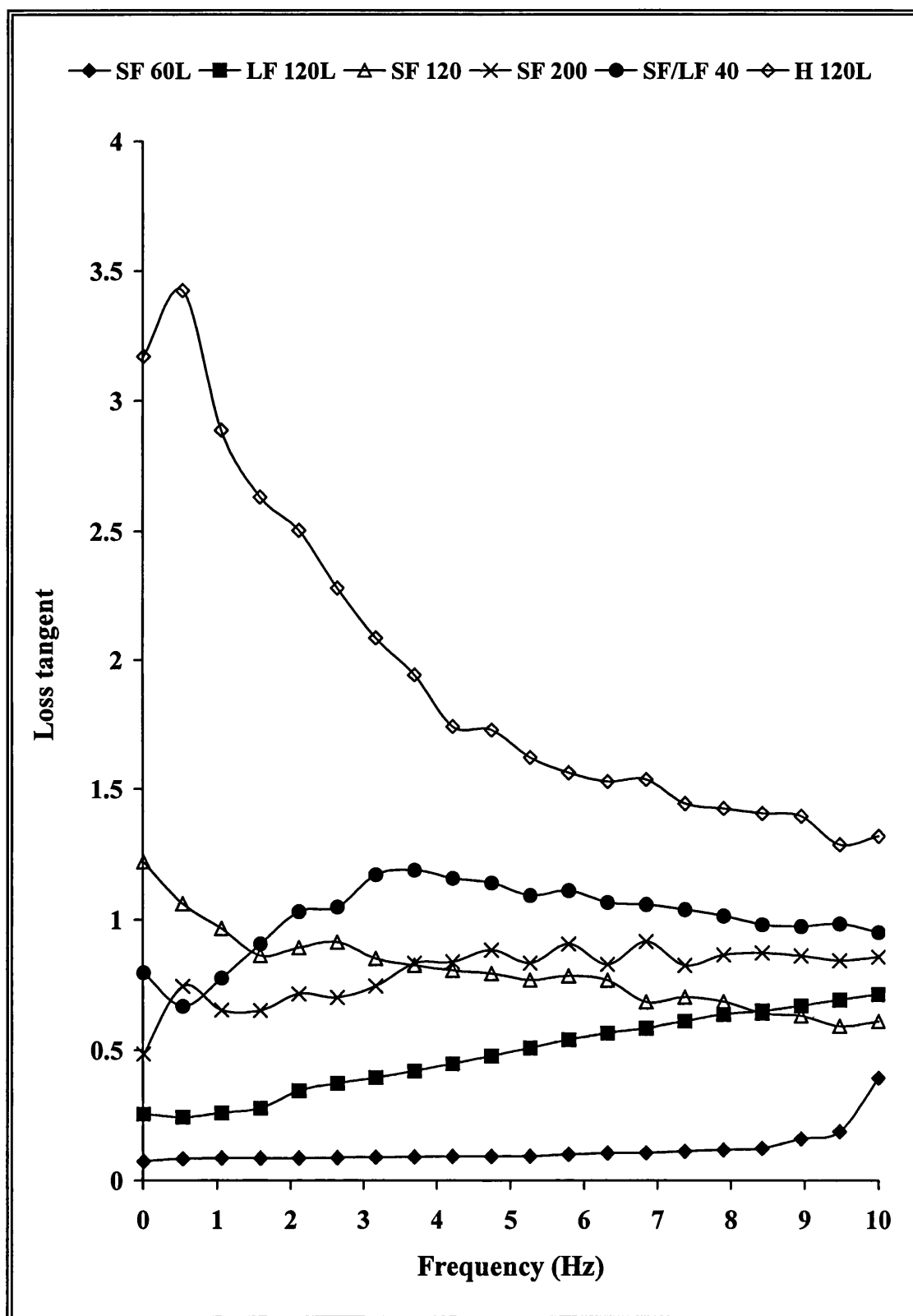


Median data of  $n = 5$  plotted.

Furthermore there was no clear relationship between the chemical compositions of the alginates and the rheological behaviour of their respective 2% w/w solutions. Due



**Figure 3.2c** Frequency dependence of the loss tangent of 2% w/w sodium alginate preparations



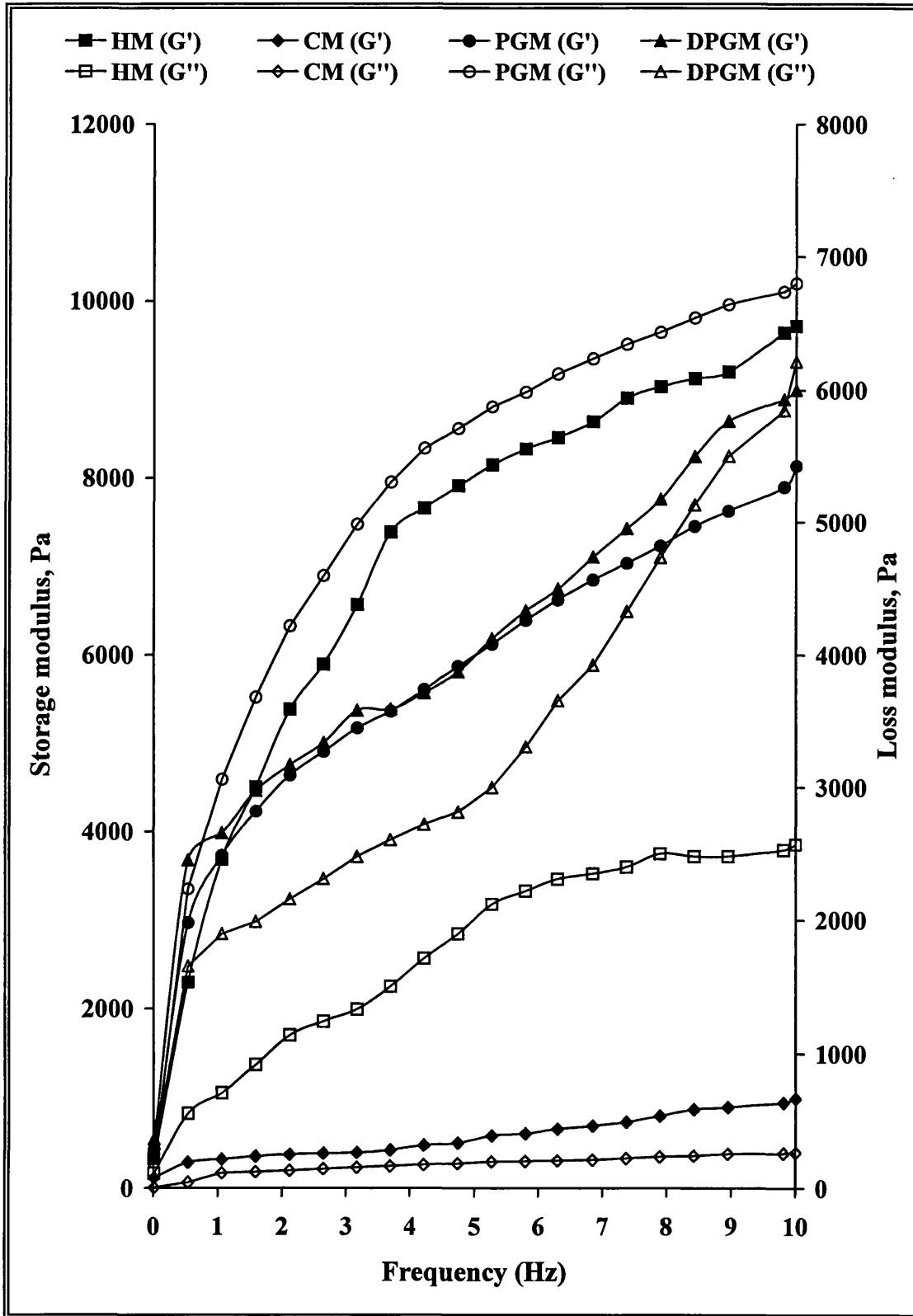
Median data of  $n = 5$  plotted.

to the low viscosity of sodium alginate LFR 5/60 (5.7 mPa.s of 1%w/w solution) attempts to perform frequency scans at concentrations similar to those of the other sodium alginate grades was not successful, as the data was very inconsistent. As the same problems were encountered when the concentration of the solution was increased to 10%w/w hence, results from those scans are not included here for comparisons. The frequency scans of alginate solutions alone showed no clear correlation between parameters such as the viscosity, the M:G ratios, and molecular weights of the sodium alginates used and both the storage and loss moduli patterns.

Earlier results (Figures 3.1a and 3.1b) indicate that the four types of mucin examined yielded very different rheological profiles. It was therefore of interest to examine how these mucins interacted with sodium alginate. In Figure 3.3, such synergistic effect with sodium alginate Protanal SF 60L is shown.

Following on from the rheological examination of mucin and alginate as individual entities, the interdependence and synergistic effects of mixing different sodium alginate batches and PGM (2%w/w and 15%w/w final concentrations, respectively) are depicted in Figure 3.4. PGM was chosen for this study because of its low storage modulus values when examined alone. In addition its commercial availability and ease of preparation makes it a reasonable choice over the other forms already described. Although no clear relationship was established between the storage moduli of the solutions of sodium alginate and their chemical compositions, there was a clear relationship between their chemical composition and their synergistic interaction with PGM. The addition of sodium alginate to the mucin preparation led to synergistic increase in the storage moduli with all grades of sodium alginates used. The greatest synergy was found to be between mucin and alginate SF 60L, with the lowest being between mucin and alginate LFR 5/60. Perhaps the most striking observation from these synergistic effects was the realisation that the trend of interaction also followed the content of mannuronate within the individual sodium alginate grade. In general, the higher the mannuronate content of a particular alginate, the greater its synergy (as an increase in storage modulus) with mucin.

**Figure 3.3: Rheological synergy between different mucin types and sodium alginate Protanal SF 60L**



The general trend of interaction of sodium alginate with Sigma mucin was in decreasing order shown below:

**SF 60L > H 120L > LF 120L > SF 120 > SF 200 > SF/LF 40 > LFR 5/60**

Similarly, the decreasing order of mannuronate content in the alginate samples used is also shown below:

**SF 60L > LF 120L > H 120L > SF/LF 40 > LFR 5/60 > SF 200 ≈ SF 120**

Examination of the above lists of mannuronate content and the extent of the corresponding alginates interactions with PGM indicates that there is a close relationship between the mannuronate content and magnitude of interaction with mucin. Although LFR 5/60 has a higher mannuronate content than SF 120 and SF 200, it has a very low viscosity (5.7 mPa.s for 1% solution), which may have contributed to the extent of its interaction with mucin. The exact mechanism by which mannuronate-rich alginates preferentially interact with mucin is not known. Nonetheless, it may be speculated that the flexible nature of the monomer as discussed in Chapter 1 allows relatively easy interpenetration and entanglements with the mucin molecules.

Hassan and Gallo (1990) and Rossi *et al.* (1994) have evaluated and quantified the magnitude of synergy between polymer-mucin interaction. The synergy was calculated as the differences between the actual viscoelastic (storage moduli) values of the mucin-polymer mixture and the theoretical values defined as the sum of the viscoelastic components of the polymer and mucin.

Thus,

$$\Delta G^I = G^I_{(mix)} - (G^I_{(polymer)} + G^I_{(mucin)}) \text{ and}$$

$$\Delta G^{II} = G^{II}_{(mix)} - (G^{II}_{(polymer)} + G^{II}_{(mucin)})$$

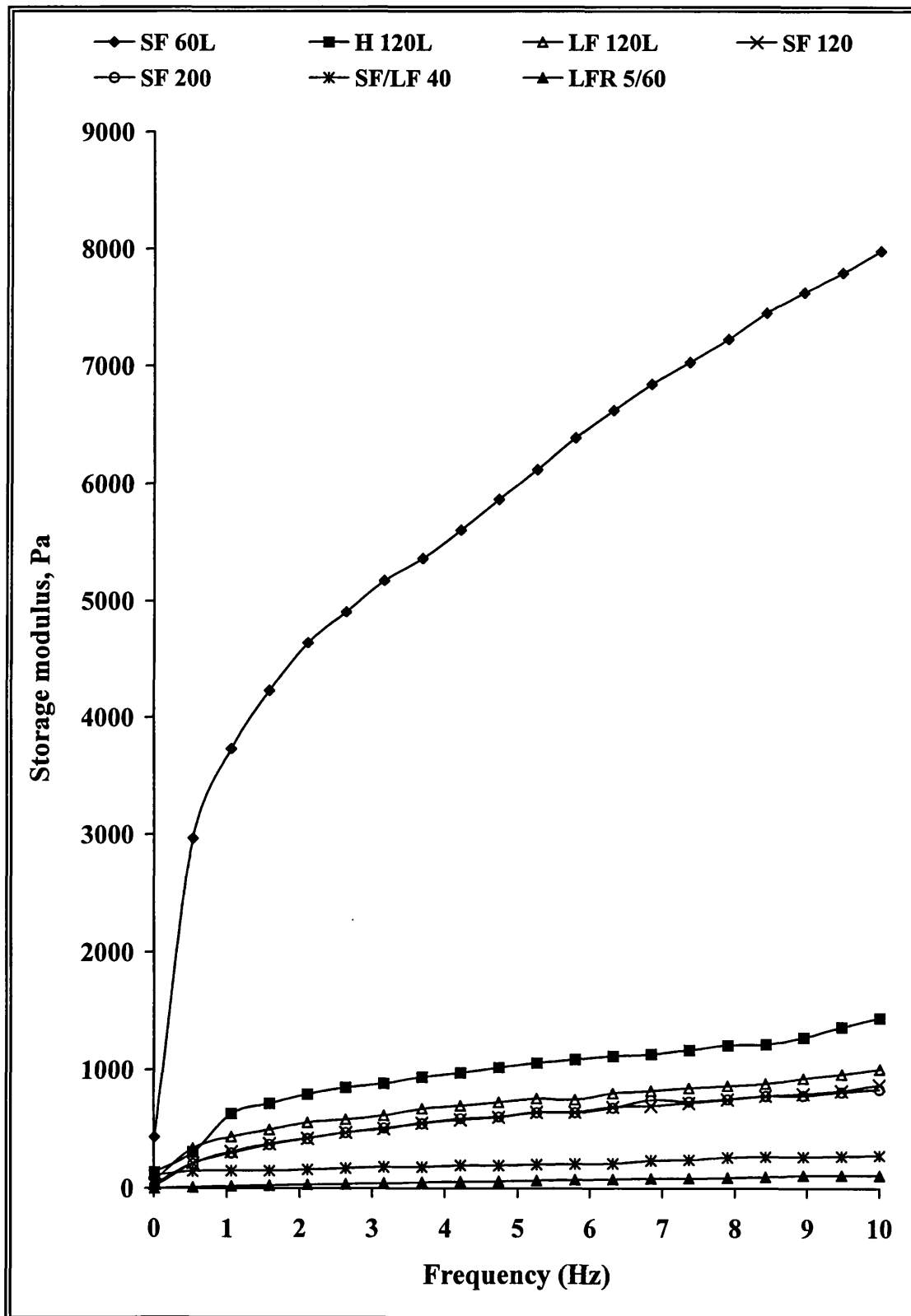
The relative rheological synergism (an expression of the relative increment in viscoelasticity) may be given by the expressions

$$\Delta G^I/G^I, \text{ where } G^I = G^I_{(polymer)} + G^I_{(mucin)} \text{ and}$$

$$\Delta G^{II}/G^{II}, \text{ where } G^{II} = G^{II}_{(polymer)} + G^{II}_{(mucin)}$$

Subsequently,

**Figure 3.4: Storage responses of various sodium alginate-PGM mixes**



All sodium alginate-PGM mixes contained 2%w/w and 15%w/w sodium alginate and PGM final concentrations, respectively. (Median data of  $n=5$  plotted.)

$$\% \Delta G = \Delta G^l / G^l_{(\text{polymer-mucin mix})}$$

When such approaches are applied to the current study, the outcome is as summarised in Table 3.3

**Table 3.3: Summary of the magnitude of interaction between mucin and alginates, at 1.587 Hz**

Alginate	$G^l$ Alginate	$G^l$ Mucin	$G^l$ Alginate-mucin	$\Delta G^l$	$\% \Delta G$	$\Delta G^l / G^l$
H 120L	75.13 ± 4.82	13.1 ± 2.13	714.6 ± 16.45	626.37	87.65	7.10
LF 120L	104.6 ± 6.34	13.1 ± 2.13	520.6 ± 10.84	402.87	77.37	3.42
SF 60L	251.8 ± 8.74	13.1 ± 2.13	4321.0 ± 47.46	3966.12	91.79	14.97
SF 120	182.9 ± 9.84	13.1 ± 2.13	420.11 ± 32.17	224.11	53.35	1.14
SF/LF 40	236.6 ± 11.23	13.1 ± 2.13	368.1 ± 12.42	118.40	67.99	0.32
SF 200	90.63 ± 6.85	13.1 ± 2.13	147.6 ± 4.85	43.87	29.13	0.42
LFR 5/60	0*	13.1 ± 2.13	24.31 ± 2.70	11.21	46.11	0.86

Mean values (± S.D); n = 5. \* No rheological data obtained for this alginate due to its low viscosity, hence the value shown is an assumed figure.

Two-way paired analysis of variance (ANOVA), unbalanced design, suggests that mannuronate-rich alginates (H 120L, LF 120L, and SF 60L) displayed significantly greater synergy with PGM than their guluronate-rich counterparts (SF 120, SF/LF 40, SF 200, and LFR 5/60) ( $p < 0.05$ ).

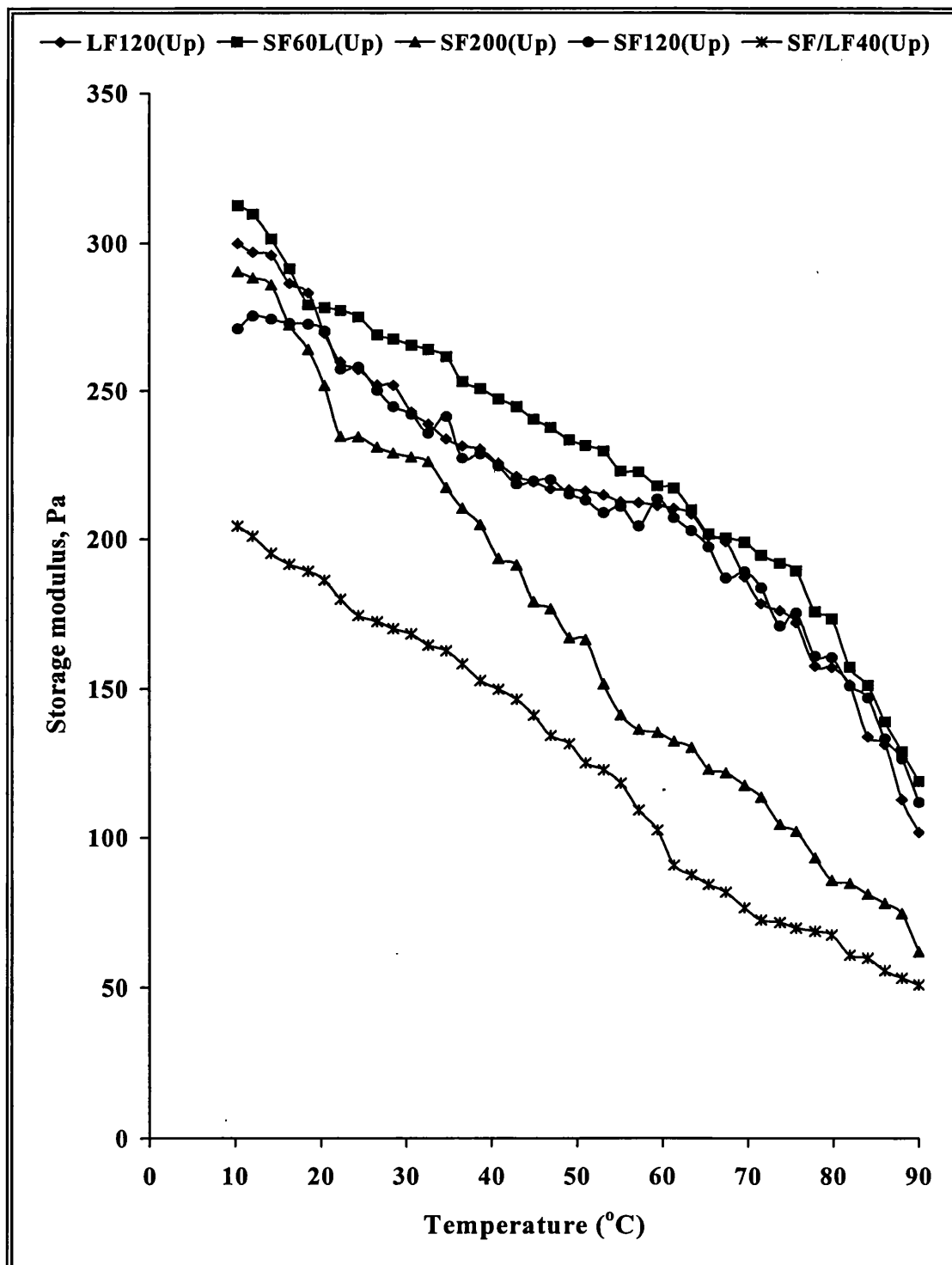
### 3.5.3 Thermorheology of sodium alginate, mucin, and alginate-mucin mixes

For systems gelling under a well-defined thermal regime, there is a characteristic 'cure' curve of  $G'$ ,  $G''$  against time (Clark 1982). For the fluid state,  $G''$  will be greater than  $G'$ . After the start of the cure experiment there is an initial lag time. Subsequently, both  $G'$  and  $G''$  begin to increase, but with  $G'$  increasing faster than  $G''$ . Consequently at a given time, there is a cross-over where  $G'$  becomes greater than  $G''$ , sometimes associated with the gel(ation) time (Clark 1982). Detection of the gel point itself has often relied on very simple measures. For example it may be judged to be when  $G'$  becomes greater than a pre-assigned threshold value, or more commonly the time when  $G'$  becomes greater than  $G''$  (or  $\tan \delta$  becomes less than 1), sometimes called the cross-over point. For the purpose of this study, the latter detection method was employed.

Although results from this study indicate synergistic interactions between all the mucin types and sodium alginate SF60L (Figure 3.3), it was found that the greatest rheological synergy was between HM and sodium alginate. However, results from the isothermal, frequency scans of mixes containing 15%w/w PGM and 2%w/w sodium alginate, and the practicability of mixing with sodium alginate justifies the choice of PGM for further investigation in the thermorheological study of alginate-mucin mixes. Temperature scans (heating and cooling) of sodium alginate solutions *per se* showed a gradual decrease in the elastic moduli with increasing temperature (Figures 3.5a & 3.5b). On cooling the alginate preparations failed to recover from both the stresses and the temperatures imposed. The elastic moduli of these were observed to be much lower than those observed during the heating process. This is an indication of the irreversibility of the thermorheological processes. The loss moduli of the alginate preparations were very inconsistent and showed no real pattern, hence, only the storage moduli are shown. The temperature scans of 15%w/w PGM alone, on the other hand, (Figure 3.5c) indicate that the gelation of mucin is a temperature dependent process. During heating, the loss modulus remained greater than the storage modulus, with very little increase in either parameter with temperature. However, during cooling, the sample had become 'gel-like' with the storage modulus being greater than the loss modulus. Such thermogelation of mucin

has previously been reported by Tamburic *et al.* (1997). Although the exact mechanism for this transition is not fully understood, it is within reason to hypothesise that during the heating process, the thermal energy available to the molecules of mucin is enough to cause uncoiling of the subunits.

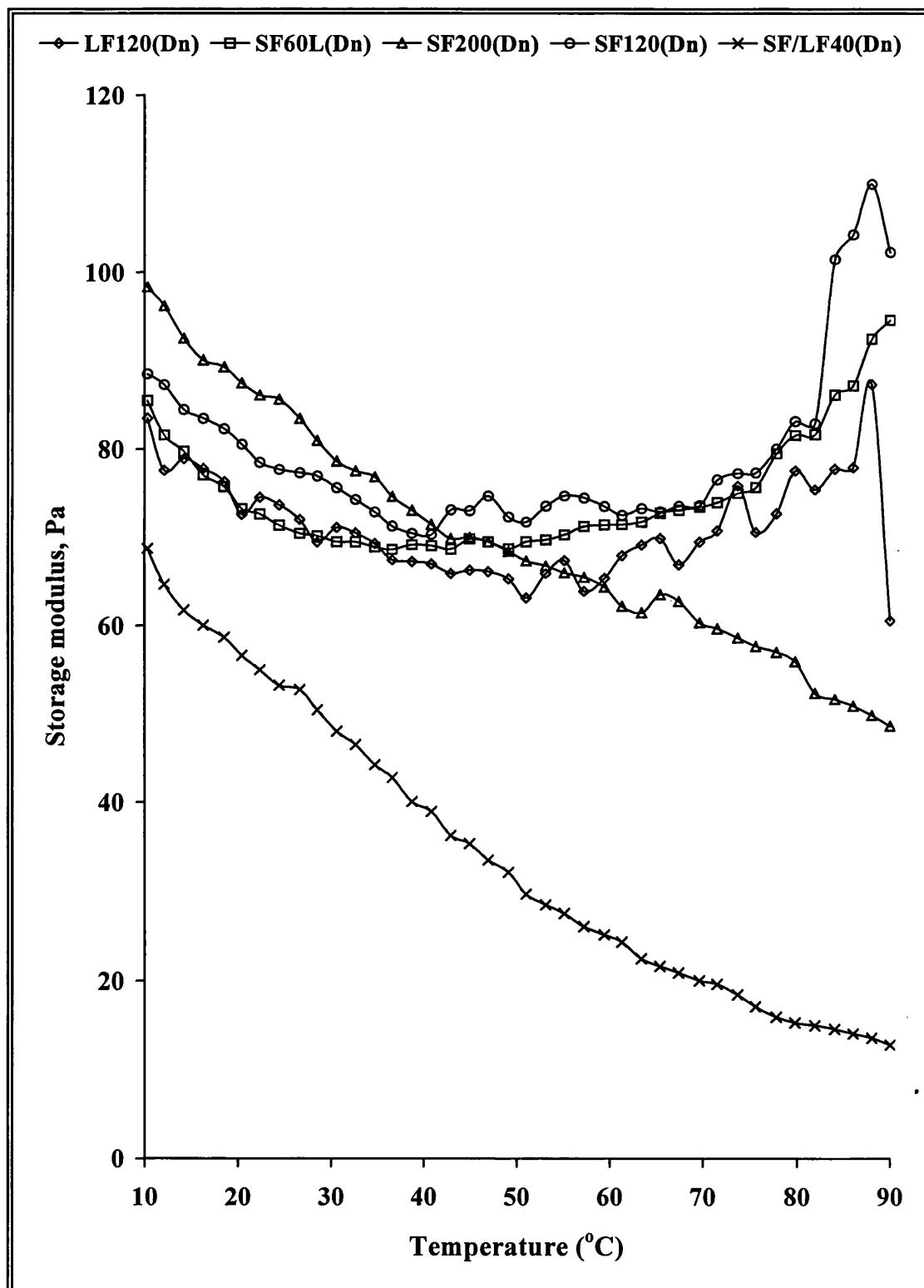
**Figure 3.5a: Thermorheological profiles 2%w/w alginate preparations during the heating cycle**





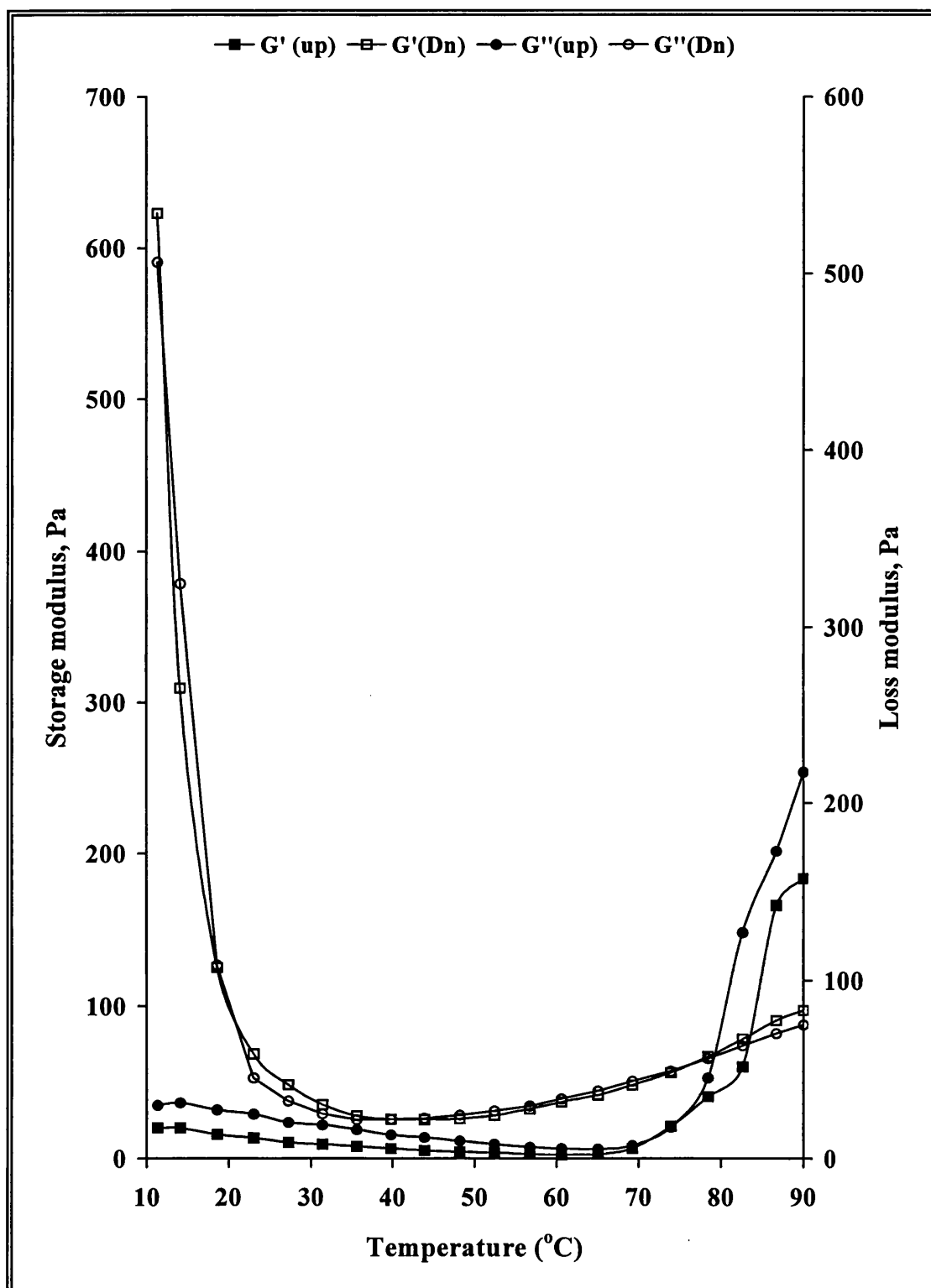
The uncoiling is then followed by a re-arrangement during the cooling phase with the molecule assuming a more compact arrangement and increasing its elastic modulus.

**Figure 3.5b: Thermorheological profiles of 2%w/w alginate preparations during the cooling cycle**



Median data of  $n = 5$  plotted.

Figure 3.5c: Storage and loss moduli of 15%w/w PGM during heating/ cooling cycles



Median data of  $n = 5$  plotted.

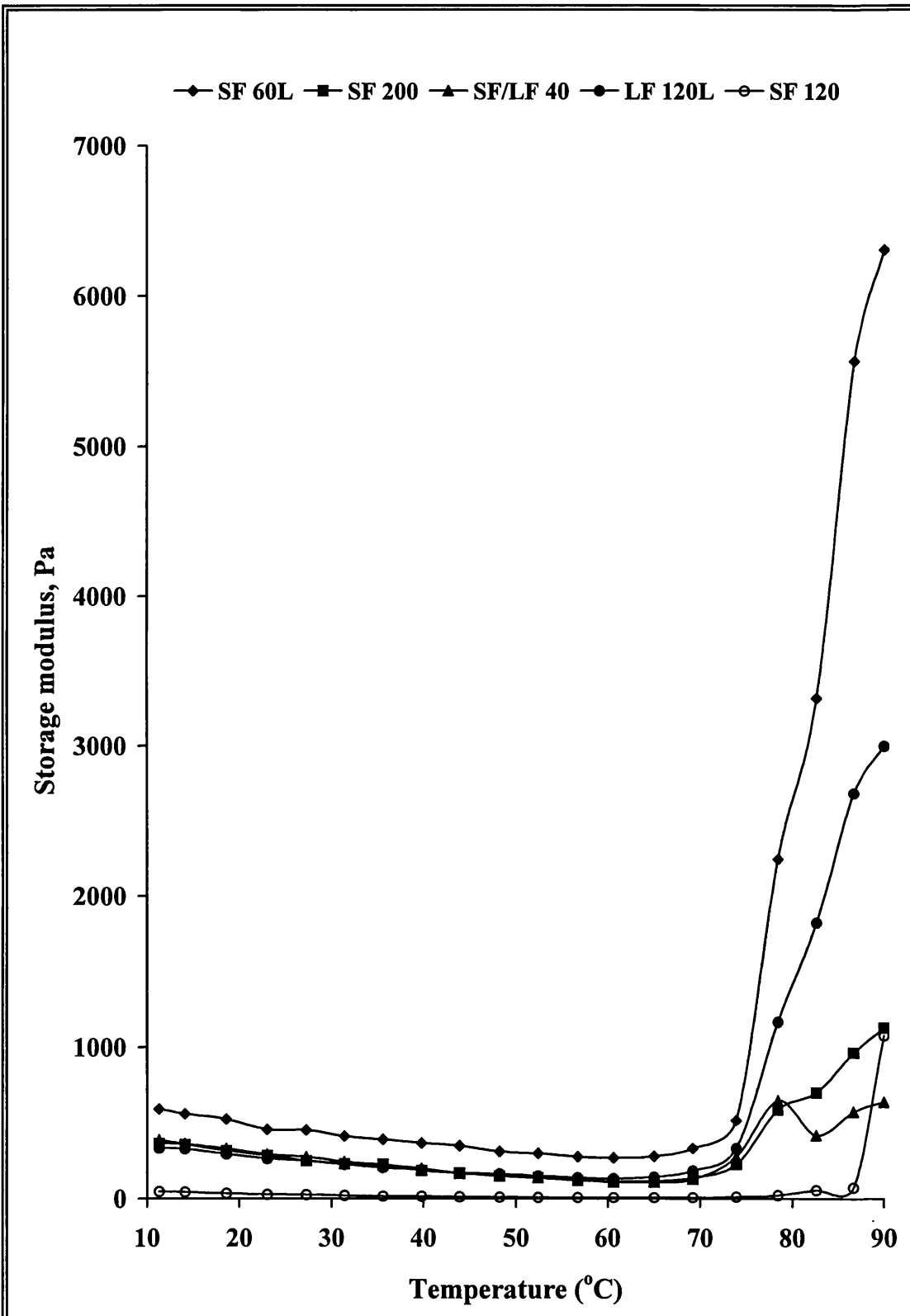
In most cases there was a drop in storage modulus between the heating and cooling cycles. This may be explained by the fact that at the end of the heating cycle, the

rheometer software had to be manually changed over to the cooling cycle and a re-equilibration of rheometer heating probes. The magnitude of the drop was also observed to be time-dependent (that is, time required to manually change over the software). The thermorheology of 2%w/w solutions of sodium alginate alone proved to be difficult to assess, as increasing temperature caused the solution to become very 'thin' thereby leading to inconsistent data points. Throughout the scanning process, all alginate solutions remained as viscous liquid, with the loss moduli being greater than the storage moduli.

In Figure 3.6a, the thermorheograms of storage moduli of various alginate-PGM mixes during heating (up curve) are shown. The addition of sodium alginate to PGM caused the temperature at which thermogelation occurred to increase to approximately 75°C from approximately 65°C for 15%w/w PGM alone. Although similar thermogelation at elevated temperatures has been reported by Tamburic *et al.* (1997) when they examined thermogelation of Carbopol<sup>®</sup>-mucin mixes, this is the first time such thermogelation has been observed with alginates. The exact mechanism(s) by which the observed thermogelation occurs is not yet known, nevertheless, degradation of mucin leading to a possible uncoiling of the individual units may play a part. In addition, dehydration of alginate-mucin mixtures at high temperatures may account for the increase in the gel properties (increase in storage moduli), although solvent traps were used to minimise such effect. The general characteristic of the alginate-mucin gels during heating is one of which there is a sudden (and continuous) increase in the storage moduli of the mixes at temperatures approximately 75°C. The increases in the storage moduli at higher temperatures also coincided with the transition from viscous preparation to gel, whereby the storage moduli were greater than the loss moduli (compare Figure 3.6a and 3.6b, respectively). As previously seen during frequency scans, temperature scans of the mannuronate-rich alginates showed the most promising synergy with mucin, with SF 60L showing the greatest synergy with PGM. The general trend in order of decreasing synergy with Sigma mucin during the heating phase was as follows:

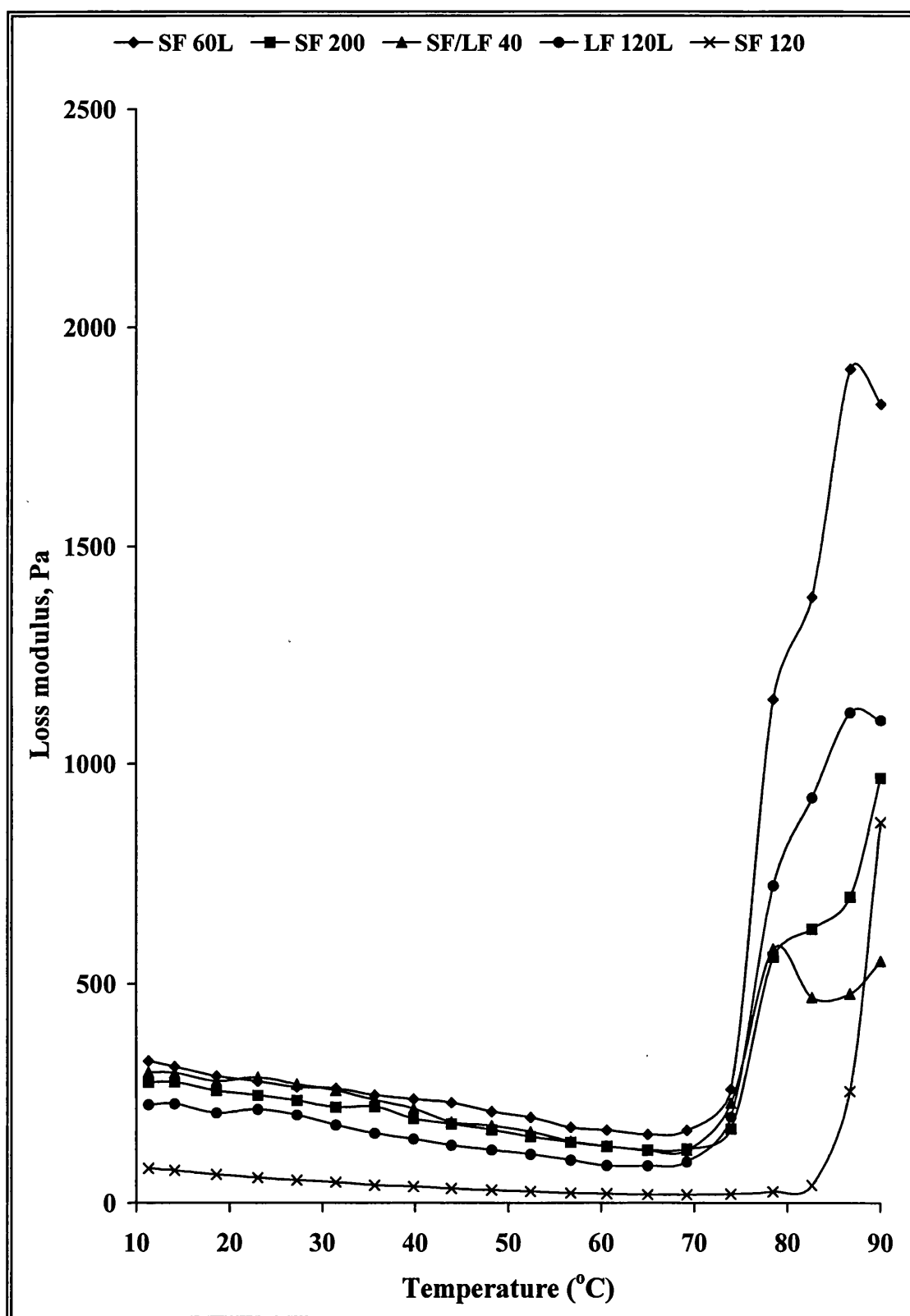
**SF 60L > LF 120 > SF 200 > SF/LF 40 > SF 120**

**Figure 3.6a:** Storage modulus dependence on temperature of alginate-PGM mixes during the heating cycle



Median data of n = 5 plotted.

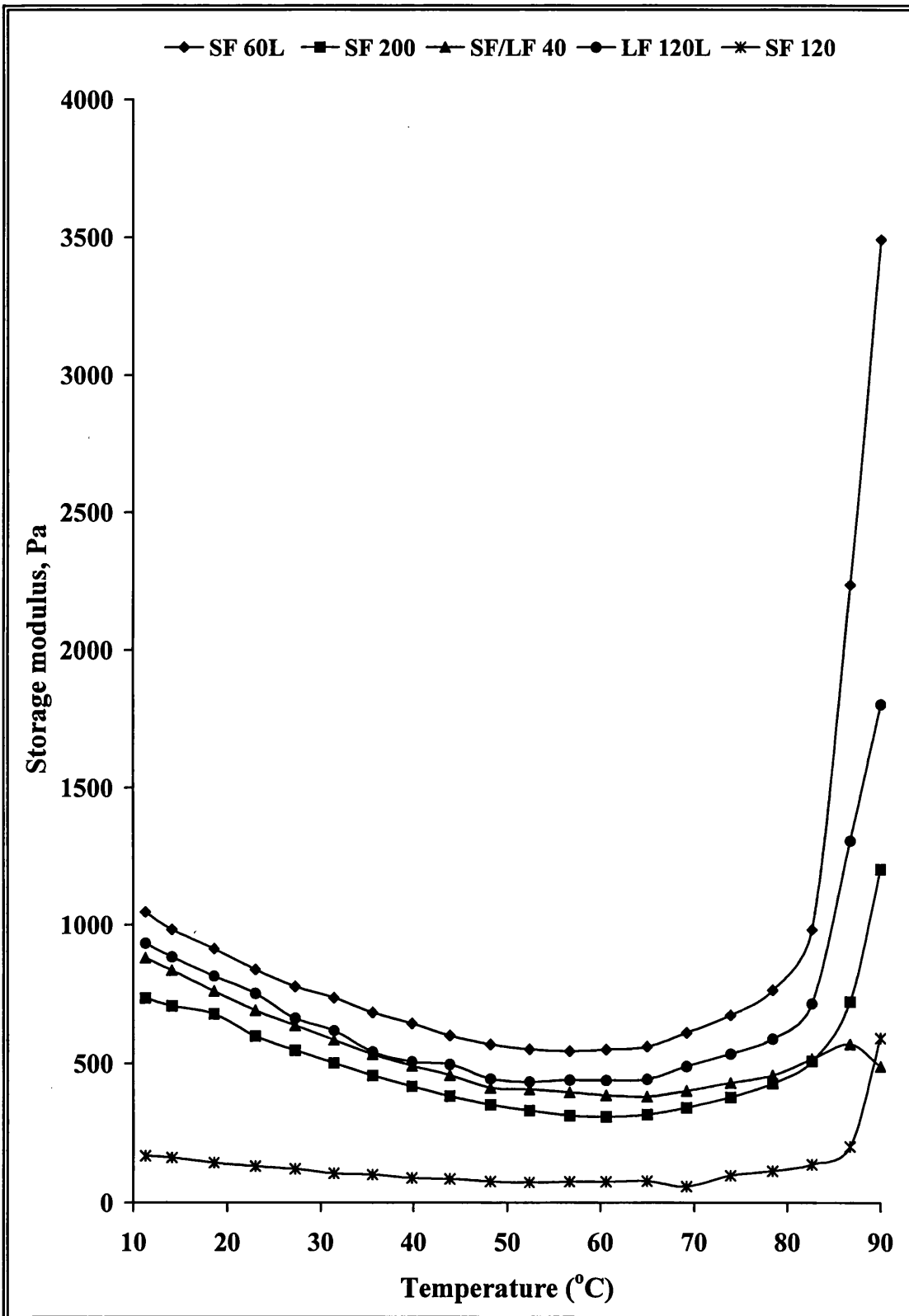
**Figure 3.6b: Loss modulus dependence on temperature of alginate-PGM mixes during the heating cycle**



Median data of n = 5 plotted.

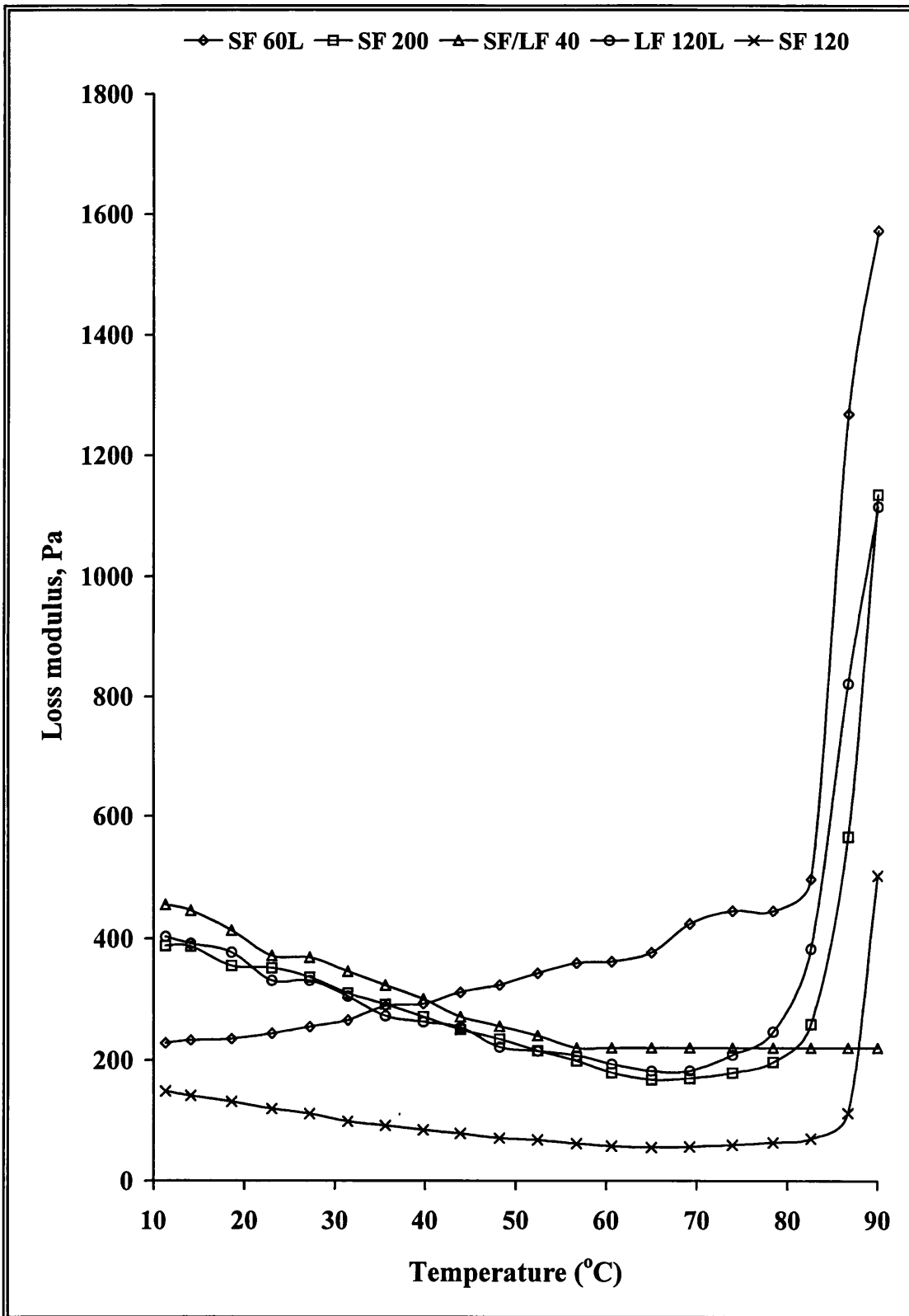
In contrast to the frequency scanning results whereby there was no apparent influence of viscosity of the individual alginates on the synergy with mucin, viscosity seems to play a role in the thermogelation with mucin. Although the overall synergy between alginates and mucin favours mannuronate-rich alginates, thermorheological profiles of these mixes also reveal the influence of the viscosities of the individual alginates. Table 3.1 indicates that the decreasing order of rheological synergy between sodium alginates and mucin also corresponds to similar order of viscosity of 1% solutions of the individual alginates. For example, within the guluronate-rich alginates used (SF 200, SF/LF 40, and SF 120) the order of magnitude of synergy with mucin was also found to correspond to their viscosities. The viscosity of a 1% solution of SF 200 (990 mPa.s) is greater than that of SF/LF 40 (413 mPa.s) which, in turn is greater than that of SF 120 (110 mPa.s). Similarly, within the mannuronate-rich alginates there was a clear correlation between viscosity and the magnitude of rheological synergy with mucin (380 and 121 mPa.s for 1% solution of SF 60L and LF 120L, respectively). That is, the higher the viscosity of the alginate grade within a group (mannuronate or guluronate-rich), the greater their synergistic interaction with mucin. One plausible explanation for this observation could be that during heating, there is the thinning of the alginate-mucin mixes, which is less pronounced with those containing high viscosity alginates. The corresponding storage and loss moduli for the alginate-mucin mixes during the cooling cycles are shown in Figures 3.6c and 3.6d, respectively. Two things are apparent from Figure 3.6c. Firstly, the loss moduli of the mixes generally followed similar trend as their storage moduli counterparts, shown in Figure 3.6b, with a sharp drop in loss moduli at approximately 75°C. However, the magnitude of loss moduli was smaller than those observed with the storage moduli, confirming the thermogelation events. Secondly, the loss moduli during cooling did not return to the initial values observed during heating. This suggests that the thermogelation observed was only partially reversible.

Figure 3.6c: Storage moduli dependence of alginate-PGM mixes during the cooling cycles



Median data of n = 5 plotted.

**Figure 3.6d: Loss modulus dependence on temperature of alginate-PGM mixes during the cooling cycle**



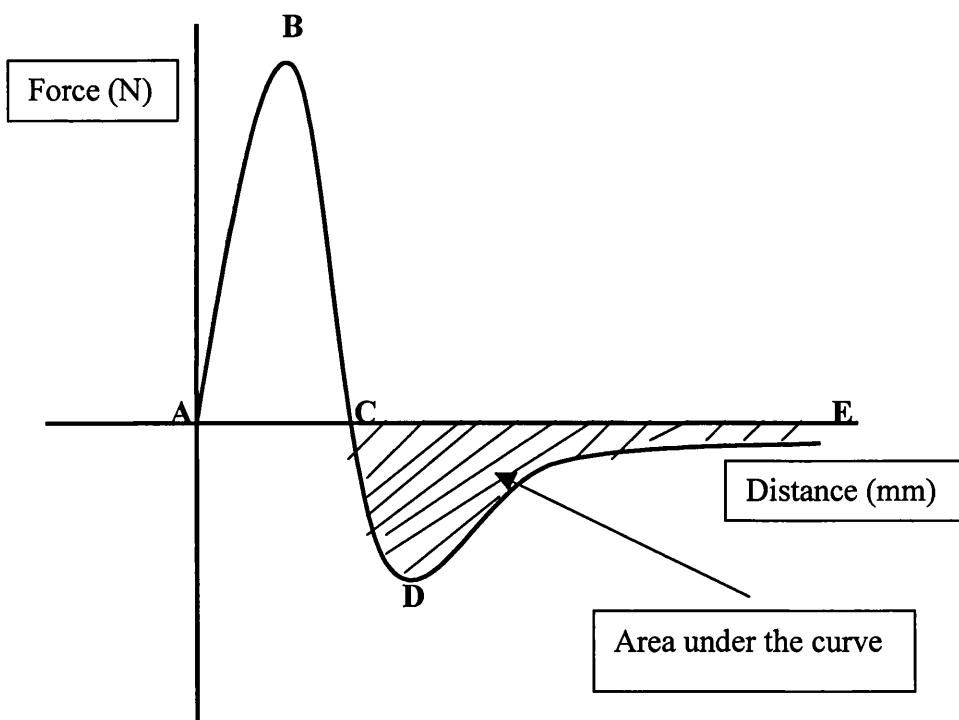
Median data of  $n = 5$  plotted.



### 3.6 Texture analysis of solid alginate formulations and mucin interactions (Work of adhesion)

The work of adhesion between the mucin and the alginate preparation was chosen as an index of the bioadhesive potential of the alginate, and is given by the area under the *Force vs. Distance* curve (AUC) (Figure 3.7).

**Figure 3.7: Schematic representation of texture analysis profiles of alginate-mucin adhesive bond**



As can be seen in Figure 3.7, the area under curve (shaded 'CDE') encompasses all the individual measurements during the detachment of the alginate preparation from, the mucin preparation. The curve 'ABC' indicates the probe attachment to the mucin preparation, with AB representing the lowering of the probe, point B, the maximum contact force between the two preparations, and BC being the probe withdrawal from the two surfaces. The point D is the maximum force of adhesion (that is, the force at which mucin and alginate preparations become totally separated). Previously, the *work of adhesion* (WOA) has been reported to be the most accurate predictor of

mucoadhesive performance (Ponchel *et al.*, 1987). Single point measurements such as the *maximum force of detachment*, although not necessarily incorrect ways of assessing the bioadhesive bond strength, do not, however, give the true measure of the bioadhesive bond. The work of adhesion (WOA) between alginate and mucin preparation was calculated as follows:

$$\text{WOA} = (\text{WOA}_{(\text{alginate/mucin})} - \text{WOA}_{(\text{alginate/de-ionised water})})$$

### 3.6.1 *Effect of the compaction force on work of adhesion (WOA)*

Due to the compressive method of preparation of compacts, it is generally expected that the porosity may influence WOA and bioadhesive characteristics of these systems. In order to establish the effect batches of 10 compacts were tested for their work of adhesion, using 30%w/w PGM gel. In Table 3.4, the net work of adhesion values indicates that the higher compaction force led to a decrease in the WOA.

From the preliminary exercise to determine the effect of compact force on the overall WOA between alginate and mucin preparations, it was decided that all compacts for further work would be made with a force of 2 tons for 60 seconds. The capping of the compacts at forces of 7.5 tons and above made them unsuitable for further work. Furthermore, maximum WOA was obtained with compacts at 2 ton for 60 seconds. The decrease of WOA with increasing compaction force may be explained as follows. Porosity plays an important role in the hydration of compacts and tablets. Hydration, in turn, is essential for bio(muco)adhesion to occur. As the force of compaction is increased the particles within the compact come closer together with very little or no inter-particulate gaps, making both bonding between particles and wetting difficult, hence the observed decrease in WOA.

**Table 3.4: Establishing compact characteristics**

Compaction force (Ton)	Appearance of compact	WOA ( $\mu\text{J}$ ) (Alginate-mucin)	WOA ( $\mu\text{J}$ ) (Alginate-water)	Net WOA ( $\mu\text{J}$ )
1	Easily broken upon handling	617.84	16.42	601.42
2	Firm, more resistance to Breaking	668.36	15.32	653.44
5	Very firm, with shiny surfaces	641.97	19.64	622.33
7.5	Very firm, shiny surface, evidence of capping	635.08	17.20	617.88
10	Extremely firm, very shiny surfaces, capping	616.67	18.47	598.20
15	Extremely firm, very Shiny surfaces, Severe capping	601.25	16.85	584.44

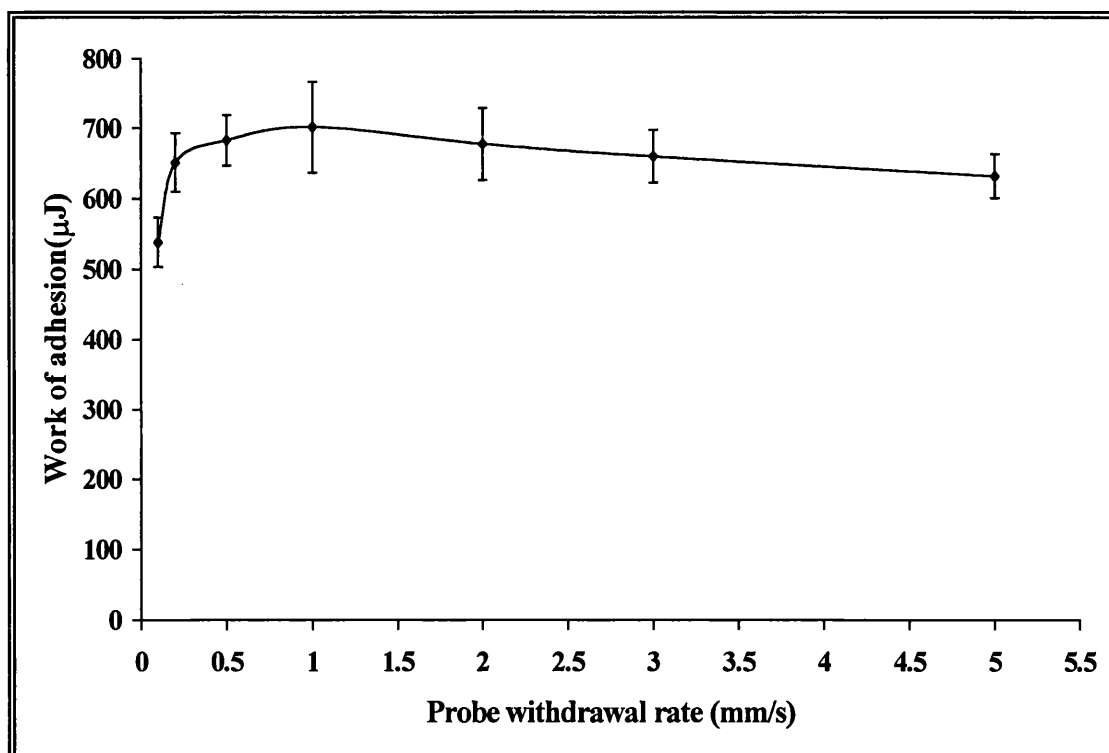
All compacts were made by compressing 250mg of Protanal LF 120L (varied compaction forces of 1-15 tons for 60 seconds). Contact time between the compacts and mucin preparation was 60 seconds in each case, probe withdrawal was set at 0.2 mm/s.

### 3.6.2 Effect of rate of probe withdrawal on WOA

The probe withdrawal rate was first shown to have an effect on net WOA between the bioadhesive polymer and the biological substrate (Ponchel *et al.*, 1987) and has since been validated (Tobyn *et al.*, 1995). In this study the WOA dependence on the rate of probe withdrawal is depicted in Figure 3.8. Analysis of the observed effect of probe withdrawal rate was not a straightforward one. There was an initial increase in

the WOA in line with the withdrawal rate of the probe. However, this effect has a maximum of 1 mm/s, after which point, the WOA decreases almost linearly with the withdrawal rate of the probe. Although both Tobyn *et al.* (1995) and Ponchel *et al.* (1987) have independently demonstrated that there is a relationship between the rate at which the probe is withdrawn and the work of adhesion, the relationship was not a simple linear one. Nonetheless, the results obtained here suggest that varying the rate of probe withdrawal during mucoadhesion testing could have a profound effect on the observed mucoadhesive index of the polymers being assessed. Using similar technique, Smart (1991) observed that large deviations occur when the probe withdrawal rates were high and partially attributed this observation to the viscoelastic nature of the bond. Although such large deviations were not observed in this study, low probe withdrawal rates were used. Unless otherwise specified, in all further studies, the probe withdrawal rate was set at 2 mm/s.

**Figure 3.8: Work of adhesion dependence on the rate of probe withdrawal**

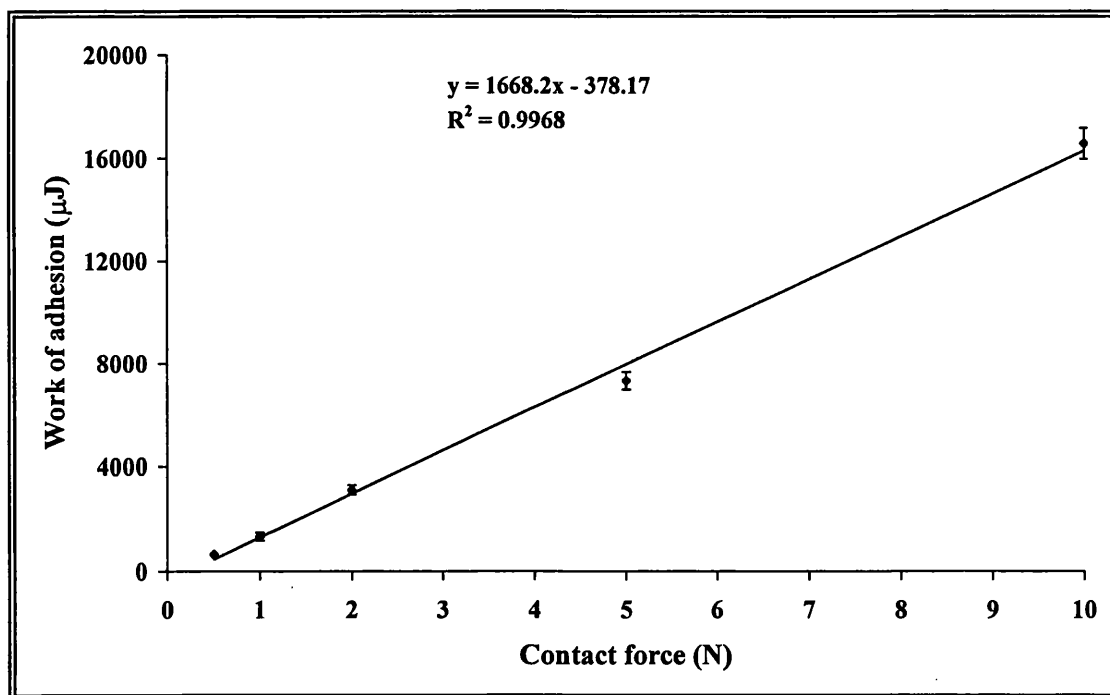


Data points are means  $\pm$  s.d.,  $n = 15$ . Compacts were made from 250mg Protanal LF 120L, contact time and force of 60s and 0.5N, respectively. 30%w/w PGM gel was used.

### 3.6.3 Effect of contact force on WOA

In Figure 3.9, the influence on observed mucoadhesion of increasing the applied (contact) force between the sodium alginate compact and PGM during the contact phase of the experiment is shown. Student t-test analysis demonstrated that increasing the applied force during the contact phase of the test leads to a significant ( $p < 0.01$ ) increase in the observed work of adhesion. Moreover, the increases in WOA were found to have a linear relationship with the applied force,  $r^2 = 0.9968$ . Similar work reported by Tobyn *et al.* (1995) also found that mucoadhesive performance of compacts of Carbopol<sup>®</sup> 934P significantly increased when the applied force was increased beyond 0.1N. However, unlike in this study, no clear linear relationship was established.

Figure 3.9: Effect of contact force on Work of adhesion



All compacts were made from 250mg Protanal LF 120L (2 ton for 60 seconds), contact time of 60 seconds, probe withdrawal rate of 0.2 mm/s,  $n = 20$ .

The increase in mucoadhesive performance of sodium alginate compacts with increase in applied force demonstrates the importance of quoting the applied forces used during a mucoadhesive test, when (and if) this system is used. In addition, this

parameter requires a close monitoring in order to avoid any further increases in the applied force during a test with a set applied force. During this set of experiments, the applied force did not vary by more than 2% of the set figure, with this value being less in the lower applied forces of 0.5N and 1.0N. The ability of lower applied forces to yield significant mucoadhesive values, has been explained in terms of a phenomenon, either a surface based or dependent on secondary chemicals (Ponchel *et al.*, 1987). It is conceivable that the pressing together of the two surfaces creates a more intimate contact between the rough surface of the mucus and the compact surface which is, due to the high compression forces, required to form these compacts, relatively smooth. Consequently, there is a greater surface area for interaction between the two surfaces. From Figure 3.9, the fitted curve/line may be written out in an equation to represent the mucoadhesive performance of sodium alginate compacts as follows:

$$\text{WOA} = 1.6682F - 0.3782 \quad r^2 = 0.9968 \quad \text{Eqn. 3.1}$$

where WOA (in mJ) is the work of adhesion dependent on the contact force F.

Park and Robinson (1985) reported that the increase in work of adhesion with respect to increase in the applied force might also depend on the nature of the polymer being examined. For a good bioadhesive polymer such as polycarbophil, the penetration of the hydrogel into the mucus layer was found to increase with the initial applied force. The relatively large deviations in WOA at higher applied forces, coupled with the ability of lower applied forces to yield significant mucoadhesive values meant that subsequent tests were performed at applied force of 0.5N, unless otherwise indicated.

#### 3.6.4 Influence of contact time on WOA

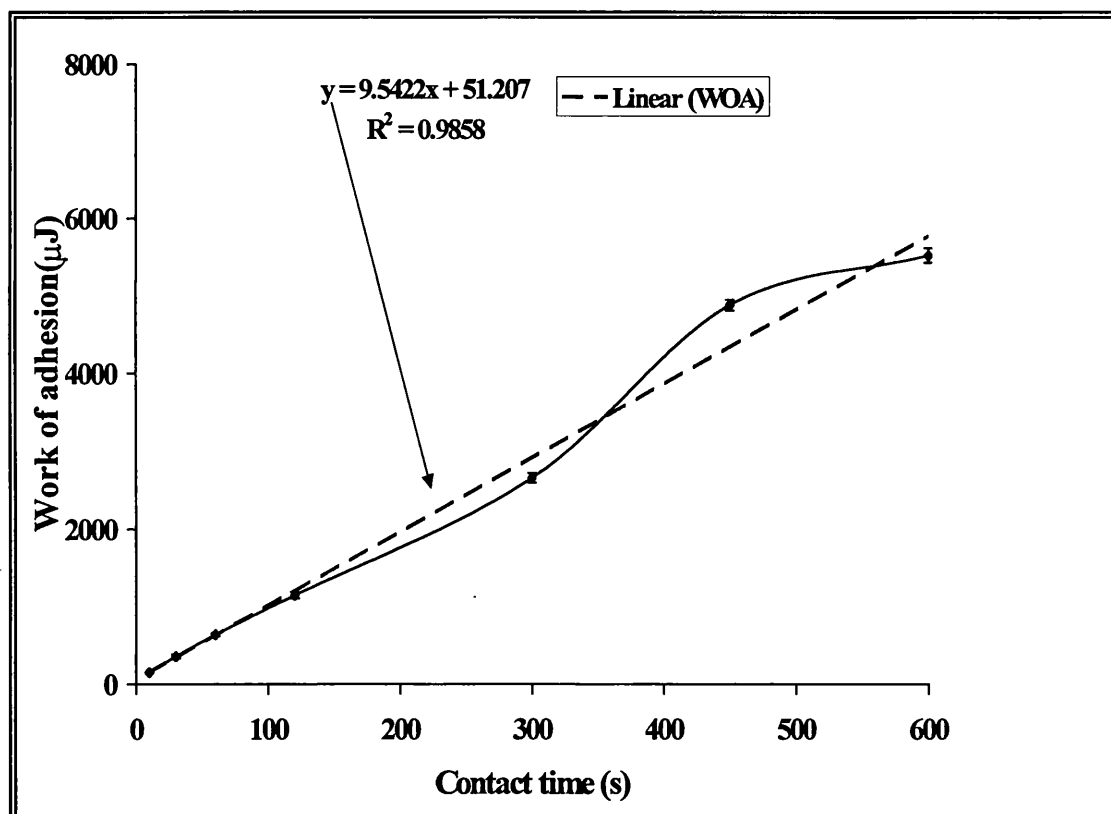
The results shown in Figure 3.10 indicate that there was a significant increase in the WOA (index of mucoadhesive performance) when the contact time between the sodium alginate compact and PGM was increased. Using a two-way ANOVA, these results were found to be statistically significant ( $p < 0.01$ ) between the time groups examined.

Further analysis of curve fitting (broken lines) suggests that the increase in mucoadhesion fit a near perfect linear equation (Excel '98). Similarly, the fitted line may be represented in equation 3.2.

$$\text{WOA} = 0.009542t + 0.05121 \quad r^2 = 0.9858 \quad \text{Eqn. 3.2}$$

where WOA has the same meaning as before and  $t$ , is the contact time in seconds.

**Figure 3.10: Effect of contact time on work of adhesion between alginate compacts and PGM**



Compacts were made from Protanal 250mg LF 120L (2 ton for 60 seconds), applied force of 0.5N, probe withdrawal speed of 0.2 mm/s,  $n = 20$

The increase in mucoadhesive performance of sodium alginate compacts with respect to contact time with PGM is not surprising if one considers some of the processes involved in mucoadhesion. Ghandi and Robinson (1988) considered the formation of an adhesive bond as a two step process. The first step involves the initial contact between the two surfaces, where chain interpenetration occurs while the second

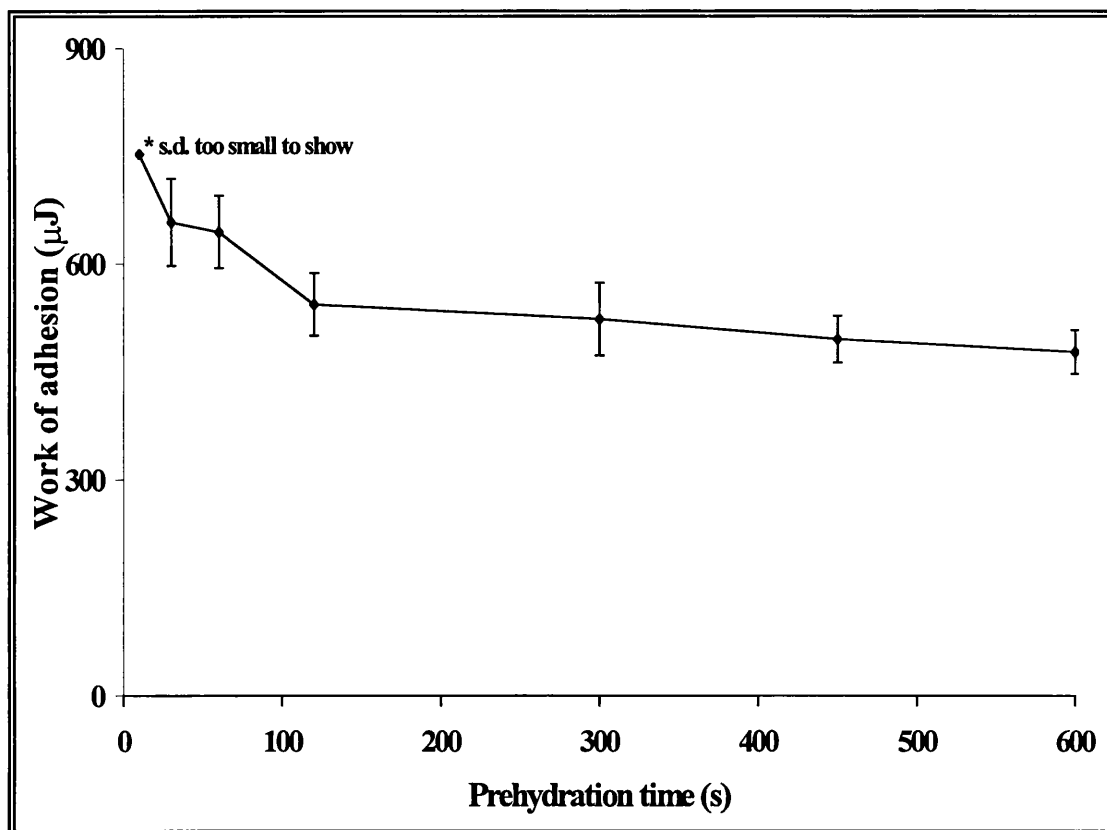
involves the formation of secondary bonds. As the time is increased, the interpenetration occurs to a depth sufficient enough to create the secondary bond such as hydrogen bonds and van der Waals forces. Using a technique based on Wilhelmy plate method for the assessment of mucoadhesion and two viscosity grades of sodium carboxymethylcellulose (SCMC) as the mucoadhesive polymer, Smart *et al.* (1984) demonstrated that the contact time has a profound effect on the mucoadhesive performance of these polymers. Similarly, Tobyn *et al.* (1995) demonstrated, using the same technique as used in this study, that the mucoadhesive performance of compacts of Carbopol<sup>®</sup> 934P was significantly increased when the contact time was increased.

### 3.6.5 Effect of prehydration of compacts on observed WOA

The effect of prehydration time on the observed bioadhesion is not as straightforward as the other parameter examined so far. Whilst Ponchel *et al.* (1987) have stated that there was an optimum prehydration time of around 10 minutes before the compact displayed maximum adhesive characteristics, Woolfson *et al.* (1992) and Tamburic and Craig (1997) have independently reported that systems with minimum prehydration displayed the greatest observed mucoadhesion. The mucoadhesion profile of sodium alginate compacts at various prehydration times is shown in Figure 3.11. The mucoadhesion performance compacts of Carbopol<sup>®</sup> 974P, Carbopol<sup>®</sup> 971P, and Noveon<sup>®</sup> AA-1 (Tamburic and Craig, 1997) and sodium alginate compacts from this study, were all found to decrease with increase in prehydration times. Sufficient water is necessary to hydrate the mucoadhesive polymer to expose the adhesive site for secondary bond formation, expand the gel to create pores of sufficient size, and mobilise all the flexible polymer chains for interpenetration. However, when the degree of hydration is high, as in increasing the prehydration time, adhesiveness is lost probably due to formation of a slippery non-adhesive mucilage in an environment of a large amount of water at or near the surface (Chen and Cyr, 1970).



**Figure 3.11: Effect of prehydration time on work of adhesion between alginate compacts and PGM**



Compacts made from 250mg Protanal LF 120L (2 ton for 60 seconds), contact time of 60 seconds, probe withdrawal rate of 2 mm/s, n = 20.

It is possible that the hydration required for maximum mucoadhesion to occur is obtained from the mucus (PGM, in this case) used for the test and therefore, any additional hydration causes the breakdown of the mucoadhesive bond(s).

### 3.6.6 The effect of different sodium alginate grades on mucoadhesion

The general physical and chemical characteristics of a putative mucoadhesive polymer may play a role in the mucoadhesive performance of formulations prepared from that polymer. In the preceding sections, the muco(bio)adhesion performance of different sodium alginate batches has been assessed in terms of their interaction(s) with different forms of mucin. It was shown that solution of sodium alginates rich in mannuronate content interacted better with mucin (rheological data) than their guluronate-rich counterparts. In Table 3.5 the effect of different sodium alginate

compacts interaction with Sigma mucin is shown. Unlike the rheological interactions, there is no clear effect of the monomer content on the observed mucoadhesive performance of the sodium alginate batches used. Nevertheless, there were significant ( $p < 0.01$ ) increases in mucoadhesive performances of all the sodium alginate batches examined with respect to increase in contact times. The mucoadhesive performances of SF 120 and LF 120L at 60 seconds contact time were significantly (one tailed, paired t-test,  $p < 0.05$ ) greater than those of LFR 5/60 and SF 60L at 60. In addition, the mucoadhesive performance of SF 120 was significantly greater than the other alginate samples used at all contact times. Although these pairs of sodium alginate batches had very different monomer contents, a close examination of the data sheet supplied by the manufacturer revealed a possible connection between these pairs. Firstly, the particle size of LF 120L and SF 120 are identical (mesh 120) and that of LFR 5/60 and SF 60L are also identical (mesh 60).

Given that all the compacts were made from the same weight (250mg) and compaction force and time (2 ton for 60 seconds), the observed closeness of the mucoadhesion performance of each pair may be attributed to the porosity of the compact. Although the exact mechanism by which these factors may affect the mucoadhesive performance is not clear, it may be postulated that the larger particle size of LF 120L and SF 120 means that the particles are held more loosely together within the compacts than those of LFR 5/60 and SF 60L. This in turn makes the hydration of these compacts (through the absorption liquid from the mucus surface) easier with the compacts of larger particle size. The superior absorption properties of the compacts of larger particles possibly leads to much stronger mucoadhesive bonds being created between the two surfaces. Secondly, the percentage dry matter (a measure on inherent water content) of each pair was found to be identical (91% and 90.6% for SF 120 and LF 120L, respectively) (85.8% and 86% for LFR 5/60 and SF 60L, respectively).

**Table 3.5: Mucoadhesion dependence on type of sodium alginate used**

Contact time (s)	Work of Adhesion ( $\mu\text{J}$ ), $n = 20$			
	<i>LF 120L</i>	<i>SF 120</i>	<i>LFR 5/60</i>	<i>SF 60L</i>
30	358.80 $\pm$ 40.23	456.28 $\pm$ 45.32	366.33 $\pm$ 38.64	372.46 $\pm$ 40.75
60	638.24 $\pm$ 58.36	800.24 $\pm$ 61.58	624.60 $\pm$ 51.43	601.64 $\pm$ 44.25
120	1144.47 $\pm$ 61.78	1357.67 $\pm$ 73.48	1078.45 $\pm$ 66.65	989.78 $\pm$ 56.34
300	2657.63 $\pm$ 65.81	3021.54 $\pm$ 88.26	2680.65 $\pm$ 71.44	2741.25 $\pm$ 78.50
450	4213.25 $\pm$ 101.24	4368.47 $\pm$ 128.32	4132.87 $\pm$ 96.72	3997.82 $\pm$ 84.75
600	5515.05 $\pm$ 121.15	5612.84 $\pm$ 132.54	5035.57 $\pm$ 114.67	4889.47 $\pm$ 102.48

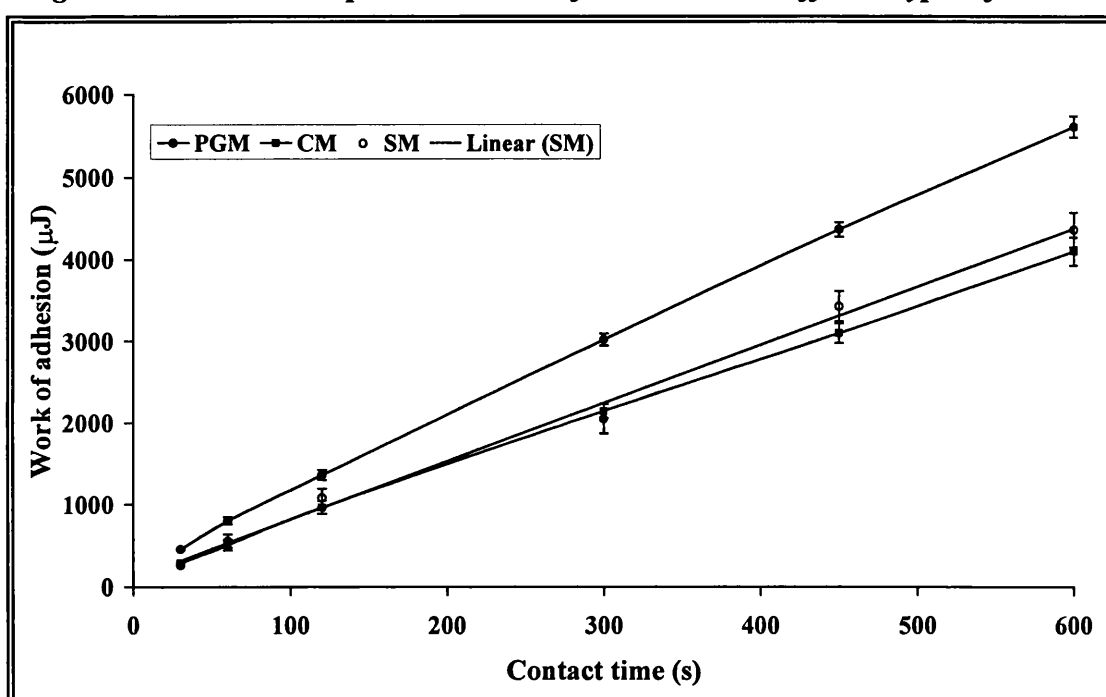
All compacts were made from 250mg of each alginate batch (2 ton 60 seconds), probe withdrawal rate of 0.2 mm/s, contact force of 0.5N, no prehydration.

### 3.6.7 Effect of different mucin (biological substrate) types on WOA

Another aspect of the bioadhesive bond between sodium alginate compacts and mucin, which may have profound effect on the observed mucoadhesion, is the nature of the mucin used. In this study, three forms of mucin (crude mucin, CM, mucosa tissue itself, SM, and porcine gastric mucin type III from sigma, PGM) preparations of which have been described above were assessed. In Figure 3.12, the WOA profiles, with respect to contact times, of adhesion between these mucin types and sodium alginate compacts (from Protanal SF 120) are shown. There were significant differences ( $p < 0.01$ ) between the three types of mucin used. The WOA of PGM, for example, was consistently higher than those of CM and SM (paired t- test,  $p < 0.001$ ).

However, there were no such differences between SM and CM, although the deviations were much higher in SM than were in CM. The deviations in work of adhesion values obtained with SM might have arisen as a result of using different pieces of tissues from different porcine sources. On the other hand, the relatively low deviations amongst CM values may be accounted for by the pooling together of all the mucus extracted from different porcine sources thereby minimising the deviations.

**Figure 3.12: Relationship between work of adhesion and different types of mucin**



All compacts were made from 250mg Protanal SF 120, contact force of 0.5N, probe withdrawal rate of 0.2 mm/s, n = 15

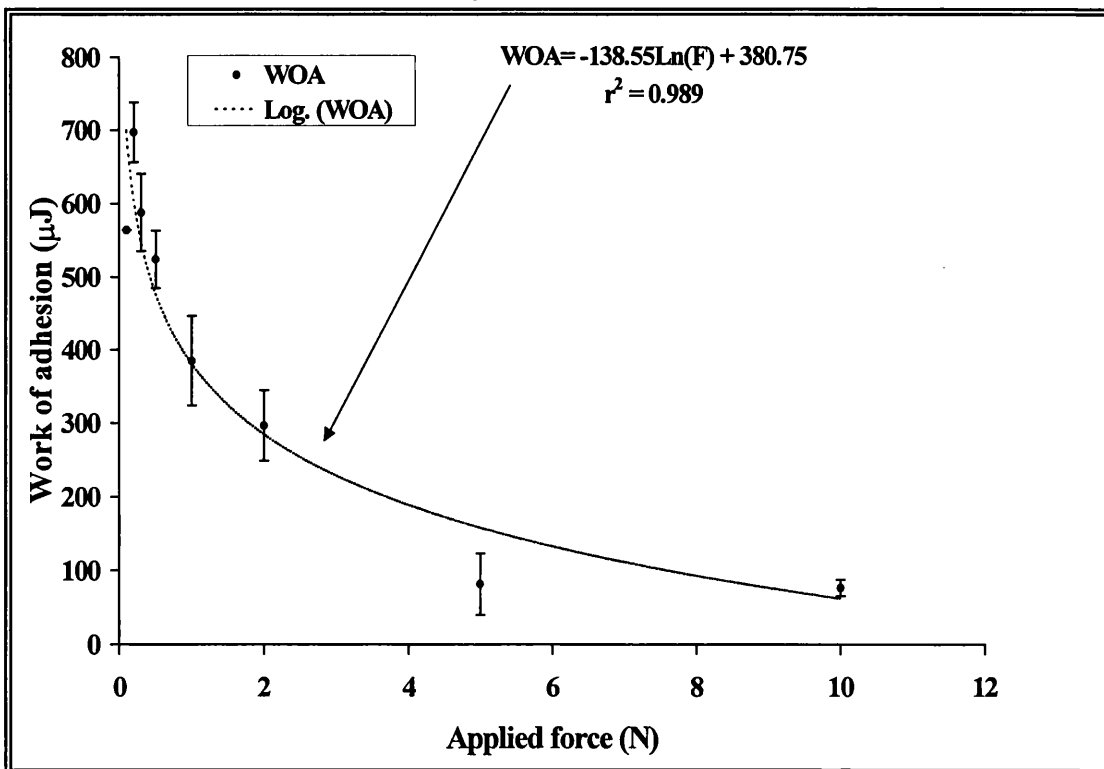
### 3.7 Mucoadhesive performance of liquid formulations of sodium alginate

The effect of formulations of sodium alginate on the work of adhesion between alginate batches and mucin preparation was examined by the inclusion of the work of adhesion between 2%w/w sodium alginate preparations. Amongst the parameters examined were the effect of mucin concentration (15%w/w and 30%w/w), contact force applied, and the nature of the polymers themselves.

### 3.7.1 Effect of contact force on the mucoadhesive performance of 2%w/w solution of Protanal LF 120L

In the previous sections, the effect of parameters such as the applied force on mucoadhesive performance of solid sodium alginate preparations has been discussed. The relationship between the mucoadhesive performance of 2%w/w sodium alginate and PGM (Figure 3.13), unlike the solid formulation was found to generally decrease with increase in applied force. Two additional applied forces (0.1N and 0.2N) were necessary in order to obtain a much clearer pattern of events. Upon increasing the applied force from 0.1N to 0.2N, there was a significant increase ( $p < 0.001$ ) in the work of adhesion. However, the WOA began and continued to fall as the applied force was increased beyond 0.2N. This fall in WOA did not fit into a linear equation, but had a reasonably good Log fit.

**Figure 3.13: Relationship between the applied force and mucoadhesive performance of sodium alginate solutions**



2%w/w Protanal SF 120 was applied to the upper probe, contact time was 60 seconds, probe withdrawal set at 0.2 mm/s, 15% w/w PGM gel used,  $n = 15$ .

One plausible reason for such observation could be that there is a threshold of applied force (possibly 0.2N) for the optimum WOA between the liquid sodium alginate formulation and the mucin gel. Owing to the fact that the two surfaces were very hydrated and reasonably smooth, very little force was required to bring them into an intimate contact with each other. Further forces only served to squeeze the formulations out of contact with each other, thereby breaking their mucoadhesive bond.

Upon increasing the concentration of PGM gel to 30%w/w, there was general significant increase in mucoadhesive performance of the sodium alginate batches examined (Table 3.6). Such increases in WOA with increase in concentration of PGM, are consistent with the belief that (de)hydration plays a role in mucoadhesive bond through interpenetration. As the 30%w/w PGM gels contained half the amount of liquid of that of the 15%w/w, the gels appeared very sticky and the pre-set distance for separation between the two surfaces had to be increased to 30 mm, instead of the 20 mm used throughout this study. The increase in WOA with concentration of PGM does not reflect in doubling of the PGM concentration, as the WOA values were more than twice those of 15% w/w PGM. These increases could be due to the fact that with the 30%w/w PGM gel, there was less amount of liquid available to the two surfaces to cause the 'slipping out' of the bioadhesive bond.

**Table 3.6: Dependence of work of adhesion on both PGM concentration and alginate type**

<i>Sodium alginate batch</i>	<i>Work of adhesion (<math>\mu\text{J}</math>), <math>n = 15</math></i>	
	<i>15% w/w PGM</i>	<i>30% w/w PGM</i>
LF 120L	734.65 $\pm$ 48.71	1764.18 $\pm$ 74.97
SF 120	697.18 $\pm$ 52.64	1434.01 $\pm$ 67.78
SF 60L	756.43 $\pm$ 57.21	1827.45 $\pm$ 77.07

Contact force = 0.2N, probe withdrawal speed = 2 mm/s, contact time = 60 seconds.

Absorption of liquid from the mucin surface to the alginate preparation was therefore greater, leading to a stronger bioadhesive bond being formed between the two surfaces. In addition, there were no significant differences between LF 120L and SF 60; however, these two alginates showed significantly higher WOA values than SF 200 (paired t-test,  $p < 0.01$ ).

### 3.8 Discussion and Conclusions

These results indicate that there are substantial rheological differences between different types of mucin, which in turn have implications on the associative interactions with different grades of sodium alginate. These associative interactions between water-soluble polymer and mucin glycoproteins can be evaluated by comparing the viscoelastic properties of mucin-polymer mixtures against those of the pure components. The ability of the higher concentrations of mucins to interact with lower concentrations of sodium alginates, evidenced by the increases in the storage moduli and decrease in loss tangent ( $\tan \delta$ ) values of the mixes, indicates that the alginate molecules possess higher numbers of interacting sites than do mucins, and can form cross-links between the mucin chains leading to gelation at sufficiently high mucin concentration. The nature of the binding sites remains unclear.

Temperature dependent rheological synergy between grades of sodium alginate and different types of mucin has also given an insight into the thermogelation event. Although not previously elucidated, it may be speculated that the thermal energy driven uncoiling and recoiling of the mucin glycoproteins might have contributed to the cross-linking of the mucin binding sites by alginate molecules. However, the exact mechanism(s) by which this binding occurs may require, perhaps, a more sophisticated approach. This approach of thermogelation of polymer-mucin mixes has previously been shown to be of importance (Tamburic and Craig, 1997) at least, to the understanding of some of the controlling factors of this type of gelation. However, the pharmaceutical implications of such events remain to be seen. Clearly, both the isothermal and temperature-dependent rheological examinations have demonstrated that there is a strong molecular interaction (possibly interpenetration)

between sodium alginate and mucin of various purity, with the underlying implications of mucoadhesion.

The use of texture analysis has contributed to the elucidation of some of the parameters, which may be responsible for the mucoadhesive performance of dosage forms. Whilst there has been a clear demonstration of the relationship between parameters such as the contact time, the applied force and the pre-hydration time and the subsequent mucoadhesive performance of sodium alginate compacts, this was less clear with respect to the liquid formulation. In the case of solid formulation, greater forces were required to establish an intimate contact with mucin for other phenomena of bioadhesion (such as interpenetration and hydration) to occur. However, such forces were not necessary in the case of liquid formulations, as the two surfaces easily form a bioadhesive bond due to the nature of the bioadhesive. As observed with the rheological data, the type of mucin used had a profound effect on the bioadhesion of the sodium alginate compacts, with the PGM yielding the greatest WOA with these compacts.

These results have highlighted some of the factors involved in the formation of bioadhesive bonds between a biological surface and a bioadhesive polymer. Furthermore, these results have indicated the potential use of sodium alginates as bioadhesives, a property of which, is examined in more detail in the next chapter, using a bioadhesive model specially designed for the purpose.

The only drawback of using this method is that it does not seem to be very sensitive to differentiate alginates with widely varied molecular weights and G/M content, even though there were significant differences in the rheological studies. This could be due to the fact that this method relies more on the surface properties of the bioadhesive polymer (compacts) rather than the molecular interactions as in rheological measurements.



**CHAPTER 4: AN EVALUATION OF ALGINATE ADHERENCE TO PORCINE  
OESOPHAGUS**

#### 4.1 Introduction

The bioadhesive properties of sodium alginate have been an area of contention, dependent on the literature source. Peppas and Buri (1985) have proposed that a good bioadhesive must have strong hydrogen bonding groups (-OH, -COOH), strong anionic charges, high molecular weight, and sufficient flexibility to penetrate the mucus or tissue crevices. Sodium alginate appears an excellent candidate for bioadhesion, according to these criteria. However, there still remain questions over the bioadhesive nature of sodium alginate. For example, while Charraeu *et al.* (1993) have stated that all alginates are 'poor' bioadhesives, Smart *et al.* (1984) and Chen and Cyr, (1970) ranked sodium alginates as 'excellent' bioadhesive. Not surprisingly, these discrepancies stem from the fact that there is lack of a universally standardised method of bioadhesion, as many methods are available which may not necessarily yield equivalent results (Park and Park, 1980).

The extrapolation of the behaviour of a bioadhesive from a test to its performance *in vivo* is not a straightforward one, since testing is generally made under a controlled environment that is different from in-use conditions (Park and Park, 1990). In addition, each test measures a particular aspect of bioadhesion and a particular property of a bioadhesive while the actual *in vivo* performance of a bioadhesive depends on various interdependent properties. Although certain test methods represent the actual *in vivo* performance of a bioadhesive better than others do, it is not clear what parameter is most suitable for evaluating the *in vivo* bioadhesive performance. It is therefore of paramount importance that various properties of a bioadhesive should be measured, and the parameters obtained compared with the actual (if known) *in vivo* performance of the bioadhesive. The performance of a bioadhesive can be evaluated by various parameters such as the adhesion strength, or duration of adhesion. Previously used methods for the assessment of the bioadhesive/mucoadhesive for the two aforementioned parameters have been discussed in Chapter 1. While the adhesive strength measurement is probably the most direct way to quantify the bioadhesive performance, perhaps the most important property of a bioadhesive is to maintain satisfactory performance in the actual in-use condition for a desired period of time. Thus the *durability* of a bioadhesive may be

the ultimate parameter that should be used to compare various bioadhesives, since the durability does not solely depend on the adhesive strength alone. The durability of bioadhesives may be evaluated by changing the experimental conditions such as changing the pH, ionic strength temperature, humidity, water content, etc. (Park and Park, 1990).

In the preceding chapter, the use of rheology to assess the alginate-mucin interactions has been discussed. The insight gained from that chapter which included (amongst other things) the understanding of some of the possible controlling factors of alginate-mucin interactions led to the further testing of such factors. With the exception of adhesion to the skin, very little attention has been given to other biological substrates. One such biological substrate is the oesophagus. Limited information is available on the adhesion to the oesophagus, although, Iooss *et al.* (1995) have reported the use of an in-house technique to assess the adhesive performance of some water soluble polymers to the oesophagus, with very encouraging results. In addition, reports on the adhesion of solutions of polymers to biological substrates are rare, and more so with respect to the adhesion to the oesophagus.

To this end, a simple 'in-house developed' *in vitro* method was used to assess various alginate grades (in terms of their molecular weights, viscosity, and mannuronate contents).

#### **4.2 *Scope and rationale of study:***

The aims of this study were to assess the adherence of solutions of several grades of sodium alginate of differing chemical compositions to porcine oesophagus. To this end it was necessary to develop a simple, yet robust model to evaluate these alginates. In addition the effects of some experimental parameters on the extent of the bioadhesive properties of these sodium alginates were also examined.

### 4.3 *Materials and methods:*

#### 4.3.1 *Materials:*

Five sodium alginate batches (Protanal SF 60L, SF/LF 40, LF 120L, H120 L and SF 200) selected from a total of nine batches, according to their polymer-mucin interactions from the previous Chapter, were used in this study. In addition, two more sodium alginate batches Protanal LFR 5/60 and LF 10L, also supplied by Pronova Biopolymers a.s., Drammen, Norway, were used as received. Other materials together with their batch numbers and suppliers are as listed below:

*From BDH, Poole, Dorset, UK*

Material	Batch number
Potassium dihydrogen orthophosphate	146A552725
Sodium hydroxide	4X500GMS
Sodium bicarbonate	346684A
Sodium chloride	5531500A
Potassium chloride	TA714938
Calcium chloride dihydrate	6346160N
Sodium dihydrogen phosphate dihydrate	4974520J

*From Sigma Chemicals, UK*

Pork mucin type II (crude)	140H4374
Fluorescent-labelled latex beads (0.026 $\mu$ m mean diameter)	94H0873

*From Avocado chemicals, UK*

Sodium Fluorescein	A2850A
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Fluorescent-labelled sodium alginate LFR 5/60 was kindly donated by Dr. Peter Ross, Dundee University, UK.

In addition, Table 4.1 lists some of the main properties suspected of having some influence on the bioadhesive properties of the alginate grades used in this study.

### **4.3.2 Methods:**

#### **4.3.2.1 Preparation of biological substrate**

Porcine oesophageal tubes were obtained fresh from the abattoir and were maintained at 2°C, using carbon dioxide (dry) ice, during transport. The oesophageal tubes were cleared of any undigested food and other materials by gently and carefully flushing them with cold de-ionised water, making sure that there was no damage to the tissues. The surrounding muscular layers were removed, exposing the oesophageal tube. The top, middle, and bottom segments (approximately first, second, and third 10 cm lengths, respectively) parts were identified and sectioned accordingly. The isolation of the oesophageal tubes was performed at 4°C in attempt to retain most of the tissues' integrity.

The pieces of oesophageal tissues were immediately flash-cooled in liquid nitrogen before they were kept at -20°C until required. A recent report (Young and Smart, 1998) has shown that oesophageal tissues prepared this way maintain most of the epithelial profiles. This is because the formation of water crystals, which often occurs during slow freezing, is prevented by the flash-cooled technique. Prior to each experiment, previously frozen tissues were allowed to slowly thaw out in the refrigerator overnight before use.

#### **4.3.2.2 Preparation of solvents for bioadhesive tests**

One litre batches of artificial saliva with similar ionic concentrations, mucin content and pH to natural saliva were prepared on each day of the experiment. The molar ratio of sodium to calcium ions is known to be critical to the swelling of alginate (Haug and Smidsrød, 1965; Smidsrød, 1996) and so particular attention was paid to the concentration of those ions. Phosphate, potassium, and chloride ions were also

identified as being in significant quantities. The formula for the artificial saliva is shown in Table 4.2.

**Table 4.1: Some significant properties of the sodium alginate used in the study**

<i>Polymer</i>	<i>Viscosity of 1% Solution (mPa.s)</i>	<i>Molecular weight (kDa)</i>	<i>FM</i>	<i>FGM.MG</i>	<i>NG&gt;1</i>
LF 120L	121	225	0.556	0.196	6.9
SF 60L	380	320	0.562	0.184	7.4
SF/LF 40	413	315	0.370	0.050	20.3
H 120L	950	350	0.540	0.190	7.0
SF 200	990	380	0.310	0.110	20.3
LFR 5/60	5.7	40	0.360	0.127	15.7
LF 10L	9.3	75	0.55	0.200	6

FM = Fraction of mannuronic acid residues, FGM.MG = Fraction of alternating sequences, NG>1 = Typical average length of guluronic acid blocks larger than 1.

The use of a phosphate buffer to adjust the pH of the solution would have introduced an unacceptably high concentration of sodium ions. Instead, the pH was brought within limits for saliva by careful adjustment of the sodium hydrogen carbonate content. Table 4.3 compares the principal ionic constituents of both natural and the artificial saliva and also compares the pHs. The figures for natural saliva are taken from Geigy tables. The mucin in solution provides a good growth medium for bacterial contamination and therefore the artificial saliva was freshly prepared, stored in a refrigerator, and used within 24 hours. To prepare the artificial saliva, sodium bicarbonate (0.42g), sodium chloride (0.43g), potassium chloride (1.49g) and sodium

**Table 4.2 Formula for artificial saliva**

<i>Ingredient</i>	Mmol/L	g/L
NaHCO <sub>3</sub>	5.0	0.42
NaCl	7.42	0.43
KCl	20	1.49
CaCl <sub>2</sub> .2H <sub>2</sub> O	1.5	0.22
NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	6.58	0.91
Mucin (type II)	-	2.7
De-ionised water to		1000 ml

dihydrogen phosphate (0.91g) were accurately weighed and dissolved in 600 ml of de-ionised water by vortex created by a mechanical mixer. Calcium chloride (0.22g) was then added. The weighed, dried pork mucin (2.7g) was dissolved in the salt solution with vigorous stirring. The solution was then made up to 1000 ml with de-ionised water. In order to minimise the breakdown of the mucin glycoproteins, the artificial saliva was kept on ice throughout the experiment, and was prepared fresh each day. The pH of the artificial saliva was found to be pH  $6.9 \pm 0.1$ .

Aliquots of 200 ml of the phosphate buffer was prepared by dissolving 5.44g of potassium dihydrogen orthophosphate in 200 ml de-ionised water, giving a concentration of 0.2M. 50 ml of this solution was mixed with 39.50 ml of 0.2M sodium hydroxide (prepared by dissolving 1.6g sodium hydroxide in de-ionised water) and making up to 200ml with de-ionised water, giving a final pH of 7.4.

**Table 4.3 Comparison of the principal constituents between natural and artificial saliva**

<i>Principal constituents</i>	<i>Natural saliva</i>	<i>Artificial saliva</i>
Sodium	19.2 mmol/L* (5.2-36)	19 mmol/L
Potassium	19.9 mmol/L* (11-27)	20 mmol/L
Calcium	1.55 mmol/L** (1.15-2.75)	1.5 mmol/L
Chloride	17 mmol/L**	30.42 mmol/L
Phosphorus	204 mg/L** (120-288)	210.6 mg/L
Mucin	2.7 g/L*** (0.8-6.0)	2.7 g/L
PH	6.4** (5.8-7.1)	6.9

\* mean of values for resting saliva and saliva at minute volume of 2 ml; \*\* value for total adult saliva; \*\*\* value from stimulated saliva, ( ) range values

#### **4.3.2.3 Preparation of sodium alginate solutions**

Serial dilutions (1 in 20 to 1 in 100) of the fluorescent-labelled LFR 5/60 (5 mg/ml) were made yielding LFR 5/60 concentrations of 0.25 mg/ml to 0.05 mg/ml, respectively. A calibration curve was then constructed, following fluorescence determination using a Perkin Elmer Spectrophotometer PE-204, coupled with a Perkin Elmer 150 Xenon power supply and PE-56 recorder. Because the calibration curves were constructed from the emission fluorescence from fluorescein-alginate mixture, the effect of quenching of fluorescence by sodium alginate had been taken into account. All fluorescence measurements were conducted at 490 nm excitation and 515 nm emission wavelengths.



In similar experiments, sodium alginate-fluorescein solutions containing 2% w/w sodium alginate and  $4 \times 10^{-3}\%$  of sodium fluorescein were prepared by dissolving the alginate powder in a solution containing 1mg sodium fluorescein (Avocado Chemicals, U.K) in 25ml de-ionised water. Following a 24-hour storage of this solution (container wrapped in aluminium foil to minimise loss of fluorescence), a 1:100 dilution of this solution was made as working stock, from which further dilutions were made for the construction of calibration curves. In the third exercise, fluorescein-labelled latex beads (0.026  $\mu\text{m}$  mean diameter) were diluted to give concentrations similar to those described above. Following the construction of calibration curves for all three fluorescein sources, mixtures of sodium alginate with fluorescein were made by adding the sodium alginate powder to solutions containing sodium fluorescein (in the case of the sodium fluorescein-labelled and sodium fluorescein powder). Alternatively, the labelled LFR 5/60 was added to the sodium alginate solution of interest. The final label concentration in each case was  $4 \times 10^{-3}\%$ .

#### ***4.3.2.4 The humidity chamber (controlled experimental environment)***

The chamber ('in-house built), depicted in Figure 4.1, was constructed from a humidity controlled oven. In its current state, the chamber consists of an insulated casing, an underneath piping system of cold and hot water (depending on the temperature settings) and a fan. The water vapour created conveys to the temperature and humidity settings and allows the required humidity to be achieved within 10 minutes of switching on the chamber. Two pieces of wicks attached to the sensors for the dry and wet bulbs ensure that the required humidity is maintained throughout the experiments. The required temperature and relative humidity (%RH) are manually set on the panel outside the chamber and the 'actual' or the attained temperature and humidity are digitally displayed on the same panel. In addition, the temperature and the humidity inside the chamber were monitored throughout the experiment with the aid of a 'dry'/'wet' bulb hygrometer placed inside the chamber. Comparisons of these sets of temperature and humidity readings were found to be in excellent agreement, indication that only very little vapour (if any at all) escaped from the chamber. The entire experiments were conducted with the chamber firmly closed in order to maintain the attained humidity, and manual handling of equipment was done

*Figure 4.1: Modified (in-house built) humidity chamber*



*Top: The modified humidity chamber in its entirety*

*Bottom: Inside view of the modified humidity chamber*



through a window made out of a double perspex sheet and wearing a pair of arm length gloves. The inside window was constantly wiped using paper towels placed inside the chamber before the experiments commenced to clear the condensation build up. A separate water reservoir (of doubly distilled water) supplies the pipes within the chamber with water to create the vapour and humidity. Placed on the top of this chamber was a peristaltic pump, whose tube is passed through a small slit made out on the outside of the chamber and connected to the perspex plate and the test tissue. The other end of the peristaltic pump tubing is fed into a reservoir of the perfusing solvent, which was also placed on the top of the chamber. For simplicity, a schematic representation of this controlled chamber is shown in Figure 4.2.

#### ***4.3.2.5 Optimising the experimental conditions***

In order to minimise the number of the possible variables which could influence the adherence of sodium alginate solutions to the porcine oesophageal tissue, some exploratory exercises were carried out to optimise the experimental conditions. These included the relative humidity within the operating chamber, which was varied from normal atmospheric humidity to a relative humidity of 98%. Furthermore, effect of high relative humidity on the oesophageal tissue itself was examined by placing strips of the porcine oesophagus on the test perspex plate and monitoring any weight change over two hours at a relative humidity of 98% (the maximum permissible humidity within the chamber). In another exercise, the angle of inclination was also varied in order to examine its effects on the adhesion of the polymer to the tissue. With a set-up such as this one, it is conceivable to expect that the viscosity of the sodium alginate solution would somehow affect its adherence to the oesophageal tissue. However, standardising the viscosities of the sodium alginate solutions would require the addition of ions such as sodium, which would ultimately change the physical characteristics of the solutions. It was therefore decided that the experiments be performed on the solutions in their natural states (in de-ionised water). This also allowed the delineation of the effect of the inherent viscosity of each alginate sample to be assessed.

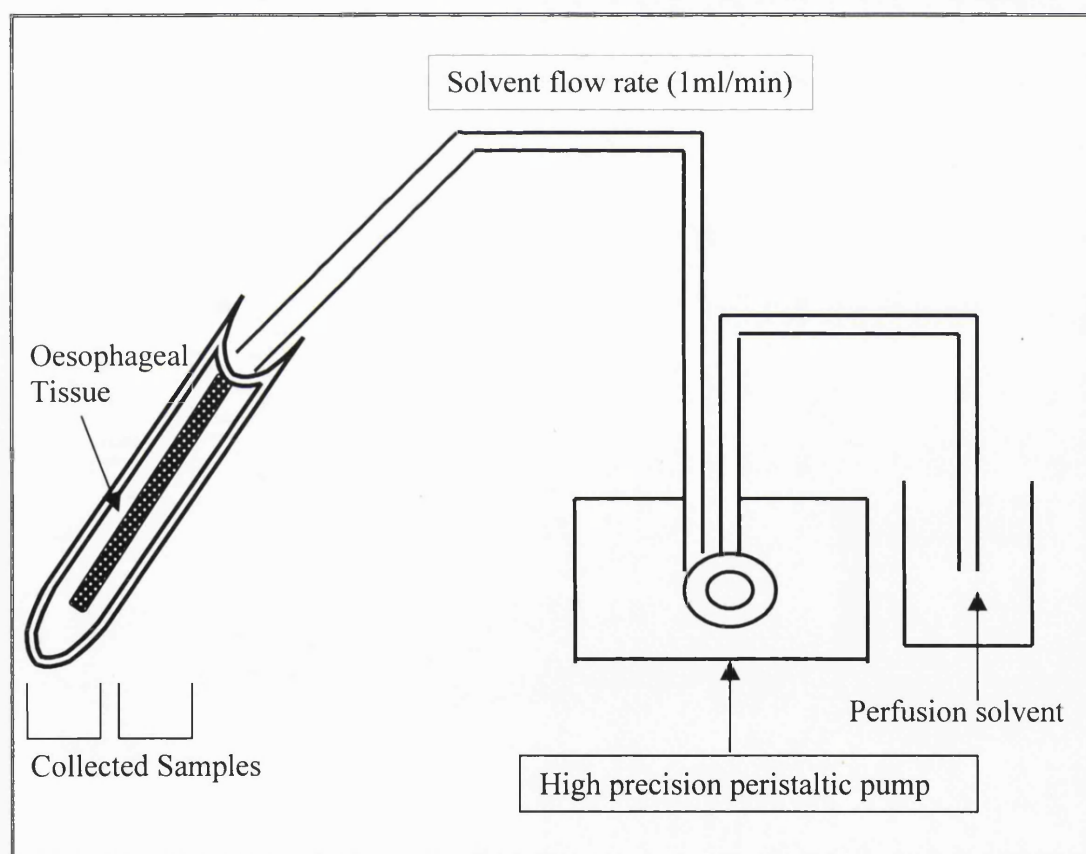
In another exercise the effect of each fluorescent label (fluorescent-labelled latex beads, fluorescent-labelled Protanal LFR 5/60, and sodium fluorescein-alginate mixtures) on the alginate solution and consequently, on the adhesion of the polymer to the tissue was examined by measuring the quantity of polymer adhered to oesophageal tissues at a 3-minute intervals, for 30 minutes. This exercise was necessary in order to decide on the suitability of each label, based on its ability to form a homogeneous mix with sodium alginate solution, cost, and the ease of handling and availability. It was anticipated that the adherence of sodium alginate to porcine oesophageal tissue might be subject to zonal variations along the entire length of the oesophagus. A set of experiments were therefore performed, whereby the adherence of a sodium alginate solution to different segments of the porcine oesophagus (designated top, middle, or bottom 10 cm) was assessed, using a single label, and at RH of 91%. The temperature within the chamber was kept at 37°C throughout each experiment. Finally, the effect of perfusing solvent on the adherence of alginates to the oesophageal tissues was also examined, by using three solvents, namely, de-ionised water, phosphate buffer pH 7.4, and artificial saliva, preparations of which, are described above.

#### ***4.3.2.6 The model bioadhesive test***

Prior to each experiment, the oesophageal tissue was thawed overnight (at 4°C) The tissue was carefully sectioned, longitudinally into strips of 90mm length and 15mm width. A strip was then carefully mounted into a groove cut into a perspex plate (using a pair of tweezers), exposing the inside (mucosa) of the oesophagus (Figure 4.2). A known weight of the 'labelled' sodium alginate mixture was applied to the mucosa with the perspex plate in a horizontal position, and was left in that position for 5 minutes. Any polymer dripping off the perspex plate was collected and accurately weighed. The polymer-coated oesophagus was then inclined at an angle of 14° to the horizontal, for a further minute, still collecting any polymer dripping off the plate. The polymer was then washed with a suitable solvent at a rate of 1 ml/min and the eluent collected at 3 minute intervals, over a 30-minute period. The collected eluents were then analysed for sodium alginate content at each time point of elution, using a fluorescence spectrophotometer at 490 nm excitation and 515 nm emission

wavelengths. This was made possible by reference to previously constructed calibration curves. A calibration curve was constructed for each labelled sodium alginate sample used. The percentage cumulative polymer recovered was subtracted from the original amount placed on the tissue. This value (the amount adhered to the tissue) was then used to express the bioadhesive potency of the individual sodium alginate. A high percentage cumulative value indicates a low bioadhesive potency of that polymer and vice versa. A fresh strip of oesophagus was used for each experiment, including all repeats.

**Figure 4.2:** Diagrammatic representation of the bioadhesive model set-up



In addition, the rate at which the polymer was perfused with the suitable solvent was assessed. It was found that flow rate above 2 ml/min created turbulence on the surface of the polymer. All experiments were therefore performed using a perfusion

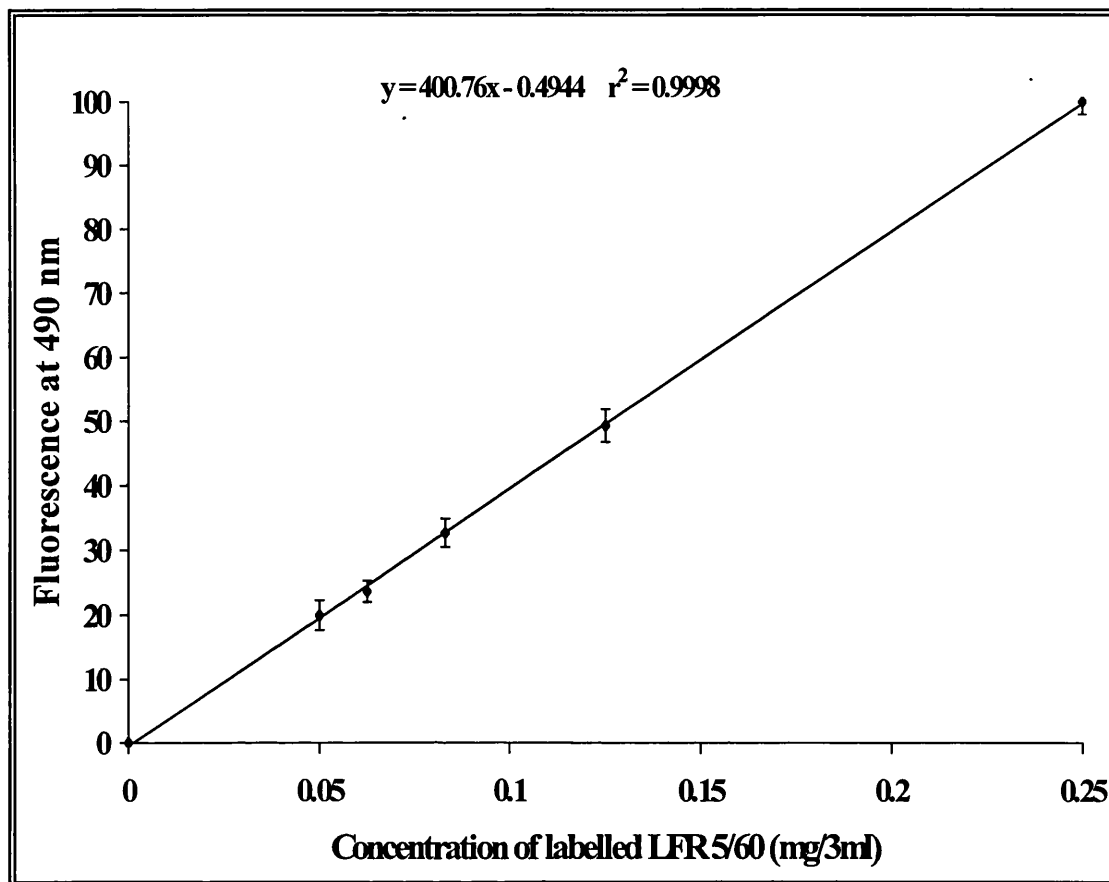
rate of 1 ml/min. In general, the amount of polymer applied to the tissue was  $1.0 \pm 0.15\text{g}$ .

#### **4.4 Results:**

##### **4.4.1 Calibration curve for the determination of amount sodium alginate eluted**

The construction of the calibration curve for each sodium alginate sample was necessary so that the amount of the polymer eluted off the oesophageal tissue could be back calculated. The effect of quenching of fluorescence by the presence of the polymer was accounted for in the individual calibration curves. In Figure 4.3, a typical calibration curve for fluorescent-labelled Protanal LFR 5/60 solution is shown, as a representative of the calibration curves constructed for each alginate sample. The dilute nature of the alginate sample allows a linear relationship between the fluorescence and the concentration of the alginate to be established, which obeys the Beer-Lambert law. In most cases, it was necessary to dilute further, the eluted and the collected samples of the labelled polymer and the actual amount back calculated. This was because the concentration of the label (and of the polymer) in the first few minutes of the collection was far too high for the spectrophotometer to read accurately.

**Figure 4.3: Calibration curve of a fluorescent-labelled alginate solution**



Data points are means  $\pm$  standard deviation, n = 5

#### **4.4.2 Effects of experimental conditions on the adherence of alginates to oesophageal tissues**

##### **4.4.2.1 Effect of the type of label used on bioadhesion**

Due to the number of experimental repeats required for good reproducibility of data for statistical analysis and the small quantity of the labelled LFR 5/60 available, the use of other labels was necessary. Labelling of each sodium alginate batch is a difficult and lengthy process with very little success rate, and large molecular-weight sodium alginates tend to pose much more problems due to the large numbers of monomers. Furthermore, sodium alginate batches of medium to high molecular weights tend to have high intrinsic viscosity, which minimises the success rate of labelling (Personal communication with Dr. Peter Ross, Dundee University). The other two 'labels' used were sodium fluorescein (powder form) and fluorescein-coated latex beads (mean diameter 0.026  $\mu\text{m}$ ), the method of preparing the alginate

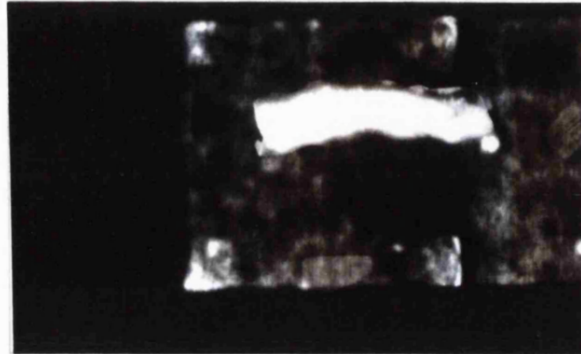
solutions is as previously been described in section 4.3.2.3. It was necessary to use very small particle-sized fluorescently labelled latex beads in order to ensure a complete mixing of the beads with sodium alginates and also, to prevent any possible phase separation between the beads and sodium alginate.

Figures 4.4a–c show pieces of oesophageal tissues, which have previously been coated with the three ‘labels’ and washed continuously (with de-ionised water) for 18 minutes. In addition, the amount of alginate recovered at each designated time point, using these ‘labels’ was also compared (Table 4.4). These results indicate that there were no significant differences between the three ‘labels’ used ( $p < 0.025$ ). It was thought that the mixing together of alginate and the ‘labels’, especially, the fluorescently labelled latex beads and sodium fluorescein would lead to phase separation between the alginate molecules and fluorescein molecules in solution. Furthermore, it was suspected that the fluorescein molecule would be ‘washed off’ leaving the alginate on the oesophageal tissues, thereby giving inaccurate results.

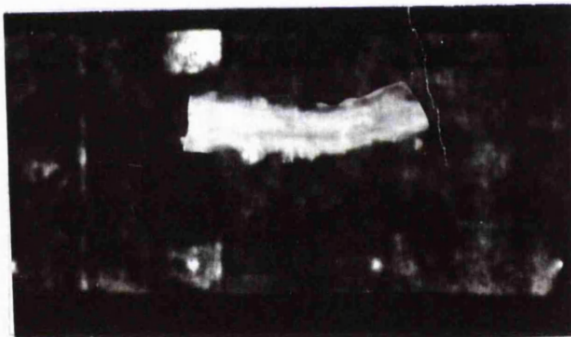
However, this was found not to be the case, as the alginate and ‘labels’ appeared to have been thoroughly mixed, remained in one phase, and eluted with alginate. Therefore, sodium fluorescein was preferentially used, unless otherwise indicated. The choice was based on the commercial availability and ease of handling. In some cases the time-dependent disappearance of the label from the mucosa of the oesophageal tissue (which coincided with the recovery in the collecting containers) were correlated with figures obtained by taking photographs of the tissue with ‘labelled-alginate’ at set time points. Photographs were obtained by using a Polaroid™ camera (loaded with an ISO 3000/36° 667 Polaroid™ film) mounted onto an UV chamber whose wavelength had been set at 365 nm. The presence of the label (and of the sodium alginate) on the mucosa was still visible after 30 minutes of washing, in most cases, an indication of adherence of sodium alginate onto porcine oesophagus.



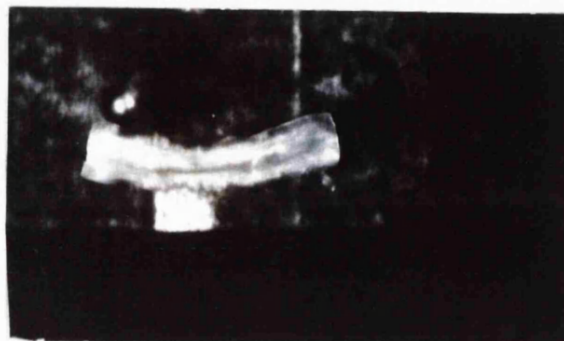
*Figure 4.4: Comparison of the effectiveness of the three labels used (SF 60L after 18 minutes of washing with de-ionised water)*



*Figure 4.4a: Label with Latex beads*



*Figure 4.4b: Label with sodium fluorescein*



*Figure 4.4c: Label with 'Labelled' LFR 5/60*

**Table 4.4: Comparison between markers for alginate adherence to porcine oesophageal tissues, at 91% RH and 14° angle**

Elution Time (min)	Percentage of sodium alginate adhered to oesophageal tissue		
	Labelled LFR 5/60	Latex-beads	Sodium fluorescein
6	83.56 ± 5.4	84.09 ± 3.9	85.14 ± 4.5
12	78.92 ± 4.6	79.10 ± 5.2	78.04 ± 3.7
18	75.23 ± 5.2	77.65 ± 4.2	76.45 ± 4.6
24	73.41 ± 5.1	76.07 ± 5.3	75.55 ± 4.8
30	71.90 ± 6.3	74.84 ± 4.7	75.10 ± 3.6

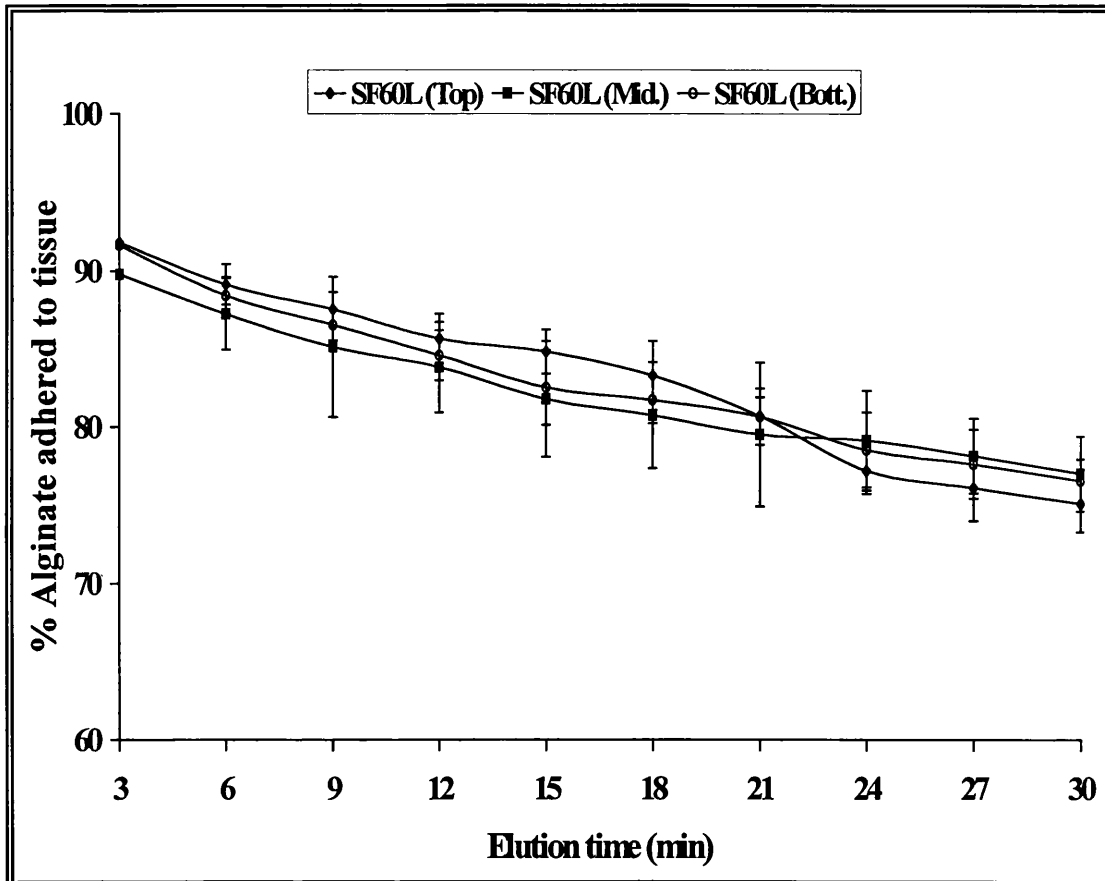
Data points are means and standard deviations of n = 5, 2%w/w Protanal LF 120 used, de-ionised water was used as the perfusing solvent.

#### 4.4.2.2 Effect of oesophageal tissue zonal differences on adherence of alginates

Given the length of the porcine oesophagus (30 – 40 cm) it was expected that there might be some histological zonal differences. These inherent differences may play a role in terms of transit of food and other materials to the stomach. If these assumptions were to hold true, then these may have implications on the adherence of sodium alginate to the porcine oesophagus as a whole. In this study, the adherence of alginates to designated segments of the porcine oesophagus was assessed. In Figure 4.5, the adherence of Protanal LF 120L to these three segments designated as the top, middle, or bottom 10-cm pieces of the porcine oesophagus is shown. There were no substantial differences between the adherence of sodium alginate to the different segments described. The variability in the data is more likely to have originated from inter-batch variability, rather than zonal differences, as tissues were from different porcine sources. These results indicate that the part of the porcine oesophagus had no

significant ( $p < 0.5$ ) effect on the adherence of sodium alginates to the oesophagus, and therefore, in all subsequent tests, the tissue pieces were randomly chosen. This minimised (and/or standardised) the variabilities between different alginate types due to zonal differences.

**Figure 4.5: Zonal adherence of sodium alginate to oesophageal tissue**



Data points are means  $\pm$  s.d. of  $n = 5$ , 2%w/w Protanal LF 120L, sodium fluorescein as marker, de-ionised used as the perfusing solvent

#### 4.4.2.3 Relative humidity and type of sodium alginate used

As part of an exploratory exercise to optimise the experimental condition, and to attempt to mimic the possible environmental conditions of within an *in situ* oesophagus, the bioadhesion of six sodium alginate batches were examined with respect to the relative humidity within the humidity chamber. In Table 4.5, the effect of varying the relative humidity on bioadhesion of these alginates is shown. In

general, the increase in %RH led to an increase in the amount of sodium alginate adhered to the oesophageal tissue. A one-way analysis of variance (ANOVA) of the effect of %RH indicates that there were significant increases in bioadhesion ( $p < 0.005$ ) when the %RH was increased from 60% to 91%, in all cases. Further statistical analyses of the data, using a two way ANOVA (balanced design with replication) to assess the significance (if any) of the same data set, of the monomer composition and the %RH on bioadhesion also indicated significance of the data, albeit at a lower significant value level ( $p < 0.01$ ). Relative humidity over 91% created too much water vapour within the chamber, making it difficult to operate. Therefore, 91% was chosen as the maximum %RH tolerable.

The exact mechanism(s) by which increases in %RH led to corresponding increases in the bioadhesive potential of sodium alginate is not known, nor has it previously been documented in the literature. The novelty of these experiments only leaves one to speculate on the probable cause of such increases in bioadhesion. A plausible explanation would be that at a higher relative humidity, the oesophageal tissue becomes more receptive to sodium alginates. This increase in adherence with respect to increase in relative humidity was also found to be more pronounced with alginates rich in mannuronate. Given the undoubtedly high humidity within the *in situ* oesophagus, these results may have profound implications in the drug delivery to the oesophagus.

In Table 4.6, the summarised bioadhesive data following washings with various solvents is shown. Clearly, there seems to be a good correlation between the polymer type (in terms of its chemical composition) and the ability to adhere to the porcine oesophagus. A close examination of this Table 4.6 reveals that sodium alginate Protanal SF 60L yielded the most promising adhering property, followed by Protanal LF 120L. Further examination into the chemical make-up of these two batches in particular (Table 4.1) indicates that these batches are both rich in mannuronate content. In addition, these alginate batches have appreciably moderate to high viscosities (360 mPa.s and 121 mPa.s of 1% solutions, respectively). There was no significant difference between these two alginate batches ( $p < 0.01$ ) following washes with all the 3 solvents used.

**Table 4.5: Adherence of sodium alginate dependence on relative humidity**

Type of sodium alginate used	Total alginate (%) adhered after 30 minutes, 14° angle		
	Atmospheric	60%	91%
Protanal H 120L	49.13 ± 4.7	55.53 ± 2.8	64.72 ± 4.7
Protanal LF 10L	33.60 ± 3.8	47.35 ± 4.5	49.55 ± 2.7
Protanal LF 120L	51.5 ± 2.9	67.8.9 ± 3.6	75.10 ± 1.6
Protanal SF 60L	65.83 ± 2.4	74.88 ± 3.8	81.56 ± 2.1
Protanal SF 200	34.79 ± 7.6	48.95 ± 5.2	54.72 ± 3.5
Protanal SF/LF 40	31.77 ± 6.4	46.44 ± 4.8	59.40 ± 2.5

Tissues were perfused with de-ionised water, n = 5.

With the exception of the perfusion with phosphate buffer, this correlation holds true, as Protanal H 120L (54%M, 950 mPa.s) was the third ranked sodium alginate in terms of its ability to adhere to porcine oesophageal tissue, although it has the greatest viscosity values. In contrast, however, alginate batches relatively poor in mannuronate content generally yielded lower adherence values. For example, SF 60L (56.2%M) showed the best overall bioadhesive properties while SF 200 (31.0%M) showed considerably weaker adhesion, although it has a very high viscosity value (990 mPa.s of 1% solution). This may indicate that viscosity alone does not directly affect the bioadhesion of sodium alginates to porcine oesophagus. Nonetheless, very low viscosities values generally yielded poor bioadhesion, as exhibited by LFR 5/60 (34%M, 5.6 mPa.s of 1% solution). In addition, LFR 5/60 showed a poorer bioadhesive properties than a similar sodium alginate batch in terms of mannuronate content, SF/LF 40 (37%M) which has a much higher viscosity value (410 mPa.s of 1% solution). The implication of this, therefore, is that viscosity may play a role in

the bioadhesion of these sodium alginate batches to porcine oesophageal tissue, albeit indirectly. Criteria for any influence of viscosity on the bioadhesion of the alginates thereof, should include amongst others factors, a reasonably high mannuronate content, and an appreciably high viscosity value.

**Table 4.6: Effects of different perfusing solvents on the adherence of sodium alginates to porcine oesophagus**

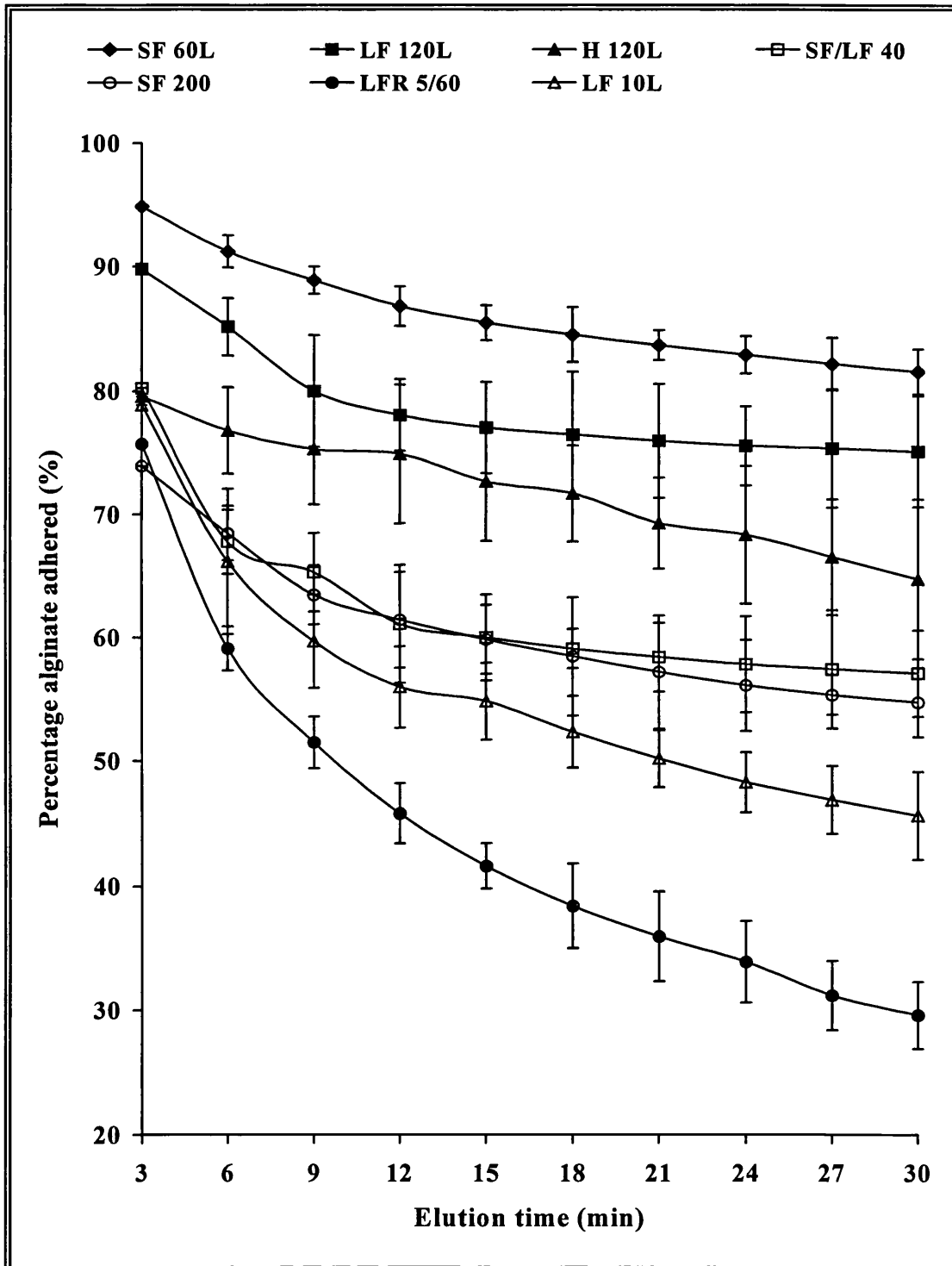
Sodium Alginate	Artificial Saliva		Phosph. Buffer pH 7.4		De-ionised Water	
	% Adherence	Rank	% Adherence	Rank	% Adherence	Rank
LF 120L	87.51 ± 1.8	1	62.36 ± 1.8	2	75.10 ± 3.3	2
SF 60L	85.43 ± 1.1	2	67.57 ± 3.5	1	81.56 ± 4.9	1
SF/LF 40	21.77 ± 6.4	5	47.44 ± 4.8	3	59.40 ± 2.5	4
H 120L	67.88 ± 4.2	3	21.68 ± 2.6	6	64.72 ± 1.2	3
SF 200	27.58 ± 3.4	6	59.13 ± 4.2	5	57.05 ± 5.3	5
LFR 5/60	17.82 ± 3.7	7	24.76 ± 4.6	7	29.60 ± 2.5	7
LF 10L	48.57 ± 4.5	4	43.26 ± 5.4	4	45.62 ± 4.2	6

Data points are means and standard deviations of n = 7

The effect of perfusing solvent on the bioadhesion of alginate batches examined (Table 4.6) is less clear than the other parameters such as the relative humidity within the chamber. Although the general trend of bioadhesion follows the results displayed in Figure 4.6, there were significant increases in bioadhesion in nearly all the sodium alginate test, when the solvent was changed to artificial saliva, which contained mucin (Table 4.6). However, the mechanism(s) by which the bioadhesion of sodium alginate to porcine oesophagus occurs still remain unknown, a possible factor for the

preference of the tissue for mannuronate-rich alginates may be the greater molecular flexibility of the mannuronate monomers as compared to their guluronate counterparts (Atkins et al., 1971).

**Figure 4.6 Adherence profile of different sodium alginate, at 91%RH**



De-ionised water used as the perfusing solvent,  $n = 5$ ,  $14^\circ$  angle of inclination.

In Figures 4.7-4.11, the adherence of sodium alginate solutions to oesophageal tissues, following washes with the 3 solvents described above are visually compared. The label appears intense during the first 6 minutes of washing with artificial saliva, and remained more intense than the other solvents throughout the period of washing. Furthermore, there was evidence of substantial adherence of sodium alginate solution to the oesophageal tissue up to 30 minutes of washing with artificial saliva (Figure 4.11a).

#### 4.5 Discussion

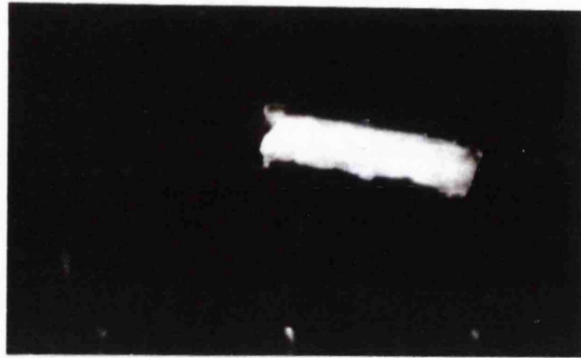
The issue of whether or not mucus (or mucin) is the responsible or indeed, necessary for adhesion of polymers to oesophagus, is one which requires a critical examination, given that there is no evidence for the presence of *in situ* mucin in the oesophagus. Nevertheless, natural saliva contains mucin, which washes down the oesophagus. The results of this study indicate quite strongly that sodium alginate preparations adhere to the porcine oesophagus to a varying degree. In this context, the term 'mucoadhesion' may not be applicable to describe the observed adhesion of these sodium alginate batches to the oesophagus. A study by Robert *et al.* (1988), in which the authors examined a range of putative bioadhesives and non-bioadhesives, indicated that all the polymers classified as bioadhesives adhered better on a mucin-free epithelium than in the presence of mucus. In addition, sodium alginate yielded higher bioadhesive values than some traditionally well known bioadhesives such as polyacrylic acid (Carbopol<sup>®</sup> 941) and sodium carboxymethylcellulose (CMCNa) of various viscosity grades. Although the method used (a modified tensiometer method, using force of detachment as an index of bioadhesion) differs significantly from the one used in this study, there are still some parallel conclusions to be drawn.

#### 4.6 Conclusions

The results from this study indicate the potential use of sodium alginate as bioadhesive polymer for site-specific drug delivery carrier. The significance of the



*Figure 4.7: Visual examination of the effect of perfusing with various solvents on the adherence of sodium alginate to porcine oesophagus (after 6 minutes)*



*Figure 4.7a: Artificial saliva*

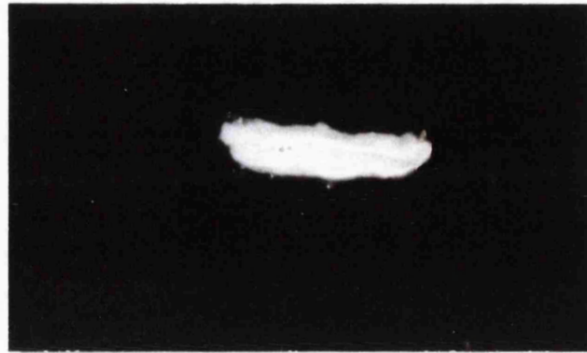


*Figure 4.7b: De-ionised water*

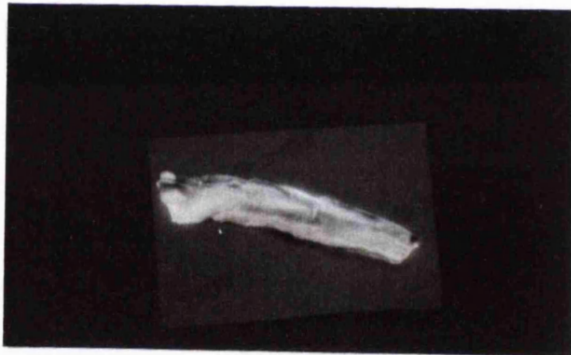


*Figure 4.7c: Phosphate buffer pH 7.4*

*Figure 4.8: Visual examination of the effect of perfusing with various solvents on the adherence of sodium alginate to porcine oesophagus (after 12 minutes)*



*Figure 4.8a: Artificial saliva*



*Figure 4.8b: De-ionised water*



*Figure 4.8c: Phosphate buffer pH 7.4*

*Figure 4.9: Visual examination of the effect of perfusing with various solvents on the adherence of sodium alginate to porcine oesophagus (after 18 minutes)*



*Figure 4.9a: Artificial saliva*

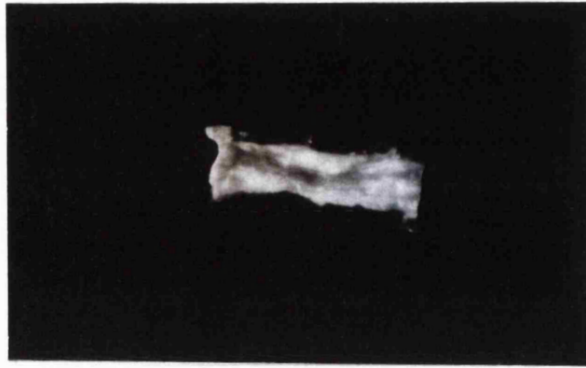


*Figure 4.9b: De-ionised water*

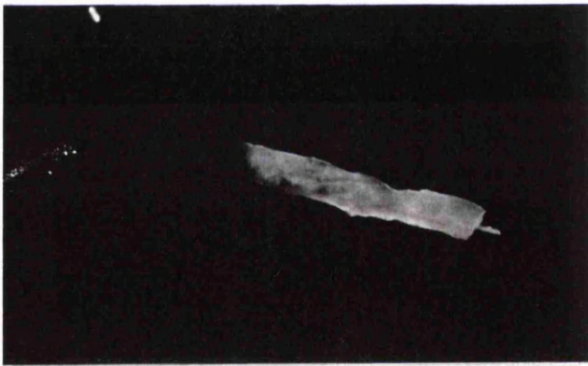


*Figure 4.9c: Phosphate buffer pH 7.4*

*Figure 4.10: Visual examination of the effect of perfusing with various solvents on the adherence of sodium alginate to porcine oesophagus (after 24 minutes)*



*Figure 4.10a: Artificial saliva*

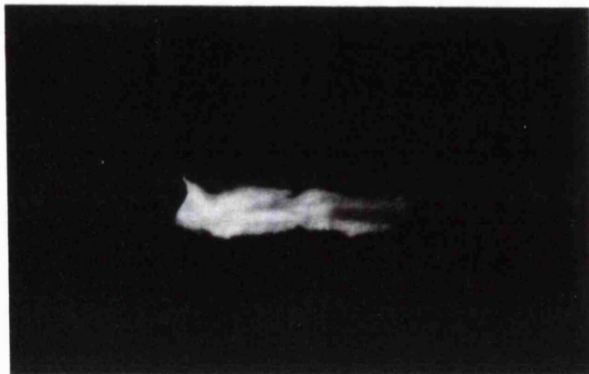


*Figure 4.10b: De-ionised water*



*Figure 4.10c: Phosphate buffer pH 7.4*

*Figure 4.11: Visual examination of the effect of perfusing with various solvents on the adherence of sodium alginate to porcine oesophagus (after 30 minutes)*



*Figure 4.11a: Artificial saliva*



*Figure 4.11b: De-ionised water*



*Figure 4.11c: Phosphate buffer pH 7.4*

increase in bioadhesive potential of sodium alginate with increasing relative humidity may hold some answers to the drug delivery to humid environments such as the oesophagus. Furthermore, the enhancement of the bioadhesive properties of the alginates in the presence of artificial saliva is an encouraging one, as the natural saliva contains similar principal ingredients and of similar pH values, although there are no enzymes in the artificial saliva, unlike the natural saliva.

**CHAPTER 5. GENERAL CONCLUSIONS**

## 5. *General Conclusions*

The following are general conclusions drawn from the results obtained during this work.

- The physical and chemical characteristics of sodium alginates have direct implications on their behaviour such as gel formation and the strength of these gels. The rheological examinations of the sodium alginates used in this work indicate that alginates rich in guluronate content yield gels that are firm, rigid and less elastic, with calcium ions. On the other hand, mannuronate-rich alginates offer gels that are more elastic but of comparatively weak strength, at similar calcium ion and alginate concentrations. These findings may have some implications in diverse areas such as food industry, where the gel characteristic is of paramount importance. Similarly, in the fields of biotechnology and medicine, the properties of alginate gels have been applied in encapsulation of drugs and drug molecules (Espevik and Skjåk-Bræk, 1996). The understanding, therefore, of the basic rheological and textural profiles may contribute to the formulation and performance prediction of the dosage forms.
- Polymer-mucus interactions are very well documented in the literature (Marriot *et al.*, 1990; Mortazavi *et al.*, 1992; Caramella *et al.*, 1994; Tamburic and Craig, 1996; Tamburic and Craig, 1997), however, relatively little has been reported on the interaction between mucin and alginates (Smart *et al.*, 1984, Founfuchat *et al.*, 1996; Madsen *et al.*, 1998). This work has demonstrated the potential bioadhesive properties of sodium alginate via their synergistic interaction with mucin. Furthermore, the interaction was shown to be dependent on the source and type of mucin used, with partially purified (homogenised) mucin extracted from the porcine stomach yielding the greatest magnitude of interaction with alginates. Another aspect of the bioadhesiveness of sodium alginates was clearly shown to be related to the monomeric composition of the polymer, with alginates rich in mannuronate content yielding greater synergistic interaction with mucin than their guluronate-rich counterparts. Higher viscosity of the individual alginates did not necessarily lead to greater interaction with mucin, except within a group of mannuronate-rich or guluronate-rich alginates, whereby higher viscosity values corresponded with greater synergy with mucin.



In addition, temperature-dependent gelation of mucin was augmented by the addition of sodium alginates, an observation not previously reported in the literature. The thermogelation was only partially reversible, following cooling-down the previously heated mixtures.

- Further evidence of the adhesiveness of sodium alginate to biological substrates was given by their adherence to porcine oesophageal tissue. The unique work on oesophageal bioadhesion showed that the degree of alginate adhesion varied with alginate polymer structure. This finding was reflected in fact that mannuronate-rich alginates adhered longer to the oesophagus than their guluronate-rich counterparts. The increase in the adherence following perfusion with artificial saliva (which contained ingredients similar to those found in natural saliva) may have a direct consequence on the formulation of dosage forms to this part of the GI tract.

**CHAPTER 6: RECOMMENDATIONS FOR FUTURE WORK**

Further work could be carried out in order to gain a better understanding of alginate behaviour, with particular emphasis on the formulations for site-specific drug delivery:

- Advantage could be taken of the gelling properties of alginates in formulations by incorporating various drug entities within several gel systems and following their transit and release profiles within the GI tract.
- The interaction observed between sodium alginate and various forms of mucin could be taken further by examining the retention times of the alginates on the mucosal surfaces. In addition, the mucosal passing of drugs incorporated in the alginate formulations across the mucus barrier may be measured to ascertain information on not only the mucoadhesiveness of the alginates, but also on the therapeutic effects of such delivery systems.
- It would necessary to improve on current techniques of measuring the bioadhesiveness of sodium alginate to biological substrates, such as the oesophagus. One of the drawbacks of the study on the alginate adherence to oesophageal tissue has been the lack of a universally accepted method of bioadhesion to the oesophagus. It is therefore of paramount importance that the detection method of the adhesion should be robust and reliable. In addition, there is the need for a reliable method of preparing 'labelled' sodium alginate batches in large quantities for a comparison of the performance of the individual alginates.

Bioadhesion (mucoadhesion) has traditionally been thought of as involving either mucus or some elements of mucus. Although the oesophagus served as an excellent biological substrate for bioadhesive studies in this work, it is believed that there is no inherent mucin within the oesophageal tract. It would be of interest to examine the molecules (or substances) responsible for this adhesion, in particular, the alginate-mucosal interface.

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