AN INVESTIGATION INTO THE GEL CHARACTERISTICS OF XANTHAN GUM-LOCUST BEAN GUM MIXES

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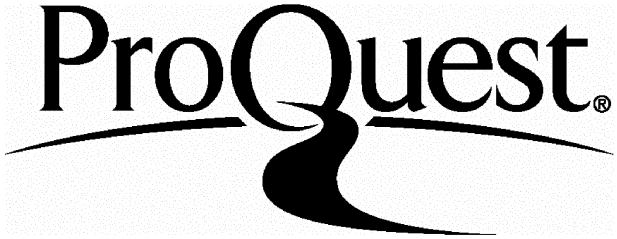

ABSTRACT

Xanthan gum and locust bean gum are two naturally occurring polysaccharides which do not form gels in isolation, but undergo a positive synergy to form thermoreversible elastic gels in combination. The aim of this project was to investigate the interaction between the two polysaccharides and factors affecting the gel characteristics of the mixes, with a view to developing their pharmaceutical uses.

The individual polysaccharides were characterised using flame emission spectrometry and nuclear magnetic resonance spectroscopy for xanthan gum and fractionation for locust bean gum. The gel strengths of the mixed systems were studied using oscillatory rheology and texture analysis with respect to temperature, fractionated locust bean gum and the inclusion of either sodium chloride or sucrose as additives. Conformational changes of xanthan gum which are thought to contribute to the mechanism of the interaction were studied using a combination of high sensitivity differential scanning calorimetry and synchrotron circular dichroism. The findings from these studies led to the development of methods for formulating controlled release tablets using a wet granulation process.

The results show that maximum synergy occurs for the 1:1 ratio between xanthan gum and locust bean gum, in which the gelation process is temperature dependent and is enhanced by heating and cooling between 20°C to 90°C. The effects of sucrose and locust bean gum fractions with a high mannose:galactose ratio increase the gel strength, whereas the inclusion of sodium chloride has no effect on the gel strength of xanthan gum-locust bean gum mixes. The thermoanalysis techniques used to determine xanthan gum conformation suggest that xanthan gum interacts with locust bean gum both in the disordered coil and ordered helical forms. These findings were employed for the formulation studies, which showed that xanthan gum conformation which is affected by different factors, depends upon an aqueous environment which in turn affects the synergy with locust bean gum and hence the release properties from the gel matrix.

This work suggests that the techniques chosen for this project are highly complimentary and a useful approach for studying the behaviour of mixed polysaccharide gel systems, which may exhibit different properties when formulated into tablet dosage forms.

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CHAPTER 1 : INTRODUCTION

1.1 CHEMICAL AND PHYSICAL PROPERTIES OF XANTHAN GUM AND LOCUST BEAN GUM

1.1.1 CHEMICAL COMPOSITION OF XANTHAN GUM

Xanthan gum is a naturally occurring extracellular heteropolysaccharide. It is secreted from *X.campestris*, which is a gram negative bacteria. This micro-organism was first isolated from the Rutabaga plant where it was found to cause black rot disease (Kennedy & Bradshaw, 1984). The bacteria secretes a polysaccharide identified as xanthan gum, which acts as a protective slime, essential for the pathogenecity of the micro-organism towards its plant host by blocking fluid flow through the xylem (Morris et al, 1983 and Holzwarth, 1976).

Today, xanthan gum is commercially produced semi-synthetically for the food, cosmetic and pharmaceutical industries by bacterial fermentation (Kennedy & Bradshaw, 1984 and Milas et al, 1985). Such a technique offers the advantage of reproducible physical and chemical properties, with a stable cost and supply (Morris, 1990b). However Kennedy & Bradshaw (1984) have emphasised that unlike other microbial extracellular polysaccharides, the composition of xanthan gum varies with the xanthomonas strain and culture conditions. In addition, most commercial samples of xanthan gum will contain variable amounts of sodium, calcium and potassium ions, which can alter the chemical and physical properties of the polysaccharide considerably.

1.1.1.1 Primary structure

The primary structure of xanthan gum is a (1→ 4)-linked-β-D-glucan cellulose backbone, with trisaccharide side chains attached at the carbon-3 position to alternate glucose residues (Morris et al, 1977, Kennedy & Bradshaw, 1984, Cuvelier & Launay, 1988 and Kitamura et al, 1991). However, other workers have postulated that the trisaccharide side chains are linked at the oxygen-3 position of alternate residues to give an overall repeating pentasaccharide sequence (Tako & Nakamura, 1985, Cairns &

Morris, 1986 and Foster & Morris, 1994a). Such repeating sequences are described by Jansson et al (1975) to be common to extracellular bacterial polysaccharides.

The actual trisaccharide side chain consists of two D-mannose residues and one D-glucuronic acid residue occurring as mixed sodium, calcium and potassium salts which are linked according to the following sequence determined by methylation analysis and uronic acid degradation (Jansson et al, 1975);

$$\beta\text{-D-Manp-}(1\rightarrow 4)\text{-}\beta\text{-D-GlcpA-}(1\rightarrow 2)\text{-}\alpha\text{-D-Manp-6-Oac}\rightarrow$$

Where:

-D-Manp- = D-mannose residues (α and β)

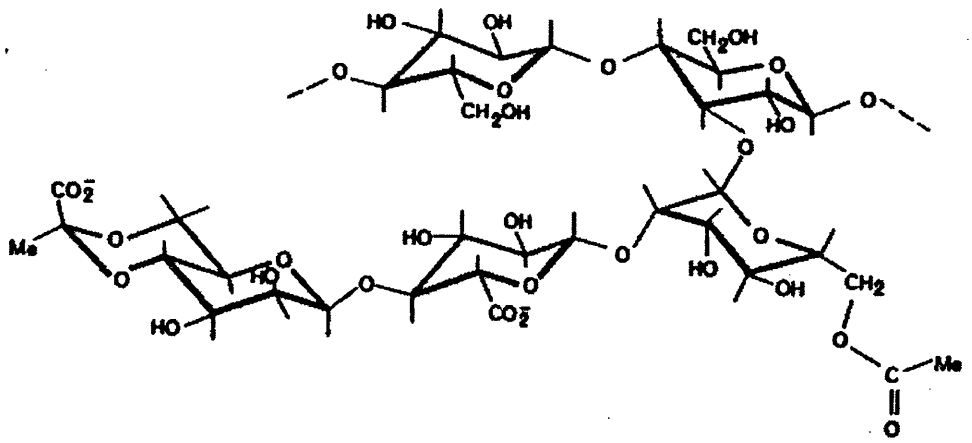
-D-GlcpA- = D-glucuronic acid residue

The α and β linked mannose residues are acetylated and pyruvated respectively which give rise to the structural heterogeneity of the polysaccharide. These groups occur in non-stoichiometric amounts and vary quantitatively, depending on the fermentation process and xanthomonas strain. The chemical structure of the xanthan repeating unit is illustrated in Figure 1.1.

Variation in the chemical constituents of xanthan gum and different fermentation conditions has led to difficulty in determining the molecular weight for this polysaccharide, although several values have been quoted ranging from approximately 2-50 million DA (Morris et al, 1983 and Holzwarth, 1976). Thus for commercially produced xanthan gum in particular, the polysaccharide will exhibit a high polydispersity in which values have been quoted from Mz/Mw~1.15 (Liu et al, 1987) to Mz/Mw~2.8 (Meyer et al, 1993).

The function of the trisaccharide side chains is proposed to modify the normal backbone geometry of xanthan gum, leading to a helical structure with 5-fold symmetry which forms the secondary structure (Brownsey et al, 1988). The secondary structure is discussed in Chapter 1.1.1.2.

FIGURE 1.1 : CHEMICAL STRUCTURE OF XANTHAN GUM REPEAT UNIT



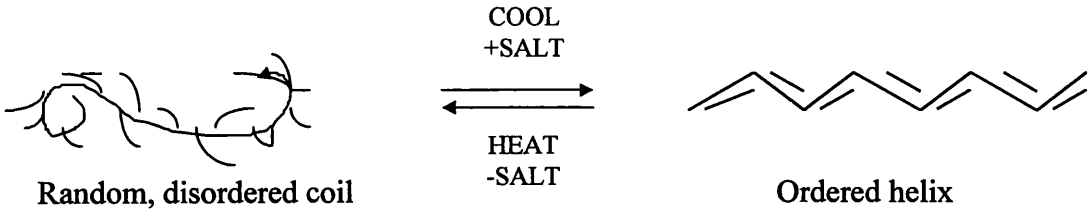
1.1.1.2 Secondary structure

Xanthan gum can exist as two different conformations in aqueous solution. The first conformation is the ordered helical form which occurs at low temperatures and in a high ionic strength environment. The second conformation is the random, disordered coil form which occurs in a low ionic strength environment or on heating xanthan gum to high temperatures (Morris, 1990a). Both conformations are shown in Figure 1.2.

In the presence of high salt concentrations, the ions screen the electrostatic repulsions between the trisaccharide side chain on xanthan gum, which protect the backbone from hydrolytic cleavage resulting in stabilization of the ordered helical form (Holzwarth & Ogletree, 1979). Increasing the temperature of the system results in melting of the ordered helix to the disordered form, which can be reversed by cooling allowing the helix to renature (Morris et al, 1977).

The ordered secondary structure of xanthan gum has been investigated using X-ray diffraction, and has been postulated to exist as a 5-fold helix in solution. It has been found using this technique that the 5-fold helix has a pitch of 4.7nm in which the branch backbone linkage is crucial in determining the helix conformation. The side chains bind non-covalently to the polymer backbone which stabilises the helix (Moorhouse et al, 1977). It has also been suggested that xanthan gum exists as the ordered helical form in the solid state (Morris et al, 1977).

FIGURE 1.2 : XANTHAN GUM CONFORMATIONS



Different approaches have been used to study the conformations of xanthan gum and to detect conformational change. Polarizing microscopy (Milas & Rinaudo, 1979), nuclear magnetic resonance techniques (Norton et al, 1984, Dea et al, 1977 and Morris et al, 1977), Differential Scanning Calorimetry (DSC) and High Sensitivity Differential Scanning Calorimetry (HSDSC) techniques (Paoletti et al, 1983, Norton et al, 1984 and Williams et al, 1991 and Craig et al, 1997) and circular dichroism (Dea et al, 1977, Morris et al, 1977, Milas & Rinaudo, 1979 and Dentini et al, 1984) have been the techniques of choice. Dentini et al (1984) used circular dichroism to detect the different xanthan gum conformations, and observed a random coil conformer for xanthan gum characterized by a peak at 198nm and a trough at 217nm. On the addition of calcium ions (increase in the ionic strength) the peak shifted to 203nm with a large rotational strength increase, and the trough shifted to 220nm with a decrease in the ellipticity, indicative of the ordered conformation.

There have been different views as to whether the ordered helix exists as a single or double helix or is composed of multistrands (Morris et al, 1983), although Rochefort & Middleman (1987) have suggested that it is difficult to predict the true structure because many factors such as the fermentation conditions, sample source and experimental conditions during the determination e.g. temperature may affect the results. In addition, it has also been suggested that there is more than one helical form (Milas & Rinaudo, 1984 and Lecourtier et al, 1986). The techniques which have been employed to determine the number of strands comprising the xanthan gum helix include light scattering experiments (Lecourtier, 1986), titration studies (Holzwarth, 1976), electron microscopy (Holzwarth & Prestidge, 1977 and Stokke et al, 1986), DSC (Paoletti et al, 1983) and HSDSC (Norton et al, 1984 and Kitamura et al, 1991). The DSC and

HSDSC studies were performed in conjunction with light scattering and/or electron microscopy studies, since DSC and HSDSC alone can not identify the number of helical strands, but is a useful tool in providing information on the transition between two states. For example, the findings from the study by Norton et al (1984) suggested that xanthan gum undergoes a single helix to single coil transition, whereas Paoletti et al (1983) suggested a double helix to single coil transition and Kitamura et al (1991) suggested that xanthan gum exists as a double helix below the transition temperature and dissociation is incomplete even at temperatures well above the transition temperature (i.e. 95°C in water).

The transition temperature of xanthan gum (Tm) has been determined using HSDSC and has been reported to be 51°C for a 1.2% xanthan gum solution in water (Williams et al, 1991) and has been described to undergo two transitions at 30°C and 80°C on the first heating and one transition at 74°C on temperature cycling (Craig et al, 1997). The peaks have been described as broad, which makes the precise midpoint of the transition temperature difficult to determine. The Tm has also been determined using optical rotation studies. For example, Holzwarth (1976) used optical rotation to determine the Tm for xanthan gum, in which a sharp decrease in measurement for a 1% xanthan gum solution in water was observed at 55°C indicative of the order-disorder transition. This order-disorder transition has been described by most workers to be first order (Kennedy & Bradshaw, 1984).

The Tm of xanthan gum is affected by the ionic strength as would be expected from the previous discussion that the addition of salt contributes to the stabilization of the ordered helical form. Consequently, the Tm is shifted to higher temperatures in the presence of salt, for example Williams et al (1991) observed a shift in the Tm from 51°C for a 1.2% xanthan gum solution in water to 84°C for a 1.2% xanthan gum solution in 0.04mol/dm^3 sodium chloride.

The transition of xanthan gum is also sensitive to the polymer concentration, cation valency, pH, molecular weight and the pyruvate and acetate content. In terms of the polymer concentration, it has been shown that if the ionic strength is kept constant, the transition is independent of the polymer concentration (Morris et al, 1977, Milas &

Rinaudo, 1979 and Morris et al, 1983), which suggests that the order-disorder transition is either unimolecular or extremely co-operative.

It has also been described that divalent ions have more of a stabilizing effect on the ordered helix compared to monovalent ions. In a study by Holzwarth (1976), it was observed that in the presence of calcium ions which are divalent, the transition temperature for xanthan gum increased by 70°C per decade for Ca^{2+} concentration compared to a 30°C per decade for Na^{+} concentration which are monovalent ions (e.g. per concentration decade = 0.1M to 1M).

The pH of the system is said to also affect the Tm of xanthan gum through stabilization of the helix. A decrease in the pH from 7 to 4 in 0.01M sodium chloride solution resulted in an increase in the Tm by 15°C, and a 40°C increase when the pH was reduced further to 3.4 (Shatwell et al, 1990). This is contrary to Rocks (1971) findings in which it was suggested that xanthan gum is stable to pH and temperature variations which enables the polysaccharide to have many applications in the food, cosmetic and pharmaceutical industries.

It has been observed by Kitamura et al (1991), that there was no systematic dependence of xanthan gum molecular weight on the Tm and enthalpy. This contrasts to studies performed by Milas & Rinaudo (1978), in which it was observed that the Tm decreased as the molecular weight of xanthan gum decreased from $3.2x10^{6}$DA to $2x10^{5}$DA. Liu et al (1987) found that the Tm for xanthan gum decreased below a molecular weight of $2x10^{5}$DA but was independent of molecular weight above $3x10^{5}$DA. This observation suggests a decrease in order.

Xanthan gum has been described to exhibit aggregation behaviour, in which it has been proposed that a xanthan gum solution contains an equilibrium between aggregated and non-aggregated molecules, which is displaced towards the non-aggregated form by shear (Launay et al, 1985 and Cuvelier & Launay, 1988). This self-association process leading to tertiary structures has been proposed to account for the high molecular weight values observed in the literature for xanthan gum (Launay et al, 1985).

Xanthan gum aggregates have been described by Cheetham & Mashimba (1988) to be insoluble, in which light scattering experiments have revealed that they are formed through side by side associations of ordered chain sequences, existing at low temperatures (Norton et al, 1984 and Tako & Nakamura, 1985). The formation of aggregates has been described to be time dependent, however it has been suggested that this observation may be partly due to the manufacturing and purification processes used for xanthan gum which precipitate and dry the polymer.

The aggregation process for xanthan gum is affected by temperature and salt (Rochefort & Middleman, 1987). Heating xanthan gum solutions disrupts the interactions between xanthan gum molecules leading to disaggregation, resulting in a filterable solution with viscosities smaller than for aggregated solutions of xanthan gum by a factor of 3 (Cheetham & Mashimba, 1988). Circular dichroism has been used to detect aggregation, in which it was found that aggregation increased as the salt concentration increased, suggesting that intermolecular attractions between neighbouring xanthan gum chains occur (Meyer et al, 1993). Tako & Nakamura (1985) also observed an increase in aggregation as salt (potassium chloride) was added to xanthan gum solutions, which suggested that salt enhances xanthan gum self-associations through pyruvate side by side dimerization or by limited double helix formation.

The acetate and pyruvate groups on the trisaccharide side chains of xanthan gum also contribute to the secondary structure. In addition, it has been suggested that quaternary structures for xanthan gum exist through the charged trisaccharide chains of associated xanthan molecules (Tako & Nakamura, 1985). These groups affect the order-disorder transition (Kitamura et al, 1991), the rheological (Smith et al, 1981) and solution properties (Jansson et al, 1985) of xanthan gum. The acetate groups are located close to the centre of the xanthan gum helix and are thought to have involvement in intermolecular interactions, whereas the pyruvate groups are thought to be involved in macromolecular associations, since these groups are located on the periphery of the helix (Kennedy & Bradshaw, 1984).

The pyruvate groups affect the secondary structure of xanthan gum by destabilizing the xanthan gum helix through electrostatic repulsions between the trisaccharide side chains, resulting in a decrease in the Tm. In addition, the removal of pyruvate groups

has also been found to cause depolymerisation of the xanthan polymer (Shatwell et al, 1990). In support of this, Smith et al (1981) suggested that pyruvate groups must promote association and structure formation by increasing the polymer-polymer affinity relative to the polymer-solvent affinity. In contrast to the pyruvate groups, the acetate groups have a stabilizing effect on the xanthan gum helix which gives rise to an increase in the Tm (Holzwarth & Ogletree, 1979 and Foster & Morris 1994a). Two possible explanations for this stabilizing effect could be due to apolar interactions between acetyl methyl groups or the acetate groups represent hydrogen bond acceptors which are involved in stabilization (Shatwell et al, 1990).

It has also been suggested that the distribution of the acetate and pyruvate groups may affect the temperature range over which the transition occurs. Thus, native xanthan gum (unpurified) which is heterogeneous i.e. contains a mixture of pyruvate poor and rich regions can cause a broadening of the melting curve, in which the pyruvate rich regions decrease the transition temperature due to an increase in the charge density. This transition curve can be sharpened by removing the destabilizing effect of the pyruvate groups, resulting in a more homogenous population of pyruvate groups (Holzwarth & Ogletree, 1979). In a different study by Shatwell et al (1990), removal of the acetate groups had no effect on sharpening the transition curve, since the distribution of acetate groups were found to be stoichiometric.

Most commercial xanthan gum have approximately 30%-40% pyruvate and 60%-70% acetate content. The degree of substitution and distribution varies between different samples, because of differences in the bacterial strains and the fermentation conditions employed. This may explain why there have been differences in result interpretation within the literature for xanthan gum structures.

1.1.2 PHYSICAL PROPERTIES

1.1.2.1 Solution properties and solubility

Xanthan gum dissolves readily in hot or cold water to form high viscosity solutions at low concentrations, atypically insensitive to a wide range of temperature, pH and salt concentrations, which makes xanthan gum an ideal thickener and suspending agent. Xanthan gum displays solution properties of commercial interest since it adopts ordered

structures in aqueous as discussed in Chapter 1.1.1.2, whereas most thickeners exist as fluctuating random coils in aqueous solution.

It has been found that the viscosity of xanthan gum is stable between 10°C to 70°C (Rocks, 1971), however it has been found that for xanthan gum solutions of concentrations less than 1% w/v, the viscosity may be reduced by temperatures greater than ambient (Pharmaceutical Codex, 1994). Jeremic et al (1999) observed in the temperature range of 25°C-70°C that the viscosity of xanthan gum solutions are stable at high shear rates, which is close to the temperature range cited by Rocks (1971). In terms of degradation, xanthan gum has been described by Rocks (1971) to be resistant to heat degradation, and thus can be held at elevated temperatures for long periods without any major changes in viscosity. In addition, xanthan gum solutions are stable under freezing conditions which is important in the food industry.

The viscosity of xanthan gum solutions are stable over a wide pH range from about pH 3-12 (Pharmaceutical Codex, 1994) although it has been quoted that this range is even wider i.e. pH 1.5-13 (Kennedy et al, 1985). This property is again important in terms of industrial use where extremes in pH are likely to be encountered.

Xanthan gum is also compatible with salts, which are necessary in industry for improving the heat stability of aqueous solutions, in which the viscosity of xanthan gum solutions are described to be unaffected by the salt concentration (Rocks, 1971 and Pharmaceutical Codex, 1994). A study by Jeremic et al (1999) also observed that there was no change in the viscosity for xanthan gum with the addition of salt in which salt concentrations of up to 10% mass were investigated.

Xanthan gum being a water soluble polymer has good 'water wicking' properties which results in fast hydration of xanthan gum molecules. This property has been exploited pharmaceutically for tablet dosage forms using xanthan gum in combination with locust bean gum (Baichwal, 1991). Xanthan gum is not generally soluble in organic solvents such as propylene glycol, although it is known to be soluble in glycerin at 65°C. This can be a disadvantage in the pharmaceutical and cosmetic industries where organic solvents are sometimes necessary.

1.1.2.2 Rheological findings

Xanthan gum forms pseudoplastic solutions. This non-Newtonian behaviour produces viscous solutions with thickening properties existing at rest (no shear rate) and less viscous solutions which can flow easily when poured (shear rate applied). This behaviour is otherwise known as shear thinning. Thus, the viscosity of xanthan gum increases as the shear rate decreases, with solutions returning to their original viscosity once the shear force is removed (Holzwarth, 1976 and Dea et al, 1977).

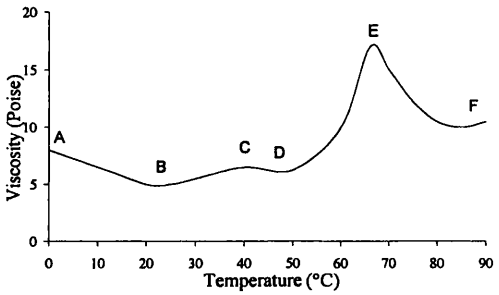
The high viscosity and shear thinning properties of xanthan gum have been attributed to the rod like properties of the molecule (Morris et al, 1983). It has been suggested that at high shear rates, xanthan gum undergoes the onset of helix melting which contributes to an increase in viscosity on heating due to non-specific molecular entanglements. These entanglements are minimal for the rigid rod conformation, but increase when xanthan gum is in the disordered state i.e. on heating. Milas & Rinaudo (1978) also support this observation, and in addition found that in the presence of salt, there was no further increase in the viscosity due to the suppression of electroviscous effects and the existence of a more or less rigid conformation. It has been proposed that once xanthan gum is in the ordered form, the viscosity is relatively insensitive to increasing ionic strength because salt stabilises the helix as discussed in Chapter 1.1.1.2 (Morris et al, 1977 and Milas & Rinaudo, 1978). Southwick et al (1982) found from viscosity studies that the disordered form is shear insensitive whereas the ordered form is shear sensitive.

It has been proposed that the intrinsic viscosity of xanthan gum is high indicative of stiff chains (Holzwarth, 1976). Shatwell et al (1990) is also in agreement with this and proposed that the disordered form may be a stiff chain rather than a random coil, since only small changes in the intrinsic viscosity over the temperature range of the transition were determined.

An alternative explanation for the shear thinning properties of xanthan gum may arise from non-permanent chain aggregation (Cuvelier & Launay, 1986b). This is because at low ionic strengths, xanthan gum shows a typical decrease in viscosity with a decrease in the hydrodynamic volume as the salt concentration is increased, but above 0.02M sodium chloride, the reverse situation occurs. This phenomenon could be due to

screening of the intermolecular electrostatic repulsions by excess salt ions leading to a self-association process, supported by the observation that at 1.7M sodium chloride, an increase in viscosity is concentration dependent which would be expected for a self-association process (Southwick et al, 1982). Dea et al (1977) also favour the aggregation explanation for the shear thinning properties of xanthan gum, in which a temperature profile for the viscosity of a xanthan gum solution at a constant rate of shear is illustrated and explained in Figure 1.3.

FIGURE 1.3 : TEMPERATURE PROFILE OF XANTHAN GUM 1%w/v SOLUTION VISCOSITY WITH NO ADDED SALT (DEA ET AL, 1977)

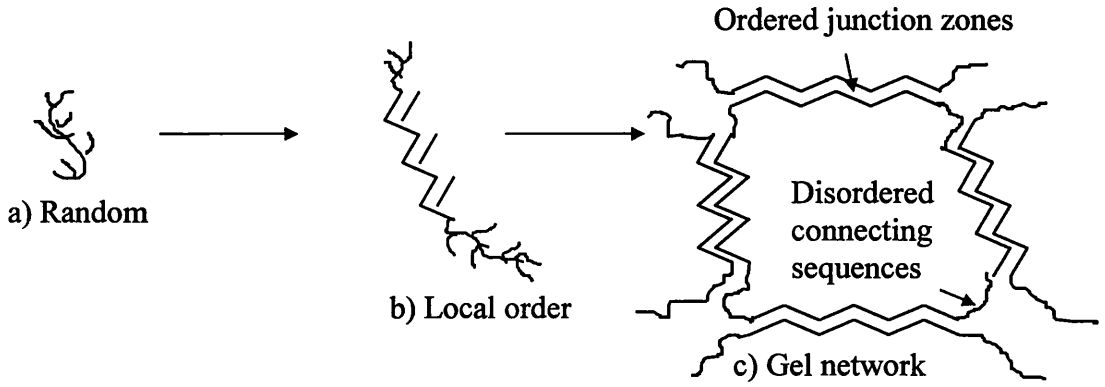


The greatest anamolous increase in viscosity (D-E) corresponds to the breakdown of native xanthan gum which form entanglements, which are more likely to occur between flexible coils than stiff rods. Once in the coil form, the molecule shows a typical decrease in viscosity with increasing temperature (E-F).

The smallest anamalous increase in viscosity (B-C) corresponds to the breakdown of rod-rod aggregates.

Other workers have suggested that the pseudoplastic behaviour of xanthan gum is due to formation of a weak tenuous gel like network (Morris et al, 1983). It has been described that the gel like properties occurring at rest may arise from lateral association of ordered chain sequences to form extended junction zones analogous to true gels, but are much weaker such that the network is broken down under stress allowing the solution to flow (Norton et al, 1984). This model is illustrated in Figure 1.4 where a) is the disordered conformer of xanthan gum, b) mechanism of local conformational ordering and c) lateral association of ordered chain sequences to give a weak gel like network.

FIGURE 1.4 : MODEL PROPOSED FOR THE PSEUDOPLASTIC BEHAVIOUR OF XANTHAN GUM



Many workers are in disagreement that xanthan gum can form true gels in isolation (Kovacs, 1973, Morris et al, 1977, Cuvelier & Launay, 1988, Zhan et al, 1993 and Lundin & Hermansson, 1995). Capron et al (1998) discovered that the reason that some workers have suggested that xanthan gum can form gels in isolation is because the rheological properties are dependent on the processing and experimental conditions of xanthan, such as the thermal and duration of treatment as well as concentration and salinity of broths. Zatz & Knapp (1984) also found that the rheological behaviour of xanthan gum such as the pseudoplasticity, weak gel properties and salt effects on the viscosity of xanthan gum were highly dependent on the concentration of xanthan gum. In support of this, Talukdar et al (1996) observed gel like behaviour for 4% and 7% xanthan gum solutions, thought to be formed through intermolecular associations. Below 4% xanthan gum concentrations, gel like behaviour was not observed.

Oscillatory rheology experiments have also been used to study the behaviour of xanthan gum, and in one such study by Rochefort & Middleman (1987) it was found that the storage modulus (G′) and loss modulus (G′′) were weakly dependent on frequency at low and high salt concentrations for a 5000ppm xanthan gum sample, indicative of a gel like structure. However, it was observed in the same study that for a 2500ppm xanthan gum solution, the storage modulus (G′) was smaller than the loss modulus (G′′) until the crossover point at a frequency of 2rad/sec was reached. Beyond this point, gel like properties were observed. As the concentration of xanthan gum decreased, the crossover point increased, suggesting a disappearance in structure. Again this illustrates

the importance of the xanthan gum concentration used in rheological studies. Oscillatory measurements have the advantage of being less likely to disrupt intermolecular associations compared to other techniques, and have been used to monitor the dynamic viscosity and rigidity of xanthan gum which both increase with temperature as mentioned previously in this chapter, again supporting the alignment and aggregation of rods. Oscillatory rheology has been used in the present work, in which the theory will be dealt with in Chapter 4.

In summary, it has been discussed in this chapter that the rheological behaviour of xanthan gum is related to the orientation and deformation of the molecules and any modification of the interchain interactions (Milas et al, 1985). These factors are largely affected by the acetate and pyruvate content, temperature, polymer concentration, pH and salt effects. In addition, the valency of any ionic species such as a salt can also affect the rheological properties of xanthan gum through crosslinking. For example, Jeremic et al (1999) found that monomeric and dimeric cations did not influence the viscosity of xanthan gum solutions, whereas trivalent cations such as Fe^{3+} are effective cross-linkers, which bind to xanthan gum and hence contribute to an overall increase in viscosity.

1.1.3 USES OF XANTHAN GUM

1.1.3.1 Biological function

It is known that bacterial cell walls are surrounded by a variety of carbohydrate polymers of different structures, whose functions are not entirely well understood. However, it has been observed for xanthan gum in solution that it has an ordered conformation which can show specific co-operative interactions with other polysaccharide chains. This finding supports a biological function, i.e. colonization of plant host by bacterial pathogen *xanthomonas* (Morris et al, 1977). This has been supported by Dea et al (1977) in which it was observed that native xanthan gum interacts specifically with plant glycans, indicating again a possible biological role in host-pathogen recognition.

Cairns & Morris (1986) have proposed that recognition and adhesion of *xanthomonas* occurs within plant vascular systems. However, the binding of xanthan to plant cell

wall components is said to occur only if helix formation is incomplete. It has also been discovered that *X.campestris* can change the degree of pyruvate substitution of its exopolysaccharide xanthan gum, in response to a change in the environment which represents a possible controlling mechanism on the rheology of the polysaccharide which may be an important survival factor for the bacterium (Smith et al, 1981).

There are four main *xanthomonas* species, namely *X.campestris*, *X.albilineans*, *X.axonopodis* and *X.fragaria*. Each species has been known to pathogenize a specific plant host (Kennedy & Bradshaw, 1984), which suggests that their function is related to the environment, e.g. some cause blight diseases of a number of crop plants such as beans, cabbage, peas and cotton (Morris et al, 1977).

1.1.3.2 General uses

Xanthan gum has a wide range of applications in many industries since it is non-toxic, biodegradable, non-immunogenic and has thickening and suspending properties. For these reasons, xanthan gum has been approved by the FDA as a food ingredient using acute short term and long term feeding studies as well as reproductive studies (Rocks, 1971). In support of this, Al-Shamkhani et al, (1991) found that xanthan gum has good blood compatibility with little haemoglobin being released over 24hrs and in addition, repeated systemic administration does not cause any adverse IgG or IgM humoral response.

Xanthan gum is widely used in the food industry, since the pseudoplastic nature of xanthan gum provides a non-gummy texture to food, which allows for good flavour detection (Kennedy & Bradshaw, 1984 and Brownsey et al, 1988). This is because when the minimum shear is reached, the viscosity of xanthan gum is temporarily broken down which allows for good flavour release (Rocks, 1971). In addition to the pseudoplastic nature of xanthan gum, the polysaccharide has good thermal stability which is useful for foods such as gravies which need to retain their heat for a long time, and in addition can improve the freeze-thaw stability of many foods, which makes xanthan gum useful for stabilizing foods such as puddings and spoonable type dressings within the food industry (Kennedy et al, 1985).

The ability of xanthan gum to form pseudoplastic solutions having texture, stabilizing effects, appearance and suspending properties even at low levels of polysaccharide has made this polymer a useful additive to many products. For example it has been found that the addition of 0.25%-0.3% xanthan gum improves the long term stability of oil in water emulsions even at elevated temperatures, because of the pseudoplastic nature of xanthan gum (Rocks, 1971). In addition, since the polymer can exhibit dispersion stability at rest and low viscosity under application, xanthan gum can be pumped easily within a food plant in the food industry (Rocks, 1971 and Kennedy & Bradshaw, 1984), and is also used commercially as a mobility controller in oil recovery processes (Morris et al, 1977) and as a viscosifier in drilling mud (Kennedy & Lloyd, 1998). Likewise, xanthan gum is also a useful added ingredient in the cosmetic industry for applications such as toothpastes and shampoos which need to flow under an applied stress but remain solid like at rest (Kennedy & Bradshaw, 1984).

In addition, xanthan gum has adhesive properties, since evaporation of aqueous xanthan gum solutions results in water soluble films that adhere strongly to glass and many metal surfaces which can also be exploited industrially (Rocks, 1971).

1.1.3.3 Pharmaceutical Applications

Most pharmaceutical applications of xanthan gum consist of oral controlled release dosage forms, aqueous suspension formulations and topical wound dressings (Holzwarth & Ogletree, 1979). It has already been mentioned that xanthan gum has excellent water wicking properties which provide fast hydration, a necessity for matrix swelling of tablets in order for drug to be released (Baichwal et al, 1991). Some of the pharmaceutical applications and studies using xanthan gum will now be discussed.

One study by Talukdar & Plaizier-Vercammen (1991) found that the drug release rate of caffein from xanthan gum tablets in water decreased as the xanthan concentration was increased from 4% to 33%, and at 6% xanthan gum concentration, a sustained release profile was observed, in which all tablets were intact for greater than 8hrs. Different variables such as the compression force (1-3kN) did not significantly affect the release rate. The addition of 0.1M NaCl, resulted in faster release within the first 30min (40%-60% of drug) due to retardation of swelling, but thereafter the release rate was linear and

much slower indicating zero order kinetics, which is in agreement with Sujja-areevath et al (1996). Increasing the NaCl concentration to 0.2M, enhanced the release even further (90% drug release within the first hour) which was overcome by increasing the xanthan gum concentration. The pH did not have a significant effect on caffein release, since a pH change from 1.2-7.4 of the dissolution medium had no effect on release rates. The method of tablet preparation i.e. direct compression and wet granulation were only found to affect the initial stages of drug release whereby tablet swelling was incomplete thus, drug release was faster from the directly compressed tablets initially due to small disintegration of the tablet. In addition, the choice of binder was found to affect the release rate of drug from the tablets.

In another study by Talukdar et al (1996), the effects of salt concentration on the release rates of caffein (soluble drug) and indomethacin (insoluble drug) from xanthan gum matrices were investigated and compared to hydroxypropylmethyl cellulose matrix tablets (HPMC). It was found that by mimicking the ionic strength environment of the gastrointestinal tract (GIT) ($0.01<\mu<0.12$), release rates of both drugs from xanthan gum matrices were higher in the medium of low ionic strength compared to a medium of high ionic strength, and the mechanism was predominantly by an erosion process, whereas release rates from HPMC matrices were found to be independent of salt concentration. This erosion mechanism for drug release is consistent with the findings from a similar study by Dhopeshwarkar & Zatz (1993), in which theophylline (insoluble drug) was used. However the mechanism for release was found to be drug dependent, since it was found for chlorpheniramine, a soluble drug, the mechanism was via diffusion. In contrast, Talukdar et al (1996) did not observe a drug dependent release mechanism when comparing caffein (soluble drug) to indomethacin (insoluble drug). Additionally, xanthan gum matrices were found to retard drug release more so than HPMC matrices in the concentration range studied (4% and 7%), probably because of the gel-like behaviour of xanthan gum solution whilst HPMC behaves as a typical polymer solution. Dhopeshwarkar & Zatz (1993) also support these results, and proposed that the use of xanthan gum matrix tablets enables smaller amounts of polysaccharide to be used to achieve a comparable sustained release profile to HPMC tablets, which has an advantage in formulating high dose drugs without excessive increase in tablet weight.

A study by Fu Lu et al (1991) used sodium alginate tablets as a comparison polymer to xanthan gum, and found that for xanthan gum tablets in both 0.1N hydrochloric acid and potassium phosphate buffer pH 6.8, release of theophylline was zero order in vitro and in vivo, which was independent of pH. On the other hand, the release of theophylline from the sodium alginate tablets was pH dependent with drug release being much faster. These findings are in agreement with a similar study by Johnson et al (1997), in which the release rates of a soluble drug promethazine were also investigated and compared to hydrochlorothiazide an insoluble drug. The differences in drug release mechanisms are said to reside in the different gel like behaviours of these two polymers i.e. xanthan gum exhibits swelling behaviour in which drug is slowly released from the dry core, whereas sodium alginate converts to insoluble alginic acid which is non-swellable and very porous in acid media, allowing drug to be released quickly depending on the pore size and the number of channels available (Fu Lu et al, 1991).

Xanthan gum has also been used as a controlled release hydrogel for an antimicrobial bicomponent system containing neomycin and furazolidone (Dumitriu et al, 1993). It was found from this study that the release of the latter drug was independent of pH whereas the converse was true for the former drug. Both drugs exhibited zero order release after 1hr, in which the gel swelling had equilibrated which determines the diffusion rate. In addition there was also some evidence of mucoadhesion, since the formed gel had a lower transit rate through the intestines compared to the incomplete gel structure. In conclusion, in vivo studies suggested a higher therapeutic efficiency for the bicomponent hydrogel compared to the free drugs i.e. without xanthan gum, in which there was 100% recovery from *Salmonella* after 2 days administration of hydrogel (250mg), compared to 70% recovery after 7 days administration with the control tests (i.e. no xanthan gum).

Another use of xanthan gum is for flocculation of pharmaceutical suspensions (Felmeister et al, 1973 and Tempio & Zatz, 1980), which is thought to be due to changes induced in either the interparticulate structure or by adsorption of polymer molecules which joins several particles.

1.1.3.4 Xanthan gum interactions with sucrose

Xanthan gum is compatible with most other hydrocolloids including starch, which is particularly useful within the food industry. This compatibility can modify the pasting properties, improve moisture retention and the textural properties of starch (Christianson, et al 1981). In addition, xanthan gum has unusually long flow behaviour which can be modified by starch in the presence of 30-35% sugar (Rocks, 1971). This leads on to the discussion of sugars, which are known to impede starch granule swelling by competing for water which in turn delays the gelatinisation temperature which may be an undesirable effect (Hester et al, 1956).

It has been shown in one study that xanthan gum and guar gum (a galactomannan) are known to diminish the delayed gelatinisation temperature of starch by combination of two opposing effects. One effect is the decrease in the gelatinisation temperature of starch with an increase in the gum concentration. The second effect is an increase in the gelatinisation temperature with an increase in the sugar concentration. Thus, a combination of these effects using these polysaccharides with sugars may bring the gelatinisation temperature close to that of starch alone. Another hypothesis which has been proposed is that sugar-water co-solvents have an antiplasticizing effect, which may be counteracted by xanthan and guar gums which is observed as a delay in the gelatinisation temperature in the presence of sugars. A further hypothesis may be that xanthan counteracts the decreased water activity of the sugar solution as compared to water alone which also manifests as a delayed gelatinisation. In addition, it has also been observed in the same study that at fixed low concentrations of starch-xanthan mixes, the viscosity increased when sucrose was increased from 10% to 30%. However, for xanthan concentrations of between 0.1% and 0.25%, the opposite in viscosity was seen as the sucrose concentration was increased. This observation may be explained by incomplete hydration of starch and xanthan which is necessary for efficient interactions to occur (Sudhakar, et al 1995).

Although there are not many reports on xanthan-sugar interactions, the findings from Sudhakar et al (1995) may be supported by other workers (Baird & Sandford, 1987), in which it was noted that xanthan-sugar solutions were non-uniform with lumps in the absence of a surfactant, which implies that xanthan hydration is restricted by sugar. In a

different study by Watanabe et al (1992), drug release from xanthan gum:locust bean gum hydrogels containing sucrose or glycerin as additive have been investigated using prednisolone as a model drug. It was found that drug release decreased in the presence of sucrose or glycerin, in which it was proposed that these sugars increase the diffusion resistance by increasing the microscopic viscosity of the hydrogels.

1.1.4 CHEMICAL COMPOSITION OF LOCUST BEAN GUM

1.1.4.1 Primary structure

Locust bean gum is a neutral, naturally occurring polysaccharide, from the seeds of *Ceratonia.siliqua* (leguminous plant family). It belongs to the galactomannan family of polysaccharides, having a molecular weight of between 300,000-360,000DA (Maier et al, 1993).

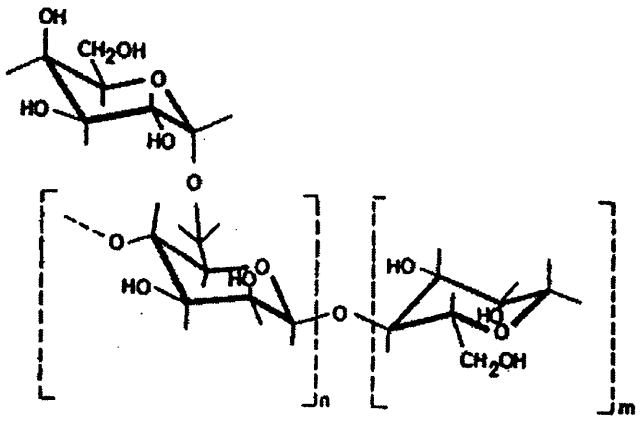
The primary structure is composed of a mannan backbone; [β-(1→ 4)-D-mannose] which is incompletely or irregularly substituted at carbon-6 position with α-D-galactose residues (Cuvelier & Launay, 1988, Zhan et al, 1993 and Lundin & Hermansson, 1995). However, some workers have suggested that substitution occurs at the oxygen-6 position rather than at the carbon-6 position (Baker & Whistler, 1975 and Cairns & Morris, 1986).

The structure of locust bean gum is illustrated in Figure 1.5 and is described as having a smooth mannan backbone, with hairy regions which are the substituted galactose residues (Morris, 1990a). It has been suggested that these hairy regions are clustered mainly in blocks of approximately 25 residues, interspersed by longer smooth regions of unsubstituted mannan backbone (Dea et al, 1977). It has been described that the average degree of substitution is approximately 1.4, and the distribution of galactose residues is irregular with unsubstituted and completely substituted regions (Cuvelier & Launay, 1988). Studies involving enzymatic hydrolysis have revealed that the side chain units are disposed in uniform blocks along the backbone, since galactomannans have the ability to interact with some polysaccharides possessing a high order of molecular association such as xanthan gum and carageenan. These blocks are thought to be composed of non-singular galactose side chains, which are separated by β-D-mannopyranosyl residues bearing no substituents (Baker & Whistler, 1975). McCleary

(1979) is also in agreement that the side chains exist as blocks on the mannan backbone in a non-singular manner compared to a random distribution. However, it has recently been shown that locust bean gum from *Ceratonia.siliqua* has neither a block nor a regular or statistically random distribution of galactose residues along the main chain. Instead, it has been proposed that a non-regular structure with a higher proportion of unsubstituted blocks of intermediate length exists, which would not fit the description of a statistically random distribution (Dea et al, 1986a).

The degree of substitution is said to depend on the plant species (Mannion et al, 1992) as well as the source and method of polymer extraction (Zhan, et al, 1993 and Lundin & Hermansson, 1995). Optical rotation studies can be used as a simple test to determine the galactomannan composition, since the rotation will change from negative to positive as the galactose content increases (Morris, 1990a). It has been determined by various workers that locust bean gum has a mannose to galactose ratio (M:G) of approximately 3.5:1 (Dea et al, 1977, Cairns & Morris, 1986 and Mannion et al, 1992). The M:G ratio and distribution pattern of galactomannans, are important determinants of synergistic gel formation with other polysaccharides such as xanthan gum (Morris, 1990a and Zhan et al, 1993). The galactose content affects the dissolution properties of the galactomannan, i.e. increasing the galactose component increases the dissolution properties of the polysaccharide (Lundin & Hermansson, 1995 and Morris, 1990a).

FIGURE 1.5 : STRUCTURE OF LOCUST BEAN GUM REPEAT UNIT



1.1.4.2 Secondary structure

The solid state of locust bean gum is said to adopt an ordered, 2-fold conformation with a repeat distance of 0.52nm per residue, as expected for an almost fully extended 1,4-diequatorially linked chain. The individual chains are packed together into flat sheets with 0.9nm spacing between chains, whilst the mannan sheets are separated by a distance of 0.72nm. The presence of the galactose side chains separates the sheets further to a distance of 0.3nm or greater. The exact spacing is sensitive to humidity, but independent of the M:G ratio. Higher degrees of substitution can be accommodated without an increase in spacing, because the first few galactose 'spacers' abolish close packing of the mannan sheets (Morris, 1990a).

When locust bean gum is dissolved in water, locust bean gum forms a disordered, fluctuating random coil (Cuvelier & Launay, 1988 and Lundin & Hermansson, 1995), although some renaturation may occur if the galactose content is low (Morris, 1990a). This random coil is difficult to observe close to the resolution obtained using the rotary shadowing technique, because when locust bean gum is sheared, parallel strands are observed, suggesting that the structure is probably formed, owing to the shearing of locust bean gum when the mica plates are put together. This indicates that when a stress is applied, the polymer is orientated which leads to visible structures. Consequently the method of preparation is very important (Lundin & Hermansson, 1995).

1.1.5 PHYSICAL PROPERTIES

1.1.5.1 Solution properties and self association

Locust bean gum is only partially soluble in cold water because the mannan backbone is insoluble in water (M:G ratio is 3.5:1) but soluble in strong alkalis (Morris, 1990a). The addition of O-acetyl groups which can be detected by infra red spectrometry, could increase the solubility of locust bean gum by hindering the packing of the polysaccharide molecules, which has been shown for polysaccharides having similar structure, the glucomannans. (Bacon et al, 1975).

Locust bean gum can be separated into cold water soluble and hot water soluble fractions with lower and higher M:G ratios respectively, by a fractionation process

(Mannion et al, 1992 and Lundin & Hermansson, 1995). This process affects the physical properties and behaviour of this galactomannan with other polysaccharides, and will be discussed in subsequent chapters.

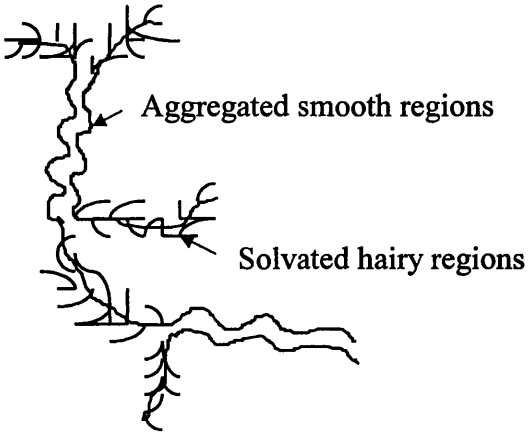
Locust bean gum has the ability to self-associate, which requires free sections of mannan backbone with a low degree of galactose substitution (Dea et al, 1986b and Mannion et al, 1992). Therefore self-association increases as the M:G ratio increases (Mannion et al, 1992), and thus the least substituted locust bean gum samples will have a greater tendency to form associations and precipitations.

It has been suggested that chain-chain association is by aggregation of the smooth mannan regions in a regular ribbon like 2-fold conformation, with hairy regions serving to solubilise the network, as illustrated in Figure 1.6 (Dea et al, 1977). In addition, the distribution of galactose groups along the main chain will also have an effect on the interactive properties of the galactomannans (Dea et al, 1986b). Freeze thaw studies of locust bean gum, showed that precipitation (i.e. self-association) of the polysaccharide required regions of a weight average length of approximately 6 consecutive totally unsubstituted D-mannose residues. Above this threshold, the interactive properties are controlled by structural features associated with totally unsubstituted D-mannan regions, whereas below the threshold, the interactive properties are controlled by structural features associated with the unsubstituted sides D-mannan backbone (Dea et al, 1986a).

1.1.5.2 Rheological properties

Locust bean gum is a non-gelling, neutral macromolecule, which is relatively stable to pH, temperature and salinity (Lundin & Hermansson, 1995). The spectrum for locust bean gum 0.01% is said to be typical of a viscous solution, since a study by Mannion et al (1992) observed that the loss modulus (G′′) was greater than the storage modulus (G′) at all frequencies (ω) studied (0.1-10 rad.sec^{-1}), with both parameters dependent on the oscillation frequency. In a different study by Doublier (1994), the viscoelastic spectrum for locust bean gum 2%w/w concentration showed that at low frequency (ω), the loss modulus (G′′) was greater than the storage modulus (G′) with both parameters varying sharply with frequency (ω) i.e. $G'' \alpha \omega$ and $G' \alpha \omega^2$. Again this is indicative of 'liquid-like' properties.

FIGURE 1.6 : CHAIN-CHAIN ASSOCIATION OF LOCUST BEAN GUM MOLECULES



However as the frequency increased, G′ω crossed G′′ω, and beyond this crossover frequency, locust bean gum exhibited 'solid-like' properties (i.e. G′>G′′). This overall behaviour at the different frequencies studied (0.01-100 rad. sec^{-1}), is typical of macromolecular solutions with topological entanglements. In summary, it was concluded from this study that the rheology of locust bean gum solutions are governed by the degree of entanglement of the individual macromolecules and show random coil behaviour, whereas xanthan gum solutions adopt a helical conformation which is much more rigid. In another study comparing locust bean gum to xanthan gum, it was suggested that the only difference between xanthan gum and locust bean gum solutions is the time scale, in which xanthan solutions have much longer relaxation times indicative of highly structured liquids compared to simple entangled macromolecular solutions as locust bean gum (Giboreau et al, 1994).

Locust bean gum solution viscosity behaves differently in the presence of salts compared to xanthan gum solutions. The viscosity of locust bean gum solutions tend to decrease with increasing salt concentration, due to a decrease in the intermolecular charge-charge repulsions which causes a contraction of the coil dimensions. This is the opposite of xanthan gum solutions.

Although it has been discussed so far that locust bean gum forms viscous solutions, locust bean gum has been known to show weak gel-like behaviour depending on certain experimental conditions. For example, fractionation techniques used by Mannion et al

(1992) which alter the M:G ratio through solubility differences, have resulted in different viscoelastic properties of the polymer. As an example, locust bean gum-35 (cold soluble/low M:G ratio) formed a viscous solution, whereas locust bean gum-80 (hot soluble/high M:G ratio) formed a soft flexible gel.

Freeze thaw processes can also precipitate weak gels of locust bean gum. The process of freezing and thawing a 2% fractionated locust bean gum (M:G approximately 4.5) has resulted in rubbery, self-supporting gels which at higher concentrations can only be broken down by autoclaving. Similar observations have been observed for unfractionated locust bean gum, although higher concentrations were needed to achieve the same results. However, these findings were not reproducible, since some samples were stored at 2°C for many days and showed no evidence of structure formation. The galactomannan solutions which did show gel-like behaviour were generally unstable, often resulting in syneresis with up to 50% water losses on a second freeze thaw cycle (Dea et al, 1977).

Locust bean gum viscosity is affected by the water activity which has been exploited to produce gels and precipitates (Morris et al, 1981). For example, the addition of 50% aqueous ethylene glycol which has the effect of lowering the water content has been shown to induce interchain associations to produce weak but cohesive gels at concentrations down to 0.2%. On the other hand, sucrose which is said to have a dehydrating action was not found to affect the viscosity of locust bean gum even at high sucrose concentrations of 40% (Launay et al, 1997).

1.1.6 USES OF LOCUST BEAN GUM

1.1.6.1 General uses

Locust bean gum is mainly employed in the food, cosmetic, and pharmaceutical industries. Since locust bean gum is a non-ionic polysaccharide, it is useful in processes where saline water or polyvalent cations need to be tolerated (Chemistry and technology of water-soluble polymers, 1983) In addition, this polysaccharide is compatible with other components and can produce stable solutions of relatively high viscosities even at low polysaccharide concentrations (Baker & Whistler, 1975). This latter property is a major requirement for thickening, binding and suspending agents which are some of the

functional roles of locust bean gum in the food and cosmetic industries (Martindale, 31st Edition, 1996 and The Merck Index, 12th Edition, 1996). Other advantages of locust bean gum include that it is natural, odourless and tasteless unless it is boiled in water, in which it acquires a leguminous taste. For these reasons, it is widely used in the food industry on its own as a coffee, chocolate and cocoa substitute (The Merck Index, 12th Edition, 1996). Its uses in the food industry are more extensive when locust bean gum is combined with certain other components which give rise to synergistic increases in viscosity and gel strength. Xanthan gum-locust bean gum mixes is one such example (Morris, 1990b), which have a very wide range of food applications such as bakery and pie-filling, puddings, dips and spreads, acidified milk gels, meat products and pet food. One disadvantage of this system is that it is very cohesive and elastic which is unsuitable for direct use in certain food products such as table jellies. However the texture can be modified by the addition of further components such as starch, carageenan, microcrystalline cellulose or high concentrations of fats or meats (Morris, 1990a).

Locust bean gum has applications in the paper manufacturing industry as a fiber bonder and is used as an additive in the mud and oil drilling industries (The Merck Index, 12th Edition, 1996). In the oil drilling industry, locust bean gum is employed to control the fluid viscosity which prevents sands or cuttings from sedimenting into bottom hole. In addition, it is also used to seal bore with temporary filter cake (Chemistry and technology of water-soluble polymers, 1983).

1.1.6.2 Pharmaceutical properties

Locust bean gum in isolation has limited pharmaceutical applications. Its therapeutic category is an adsorbent-demulcent (The Merck Index, 12th Edition, 1996). Thus, one of its uses is as an additive in medicines to control diarrhoea and vomiting (Martindale, 31st Edition, 1996). Since the polysaccharide can form viscous solutions, it is sometimes used as a substitute or adulterant to tragacanth which is used as a stabiliser in oral emulsions and suspensions. In addition, tragacanth and similarly locust bean gum can be used as excipients in tablets, medicated lozenges due to the demulcent properties in addition to formulating external lotions and creams (The Pharmaceutical Codex, 1979).

1.2 CHARACTERIZATION OF XANTHAN GUM-LOCUST BEAN GUM MIXES

1.2.1 Physical properties of the gels

It has been discussed in Chapters 1.1.2.2 and 1.1.4.4 that xanthan gum and locust bean gum respectively are both non-gelling polysaccharides. However, when these two polysaccharides are mixed together, xanthan gum-locust bean gum mixtures have been reported by many workers to form thermoreversible, elastic gels (Kovacs, 1973, Dea et al, 1977, McCleary, 1979 and Brownsey et al, 1988). It has been observed that the addition of locust bean gum even in low concentrations to a solution of xanthan gum, forms firm, rubbery gels (Dea et al, 1977). These gels are described as 'true gels' since they do not flow, nor do they recover from mechanical damage (Dea et al, 1977) and are firm and cuttable (Rocks, 1971).

Texture analysis studies have been used to study the behaviour of these gels, in which a plot of stress (force applied to gel) against strain (deformation) showed an almost power function curve, as opposed to a linear curve as for other natural polysaccharides. The shape of this curve supported that xanthan gum-locust bean gum gels are elastic, and after a certain amount of penetration, the stress applied to the gel encountered a second resistance gradient. In addition, it was observed that the most elastic gels occurred for mixtures between the ratios of 1:9-9:1, whereas the gels with the most dramatic break from linearity occurred for 1:1 ratios (Kovacs, 1973). Sedimentation experiments by Luyten et al (1993), have been used to investigate the gel structure of xanthan gum-locust bean gum mixes. Slow sedimentation was observed, which suggested the existence of a yield stress, combined with a further slow breakdown of the xanthan-locust bean gum network.

Electron microscopy studies have also been carried out to investigate the structural properties of these gels. Mixtures subjected to room temperature as well as heating and cooling were both studied. Under both sets of conditions, the xanthan gum-locust bean gum network had been formed from xanthan supermolecular strands, and the addition of locust bean gum did not appear to influence the xanthan gum structure, i.e. it was not possible to detect an interaction between the two polysaccharides at the molecular level. (Lundin & Hermansson, 1995). Polarizing microscopy has also been used to study the

gel structure of xanthan gum-locust bean gum mixes, in which it was found that xanthan gum in the presence of locust bean gum (and other galactomannans) displayed a stronger anisotropy than for xanthan gum alone, which suggested that the galactomannans had a concentration effect on xanthan gum (Schorsch et al, 1995).

1.2.2 Models proposed for the interaction

Various models have been suggested for the synergy between xanthan gum and locust bean gum with consequent gel formation. These models can be generally categorised into two types of interactions which may occur between two or more different polysaccharides according to Dea et al (1977), which are described below;

(i) Mutual exclusion of incompatible molecules with a consequent increase in the effective concentration of both polymers.
(ii) Energetically favourable association of structurally and sterically regular chain segments.

The mutual exclusion theory has been favoured for xanthan gum-locust bean gum interactions by some workers such as Rocks (1971) and Schorsch et al (1995). However, most workers instead support energetically favourable associations/intermolecular binding between polysaccharide chains (Dea et al, 1977, Cairns & Morris, 1986 and Zhan et al, 1993).

The intermolecular binding theory can be mainly divided into two types, in which one theory involves the xanthan gum ordered helix binding to the locust bean gum molecule, and the second theory involves the disordered chain segments of xanthan gum interacting with the locust bean gum molecule. These conformational changes in polymer structure, which accompany gel formation can be monitored using a variety of techniques such as optical rotation, circular dichroism, differential scanning calorimetry (DSC) and Nuclear Magnetic Resonance (NMR) (Dea et al, 1977). In this section, the different theories will be discussed with reference to some of the techniques mentioned.

Early workers have attributed the gelation process to an intermolecular binding between the xanthan helices and unsubstituted regions of the galactomannan backbone. This

concept has led to the theory of junction zones in more recent years, supported by Cuvelier & Launay (1986b) and Copetti et al (1997), which are highly ordered intermolecular associated regions formed between the smooth regions of the galactomannan backbone and the ordered xanthan molecule, resulting in the build up of complex networks (Kovacs, 1973).

Optical rotation measurements have been used to monitor the interaction between xanthan gum and locust bean gum, in which it was found in one study that optical rotation shifts showed that the binding is linked to helix formation and does not require a perfect helix, although it must be essentially complete (Dea et al, 1977).

High Sensitivity Differential Scanning Calorimetry (HSDSC) has been used to study the conformational behaviour of xanthan gum in combination with locust bean gum, in which it was found in one study that the xanthan gum ordered helix interacts with locust bean gum both in the presence and absence of salt, since gelation occurred below the transition temperature (Tm) of xanthan gum in both environments (Williams et al, 1991).

More recently, different workers have proposed variations on the early model. For example, as the tertiary structure of xanthan gum has been described by some to be a 5-fold single stranded helix, its side chains are thought to insert into adjacent unsubstituted segments of the locust bean gum backbone, which extends into a 2-fold ribbon like structure similar to a lock and key model (Tako et al, 1984 and Tako & Nakamura, 1985).

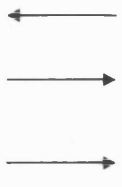
However, other workers are in disagreement with the ordered helix theory, instead favouring the disordered chain theory. For example, Cairns & Morris (1986) found that denaturation of the helix was necessary for intermolecular associations with locust bean gum and hence gelation to occur. In support of this, it was found that in the presence of sufficient calcium chloride which stabilises the ordered helix, no gels were formed which suggests that the interaction only occurs if xanthan gum is in the coil form. Additionally, it has also been suggested that since gelation only occurs under conditions in which the xanthan helix is denatured, the intermolecular binding may involve co-

crystallization of sections of the denatured xanthan molecule with structurally similar segments of the galactomannan chains (Cairns & Morris, 1986 and Cairns et al, 1987). Cairns & Morris (1986) also proposed that the binding between xanthan gum and locust bean gum is specific (from X-ray diffraction data), and this maybe accounted for by structural similarities between the two polysaccharide backbones, since X-ray diffraction patterns have shown that the first meridonal reflection for the mixed junction zones corresponded to an interplanar spacing of 0.52nm, which is characteristic of cellulose or mannan structure. This supports the theory that the binding occurs between the two backbones, and in order to achieve a spacing of 0.52nm, the xanthan side chains need to be staggered. Optical rotation studies were also performed in conjunction, and results suggested that only small segments of both backbones are involved in binding, with the remaining xanthan segments reforming the helical conformation. Cheetham & Mashimba (1988) concluded that one galactomannan chain is sandwiched between two xanthan chains (disordered, flat ribbons) and in agreement with Cairns & Morris (1986), the xanthan side chains are arranged to form a repeat distance of 0.52nm which are staggered and stacked above and below the locust bean gum backbone. The galactomannan side chains are substituted on one face only, due to steric interactions with xanthan side chains as shown in Figure 1.7, which is in agreement with Morris (1990a). Thus, it was discovered that the difference in reactivity between sparsely and highly substituted galactomannans is attributed to the inability of highly substituted chains to fit into the space between adjacent xanthan molecules. Dea et al (1977), also found correlations between the galactomannan composition and the extent of the interaction with xanthan gum i.e. the interaction involved the unsubstituted or relatively unsubstituted parts of the mannan backbone. In a later study, it was discovered that the distribution of these galactose groups determined the interactive properties of the galactomannans (Dea et al, 1986a).

A 2-fold model is a possible candidate for the xanthan conformer in the xanthan-locust bean gum interaction, according to Millane & Bowei (1990), since interactions may be more energetically favourable compared to the 5-fold state of xanthan gum. This model is compatible with the model proposed by Cairns & Morris (1986), since the interplanar spacing of 0.526nm is similar to the value of 0.52nm obtained from the study by Cairns & Morris (1986). Thus, the 2-fold state of xanthan may represent the energy minima for

the xanthan-locust bean gum complex, whereas the 5-fold state may be the most energetically favourable conformer for xanthan gum alone.

FIGURE 1.7 : MODEL PROPOSED BY CHEETHAM & MASHIMBA (1988) FOR THE INTERACTION BETWEEN XANTHAN AND LOCUST BEAN GUM



Experiments have shown that locust bean gum must be present when xanthan gum is in the disordered form and before xanthan chains self-associate (Cheetham & Mashimba, 1988, Morris, 1990a and Zhan et al, 1993), which can be achieved by increasing the temperature or decreasing the ionic strength (Foster & Morris, 1994a). For example, Cheetham & Mashimba (1988) observed gelation between xanthan gum and locust bean gum for a mixture containing potassium chloride mixed at high temperatures which was subsequently cooled. This is because heating the mixture melts the xanthan helix to the disordered coil which can interact with locust bean gum as well as salt, in which the cooling process rearranges the molecules from random coils to a more orderly conformation containing the junction zone regions on the galactomannan backbone. However, if xanthan gum is heated with potassium chloride first before adding locust bean gum followed by cooling, no gels are formed because the salt stabilises the xanthan helix prior to the addition of galactomannan.

Other workers have suggested that xanthan gum can interact with locust bean gum when it is in the ordered and the disordered state. For example, Mannion et al (1992) proposed that xanthan gum and locust bean gum can interact by two distinct mechanisms in which one takes place at room temperature and is weakly dependent on the galactose content. These gels are weak and flexible and are said to contain the xanthan helix in the post interaction product, whereas the second interaction takes place on heating the mixture above the Tm of xanthan gum. These gels are much stronger and are highly dependent upon the galactose content of locust bean gum and involve the

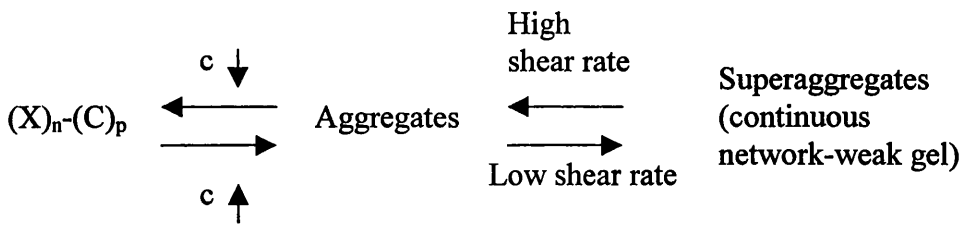
disordered form of xanthan. In contrast, Casas & García-Ochoa (1999) also determined that xanthan gum in both conformational states can interact with locust bean gum, but the interactions were greater when xanthan was in the ordered conformation (40°C) as shown from viscosity studies. In addition, the interaction was further enhanced by increasing the dissolution temperature of locust bean gum to 80°C which increased the M:G ratio, in agreement with Mannion et al (1992). Again this emphasizes the importance of the preparation temperature. In contrast to Mannion et al (1992), Lundin & Hermansson (1995), found that the rheological properties at 20°C for xanthan-locust bean gum mixtures subjected to heating and cooling cycles between 30°C-80°C, were always dependent on the M:G ratio. In agreement with Mannion et al (1992), mixtures containing a high M:G ratio (LBG-80) showed the greatest synergy at temperatures above 60°C. The gelation temperature (Tg) was 53°C for mixtures which contained a high M:G ratio and 40°C for mixtures which contained a low M:G ratio. The gelation process was slower for the latter gel, whereas the interaction was faster for mixtures containing the higher M:G ratio resulting in more elastic gels. The differences observed in the extent of interactions with LBG-35 and LBG-80, were thought to be due to differences in their galactose content, i.e. since LBG-35 is highly substituted, it can only interact with the xanthan superstrands at the surface (i.e., LBG-35 is excluded from the xanthan aggregates). In contrast LBG-80 has less galactose substitution, and so it can interact with the xanthan superstrands at the surface and bind to the xanthan helices (i.e., LBG-80 is included in the xanthan aggregates). These findings are in agreement with McCleary (1979), but disagree with the results from Mannion et al (1992), in that mixtures had to be heated to approximately 70°C before they became sensitive to the M:G ratio.

In a different study by Foster & Morris (1994b), it was observed that the onset of gelation occurred at approximately 60°C in the presence and absence of sodium chloride. In the presence of 30mM sodium chloride, the Tm was approximately 70°C suggesting that xanthan gum must have been in the ordered conformation when gelation began. However in the absence of sodium chloride, the Tm was 45°C suggesting that xanthan gum must have been in the disordered state when gelation began. This study showed that conformational rearrangement of the xanthan helices to accommodate binding interactions with other polysaccharides is reasonable, and the formation of mixed gels can occur under conditions in which xanthan is ordered and disordered. In

support of this, Morris (1990a) found that some gels contained ordered helices with disordered chains. These studies have shown that the presence of disordered xanthan is not a necessary requirement for interactions to occur. Instead it has been proposed that the thermodynamic stability of the heterotypic mixed junction zones in comparison to the 5-fold helix structure of xanthan is more important. For example, if xanthan is ordered at the time of mixing with locust bean gum, the conformation will be pulled over to the geometry required for efficient binding with the co-synergist. If xanthan is disordered at the time of mixing, the same heterotypic structure will be formed directly in competition with the normal 5-fold helix (Foster & Morris, 1994b).

It has also been suggested that the interaction between xanthan and locust bean gum involves a self-association or aggregation mechanism. Cuvelier & Launay (1988) demonstrated that a weak gel structure exists at low concentrations. Ageing of this gel results in formation of superaggregates which are shear sensitive as opposed to the stable aggregates. This results in network formation as illustrated in Figure 1.8.

FIGURE 1.8 : AGGREGATION MECHANISM PROPOSED BY CUVELIER & LAUNAY (1988) FOR XANTHAN GUM-LOCUST BEAN GUM INTERACTION



1.2.3 Factors affecting gel strength

The gel strength of xanthan gum-locust bean gum systems are affected by several factors such as the pH, galactose composition and chain length of locust bean gum, preparation temperature, polymer ratio, total polymer concentration, the degree of acetate and pyruvate groups within xanthan gum, and ionic strength. Some of these factors have already been discussed in Chapter 1.2.2 to support the models proposed for the interaction, and will now be covered in greater detail in this section.

It has been reported by some workers that the gel strength of xanthan gum-locust bean gum mixtures is affected by pH, whereby the maximum gel strength occurs between pH 6-8, and decreases outside this range. This maybe the result of fewer junction zones available, due to conformational changes, which would make interactions less favourable. In addition it has been found that the higher the xanthan-locust bean gum ratio, the greater the pH stability, because xanthan gum is stable over the entire pH range, whereas locust bean gum is less acid stable (Kovacs, 1973). In contrast to Kovacs (1973) findings, Baichwal (1991) described xanthan-locust bean gum mixtures as being relatively insensitive to the pH, and thus found the dissolution profiles for propranolol release all very similar with variable pH.

The gelation process is sensitive to the Mannose:Galactose ratio (M:G) of locust bean gum (Morris, 1990a) which has already been mentioned in the previous section. In general, the strongest interactions occur for galactomannans consisting of a high M:G ratio (Dea & Morrison, 1975, McCleary, 1979 and Cheetham et al, 1986), since increasing the mannose component increases the unsubstituted regions of the backbone which are thought to be the binding sites for xanthan gum (McCleary, 1979, Mannion et al, 1992 and Lundin & Hermansson 1995). In sterically favourable terms, accommodating the large trisaccharide xanthan side chains into the galactomannan crystallites would create distortion of the galactomannan lattice, unless the galactose content is decreased, to increase the accommodation (Cairns & Morris, 1986). Thus, it has been observed by Zhan et al (1993) that locust bean gum fractions with a higher M:G will accommodate more xanthan which is supported by an increase in the storage modulus (G′).

The chain length of the galactomannans is also critical in the formation of the 3-D gel network. Findings have suggested that the interaction does not require long, contiguous, unsubstituted D-mannose residues, but requires galactosyl residues to be located on one side of the main chain, which may also serve as junction zones. This maybe in addition to interactions with the galactose free sections (McCleary, 1979). Additionally, the distribution of galactose free residues also influences the gel strength. For example, locust bean gum from *C.siliqua* (25% galactose) forms a stronger synergy with xanthan, compared to locust bean gum from *C.pulcherinia* (24% galactose) because enzymatic hydrolysis experiments have shown that there is a good proportion of non-random

unsubstituted blocks from *C.siliqua* compared to *C.pulcherinia* which has a random block distribution. This implies that there will be fewer blocks suitable for interactions for locust bean gum from *C.pulcherinia* (Dea et al, 1986b).

The gel strength is greatly enhanced by heating followed by cooling (Cairns & Morris, 1986, Mannion et al, 1992, and Zhan et al, 1993). Cairns & Morris (1986) found that heating followed by cooling was a prerequisite in order for gels to form between xanthan gum and locust bean gum, since it was observed that they did not form gels at room temperature. In fact gelation only occurred if the mixtures were heated to 95°C (above the Tm) and cooled to room temperature. In a different study by Zhan et al (1993), it was found that as the preparation temperature (Tp) was increased, the G′ also increased which closely followed the degree of disordering of the xanthan molecules. However, it is important to note that the mixtures used in this study had been previously heated to 85°C followed by overnight cooling prior to measurements. Casas & García-Ochoa (1999) found that if the individual polysaccharides were heated separately at 'optimum' temperatures (40°C for xanthan gum i.e. below the Tm and 80°C for locust bean gum i.e. high M:G ratio) followed by mixing them together at 25°C, the synergy could be enhanced. This approach is different to the other studies, in that the polysaccharides are not mixed together under the same set of conditions.

The polymer ratio also has a great influence on the extent of the interaction between xanthan and locust bean gum. Many workers have reported that the maximum synergy occurs for a 1:1 xanthan-locust bean gum mixture (Williams et al 1991, Mannion et al, 1992 and Doublier, 1994), in which it has been proposed that there are an equal number of junction sites available on each polymer molecule (Kovacs, 1973). Other workers have found different ratios for the optimum synergy between the two polysaccharides. For example, it has been observed that the maximum synergy occurred for a 1:2 xanthan gum-locust bean gum ratio, suggesting that there are twice the number of junction sites available on each xanthan molecule as on the locust bean gum (Tako et al, 1984). Casas, & García-Ochoa (1999) are also in agreement with this ratio, although they found that this ratio could deviate depending on the experimental conditions. In support of this, Craig et al (1997) found that maximum synergy occurred at a ratio of 1:9 for an unheated xanthan gum-locust bean gum mixture, which deviated to approximately 3:7

for a xanthan gum-locust bean gum mixture which had been heated to 70°C-80°C and cooled to room temperature.

It has also been found that if the polymer ratio is kept constant and the total polymer concentration is increased, the gel strength also increases (Mannion et al, 1992). However, Casas & García-Ochoa (1999) investigated the effect of total polymer concentration on viscosity, and found that a total polymer concentration of 1.5kgm^{-3} gave the greatest viscosity reading, which may imply that there are restraints to gel structure formation or molecular interaction posed by high polysaccharide concentrations. It has also been observed that if a 1% xanthan-locust bean gum gel contains at least 20% of either polysaccharide, then an increase in the locust bean gum concentration to the total gum concentration does not have a significant effect on the gel strength (Rocks, 1971).

The acetate content of xanthan gum has been shown to greatly affect the degree of interaction with the galactomannans. Stronger interactions result between acetate free xanthan and the galactomannans, which may be due to greater side chain mobility and/or greater backbone flexibility, thus promoting optimum geometry necessary for maximum synergy with locust bean gum (Foster & Morris, 1994a and Tako et al, 1984). These findings suggest that the xanthan side chains could be responsible for the interaction (Tako et al, 1984 and Cuvelier & Launay, 1986b). In contrast, Dea et al (1977), observed that the deacetylation of xanthan gum only marginally improved the gel strength with locust bean gum.

The gelation process of xanthan gum-locust bean gum mixtures is greatly affected by salts and cations which has been discussed to some extent in Chapter 1.2.2. This will now be discussed further with reference to other peoples work in this section. Bresolin et al (1998) found that by increasing the salt concentration the extent of the interaction between xanthan and locust bean gum decreased because less disordered xanthan chain segments were available. In support of this finding, Tako et al (1984) also observed a decrease in the dynamic viscoelasticity measurements in the presence of sodium chloride and magnesium chloride at concentrations as low as 0.1%. This was thought to be due to ionic repulsions on the charged trisaccharide side chains. A different explanation could be that since it has been demonstrated that salt induces aggregation of

xanthan helices, this decreases the synergistic interaction with locust bean gum, since there is less capacity for xanthan to bind with locust bean gum in which the xanthan aggregates are less able to adjust conformation to the geometry required for efficient packing within heterotypic junctions (Ross-Murphy et al, 1983). In a different study, homogenous gels were formed in water between disordered xanthan chains and locust bean gum. However in salt solution, these polysaccharides did not form gels but formed gel islands, which could be converted back to homogenous gels by heating above the transition order-disorder temperature of xanthan gum (Cheetham & Mashimba, 1988). In contrast to these studies, Williams et al (1991) and Zhan et al (1993) found that for a given ratio of xanthan gum-locust bean gum, the melting temperature and gel strength were independent of the ionic strength.

1.2.4 Rheological behaviour

There are different rheological techniques which can be used to characterize the different types of gels that may be formed under certain conditions. For example, thermoreversible gels can be characterized by their melting point and setting temperature, because they tend to exhibit sharp melting and setting behaviour over a narrow temperature range. Firm gels however, are usually characterized by the storage modulus (G′) or the yield stress, whereas weak gels and viscoelastic fluids can be monitored by oscillatory or dynamic viscosity measurements, and any breakdown in their structure can be monitored using rotational methods (Dea et al, 1977). In this section, the rheological behaviour of xanthan gum-locust bean gum mixes will be discussed with reference to other workers findings.

It has been found using oscillatory rheology that the mechanical spectrum for a cold mixed xanthan gum-locust bean gum mixture ($0.1 g litre^{-1}$) in one study was typical of a gel, since the storage modulus (G′) was greater than the loss modulus (G′′) at all frequencies (ω) studied, with both parameters being less dependent on ω compared to locust bean gum alone. In addition, the G′′ was approximately constant throughout, suggesting that the synergy was primarily due to an increase in either the quantity or duration of cross-linking between the two polymers (Mannion et al, 1992). Furthermore, heating the mixtures to 60°C followed by cooling to room temperature significantly increased G′ for mixtures containing locust bean gum fractions greater than

35°C (LBG35). This suggests that either the heating process allows more complete molecular mixing or the higher locust bean gum temperature fractions are more heterogeneous as a result of increased self-association. Another possible explanation is that the input of energy from heating may facilitate additional interactions not possible at room temperature. In a different study by Doublier (1994), oscillatory shear and creep-recovery measurements were performed on various xanthan gum-locust bean gum mixes. It was observed that for a 1:99 xanthan gum-locust bean gum mixture, the system displayed solid like characteristics at low ω and relatively long times in the creep experiments, indicative of a weak gel. Thus it was concluded that the presence of even low concentrations of xanthan gum transforms a locust bean gum system from a macromolecular solution to a structured system with gel like properties. Further experiments investigating polysaccharide ratio provided more information on the interaction. For example, the spectrum for a 70:30 xanthan gum-locust bean gum mixture was also found to be typical of a gel (G′>G′′). However, instead of observing one flat plateau curve, two plateau regions separated by an inflection point coinciding with the maxima in the G′′ trace were observed. This was thought to be attributed to a relaxation process, related to two types of molecular interactions in the medium. Therefore, the gel could have been the result of the formation of two networks. Thus, it was concluded that on mixing the two polysaccharides, each macromolecule was excluded from the volume occupied by the other polysaccharide, which would result in two separate phases, each one being enriched by a polysaccharide (mutual exclusion theory). A high concentration of galactomannan would occupy most of the volume, but since xanthan has the major role in the interaction, it contributes to a continuous network, resulting in a bicontinuous 2-phase system.

Copetti et al (1997) also used oscillatory rheology and observed two inflection points in the complex modulus (G*) with temperature. This result is indicative of a two step process, in which it was suggested that the first step could be ascribed to the melting of the mixed junction zones whereby xanthan associates with locust bean gum in its helical form. The second step occurring at a higher temperature could be due to the conformational transition of the xanthan chains to the disordered form, such that the mixed gels may contain both heterotypic xanthan gum-locust bean gum and homotypic xanthan-xanthan junction zones with xanthan retaining its helical form. Additional xanthan–xanthan interactions have also been suggested by Cuvelier & Launay (1986b).

Zhan et al (1993) also used oscillatory rheology measurements and observed that in contrast to the findings by Copetti et al (1997), the level of xanthan gum-locust bean gum interaction is related to the degree of disordering of the xanthan molecule at the preparation temperature which was measured using G′.

Cuvelier & Launay (1988) used shear viscosity studies for low concentrations of xanthan gum-locust bean gum mixtures. It was found that for a 6:4 xanthan-locust bean gum mixture (total polymer concentration = $0.01 g dl^{-1}$), marked thixotropic behaviour was observed, due to formation of a weak gel like structure sensitive to shear (i.e., shearing at $70 s^{-1}$ for 3min resulted in structure breakdown). The thixotropic behaviour was observed at the overlap parameter ($c[\eta]$) to be much lower than that corresponding to the coil overlap. This may imply that full occupancy of available space by polymer coils had occurred (Launay et al, 1986).

Sedimentation studies have also been performed on xanthan-locust bean gum mixtures. Slow rates were observed which could have been due to lumps of gel causing the local viscosity to be higher and the creation of a yield stress resulting in thixotropy. Above a certain yield stress, deformation occurred at a constant rate as a function of time, and an apparent viscosity could be calculated because the gel could yield and break. The gels exerted an apparent yield stress, which was poorly reproducible, but above this yield stress, the viscosity was low and so the mixture flowed. This yielding process occurred where there were inhomogeneities within the gel, which depended on the applied stress, shear rate and shear time, although it must be stressed again that these results were poorly reproducible. In these sedimentation experiments, it was observed that the second bead moved faster after the first bead had fallen, suggesting that the gel structure had been partly irreversibly broken down due to the passage of the first bead. This would imply that no energy is needed for the yielding process. This was accompanied by an irreversible decrease in the apparent viscosity (i.e., the structure had broken down) (Luyten et al, 1993).

1.2.5 Biological role

It has been suggested in Chapter 1.1.3.1 that the role of xanthan gum is involved in host-pathogen relationships (Morris et al, 1977 and Cairns & Morris, 1986). One of the

reasons to supports this, is that xanthan gum has the ability to interact with polysaccharides containing a β-1,4 linked backbone, which is a component of plant cell walls, and is the major constituent of the galactomannan and glucomannan polysaccharides. This interaction may arise through co-crystallization of structurally similar segments, and data has provided evidence that the binding of xanthan to plant cell wall components can only occur if helix formation is incomplete (Cairns & Morris, 1986). Thus, the product of the synergy, may be used as a model for recognition in certain host-pathogen interactions, since xanthan gum excreted from microbial cells act as a protective barrier against dry weather conditions and represent a barrier against bacteriophage attack. In addition, xanthan gum may also be involved in recognition of appropriate sites on the plant host for colonization of bacteria (Dea et al, 1977 and Kennedy & Bradshaw, 1984).

1.2.6 Pharmaceutical uses

The xanthan gum-locust bean gum interaction has been exploited pharmaceutically for controlled release systems, since the synergy results in gels which can be used as hydrophilic matrices to retard drug release. In this chapter, two products which are used pharmaceutically will be discussed, namely Buccastem (Reckitt & Colman) and TIMERx (Penwest) and in addition, other studies which show the pharmaceutical potential of the synergy will also be discussed.

Buccastem is a buccal dosage form containing prochlorperazine which is used to treat vertigo and control nausea and vomiting. Prochlorperazine belongs to the phenothiazine class of drugs and has a bitter taste, local anaesthetic properties and in the liquid form is sensitive to light. Using conventional tablets to formulate the drug (e.g. Stemetil), prochlorperazine has poor bioavailability leading to variable blood levels in humans. The invention of Buccastem was derived from evidence that higher blood levels of the drug could be achieved via the sublingual route, but due to the unpalatable taste and anaesthetic properties of the drug, a sublingual formulation could not be formulated and so a buccal tablet was developed to overcome these problems.

The invention employs xanthan gum and locust bean gum in combination with a sugar, in which it was found that xanthan gum:locust bean gum in a weight ratio of 3:1-1:1

produced slower release than the individual polysaccharides or a 1:3 weight ratio. In addition it was determined that the preferred sugar being sucrose, is thought to improve the gelling and disintegration properties of the tablets. The locust bean gum is of the cold-water dispersible type such as Meprodyn 200 (Meyhall Chemical A.G, Switzerland), since this product is more soluble at low temperatures. The preferred method of formulation is wet granulation as opposed to direct compression, which includes lubricants, glidants and a disintegrant in the process. The buccal tablets gel by water absorption to give soft hydrated tablets, which can be retained in position for up to 2hrs, providing controlled release of drug by diffusion. In comparison, it was found that tablets containing no gums did not gel, but remained in position until the tablets fully hydrated before disintegrating. By using Buccastem, the bioavailability of prochlorperazine in man is increased by 2-fold compared to the oral route (Sugden, 1986). A similar study carried out by Sugden (1987) to investigate the delivery of etorphine from buccal tablets was performed, in which it was which claimed that the bioavailability and analgesic properties of the drug were improved using the buccal route. The formulation giving the best slow release profiles was the same as that used for Buccastem.

TIMERx is a sustained release pharmaceutical excipient which can be blended with a wide range of therapeutically active medicaments and tabletted by direct compression (Baichwal et al, 1991). Such technology avoids the need for other excipients and reduces the experimentation time to optimize formulation. The formulation for TIMERx exploits the xanthan gum-locust bean gum synergy which forms the hydrophilic material. In addition, an inert filler is required in which dextrose is the preferred choice. It was determined that the optimum ratio for xanthan gum:locust bean gum is 1:1 to 4:1 which is similar to that used for Buccastem to give a slow release granulation.

Xanthan gum undergoes fast hydration and in combination with locust bean gum, the synergy produces gels of high strength, both properties of which are necessary for a slow release system. The TIMERx system is manufactured by a wet granulation method in which it has been proposed that an aqueous solution is necessary for locust bean gum to cross link xanthan gum to form the matrix. Drug is released from the matrix through contact of the dosage form with the gastrointestinal fluids which produces tablet

swelling with consequent formation of a hydrophilic gel matrix. The swelling of the gel matrix results in a decrease in density which allows the matrix to float on the stomach contents, to provide a slow drug delivery per unit time by dispersion or erosion of the outer portions of the matrix, hence drug release is zero order. In addition, it has also been suggested that another mode by which a constant rate of drug delivery maybe obtained could be a result of the mucoadhesive property of xanthan gum, which may loosely interact with mucin. Dissolution studies have revealed that the greater the solubility of the medicament, the greater the amount of hydrophilic material is needed to produce a slow release profile. Additionally it was determined that by varying the ratio of the drug:hydrophilic material and/or the total tablet weight, different slow release profiles can be achieved. In addition, biphasic release profiles can also be obtained.

Follow on studies which have been published have found that the granulation method has a large influence on the granule properties and compact strength of TIMERx. For example, dry mixing of the components leads to the production of weak compacts as do granules produced in a fluid bed granulator, because the method incorporates a lot of air into the granules, whereas the use of a high speed mixer granulation with a wet granulation stage produces strong tablets, because the greater shear allows the components to interact more intimately in the formation of the granule. In the same study, it was also found that formulations containing a mixture of components are weaker than those containing single components, and that the inclusion of dextrose may act as a binder (Tobyn et al, 1996a). Further work using Electron Spin Resonance (ESR) found that a dry mixing process of either the components or the preformed granules is insufficient to effectively disperse a low dose crystalline agent within the matrix. Other findings from this study included an ideal distribution of compound throughout the matrix where the compound is dissolved in the granulating fluid prior to granulation. Additionally, it was found that the interaction is unaffected by the granule drying method and does not occur during the tabletting process (Tobyn et al, 1996b).

Other studies have been performed to look at the drug release behaviour from xanthan gum-locust bean gum gels. A study by Mannion et al (1991) showed that ibuprofen release depended on the ratio of xanthan gum:locust bean gum, whereby the slowest release coincided with the maximum rheological synergy which occurred at a polymer ratio of 70:30. In addition, it was suggested that since G` was linearly related to time for

50% drug release, the gel strength determined release rather than the viscosity at the surface gel layer, i.e. an erosion mechanism for drug release was postulated which is in agreement with Baichwal et al (1991).

Watanabe et al (1992) investigated the effects of xanthan gum-locust bean gum concentration and additives on the release of prednisolone. The results showed that the apparent release rate of prednisolone from the hydrogels decreased as the gum concentration increased, which suggested that the diffusion of drug molecules was mainly controlled by the density of the three dimensional network structure in the matrix. The effects of glycerin and sucrose significantly lowered drug release, in which Electron Spin Resonance (ESR) was used to measure the microscopic viscosity of the hydrogels. The results from ESR suggested that glycerin and sucrose may increase the diffusional resistance by increasing the microscopic viscosity of the hydrogels.

In vivo studies in rabbits using rectal gels containing buprenorphine and xanthan gum:locust bean gum have been investigated, in which it was found that the maximum plasma concentration of buprenorphine gradually decreased with an increase in the gum concentration. Furthermore, absorption was rapid and side effects reduced using the rectal route (Watanabe et al, 1996).

1.3 OBJECTIVES

The main objective of this study was to investigate the mechanism for the interaction between xanthan gum and locust bean gum with particular respect to the conformation of xanthan gum during the interaction. Although a lot of work has been done in this area, there are no firm conclusions, which is why this area still needs to be explored, and was also necessary in order to explore other areas during the course of the Ph.D. A combination of complimentary techniques were used throughout the Ph.D. High Sensitivity Differential Scanning Calorimetry (HSDSC) and Synchrotron Circular Dichroism (SCD) were useful techniques to study conformation and factors affecting the conformational behaviour of xanthan gum with locust bean gum. The main factors which were studied were temperature, polysaccharide ratio, sodium chloride and sucrose. Temperature played an important role in this work because it affects the synergy and hence gel strength of xanthan gum:locust bean gum systems as discussed in

the introduction. For these reasons, oscillatory rheology and texture analysis were employed. Similarly, the polysaccharide ratio which is a grey area within the literature was also investigated using these techniques to give an insight into the interaction. The incorporation of sodium chloride into xanthan gum:locust bean gum mixes has been studied extensively, so this variable was only briefly studied to determine its effects on the transition temperature of xanthan gum. However, sucrose is a sugar additive which together with xanthan gum:locust bean gum mixes has not been extensively studied, particularly within the pharmaceutical field. As a contribution to new areas of research, the effects of sucrose on this synergistic interaction were investigated. From these findings, a further objective of the study was to determine new ideas and methods for pharmaceutical uses of these systems.

CHAPTER 2

CHAPTER 2 : MATERIALS

2.1 MATERIALS

2.1.1 Xanthan gum

Xanthan gum samples were supplied by Pronova Biopolymer (Disatec S.A. France) under the trade name of Proxan. Only one batch was used throughout the study namely B.N. 9726001, to eliminate batch to batch variability. Batch variation can significantly alter results, and is a particular problem when dealing with samples originating from natural sources. Although xanthan gum is a semi-synthetic polysaccharide produced by fermentation, inter batch variation is still important as the acetate and pyruvate content can vary considerably, as discussed in Chapter 1.1.1.2. The samples were stored in a cool, dry place away from strongly oxidising material as recommended by the material safety data sheet. Table 2.1 is the certificate of analysis from Pronova which shows the physical properties of xanthan gum. The pyruvic acid determined using the pharmacopoeia test (USPXX, 1980), refers to the pyruvate groups on the xanthan side chains. Since the analysis values depend on the techniques/instruments and experimental conditions, this work was not repeated during the course of the study. Furthermore, Pronova Biopolymer is one of the few companies which supply extensive analysis data on naturally occurring polysaccharides. Therefore any values for the physical properties of xanthan gum in subsequent chapters will be quoted from Pronova Biopolymer (Disatec S.A. France) as in Table 2.1.

2.1.2 Locust bean gum

Locust bean gum samples were also obtained from Pronova Biopolymer (Disatec S.A. France) under the trade name of Isocarb. The product is naturally occurring, obtained by grinding of the endosperm of the locust bean seed. Thus, for similar reasons as discussed above, only one batch of locust bean gum was used throughout the study (B.N. 1096). The samples were stored in a similar manner to xanthan gum i.e. in a cool, dry place away from heat and solvents as recommended by the material safety data sheet, since this is a combustible polysaccharide. Table 2.2 is the certificate of analysis from Pronova which shows the physical properties of locust bean gum. Any values which may be quoted in further chapters regarding locust bean gum physical properties, will be from Table 2.2 for the same reasons as stated above in Chapter 2.1.1.

TABLE 2.1 : CERTIFICATE OF ANALYSIS FOR XANTHAN GUM (B.N. 9726001) FROM PRONOVA BIOPOLYMER (DISATEC S.A. FRANCE)

DETERMINATIONS	SPECIFICATIONS	RESULTS
ASPECT	FINE POWDER	IN CONFORMITY
COLOR	WHITE TO TAN	IN CONFORMITY
ODOUR	WEAK	IN CONFORMITY
PARTICLES<250µ	100% MINI	IN CONFORMITY
PARTICLES<175µ	95% MINI	IN CONFORMITY
LOSS ON DRYING	6 TO 12%	9.4%
ASH	6.5 TO 16%	11.3%
VISC.1% SOL IN KCl1%	1200 TO 1600 CPS	1600 CPS
pH 1% SOL	6 TO 8	7.1
ISOPROPANOL	500 MG/KG MAX	<100
ASSAY	91 TO 108%	IN CONFORMITY
PYRUVIC ACID	1.5% MINI	IN CONFORMITY
HEAVY METALS (Pb)	20 MG/KG MAX	< = 5
LEAD	5 MG/KG MAX	< = 5
ARSENIC	2 MG/KG MAX	< = 1
TOTAL PLATE COUNT/G	1000 MAX	IN CONFORMITY
YEAST MOULD/G	100 MAX	<5
PATHOGENS	NEGATIVE	IN CONFORMITY
S.AUREUS	NEGATIVE BY TEST	IN CONFORMITY
PSEUDOMONAS AERUG.	NEGATIVE BY TEST	IN CONFORMITY
SALMONELLA SP.	NEGATIVE BY TEST	IN CONFORMITY
C. PERFRIGENS	NEGATIVE BY TEST	IN CONFORMITY
COLIFORM E. COLI.	NEGATIVE BY TEST	IN CONFORMITY

TABLE 2.2 : CERTIFICATE OF ANALYSIS FOR LOCUST BEAN GUM (B.N. 1096) FROM PRONOVA BIOPOLYMER (DISATEC S.A. FRANCE)

DETERMINATIONS	RESULTS	GUARANTEE
TVC/G	200	5.0 UFC
YEAST/G	50	500 UFC
MOULD/G	50	500 UFC
E.COLI/G	NOT DETECTED	ABSENCE
SALMONELLA/ SHIGELLA/25G	ABSENCE	ABSENCE
PROTEINS (%)	7.4	<8%
ACID INSOLUBLE MATTER (%)	1.6	<5%
FATS %	0.8	<1%
ASH (%)	0.9	1.2%
HEAVY METALS PPM	<20	<20 PPM
GALACTOMANAN (%)	77.9	>75%
VISCOSITY AT 25°CPS	2850	>2800 CPS

2.1.3 Sodium Chloride

Sodium chloride was used as an additive in some polysaccharide mixes to investigate the effects on the transition temperature of xanthan gum and gel strength of certain systems. The sodium chloride used was AnalaR grade (99.99% purity) purchased from B.D.H., B.N. K23430732 645.

2.1.4 Sucrose

Sucrose as an additive was chosen to be studied, because it is the preferred sugar in the formulation of Buccastem (Sugden, 1986) for reasons which are unknown. In addition, not much research has been carried out on the effects of sucrose on xanthan gum-locust bean gum mixtures as mentioned in Chapter 1.3. The sucrose used was obtained from B.D.H. AnalaR, B.N. K23034086 644.

2.1.5 Pulverised Sucrose (powdered)

Pulverised sucrose was used as a filler in the tabletting studies to investigate the potential of xanthan gum-locust bean gum mixes for pharmaceutical uses. Powdered sucrose was chosen instead of granular sucrose (Chapter 2.1.4) used in the studies investigating sucrose as an additive, because of less segregation problems when dry blending with other excipients (similar particle size distributions). The powdered form was obtained from the British Sugar Corporation, B.N. 4090D32V.

2.1.6 Lactose

Lactose DC was chosen as the comparative filler to sucrose in the tabletting work. Lactose DC was chosen (DC=Directly Compressible) because of its improved powder flow and compatibility with other excipients. Lactose DC was obtained from Meggle, B.N. L9822.

2.1.7 PVP K30

Polyvinylpyrrolidone (PVP K30) was the choice of disintegrant in the formulation studies for the tablets, and is used in the Buccastem formulation. PVP K30 was obtained from BASF, B.N. 82-5927.

2.1.8 Magnesium Stearate

Magnesium stearate was the chosen tablet lubricant. This was acquired from Akcros Chemicals, B.N. 3310.

2.1.9 Talc

A glidant was incorporated in addition to a lubricant to prevent 'sticking' during the tabletting process. The talc used was supplied by Hays Chemicals, B.N. 307419T472.

2.1.10 Theophylline

Theophylline was the selected model drug for the dissolution studies, to investigate drug release from the different tablet formulations containing xanthan gum and locust bean gum. Theophylline was chosen because it has a high melting point (270-274°C), thus enabling different drying temperatures to be used in the formulation process, and additionally is compatible with a wide range of excipients, particularly with the ones chosen for the tabletting studies. Solutions of theophylline are also stable over a wide range of pH values except at the two extreme ends of the scale. Although theophylline is not a very soluble drug in water (1 in 120 parts at 25°C), it is soluble enough for the purposes of the dissolution studies, and in addition theophylline is a good choice of drug since it can be detected in the ultra violet absorption spectrum at 270nm (Clarkes-Isolation and identification of drugs, 1986). The theophylline used was purchased from Sigma Chemicals, B.N. 68H0610.

2.1.11 Methylparabens

Methylparabens was necessary as an antimicrobial preservative in some of the formulation studies. It is especially effective against moulds and yeasts which was a problem in the drying processes at 35°C over long time periods. Methylparabens (NipaGinM - fine) was obtained from Nipa Laboratories, B.N. M2Y899.

2.1.12 Deionised water

AnalaR grade deionised water was used throughout the Ph.D. project to make up any solutions. This was purchased from B.D.H. and was preferred over deionised laboratory water because it has consistent, known chemical properties. This was important since the conformation of xanthan gum is known to be affected by certain cations. Table 2.3 shows a list of the maximum limits of impurities.

TABLE 2.3 : MAXIMUM LIMITS OF IMPURITIES FOR DEIONISED WATER (AnalaR)

RESIDUE ON EVAPORATION AT 110°C	1ppm
CHLORIDE (Cl)	0.1ppm
NITRATE (NO_3)	0.3 ppm
PHOSPHATE (PO_4)	0.01 ppm
SILICATE (SIO_2)	0.02 ppm
SULPHATE (SO_4)	1 ppm
ALUMINIUM (Al)	0.01 ppm
AMMONIUM (NH_4)	0.01 ppm
BARIUM (Ba)	0.005 ppm
CADMIUM (Cd)	0.005 ppm
CALCIUM (Ca)	0.05 ppm
CHROMIUM (Cr)	0.005 ppm
COBALT (Co)	0.005 ppm
COPPER (Cu)	0.005 ppm
IRON (Fe)	0.005 ppm
LEAD (Pb)	0.005 ppm
MAGNESIUM (Mg)	0.05 ppm
MANGANESE (Mn)	0.005 ppm
MOLYBDENUM (Mo)	0.005 ppm
NICKEL (Ni)	0.005 ppm
POTASSIUM (K)	0.05 ppm
SODIUM (Na)	0.1 ppm
STRONTIUM (Sr)	0.005 ppm
ZINC (Zn)	0.01 ppm
SUBSTANCES REDUCING PERMANGANATE (O)	0.08 ppm

2.2 PREPARATION OF SOLUTIONS AND MATERIALS

2.2.1 Preparation of polymer solutions (xanthan gum, locust bean gum and in combination)

Xanthan gum solutions were prepared by slowly adding the correct quantity of the powder form to deionised water under mechanical stirring (paddle blade) at 1150rpm for 30min to dissolve all the powder. This was performed either at room temperature or at higher temperatures (usually 90°C) in a temperature controlled water bath using aluminium foil covering to prevent water losses.

Locust bean gum solutions were prepared in the same way, except for the lower stirring speed of 800-1000rpm depending on the temperature and concentration because of lower viscosities in comparison to xanthan gum solutions.

Xanthan gum-locust bean gum solutions were prepared by firstly dry blending small amounts (10-20g) of the dry polysaccharide powders in varying proportions in a turbula mixer for 20min to form a homogenous mix. The correct quantity of the mixed powder was then added to deionised water under mechanical stirring (paddle blade) at 1150rpm for 30min to dissolve all the powder. Unless otherwise stated in further chapters, the total polysaccharide concentration used in the experiments was 1%w/w. This was because higher concentrations created homogeneity problems whereas lower concentrations gave low readings which became 'off scale' for some of the polysaccharide solutions studied using rheology.

All polysaccharide solutions were stored at 8°C for 24hrs prior to any measurements, to allow adequate hydration and gelation effects, since interactions are time dependent for these polysaccharide systems.

2.2.2 Preparation of sucrose solutions with polymer(s)

Sucrose solutions were made prior to dissolving the polysaccharides to form a total concentration of 1%w/w solutions. The required quantity of sucrose was gradually added to deionised water under magnetic stirring for 30min or until all the sucrose had dissolved, which depended on the %w/w of sucrose solution (0.5-15%w/w) prepared.

Polymer-sucrose solutions were then prepared and stored as described in Chapter 2.2.1 using the sucrose solution to replace the deionised water.

2.2.3 Preparation of sodium chloride solutions with polymer(s)

Sodium chloride solutions were made in a similar manner to the sucrose solutions prepared in Chapter 2.2.2. Since the literature quotes 'millimolars' (mM) of sodium chloride used as an additive with xanthan gum solutions, similar concentrations were used (i.e. 10mM, 20mM and 30mM) to study the transitional behaviour of xanthan gum. These were first prepared in volumetric flasks before undergoing magnetic stirring (Mwt of NaCl = 58.44). Polymer-sodium chloride solutions were then prepared and stored as described in Chapter 2.2.1 using the sodium chloride solution to replace the deionised water.

CHAPTER 3

CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

3.1 INTRODUCTION

This chapter describes the techniques undertaken to characterise some of the basic chemical properties of the materials under study. In specific terms, three issues have been addressed, namely the pyruvate and acetate content of xanthan gum, the cation content of xanthan gum and the M:G ratio of locust bean gum.

The degree of pyruvate and acetate substitution within xanthan gum was important to characterise, because as mentioned in Chapter 1.1.1.2 this can vary considerably according to the different strains and processing conditions (Kennedy et al, 1985 and Shatwell et al, 1990) which in turn affects the stability of the secondary structures of xanthan gum. Characterisation has previously been carried out using High Pressure Liquid Chromatography (HPLC) (Slonekar & Orentas, 1962 and Cheetham & Punruckvong, 1985) and chemical methods such as lactate dehydrogenase experiments (Duckworth & Yaphe, 1970), 2,4 DNPH assays (Koepsell & Sharpe, 1952) and hydroxamic acid procedures (Hestrin, 1949) have also been applied. A more sophisticated technique which has been employed for pyruvate and acetate analysis is Nuclear Magnetic Resonance (NMR). This technique has widely been used because it is simple to use and easy to identify different groups within a sample (Jansson et al, 1975, Morris et al, 1977, Rinaudo et al, 1983 and Lopes et al, 1992). This technique was therefore used to characterise the pyruvate and acetate groups within xanthan gum.

One other important factor of xanthan gum that needs to be characterised is the salt content, which again varies between commercial samples and affects the conformational behaviour of this polysaccharide (Milas & Rinaudo, 1979 and Shatwell et al, 1990) which consequently affects interactions. The salt content was not included in the certificate of analysis from Pronova (Table 2.1) so it was important to quantify certain cations. Since most studies discuss the effects of sodium cations (Na^{+}) and calcium cations (Ca^{2+}) on the transitional behaviour of xanthan gum (Kennedy & Bradshaw, 1984 and Morris, 1990b), these two cations were determined. This has previously been done using conductivity measurements (Luyten et al, 1993) and running ashed samples dissolved in acid through an Inductively Coupled Argon Plasma (ICP) unit (Rochefort & Middleman, 1987). In this project, the use of Flame Emission Spectrometry (FES)

will be used to characterise the cationic content, since it is a quantitative and simple technique sensitive enough to detect common metals.

With respect to characterising locust bean gum, the M:G ratio was determined, since this factor affects the interactive properties with xanthan gum as detailed in Chapter 1.2.2. This is because it has been reported that the unsubstituted regions of the mannan backbone (i.e. the smooth regions of the molecule) interact with xanthan gum (Dea & Morrison, 1975 and Dea et al, 1977 and Cheetham & Mashimba, 1988). The M:G ratio can be increased by a fractionation technique described by Gaisford et al (1986). This technique separates locust bean gum into different M:G ratios according to the minimum temperature at which water solubility can be attained. This method has been used by Mannion et al (1992) and Lundin & Hermansson (1995) and has similarly been used to fractionate the locust bean gum used in this study.

3.2 DETERMINATION OF THE ACETATE AND PYRUVATE CONTENT OF XANTHAN GUM USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

Nuclear magnetic resonance (NMR) is a sensitive technique for determining different chemical groups within complex molecules such as polysaccharides. This is because every nuclide has a characteristic magnetic moment for a given magnetic field strength.

NMR has been used to characterise xanthan gum, in which most of the analysis has been qualitative. Acetate and pyruvate peaks at around 2.1ppm and 1.5ppm respectively have been identified (Jansson et al, 1975, Morris et al, 1977 and Lopes et al, 1992). In the past, NMR was not a very sensitive technique compared to other analytical techniques such as liquid chromatography, and this together with the high viscosity of xanthan gum has led to poor resolution in quantitative analysis. However, NMR instruments have improved in recent years due to high-powered superconducting magnets and the introduction of a new family of instruments called Fourier transform NMR. Since a solution of xanthan gum in the ordered conformation has a high viscosity and produces strong dipolar interactions between proton or carbon nuclei, causing severe line broadening, NMR spectra cannot be detected under the usual high resolution conditions (Morris et al, 1977 and Hall & Yalpani, 1981). However, Rinaudo et al (1983) has tried to overcome this problem by first partially depolymerizing the

polymer to decrease the viscosity before carrying out proton NMR (H^1 NMR). This process increased the resolution of the spectrum and hence a quantitative analysis was determined. Thus, the acetate and pyruvate content is greatly influenced by the handling of the polymer (purification, heating etc.) in addition to the strain and culture conditions as described in Chapter 1.1.1.2.

Thus, in this chapter, H^1 NMR was used to identify and quantify the acetate and pyruvate groups within xanthan gum. Since xanthan gum as supplied by Pronova was used throughout the following studies, H^1 NMR was performed on untreated xanthan gum.

3.2.1 Basic principles of NMR

Molecules possess atomic nuclei which are in turn magnetic. These nuclear magnetic moments are sensitive to their surroundings, yet interact very weakly with them. Thus, when a nucleus is placed in a magnetic field, it adopts one of a small number of allowed orientations of differing energies. Generally, the magnetic moment can point in the same direction of the field or against it. These two orientations can be differentiated because they are separated by an energy difference (ΔE). This energy difference can be measured by applying electromagnetic radiation of a frequency (ν), which causes nuclei to 'flip' from the lower energy level to the higher level, provided the resonance condition in Equation 3.1 is satisfied ;

$$\Delta E = h\nu \qquad \text{Equation 3.1}$$

Where:

ΔE = energy difference

h = Planck's constant

ν = frequency of electromagnetic frequency

Since every nuclide e.g. ^1H has a characteristic magnetic moment for a given field strength, the ΔE and hence resonance frequency can be determined. The energy difference (ΔE) depends on the strength of the interaction between the nucleus and the field i.e. on the size of the nuclear magnetic moment and the strength of the magnetic field. It also depends on the chemical environment of the nucleus in a molecule, an

effect otherwise known as the *chemical shift*. For example, for the molecule CH_3CH_2OH, three different resonance frequencies and hence three separate peaks can be identified by their integrated areas which are in the ratio 3 :2 :1. This ratio reflects the number of protons of each type, i.e. CH_3, CH_2, OH.

For a molecule to be suitable for NMR analysis, the nuclei have to possess an angular momentum and hence magnetic moment. The angular momentum of a nucleus is an intrinsic property known as *spin*, whose magnitude can be expressed as shown in Equation 3.2 ;

$$[I\,(I+1)]\ ½\hbar \qquad \text{Equation 3.2}$$

Where:
I = spin quantum number of a nucleus - 0, ½, 1, 1½, 2.... (Quantum numbers greater than 4 are rare).

Thus, if nuclei have I = 0, e.g. ^{12}C, such nuclei have no angular momentum and hence no NMR spectra. The spin quantum number is determined by the number of unpaired protons and neutrons, hence ^{12}C has even numbers of protons and neutrons.

The magnitude of the effective field experienced by each group of nuclei can be expressed as follows in Equation 3.3;

$$H_{eff} = H_o\,(1-\sigma) \qquad \text{Equation 3.3}$$

Where:
H_{eff} = effective field
H_o = applied field
σ = shielding constant (positive or negative number)

Chemical shifts enable different types of protons to be distinguished from one another by the position of the peaks on the spectrum. This depends largely on the shielding parameters, i.e. field induction (electrons shield the nucleus which opposes the applied field, consequently the applied field needs to be increased in order to achieve resonance

and hence better resolution). The value of the shielding constant depends on hybridization and the electronegativity of the groups attached to the atom that contains the nucleus in question. For example, elecronegative groups reduce the shielding effect on a nucleus causing a downfield shift in the peak.

Since different NMR spectrometers have different field strengths, the resonance position is expressed with respect to the resonance of a reference compound. For proton spectra in non aqueous media, the reference used is usually tetramethyl silane (TMS) whose position is assigned to a single sharp peak at zero on the δ scale. In this way the measurements of a sample can be converted to the standard units of ppm or δ. Using a reference also enables the degree of shielding or deshielding to be determined. For example, a positive δ suggests a greater degree of shielding compared to the reference.

NMR can be used for quantitative analysis since the area under a peak is proportional to the number of protons responsible for the absorption. In sophisticated NMR instruments, a device which integrates the peaks is incorporated, which calculates the height of each step which is proportional to the number of nuclei in the spectrum.

3.2.2 Method

The method used for NMR analysis was similar to that used by Rinaudo et al (1983). A Bruker NMR spectrometer was used to obtain proton spectra for xanthan gum 1% w/w at 25°C using D_2O as the reference. Chemical shifts were referred to D_2O which resonates at 4.70ppm on the δ scale. The applied magnetic field was set at 500MHz to obtain high resolution using a pulse width of 4ppm, sweep width of 6024Hz and a zero relaxation delay. A large number of scans (24161) were accumulated, to observe the peak intensities for quantitative analysis using a 16K memory.

Xanthan gum 1%w/w solution, was prepared for NMR analysis by the addition of 1g of untreated xanthan gum powder to 100ml of deuterium oxide (D_2O) as described in Chapter 2.2.1. Deionised water was replaced with D_2O, otherwise the protons in water would interfere with the spectrum interpretation.

3.2.3 Results

The spectrum for xanthan gum 1%w/w is shown in Figure 3.1, with the peak integrals at the bottom and the chemical shifts shown at the top. According to Rinaudo et al (1983), a peak at 1.40ppm and a peak at 2.09ppm were identified as pyruvate and acetate groups respectively. In Figure 3.1, two peaks are observed at similar chemical shifts (1.44ppm and 2.03ppm) which are assigned to the pyruvate and acetate groups respectively.

The size of the integrals were used to determine the percentage of acetate and pyruvate groups within 1%w/w xanthan gum using Equation 3.4 (peak is proportional to the number of protons present). The values calculated are 21% for pyruvate and 5% for acetate which corresponds quite closely to the results from the work carried out by Rinaudo et al (1983) for the pyruvate content (23%). However, the acetate content calculated in the present study is much lower compared to Rinaudo et al (1983) (100% acetate content). A likely explanation for these differences could be the form of xanthan gum used i.e. partially depolymerised (Rinaudo et al, 1983) compared to untreated xanthan gum (present study) as well as batch and supplier variation. The results in the present study using NMR analysis, contrast to the percentage pyruvate from the certificate of analysis supplied by Pronova Biopolymer (Disatec S.A France) which is a minimum of 1.5% (Table 2.1). This difference could be due to the techniques used, i.e. the pyruvate content in Table 2.1 was determined by chemical treatment of xanthan gum followed by UV absorbance detection using a spectrophotometer (USPXX, 1980).

$$\% \text{ Chemical group} = (\text{Chemical group integral}/\text{Total sum of integrals})100 \qquad \text{Equation 3.4}$$

Where:

Chemical group = acetate or pyruvate

Therefore:

In a xanthan gum 1%w/w solution, there is 21% pyruvate and 5% acetate.

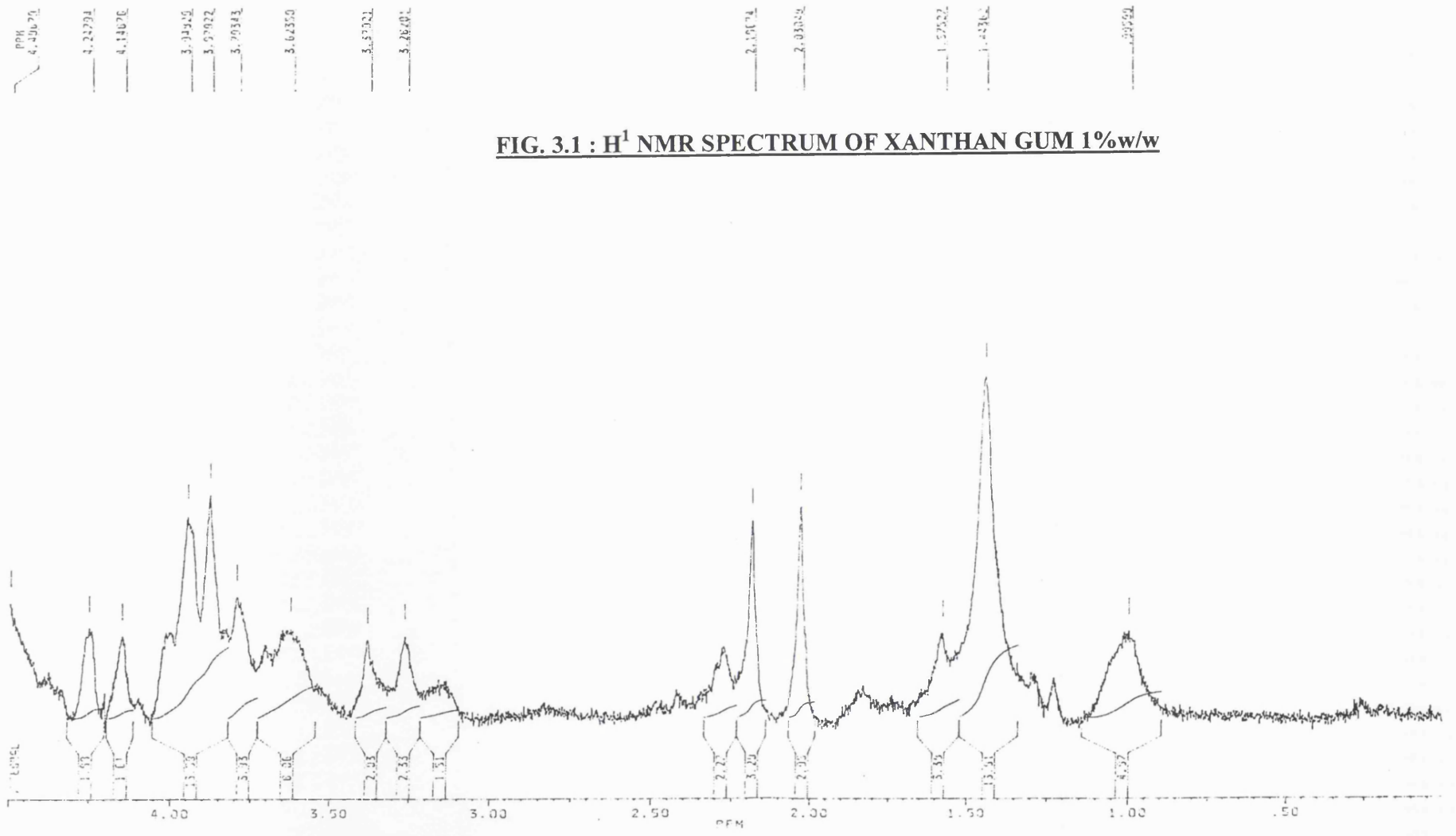


FIG. 3.1 : H^1 NMR SPECTRUM OF XANTHAN GUM 1%w/w

3.3 DETERMINATION OF THE SODIUM AND CALCIUM CONTENT OF XANTHAN GUM USING FLAME EMISSION SPECTROMETRY (FES)

Flame Emission Spectrometry (FES) is a simple, inexpensive and sensitive technique for detecting common metals. Such metals include, the alkali, alkaline earth such as sodium and calcium respectively, and several transition metals for example iron and copper. In addition, this technique can also be used to detect certain non-metals such as hydrogen, carbon and the halogens (Willard et al, 1988).

In this chapter, FES was used to quantify the sodium ions (Na^{+}) and calcium ions (Ca^{2+}) within xanthan gum, since cations can affect the conformational behaviour and hence the interactive properties of the polysaccharide (Bresolin et al, 1998).

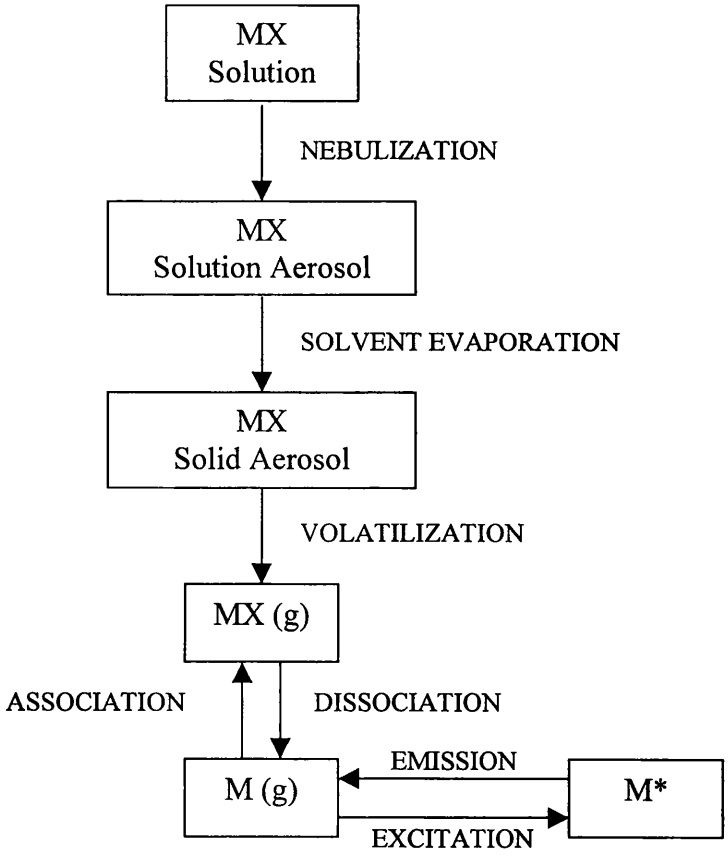
3.3.1 General principles of FES

The basis for FES is as follows. Electrons in the ground state can be promoted to higher energy levels by excitation of the atoms via electrical or thermal methods. When these electrons revert to the ground state or any intermediate energy level, the absorbed energy or radiation is emitted from the excited atoms which can be measured. The intensity of the emission for any particular element is proportional to the concentration and is characteristic of the sample components. Thus, FES is a quantitative and qualitative technique.

The flame itself is usually a common source of exciting electrons and a means of promoting them to higher energy levels. The flame temperature should be between 2000°C and 3000°C for most elements and can be achieved by burning mixtures of gases such as air and methane or air and acetylene. The exact temperature depends on the fuel:oxidant ratio and is usually highest for a 1:1 mixture. The preferred fuel : oxidant for detecting alkali metal elements is a mixture of air (oxidant) and acetylene (fuel). The sensitivity is dependent upon the flame geometry and is at a maximum for samples positioned in the higher wider part of the flame. However, the flame can also be a major source of noise which in turn can affect the detection limits, usually in the order of 0.1-10ppm for most metals. Thus, flame background emission should be minimised for sufficient resolution of the instrument.

The instrument for FES otherwise known as the flame photometer or spectrophotometer, consists of five components, namely a) nebulizer, b) burner, c) optical system, d) detector and e) recorder. When using FES, the sample must be first dissolved in a solution so that it can be nebulised for introduction into the flame ready for desolvation, vapourisation and atomisation all in rapid succession. Nebulisation is important for converting the liquid sample into a fine aerosol which is steadily introduced into the flame. Once the aerosol is in the flame, it desolvates and vapourises. The flame itself is usually the atomizer for FES, in which the atomization step converts the dissolved element into the free atoms in the ground state for FES analysis, as illustrated in Figure 3.2 (Willard, 1988).

FIGURE 3.2 : SCHEMATIC DIAGRAM FOR ATOMIZATION IN FES



Once the radiation is emitted, it then passes through a monochromator which is usually a selected filter for separate elements in simple instruments or a grating device in more sophisticated instruments. The monochromation step isolates the specific wavelength for the analysis, and a photodetector measure the power of the selected radiation which is then amplified and sent to a readout device, meter, recorder or microcomputer system.

The wavelengths usually fall within the visible or ultraviolet region, and the resulting photons are detected by photomultiplier tubes.

3.3.2 Method

Flame Emission Spectrometry (FES) is a trace level analytical technique, which meant that it was very important that all glass ware used to prepare samples were thoroughly cleaned. Since tap water can contain nearly 50ppm of Na^+, a protocol for cleaning glass ware was developed.

Volumetric flasks (100ml) and various size graduated pipettes used to prepare Ca^{2+} and Na^+ standards were soaked overnight in 10%v/v HCl. The glass ware was then thoroughly rinsed with deionised water to remove any acid followed by overnight drying in a 60°C oven. Likewise, beakers used to prepare xanthan gum samples were prepared in the same manner.

Since xanthan gum forms highly viscous solutions, low concentrations (500ppm xanthan gum solution for Ca^{2+} and 5ppm xanthan gum solution for Na^+) were prepared for FES analysis of Na^+ and Ca^{2+}. The 500ppm and 5ppm xanthan gum solutions were prepared in clean 250ml glass beakers using deionised water up to 100ml. The mixing method employed was the same as that described in Chapter 2.2.1. A stock solution of Ca^{2+} (100ppm) was used to make up known standards of Ca^{2+} ions ranging from 0.2-1ppm, which were measured using FES in order to construct a calibration curve. Similarly a stock solution of Na^+ (100ppm) ranging from 0.1-1ppm was used to make up known standards to obtain a calibration curve.

The spectrophotometer used for FES analysis was a Perkin Elmer-280 (PE-280 model). The gases burned to form the flame temperatures were a mixture of air and acetylene. The wavelengths for Ca^{2+} and Na^+ are 422.7nm and 589nm respectively, which were set on the machine accordingly by aspirating a strong solution of each cation (100ppm-stock solution) and adjusting the gain to achieve maximum deflection on the lamp/energy meter. The known standards were sprayed into the flame starting with the highest concentration first, and an average of 3 readings per standard was taken as the measurement. It was important to aspirate with water and wipe the capillary with a tissue after aspirating each solution to prevent contamination. After the standards were

measured and the calibration curves constructed were satisfactory, the xanthan gum solutions were sprayed into the flame and an average of 3 readings for each cation were determined.

3.3.3 Results

The calibration curves for Ca^{2+} and Na^{+} are shown in Figures 3.3 and 3.4, supporting a linear relationship between concentration and emission. The curve for Na^{+} is less linear than for Ca^{2+}, which is probably due to the presence of large amounts of Na^{+} in the atmosphere.

The results for the Ca^{2+} and Na^{+} content in xanthan gum are shown in Table 3.1, expressed as both parts per million (ppm) and percentage (% w/w) which were calculated as described in Appendix 1. With respect to a study by Rochefort & Middleman (1987), the Na^{+} content was calculated as 150ppm for a 5000ppm xanthan solution, which is equivalent to 300ppm for a 1%w/w xanthan solution. This value is close to the findings in the present study (342ppm), even though the cation content was determined by running ashed samples dissolved in acid through an Inductively Coupled Argon Plasma (ICP) unit (Rochefort & Middleman, 1987). However, the Ca^{2+} content calculated by Rochefort & Middleman (1987) is much higher (40ppm for a 1%w/w xanthan solution) compared to the findings in the present study (10ppm for a 1%w/w).

TABLE 3.1 : THE Ca^{2+} AND Na^{+} CONTENT DETERMINED BY FES ANALYSIS FOR A 1% w/w XANTHAN GUM SOLUTION

	Ca^{2+} ppm	Na^{+} ppm	Ca^{2+} % w/w	Na^{+} % w/w
XANTHAN GUM 1% w/w	10	342	0.001	0.03

3.4 FRACTIONATION OF LOCUST BEAN GUM FOR M:G RATIO DETERMINATION

Locust bean gum can be separated into cold and hot water soluble fractions having low and high M:G ratios respectively by a fractionation technique described by Gaisford et al (1986). The technique provides fractions according to the minimum temperature at

which water solubility can be attained. Fractionation has been used to obtain specific fractions having various M:G ratios, in which the M:G ratio has been determined by gas liquid chromatography (GLC) analysis of their alditol acetate derivative. (Mannion et al, 1992 and Lundin and Hermansson, 1995). This process has provided information on the mechanism for the interaction with xanthan gum as discussed in Chapter 1.2.2.

In the present work, the fractionation technique by Gaisford et al (1986) has been used to separate locust bean gum into two fractions, one having a low M:G ratio (LBG35) and the other having a high M:G ratio (LBG80). These two fractions were analysed for their M:G content using two methods (i) hydrolysis followed by GLC of the alditol acetates and (ii) H^1 NMR. The fractions were then mixed with xanthan gum as described in Chapter 2.2.1 at 25°C for rheological and HSDSC measurements to gain an insight into the mechanism for the interaction.

3.4.1 Method

The fractionation technique used by Gaisford et al (1986) has been slightly modified for the present work to obtain two fractions of locust bean gum with different M:G ratios. The first fraction is high M:G ratio i.e. LBG80 which is soluble between 35°C and 80°C, whereas the second fraction is low M:G ratio i.e. LBG35 which has a solubility of less than 35°C.

Locust bean gum (20g) was heated in 200ml 80%v/v ethanol at 90°C/15min in a water bath, followed by cooling in an ice bath for 15min and excess ethanol decanted. The residue was transferred into a funnel lined with high strength filter paper (113V/32cm) and washed with 200ml of 80%v/v ethanol. The washed residue was transferred into 2L deionised water and suspended using a silverson mixer for approximately 10min. The suspended residue was then separated into four 500ml beakers and left to hydrate in a 35°C water bath for 1hr. Insoluble material was separated from the soluble matter after hydration by centrifuging at 35°C at 5000rpm/30min. The solubilised locust bean gum was collected in a 2L volumetric flask and poured into 4L of 80%v/v ethanol to form the precipitate. The precipitate was collected by filtration via a large funnel lined with nylon, with subsequent washing in deionised water to remove the ethanol.

FIG. 3.3 : CALIBRATION CURVE FOR Ca^{2+} IONS

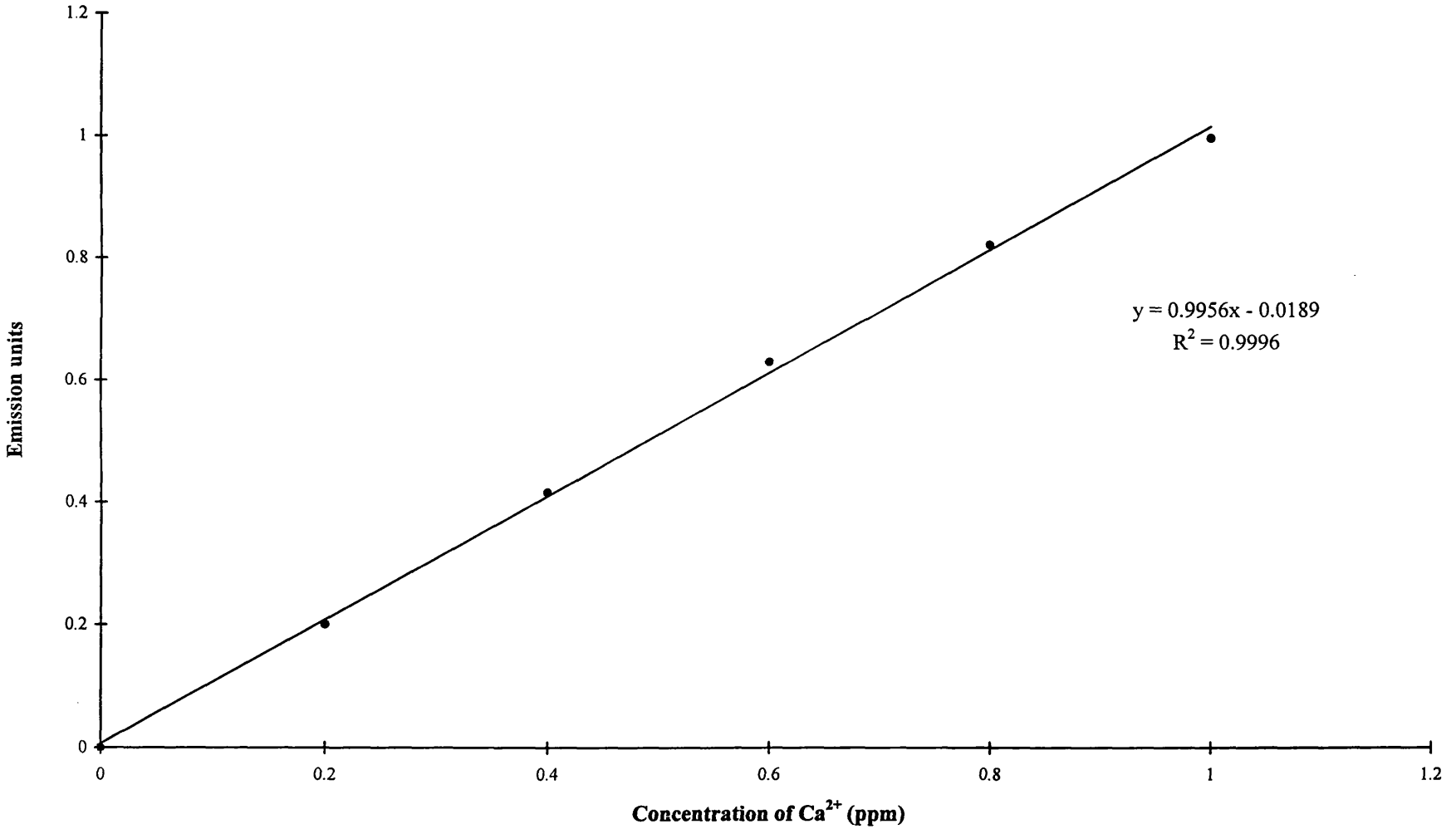
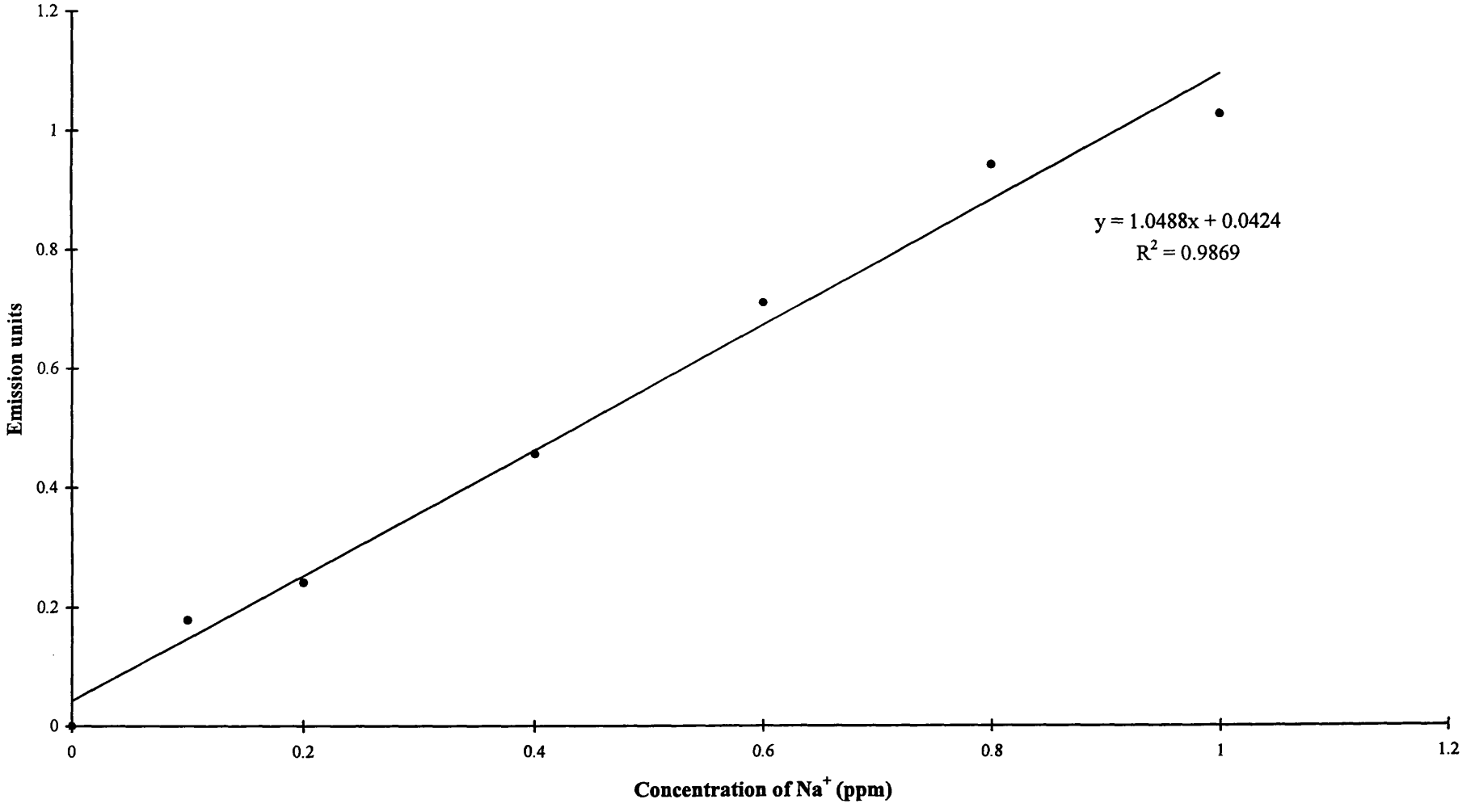


FIG. 3.4 : CALIBRATION CURVE FOR Na^+ IONS



The washed precipitate was then transferred onto a watch glass and left in a fume cupboard overnight at 25°C to dry off most of the ethanol. After 24hrs, the precipitate was transferred to a 50°C oven for 1-2 days until dry. The dry precipitate was then broken into small pieces by hand, and subsequently milled through a 3mm mesh size followed by a 1mm mesh size to form a powder using a Glen Creston hammer mill (14-580s, 240V, 50Hz). This dry powder was equivalent to LBG35 which was ready for M:G ratio analysis.

Meanwhile, the insoluble portion from centrifugation was suspended in 2L deionised water and left to hydrate in a water bath at 80°C/1hr. The solubilised form was centrifuged at 80°C and treated identical to the first locust bean gum fraction that resulted in LBG35. The final dry powder represented LBG80 which was ready for M:G ratio analysis in the same way as for LBG35. For each fraction, this method was repeated four times so that there were four samples per fraction for the M:G ratio analyses.

The two different techniques used to determine the M:G ratios were carried out at Reckitt & Colman for the hydrolysis followed by GLC of the alditol acetates and at the Norwegian Biopolymer laboratory NOBIPOL for the H^1 NMR method. The first method was based on work by Englyst et al (1992) which involves a complex procedure originally used to determine the dietary fibre content as non starch polysaccharides by GLC. The procedure has been summarised into two main parts (i) sample preparation and (ii) acid hydrolysis and GLC of the alditol acetate, as shown in Figures 3.5 and 3.6 respectively. A full description of the method is given in Appendix 2.

The second method used to identify the mannose and galactose residues was H^1 NMR. Mannose and galactose peaks could be quantitatively assessed by expressing the integrals of certain peaks to the total integration of all peaks. A Bruker NMR spectrometer was used at 84°C using D_2O as the reference and the locust bean gum fractions as the sample. The applied magnetic field was set at 300MHz and 64 scans were recorded.

3.4.2 Results

The results for the M:G ratio analysis of fractionated locust bean gum in the present work are shown in Table 3.2 and 3.3 using the methods carried out by Reckitt&Colman (acid hydrolysis and GLC of the alditol acetate) and NOBIPOL (H^1 NMR) respectively. Notice that the values for each repeat fraction are similar using either method, which shows that the fractionation technique is reproducible. The results using the two different analysis methods are compared in Table 3.4. Although the acid hydrolysis and GLC of the alditol acetate method gives slightly higher average values for LBG35 and LBG80 compared to the H^1 NMR method, the difference in magnitude for each fraction is the same (i.e. x1.08). Additionally, the difference in the average M:G ratio for each fraction analysed by the same method is identical by a factor of 1.46. Figures 3.7 and 3.8 show a typical H^1 NMR spectra for LBG35 and LBG80 respectively, with the integrals of the peaks shown at the bottom which were necessary for the M:G ratio determination.

The yields of each fraction were determined before the M:G ratio analyses and are shown in Table 3.5. The yields were very low particularly for LBG80 i.e. the last extraction considering that 20g of unfractionated locust bean gum was used for the fractionation process. Thus, this presented problems for subsequent studies investigating fractionated locust bean gum interactions with xanthan gum.

3.5 DISCUSSION

Characterisation of xanthan gum and locust bean gum was necessary in order to understand and establish the chemical nature of the individual polysaccharides used in the present work. Such findings were required in order to help determine the mechanism for the interaction, since different factors are known to affect the interaction process between xanthan gum and locust bean gum.

FIG. 3.5 : SUMMARY OF SAMPLE PREPARATION PROCEDURE FOR ACID HYDROLYSIS (RECKITT & COLMAN PROTOCOL)

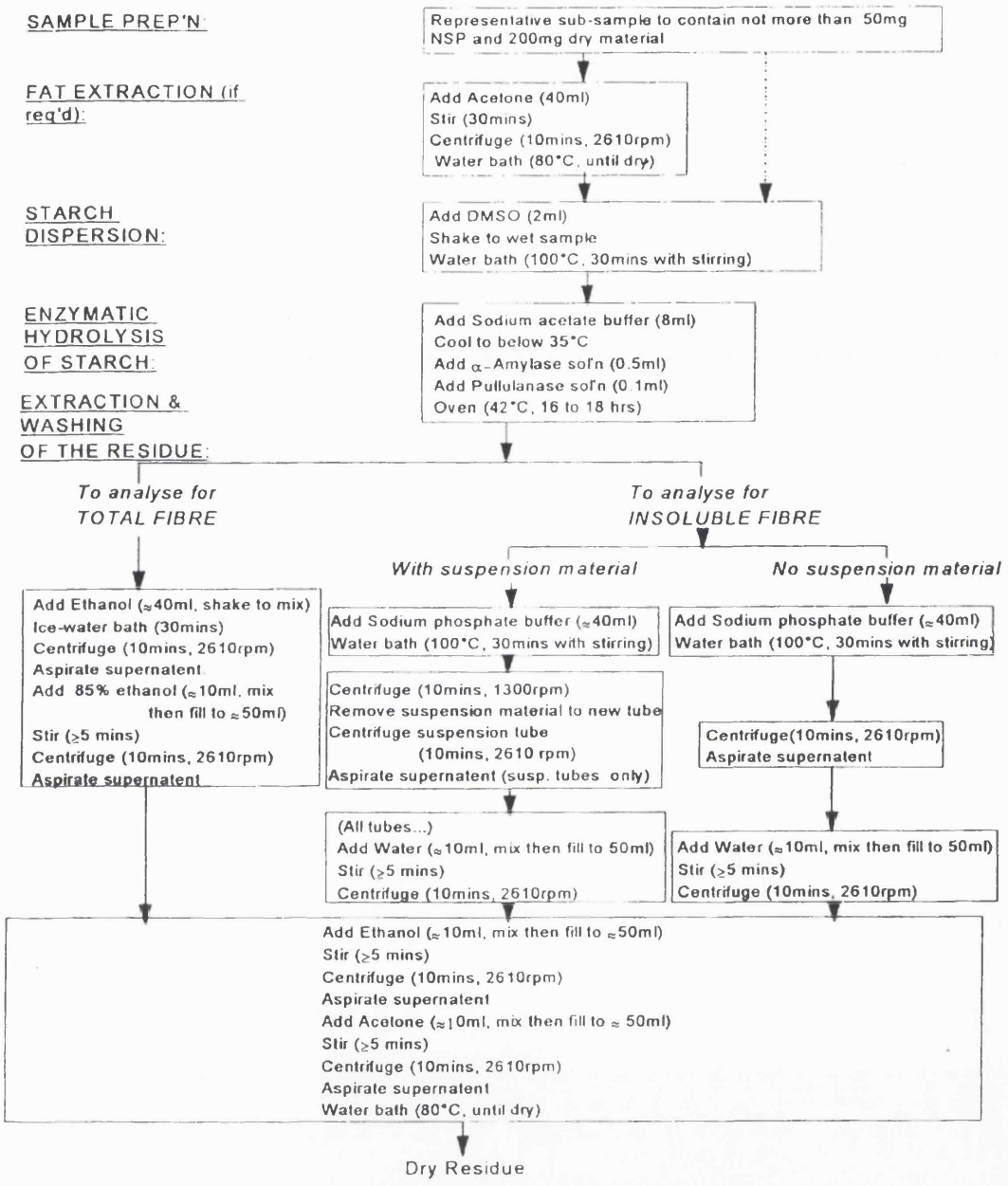
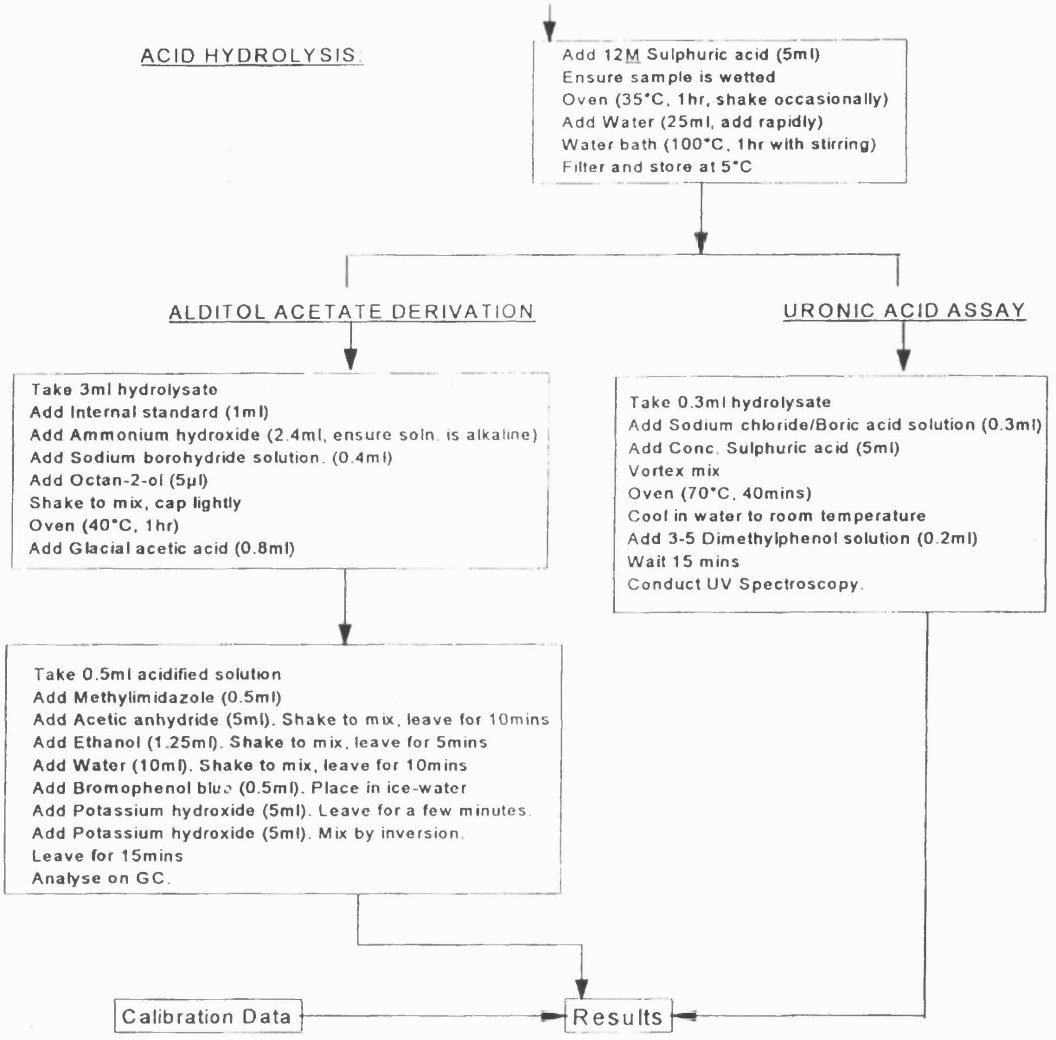


FIG. 3.6 : SUMMARY OF ACID HYDROLYSIS AND GLC OF ALDITOL ACETATE METHOD (RECKITT & COLMAN PROTOCOL)



Determination of the Na^+ and Ca^{2+} content for xanthan gum 1%w/w, provides an insight for xanthan gum structure in subsequent studies in the present work.

Although the results for acetate and pyruvate determination using H^1 NMR were different compared to the certificate of analysis from Pronova (Table 2.1), these values will be used as a reference where necessary in subsequent chapters. Differences arise due to many factors such as the xanthan gum batch, supplier or treatment process prior to measurements, in addition to the technique used for analysis.

Finally, there is good agreement between the two different techniques used to determine the M:G ratio in the ranking of the locust bean gum fractions (Table 3.4), but unfortunately the low yields reduce the number of measurements permitted.

TABLE 3.2 : M:G RATIO OF FRACTIONATED LOCUST BEAN GUM DETERMINED BY ACID HYDROLYSIS AND GLC OF THE ALDITOL ACETATE

LBG FRACTION	M%	G%	G+M%	M:G RATIO	MEAN M:G RATIO	MEAN M:G RATIO
LBG35 (1a)	62.17	20.51	82.68	3.03	3.21	3.21
LBG35 (1b)	63.08	18.66	81.74	3.38		
LBG35 (2a)	61.54	19.71	81.25	3.12	3.22	
LBG35 (2b)	60.07	18.10	78.17	3.32		
LBG35 (3a)	69.43	22.47	91.90	3.09	3.19	
LBG35 (3b)	61.09	18.57	79.66	3.29		
LBG35 (4a)	61.80	19.32	81.12	3.20	3.23	
LBG35 (4b)	60.22	18.54	78.76	3.25		
LBG80 (1a)	69.02	14.91	83.93	4.63	4.72	4.70
LBG80 (1b)	68.85	14.31	83.16	4.81		
LBG80 (2a)	68.94	14.38	83.32	4.79	4.84	
LBG80 (2b)	67.12	13.76	80.88	4.88		
LBG80 (3a)	69.12	15.24	84.36	4.54	4.57	
LBG80 (3b)	64.84	14.12	78.96	4.59		
LBG80 (4a)	72.11	15.53	87.64	4.64	4.67	
LBG80 (4b)	70.44	15.02	85.46	4.69		

M = Mannose

G = Galactose

a and b = Results are in duplicate

TABLE 3.3 : M:G RATIO OF FRACTIONATED LOCUST BEAN GUM DETERMINED BY H^1 NMR

PEAK NO.	PPM	RESIDUE	PROTON	ANOMER	AVERAGE INTEGRAL OF LGB 35	AVERAGE INTEGRAL OF LGB 80	*AVERAGE F(M)		**AVERAGE F(G)		***M:G RATIO	
							LBG 35	LBG 80	LBG 35	LBG 80	LBG 35	LBG 80
A	5.18	M	H-1(Red)	α	1.00	1.68	0.75	0.812	0.25	0.19	2.98	4.34
B	5.04	G	H-1	(α)	11.70	6.23						
C	4.90	M	H-1(Red)	β	0.58	0.80						
D	4.75	M	H-1	(β)	33.23	21.09						
E	4.73	M	H-1 (Non-red)	(β)	Peak D+E integrated as one peak	3.48						

*F(M) = (A+C+D+E)/(A+B+C+D+E) **F(G) = 1-F(M)
***M/G = (A+C+D+E)/B

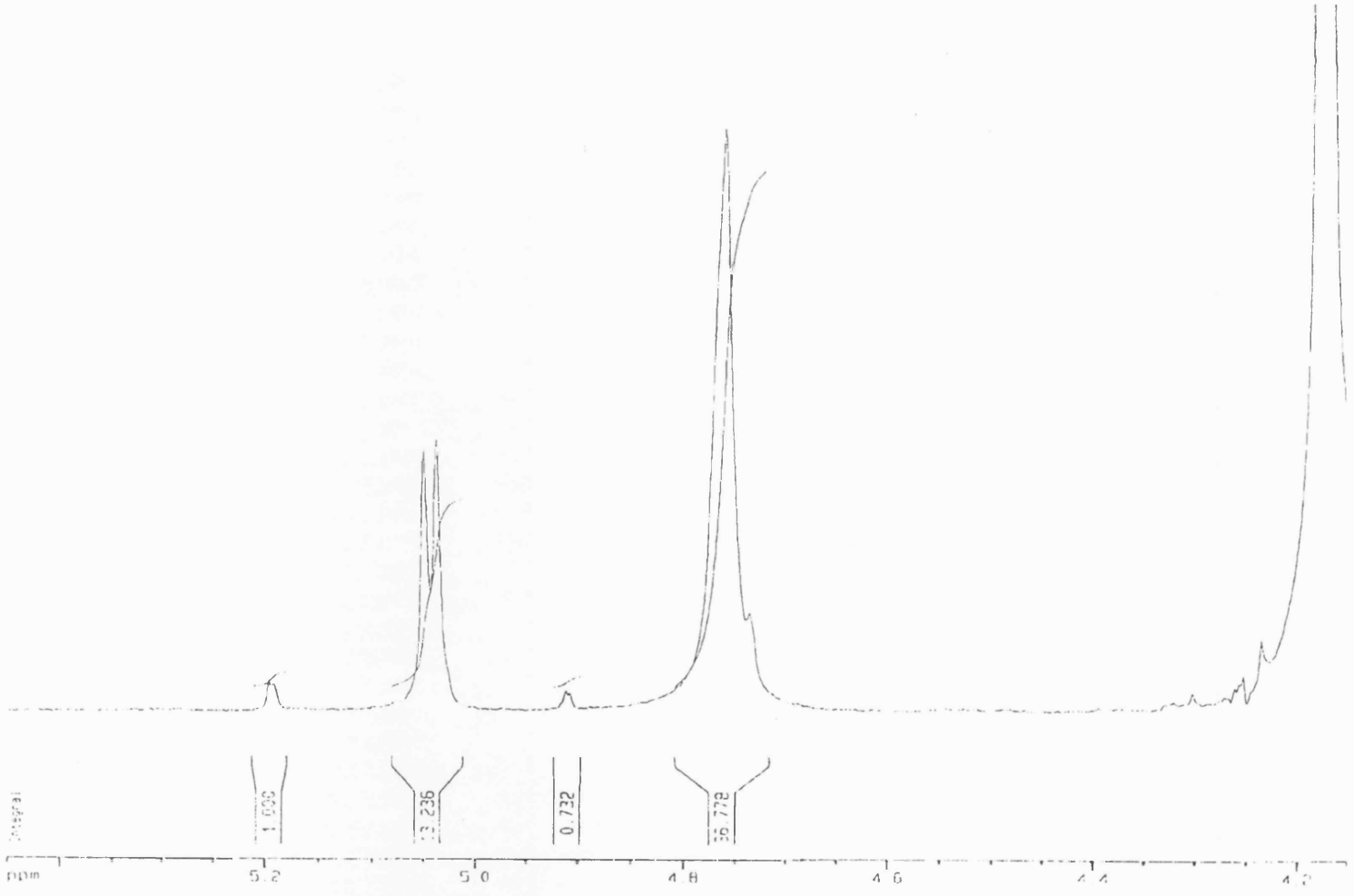
TABLE 3.4 : COMPARISON OF M:G RATIOS OF LBG FRACTIONS OBTAINED BY TWO DIFFERENT TECHNIQUES

	ACID HYDROLYSIS METHOD	H^1 NMR METHOD
LBG35 M:G Ratio	3.21	2.98
LBG80 M:G Ratio	4.70	4.34

TABLE 3.5 : YIELD (%) OF LOCUST BEAN GUM AFTER FRACTIONATION

LBG FRACTION	WEIGHT (G)	YIELD (%)
LBG35 (1)	3.72	18.6
LBG35 (2)	3.67	18.4
LBG35 (3)	4.16	20.8
LBG35 (4)	3.71	18.6
LBG80 (1)	1.34	6.7
LBG80 (2)	1.15	5.8
LBG80 (3)	1.26	6.3
LBG80 (4)	1.25	6.3

FIG. 3.7 : H^1 NMR SPECTRUM FOR M:G RATIO DETERMINATION OF LBG35

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PROCNO                   1

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TD                  32768
SOLVENT               D2O
NS                     64
DS                      4
SWH              3591.954 Hz
FIDRES           0.109618 Hz
AQ               4.5613556 sec
RG                   574.7
DW                 139.200 usec
DE                   4.50 usec
TE                  357.0 K
D1              1.00000000 sec
P1                   10.50 usec
DE                   4.50 usec
SFO1          300.1311506 MHz

F2 - Processing parameters
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SF            300.1301634 MHz
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SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
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CY                  500.00 cm
F1P                  5.450 ppm
F1                 1635.71 Hz
F2P                  4.150 ppm
F2                 1245.54 Hz
PPMCM             0.06047 ppm/cm
HZCM             18.14740 Hz/cm
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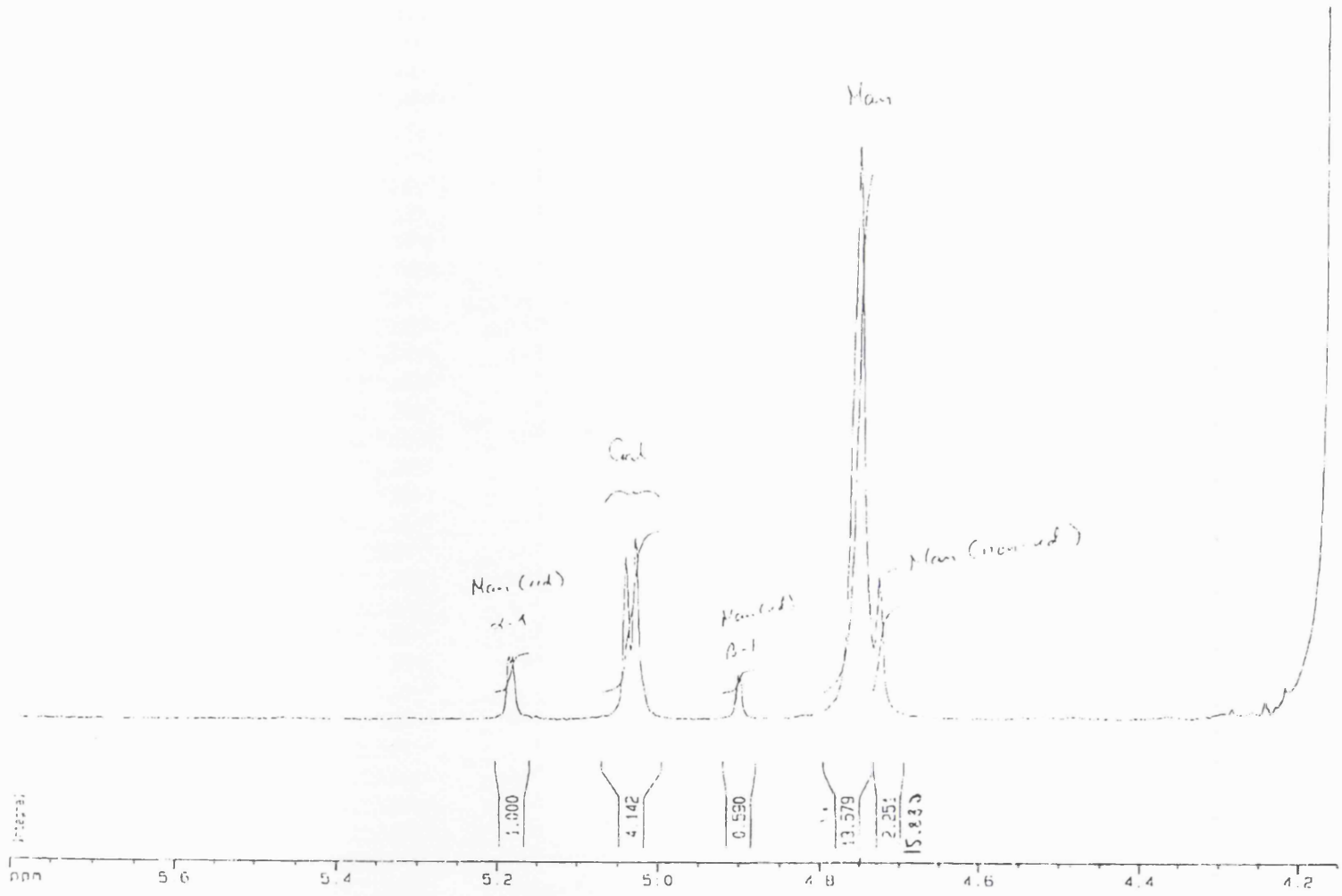
FIG. 3.8 : H^1 NMR SPECTRUM FOR M:G RATIO DETERMINATION OF LBG80

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PULPROG                 zg30
TD                     32768
SOLVENT                  D2O
NS                        64
DS                         4
SWH                3591.954 Hz
FIDRES            0.109618 Hz
AQ                4.5613556 sec
RG                     574.7
DW                   139.200 usec
DE                     6.00 usec
TE                    357.0 K
D1                 1.00000000 sec
P1                    10.50 usec
DE                     6.00 usec
SFO1          300.1313506 MHz
NUC1                     1H
PL1                   -6.00 dB

F2 - Processing parameters
SI                     16384
SF          300.1301691 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00

1D NMR plot parameters
CX                    21.50 cm
CY                   350.00 cm
F1P                   5.800 ppm
F1                 1740.76 Hz
F2P                   4.150 ppm
F2                 1245.54 Hz
PPMCM             0.07674 ppm/cm
HZCM              23.03325 Hz/cm
```

CHAPTER 4

CHAPTER 4 : RHEOLOGICAL BEHAVIOUR OF THE MIXED POLYSACCHARIDES

4.1 INTRODUCTION

It has been reported that xanthan gum and locust bean gum are both non-gelling polysaccharides (Morris et al, 1977 and Zhan et al, 1993). Xanthan gum has been described to exhibit pseudoplastic behaviour as discussed in Chapter 1.1.2.2, whereas locust bean gum displays properties typical of macromolecular solutions (Ferry, 1980). Locust bean gum forms highly viscous solutions at low concentrations, but to a lesser extent compared to xanthan gum. However, mixing these two polysaccharides together results in the formation of thermoreversible, elastic gels through synergistic interactions between the unsubstituted regions of the locust bean gum backbone and either the ordered helix (Williams et al, 1991) or disordered form of xanthan gum (Zhan et al, 1993) or possibly even both forms (Mannion, et al, 1992). Thus, the rheological properties of the mixed polysaccharide systems are important to characterise in assisting with the determination of the mechanism for the interaction. One of the implications of this synergy is the potential for these systems to be developed into products with wide applications. For example, pharmaceutical products such as Buccastem (Sugden, 1986) and TIMERx (Baichwal, 1991) exploit the xanthan gum-locust bean gum synergy for controlled release properties.

The gel strength or texture of the mixed polysaccharide system can vary considerably depending on many factors such as temperature and ionic strength as discussed in Chapter 1.2.3, which may be related to the interaction mechanism. The term 'gel' is widely used in papers, sometimes incorrectly. Although definitions of the term with different criteria do exist (Atkins, 1990), a universal definition has yet to be agreed upon. However, Almdal et al (1993) have proposed an appropriate definition stating the criteria. They limit the definition to the following;

(i) Soft solid or solid-like material of two or more components one of which is a liquid present in a substantial amount.

(ii) Exhibit a storage modulus with a pronounced plateau extending to times at least of the order of seconds, with a loss modulus considerably smaller than the storage modulus in the plateau region.

Different rheological techniques and texture analysis studies have been used to investigate the gelation properties of xanthan gum-locust bean gum mixes ; the latter technique will be discussed in Chapter 5. In terms of rheological characterisation, different techniques can be applied depending on the properties of the system, for example Dea et al (1977) suggested that thermoreversible gels are best characterised by their melting point and setting temperature, firm gels by their storage modulus or yield stress through oscillatory measurements and weak gels/viscoelastic fluids are best monitored using oscillatory or dynamic viscosity measurements. In the past, dynamic viscoelasticity measurements have been used by Tako et al (1984) on xanthan gum-locust bean gum mixes, whereby it was found that a maximum occurred for a 1:2 xanthan gum-locust bean gum mixture. Doublier (1994) used a combination of oscillatory shear and creep-recovery measurements and found that even the small addition of xanthan (1:99 xanthan gum-locust bean gum) resulted in weak gel characteristics at low frequency. Copetti et al (1997) observed two inflection points in the complex moduli vs. temperature profile, indicative of a two step process, the first step was ascribed to the melting of the mixed junction zones of xanthan helices associating with locust bean gum, and the second step at the higher temperature corresponded to the conformational transition of xanthan gum.

In the present study, oscillatory rheology was the chosen technique to investigate the behaviour of xanthan gum-locust bean gum mixes with respect to temperature, sodium chloride, sucrose and polysaccharide ratio as a means to gaining an insight into the mechanism for the interaction. This technique works on the principle of dynamic testing unlike some other methods such as creep studies which are static tests. Additionally, oscillatory rheology is a good measure of the viscous and elastic components of a sample simultaneously i.e. the 'viscoelasticity' which applies to gel systems.

4.1.1 General Rheology

Rheology can be traced back to 1687, in which Newton attempted to quantify flow as 'the resistance which arises from the lack of slipperiness originating from a fluid.' Rheology as a science was officially accepted in 1929 when the American Society of Rheology was founded. Rheology in simplistic terms is the study of the deformation of

materials which includes 'flow'. Newton showed that the rate of flow is proportional to the applied stress which can be expressed by Equation 4.1.

$$\eta = \sigma/\gamma \qquad \text{Equation 4.1}$$

Where:

σ = shear stress (force per unit area) Pascals (Pa)

γ = shear rate (for flow - units of time i.e. per second ; 1/s) or strain (for deformation – displacement ; no units)

η = viscosity - Pascals per second (Pa.s)

Materials which obey Newton's law in Equation 4.1 are described as Newtonian fluids, in which their viscosity is independent of shear rate. Those which do not obey the relationship are described as non-Newtonian which includes most materials.

There are several types of deviations from Newton's law when describing materials (mainly fluids). The first one is pseudoplastic flow otherwise known as shear thinning, whereby it has been described by some workers such as Rocks (1971) to apply to xanthan gum. In this situation, the viscosity decreases as the shear rate increases which can be expressed by the power law model in Equation 4.2.

$$\sigma = K\gamma^n \qquad \text{Equation 4.2}$$

Where:

σ = shear stress

K = viscosity coefficient

n = shear rate index ($n<1$)

The viscosity that can be calculated from this slope is called the apparent viscosity (η'), and needs to be quoted with a shear stress or shear rate to have significance. Thus, it is difficult to quantify pseudoplastic flow particularly at high shear rates where the graph becomes linear, as the equation is only an approximation.

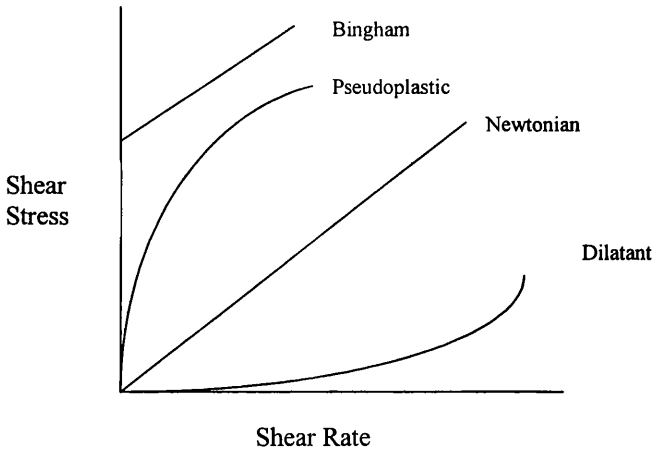
A second deviation from Newtonian flow is dilatant flow, otherwise known as shear thickening. This is the opposite of pseudoplastic flow, i.e. the viscosity increases as the shear rate increases, and is less common compared to pseudoplastic flow. Dilatant flow can also be characterised by Equation 4.2, the only difference being that n>1.

N.B - For Newtonian fluids, n = 1 for Equation 4.2.

Another type of non-Newtonian flow is plastic flow or bingham flow. In this situation, the material will only flow once a critical stress is reached otherwise known as the yield stress. Thus, above the yield stress the material will flow whilst below it no flow occurs. Once the material flows, it may display shear thinning or shear thickening behaviour as described above.

In summary, the different types of flow behaviour are illustrated in Figure 4.1.

FIGURE : 4.1 TYPES OF FLOW BEHAVIOUR



In comparison to fluids, a perfect elastic solid will obey Hooke's law which states that stress is proportional to strain (Equation 4.3). Thus in practice for an ideal solid, it will deform reversibly with a strain proportional to stress, and recover to its original shape immediately once the stress is removed (liquids on the other hand do not recover on removal of stress because they have 'flowed').

$$G = \sigma/\gamma \qquad \text{Equation 4.3}$$

Where:

σ = shear stress (Pa)

γ = strain (no units)

G = elastic modulus

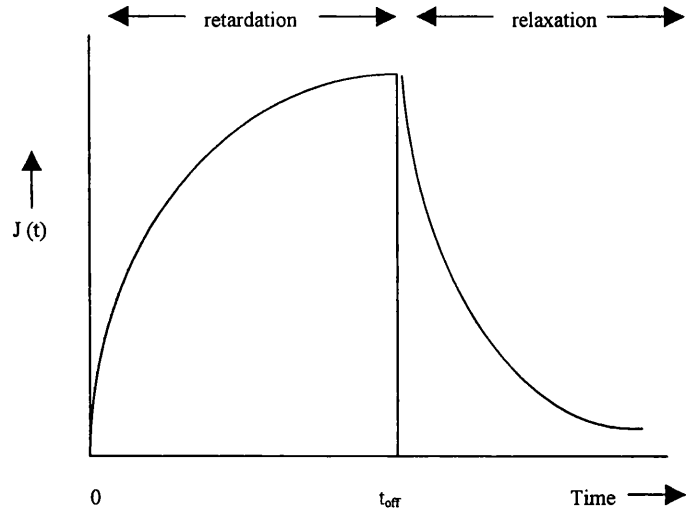
In reality, many materials are neither perfect solids nor perfect liquids, but are somewhere in between the two extremes, and are referred to as viscoelastic materials. Such materials display both solid and liquid like behaviour in varying proportions.

Viscoelasticity can be measured by applying a deformation and measuring the stress required to maintain it with time (stress relaxation), or applying a small stress and measuring the deformation with time (creep). Alternatively, oscillatory rheology can be used to measure viscoelasticity, by alternating the application and removal of a stress to produce a sine wave. The viscoelastic material is thus subjected to a sinusoidal stress wave which produces a sinusoidal strain wave.

In creep and oscillation studies, measurements should be performed in the 'linear viscoelastic region' which needs to be characterised for every sample, and is the range of stresses (or displacements) in which the sample is probed e.g. ; stretched/compressed without its structure being destroyed. Linear viscoelastic regions are manually determined using stress or torque ramps.

Creep measurements as mentioned earlier in this chapter are static tests and results are usually expressed as compliance (J), which is an indicator of the ease of obtaining a deformation. The technique involves applying a small, constant stress on the sample (retardation step) until the rate of change of strain is constant after a period of time. The stress is then removed and the rate and extent to which the sample recovers is monitored (relaxation step). This is illustrated in Figure 4.2.

FIGURE 4.2 : TYPICAL CREEP CURVE FOR A VISCOELASTIC MATERIAL



Creep studies are useful for predicting the behaviour of materials in response to very low stresses such as viscoelastic properties, film sagging, levelling, sedimentation of suspensions on storage, creaming of particulate dispersions, surface tension and gravitational behaviour since the stresses used in these tests are very low.

In contrast to creep studies which are static tests, oscillatory rheology is a form of dynamic testing which provides information about the changing structure throughout the test over short time scales, whereas creep measurements are designed for looking at samples changing over long time scales. Thus, oscillatory rheology can provide measurements relatively quickly compared to creep studies. In oscillatory rheology, a stress is applied to the sample in a sinusoidal pattern by alternating the application and removal of the stress, and the resulting strain/deformation is measured. It can measure both the viscous and elastic behaviour of a system simultaneously unlike creep measurements, and is useful for detecting interactions within the sample and molecular mechanisms which give rise to the structure.

In oscillatory rheology, a sinusoidal stress wave will produce a sinusoidal strain wave in phase ($\delta = 0$) for an ideal Hookean solid, which can be expressed in Equation 4.4. Although the strain wave is in phase, it may be of different amplitude.

$$\sigma = A \sin \omega t \qquad \text{Equation 4.4}$$

$$\gamma = B \sin \omega t$$

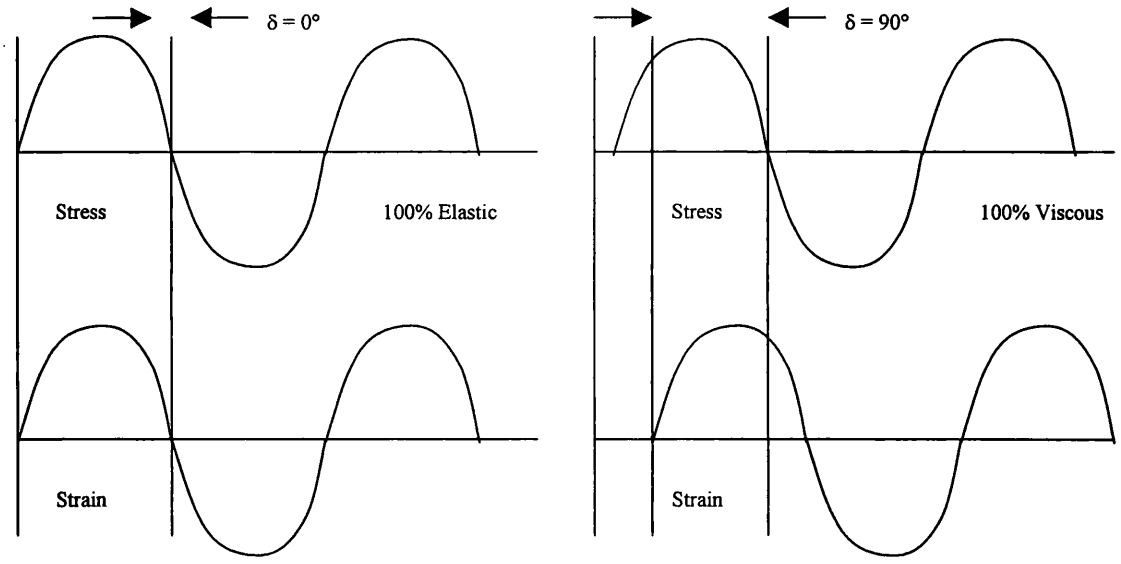
Whereas for an ideal Newtonian liquid, a sinusoidal stress wave will produce a sinusoidal strain wave which is out of phase by exactly 90° ($\delta = 90°$), since stress is directly proportional to the strain rate (Equation 4.5).

$$\sigma = A \sin \omega t \qquad \text{Equation 4.5}$$

$$\gamma = -B \cos \omega t$$

Phase angle plots in Figure 4.3 illustrate both extremes.

FIGURE 4.3 : PHASE ANGLE PLOTS OF OSCILLATION



Thus, for viscoelastic materials which have both liquid and solid properties, they will produce phase angles between $0° < \delta < 90°$.

Oscillatory rheology measurements can be expressed by several different viscoelastic parameters. The storage modulus otherwise known as the elastic or rigidity modulus (G′) refers to the elastic component of a system. It represents the elastic storage of energy, since the strain and hence structure of the elastic component is recovered once the stress is removed. In essence, it is a measure of the difficulty in obtaining a deformation, whereas this is the opposite for compliance (J) used in creep studies. The storage modulus gives information on how well a material is structured, thus G′ will be high for highly elastic or structured systems. The storage modulus can be expressed as follows;

$$G' \text{ (Pa)} = \text{Stress/Strain}$$

Whereas compliance is the inverse ;

$$J = \text{Strain/Stress}$$

The loss modulus otherwise known as the viscous modulus (G′′) expressed in units of Pa, refers to the viscous component of a system, and is a measure of the viscous dissipation (loss) of energy due to flow, in which strain is irreversible. Thus, G′′ will be high if a system is mainly viscous. The loss modulus can be used to derive another parameter known as the dynamic viscosity (η') which has units of Pa.s, and can be expressed in Equation 4.6 ;

$$\eta' = G''/\omega \qquad \text{Equation 4.6}$$

Where:

ω = angular velocity

Thus, the loss modulus and dynamic viscosity will always run parallel when shown on the same axes of a graph.

The sum of the loss and storage moduli gives rise to the complex modulus (G*) which incorporates an imaginary number (i) (Equation 4.7).

$$G^* = G' + iG'' \qquad \text{Equation 4.7}$$

Finally, a parameter known as tan δ is sometimes used which is derived from the following expressions;

Since:

$$G' = (\sigma/\gamma)\cos\delta$$

$$G'' = (\sigma/\gamma)\sin\delta$$

Thus:

$$G''/G' = \tan\delta$$

Therefore, if tan δ is less than 1, this implies that G′ is greater than G′′.

Oscillatory rheology is a non-destructive test which can be performed as amplitude (stress), frequency, time and temperature sweeps. Such studies can provide information

on viscoelasticity, the physical structure and any changes in structure, particularly with time or with temperature.

4.2 POLYSACCHARIDE RATIO EFFECTS ON THE SYNERGY BETWEEN XANTHAN GUM AND LOCUST BEAN GUM AT ROOM TEMPERATURE

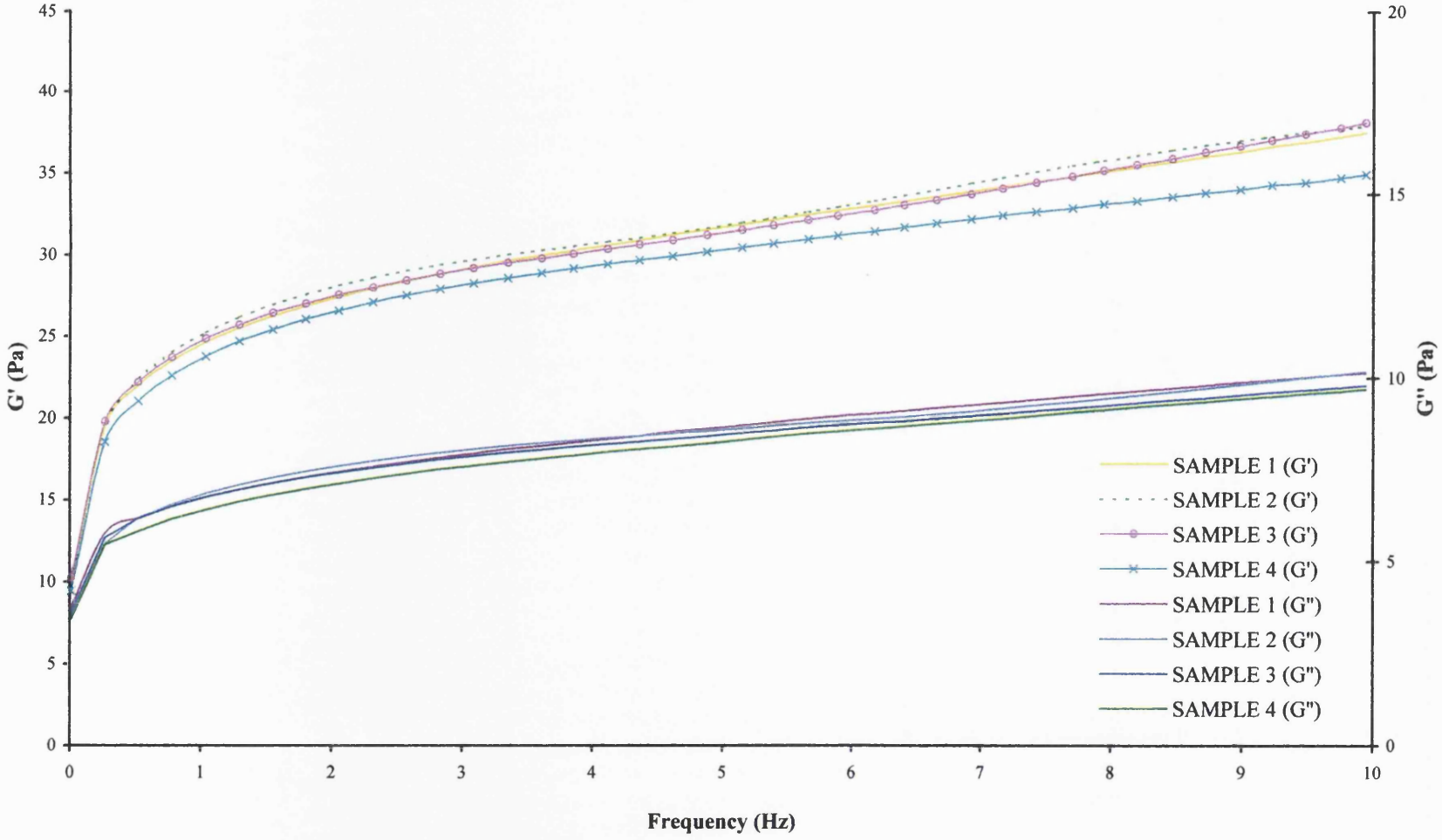
The synergy between xanthan gum and locust bean gum is affected by the polysaccharide ratio as discussed in Chapter 1.2.3. For example, most workers have found that maximum synergy occurs for a 1:1 polysaccharide ratio (Williams et al, 1991 and Mannion et al, 1992), although Craig et al (1997) observed maximum synergy for the 1:9 unheated xanthan gum:locust bean gum ratio. In this chapter, frequency (time) studies were employed to study the behaviour of different xanthan gum-locust bean gum mixes at room temperature.

4.2.1 Method

Oscillatory rheology measurements were made on 1%w/w xanthan gum-locust bean gum mixes of varying ratios. The mixes were prepared in deionised water at 25°C as described in Chapter 2.2.1. Frequency scans were performed to investigate the behaviour of the samples at room temperature, by observing the storage and loss moduli and their dependency on frequency (i.e. time).

Measurements were obtained using an AR-1000 rheometer (TA Instruments), equipped with a steel 6cm cone and plate geometry (angle=2°, serial number 980848) with a solvent trap attached to minimise any dehydration effects. Frequency scans were performed between 0.01Hz and 10Hz at 25°C in the 'linear viscoelastic region' (LVR:controlled displacement value of $5.0x10^{-4}$) on four fresh samples per ratio with good reproducibility each time (see Figure 4.4). The LVR was determined from torque ramps for a series of different polysaccharide mixes at different temperatures. A torque ramp is a stress amplitude sweep which involves measuring the moduli (usually the G′) with increasing stresses. At a certain range of stresses which are applied to the sample, the moduli will be independent of the stress amplitude which is otherwise known as the LVR. Stresses below the LVR are too low to stretch/compress the sample, whereas

FIG. 4.4 : REPRODUCIBILITY OF FOUR SAMPLES OF XANTHAN GUM-LOCUST BEAN GUM (8:2) RATIO 1%w/w MIXES



stresses above the LVR will destroy the structure. Thus, the moduli plummet at stresses greater than the LVR. The LVR value of $5.0x10^{-4}$ was chosen, since this value fell well within the linear region which was common to all samples tested.

Correct loading of each sample onto the bottom plate is important for obtaining accurate measurements and minimising 'edge effects'. For example, if the cone is under filled this creates higher stresses which may partially destruct the sample. Conversely, over filling with a very viscous sample can lead to shearing of the edges, a phenomenon known as 'viscous drag'. In addition, the use of pipettes or syringes are not advisable, since this has the effect of pre-shearing the sample before measurements, leading to inaccuracies in the results. Thus, samples were loaded onto the plate using a small spoon such that it was found that one full spoon filled the cone completely once the top plate was lowered to a fixed gap of 70microns.

4.2.2 Results

The rheological profiles for different ratios of xanthan gum-locust bean gum mixtures prepared at 25°C are illustrated in Figures 4.5 to 4.8. Figure 4.5 shows the storage moduli (G′) values for xanthan gum and xanthan gum-locust bean gum ratios 9:1-6:4, whereas Figure 4.6 shows the storage moduli (G′) values for xanthan gum-locust bean gum ratios 5:5-1:9. Similarly, Figures 4.7 and 4.8 show the loss moduli (G′′) values as a function of frequency. In all cases, the moduli show only a small dependency on frequency which suggests that these mixtures are highly structured.

Results for locust bean gum 1%w/w were not reproducible which is why they have not been included in the graphs. This is likely to be due to the low viscosity of the sample which can not be accurately measured using the oscillatory mode.

The general trends for both storage moduli (G′) and loss moduli (G′′) with respect to the xanthan gum-locust bean gum ratio are displayed in Figure 4.9. The storage modulus is greater than the loss modulus for each mixture and the magnitude of the moduli are approximately proportional to the concentration of xanthan gum. Thus, the highest G′ value is for xanthan gum 1%w/w, whereas the lowest G′ value is for xanthan gum-locust bean gum 1:9 ratio 1%w/w.

FIG. 4.5 : FREQUENCY SCANS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w

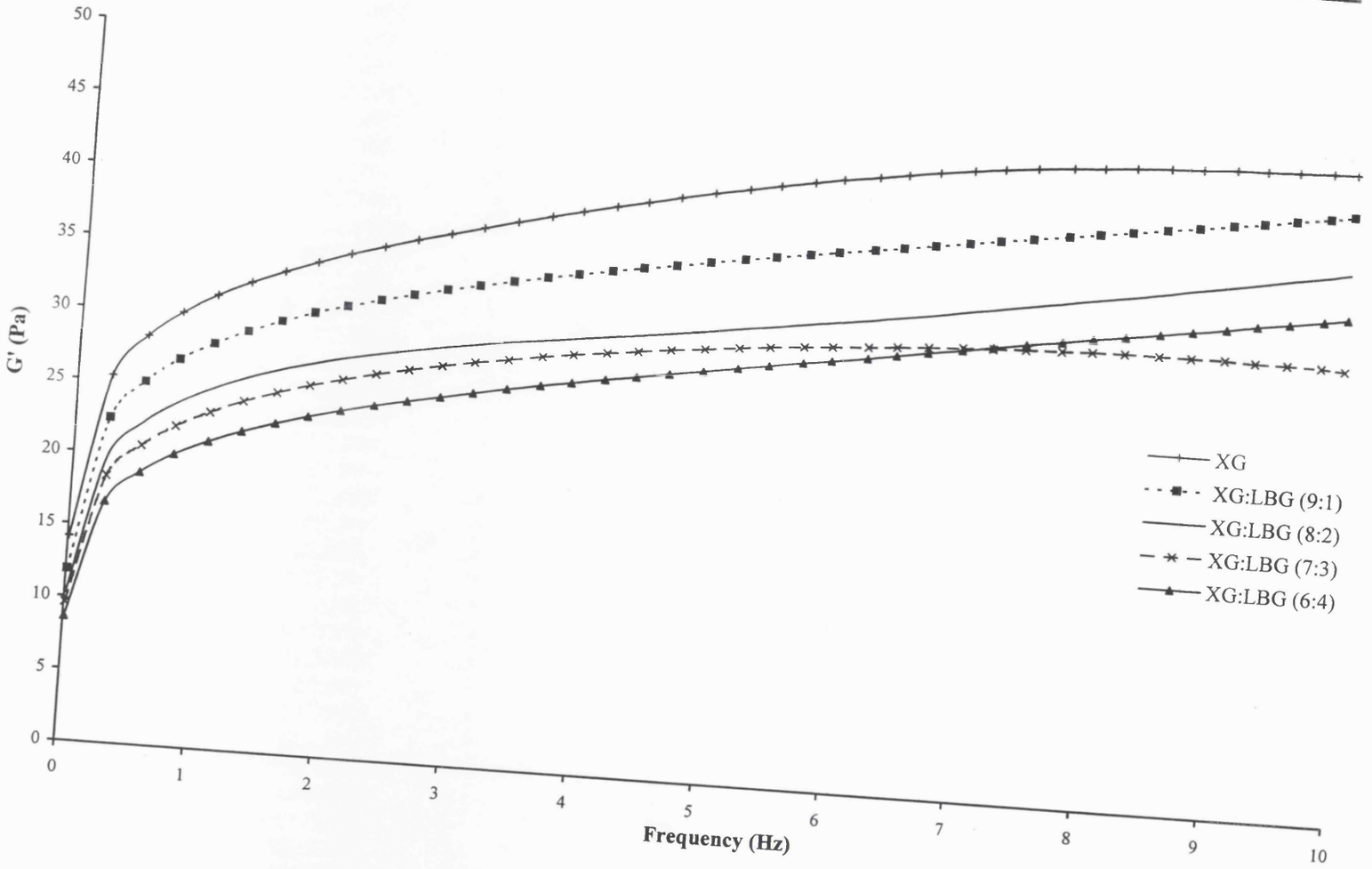


FIG. 4.6 : FREQUENCY SCANS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w

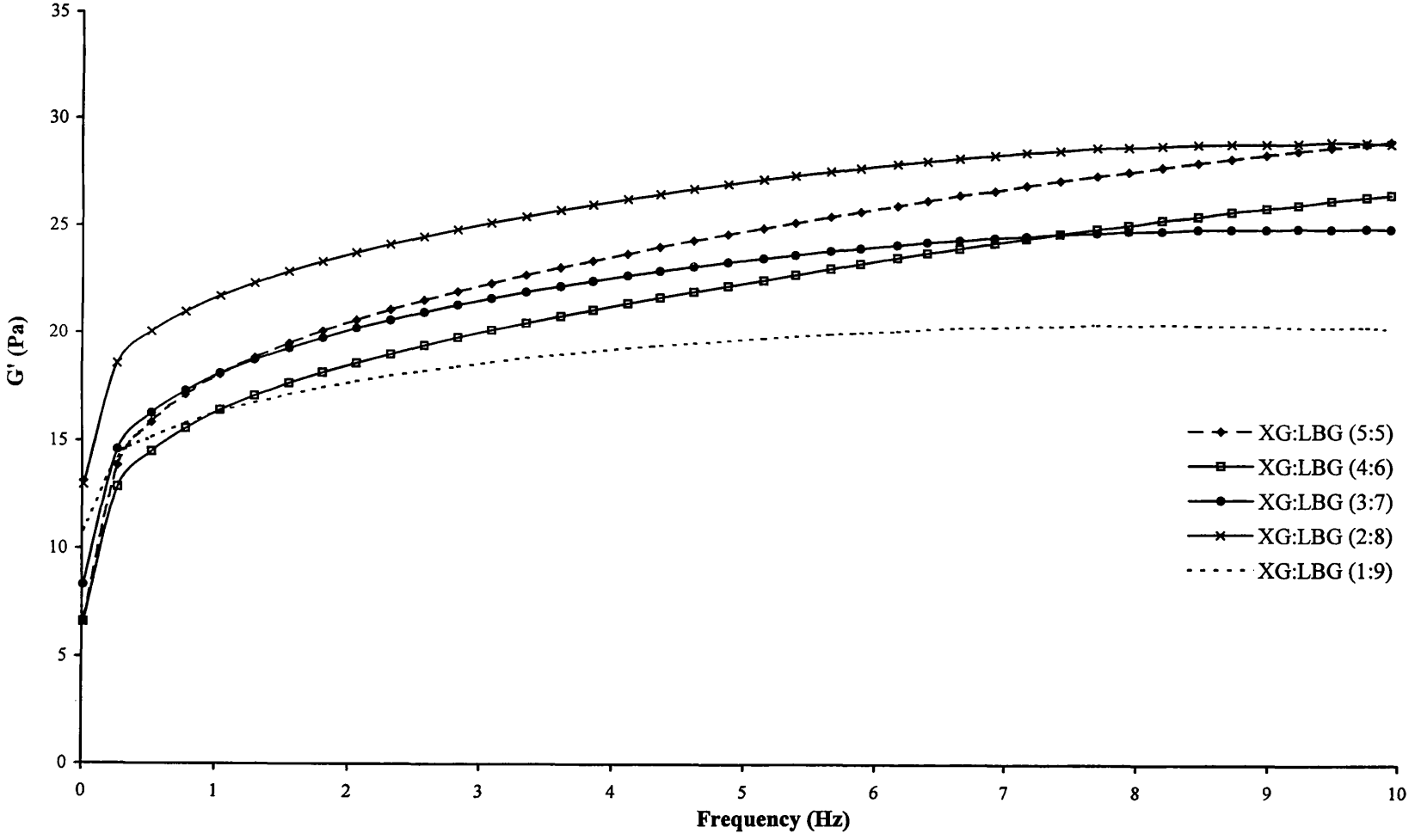


FIG. 4.7 : FREQUENCY SCANS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w

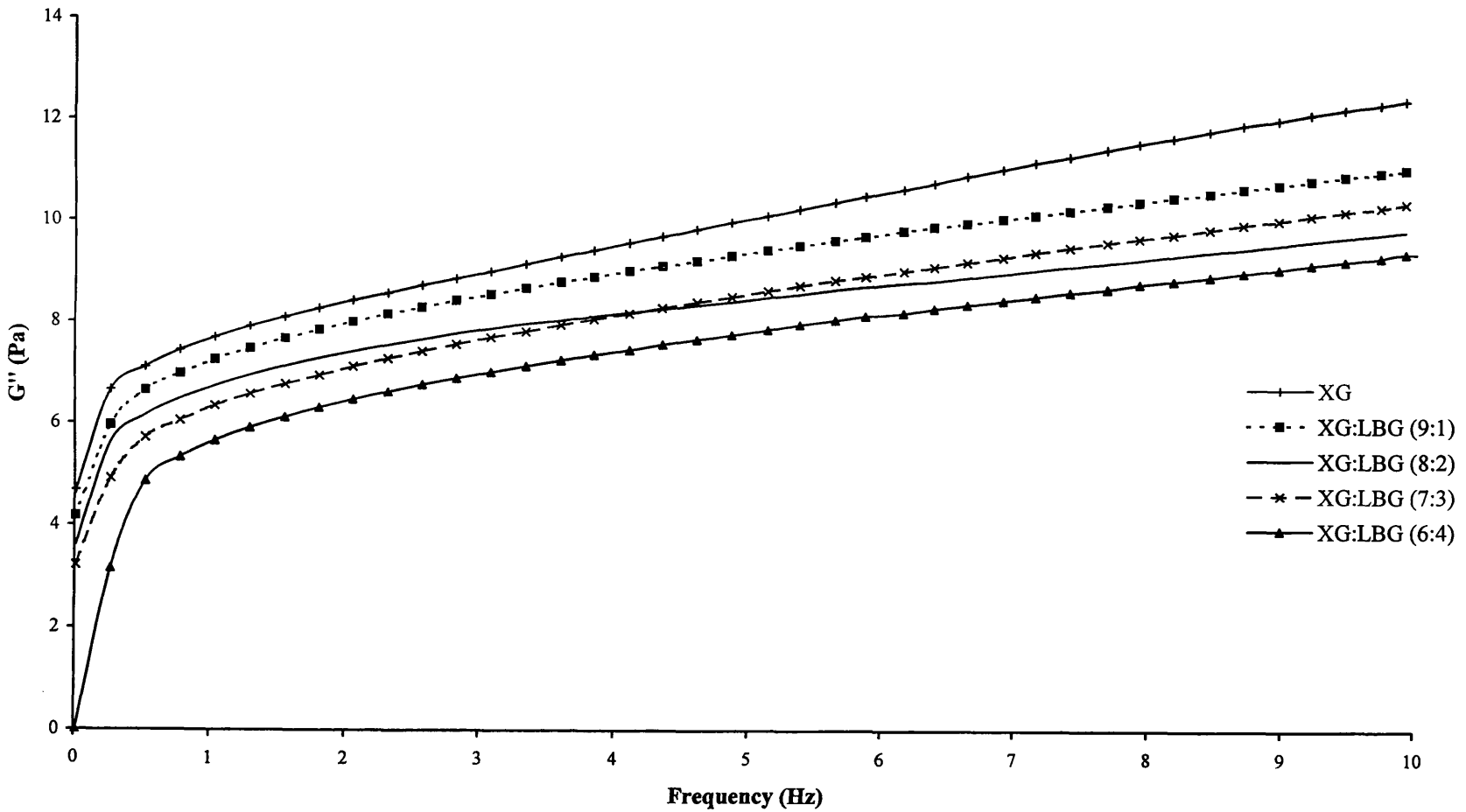


FIG. 4.8 : FREQUENCY SCANS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w

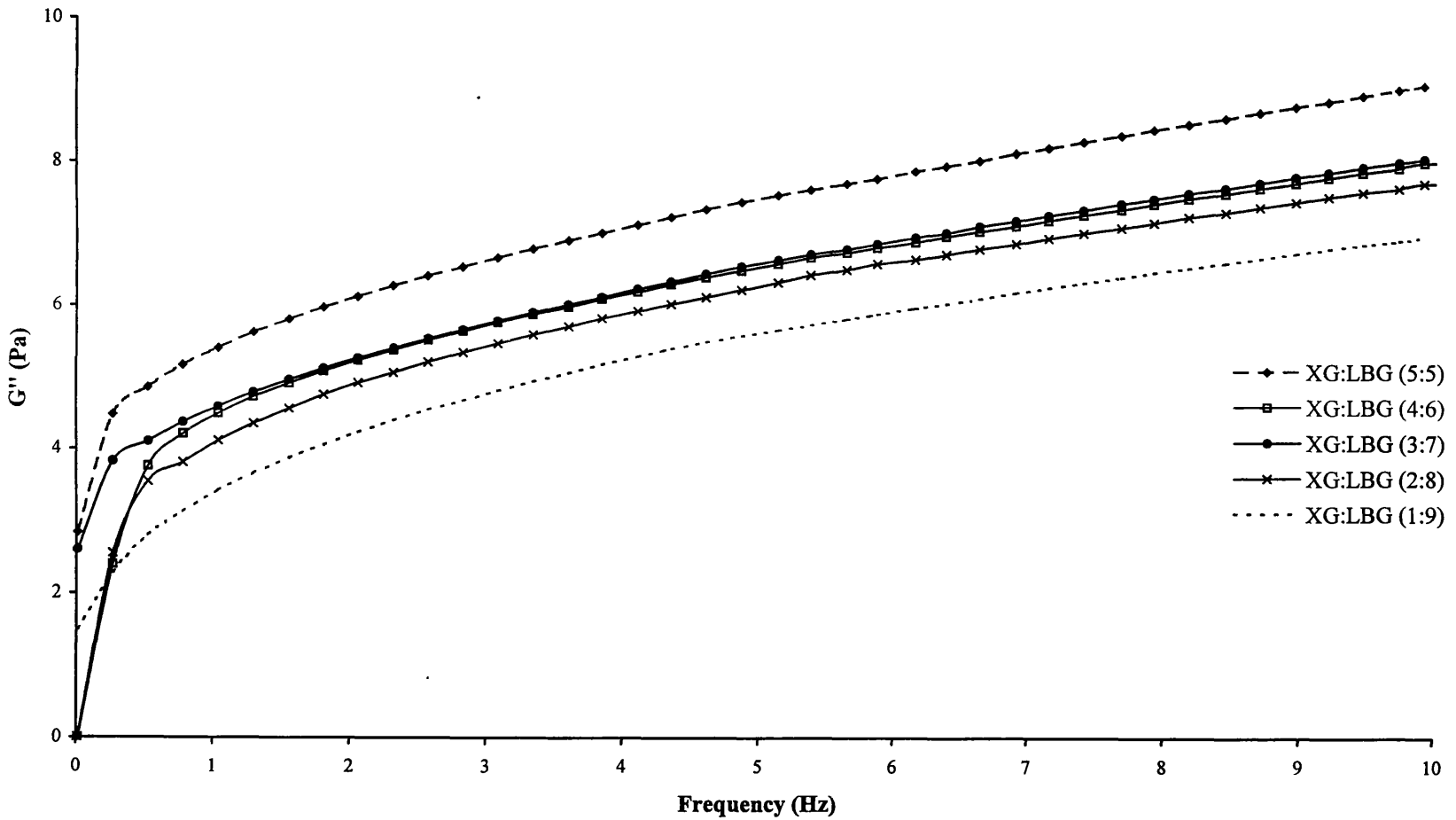
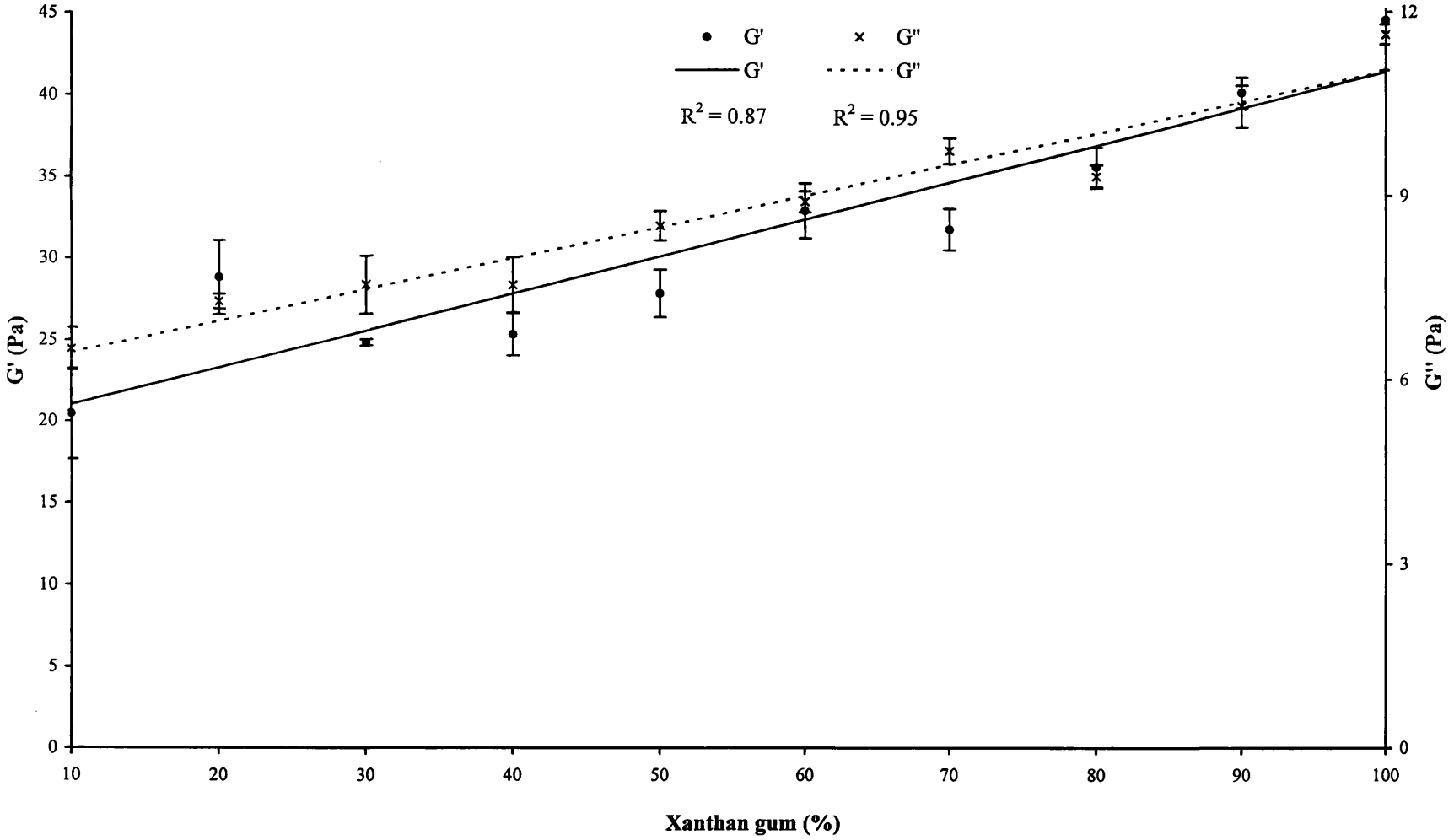


FIG. 4.9 : COMPARISON OF THE STORAGE AND LOSS MODULI FOR XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w AT 8.192 Hz



4.3 THE EFFECT OF TEMPERATURE ON XANTHAN GUM-LOCUST BEAN GUM MIXTURES

The gelation process between xanthan gum and locust bean gum is greatly affected by temperature (Zhan et al, 1993), in which it has been much debated as to whether the synergy between xanthan gum and locust bean gum depends on heating to high temperatures first (95°C) followed by cooling to ambient, as proposed by Cairns & Morris (1986). However, Mannion et al (1992) is in disagreement with this proposal, observing synergy at room temperature in addition to at high temperatures, although much weaker gels were formed at room temperature. Nevertheless, it is important to note from the work by Mannion et al (1992) that all individual solutions had been heated to 80°C for 30min followed by overnight cooling, prior to mixing the two polysaccharides together at room temperature before rheological testing. Thus, the sample preparation temperature is likely to affect the rheological properties of the final mixes leading to possible artefacts in results.

Since temperature is known to affect the conformational behaviour of xanthan gum as discussed in Chapter 1.1.1.2, xanthan gum structure must play an important role in the mechanism for the interaction with locust bean gum and the resulting gel characteristics. Thus, the effect of temperature on the rheological properties of xanthan gum-locust bean gum mixtures was extensively investigated in the present work.

4.3.1 THE EFFECT OF TEMPERATURE ON XANTHAN GUM-LOCUST BEAN GUM MIXTURES PREPARED AT 25°C

4.3.1.1 Method

Oscillatory rheology measurements were performed on 1%w/w xanthan gum-locust bean gum mixes prepared at 25°C as described previously in Chapter 2.2.1.

The effect of temperature on 1%w/w xanthan gum-locust bean gum mixes of varying ratios was investigated in a similar manner to that described in Chapter 4.2.1, except that the temperature mode was applied as opposed to the frequency mode using the AR-1000. Measurements were performed at a fixed frequency of 1Hz in the LVR ($5x10^{-4}$).

Measurements were taken during sample heating from 20°C to 90°C at a rate of 2°C per minute, followed by cooling at the same rate from 90°C to 20°C. In this way, the temperature course of the gelation process could be monitored directly.

A gap compensation test was performed prior to the temperature studies, to correct for any changes in gap size due to sample expansion on heating and contraction on cooling. This is a calibration type test which measures the gap in microns at different temperatures used in the temperature studies (between 20°C and 90°C). This is performed several times for heating and cooling at different 'backoff' values which compensate for expansion and contraction on heating and cooling respectively, until a fit regression (R) as close to 1 as possible is achieved. In the present study, the gap compensation values obtained were 0.59 for heating and 0.44 for cooling, which were entered into the temperature ramping program prior to measurements.

It was important to minimise any dehydration effects by using the solvent trap which reduces thermal gradients at high temperatures by creating a closed environment. Additionally, the use of the steel 6cm cone and plate geometry is advantageous when high temperatures are encountered, because steel has a low coefficient of thermal expansion.

4.3.1.2 Results

The results for the mixtures prepared at 25°C are split into two sets of different ratios (locust bean gum plus xanthan gum-locust bean gum mixes 1:9-5:5 and xanthan gum plus xanthan gum-locust bean gum mixes 6:4-9:1). Each set is then split into heating and cooling profiles for clarification (Figures 4.10 - 4.17).

The shapes of the heating curves tend to be dependent on the ratio of the polysaccharide mixture (Figures 4.10 and 4.12), in which it is observed that the storage modulus for each mixed system is increased after cooling the mixtures from 90°C to 20°C (Figures 4.11 and 4.13). This suggests that a positive synergy occurs between xanthan gum and locust bean gum resulting in more structured systems. The greatest increase in G′ after heating and cooling is observed for the 5:5 ratio (75Pa) i.e. the 1:1 ratio which is in agreement with most workers (Cairns & Morris, 1986 and Williams et al, 1991). However, the G′ at 20°C for the individual polysaccharide mixtures decreased slightly

for xanthan gum and remained the same for locust bean gum after heating and cooling compared to measurements taken before hand. This observation may suggest that the individual mixtures do not undergo any permanent changes with temperature.

A cross over point between the heating and cooling curves is observed for all ratios (not all graphs are included; see Figures 4.16 and 4.17 as examples) but not for the individual polysaccharides (Figures 4.14 and 4.15). The temperature at which the curves coincide is around 50°C for most ratios, but is lowest for the xanthan gum-locust bean gum (9:1) ratio (38°C – Figure 4.16) and is highest for the xanthan gum-locust bean gum (5:5) ratio (55°C – Figure 4.17). This cross over point may imply that new structures are formed after heating and cooling between 20°C and 90°C at the cross over point temperature.

4.3.2 THE EFFECT OF TEMPERATURE ON XANTHAN GUM-LOCUST BEAN GUM MIXTURES PREPARED AT 90°C

4.3.2.1 Method

The temperature program and method were identical to that described in Chapter 4.3.1.1. The mixtures were prepared at 90°C according to the procedure described in Chapter 2.2.1. The heated solutions were quickly poured into circular 6cm x 0.5cm plastic culture plates before setting into gel discs at 8°C/24hrs prior to measurements. The gel discs were then placed on top of the 6cm steel cone, and excess gel was cut away from the sides of the cone once it had been lowered to a fixed gap of 70microns. In this way it was ensured that the gel discs had fixed dimensions so results could be compared directly.

4.3.2.2 Results

The results for the mixtures prepared at 90°C are different to those prepared at 25°C, in that the storage modulus after cooling from 90°C to 20°C is lower than the initial storage modulus at 20°C i.e. before heating (Figures 4.18-4.20). The highest storage modulus is again observed for the xanthan gum-locust bean gum (5:5) ratio, and the storage moduli values for the xanthan gum-locust bean gum (1:9) ratio prepared at both 25°C and 90°C after cooling to 20°C are approximately the same (30Pa).

FIG. 4.10 : RHEOLOGICAL PROFILES OF DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w PREPARED AT 25°C ON HEATING

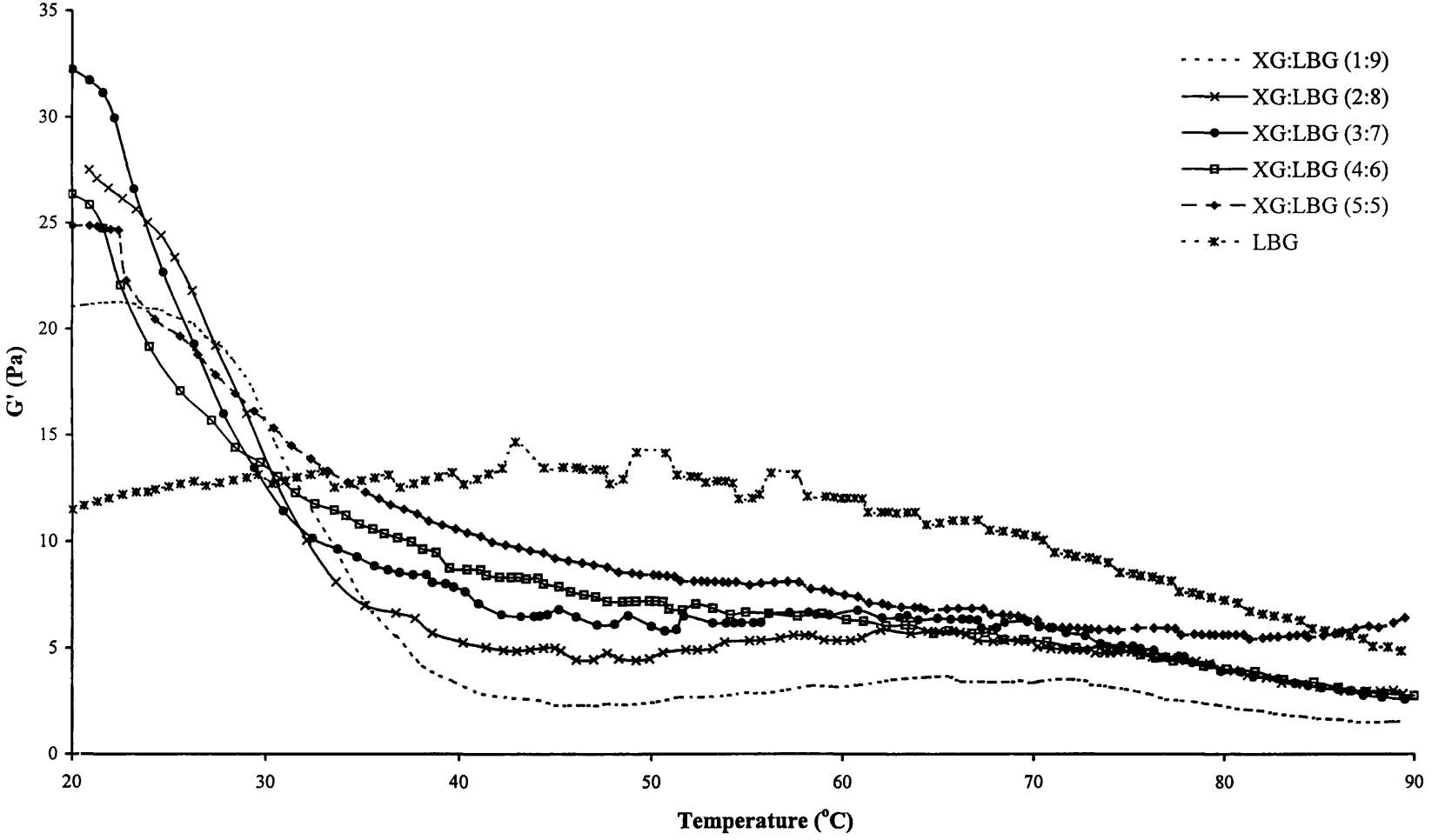


FIG. 4.11 : RHEOLOGICAL PROFILES OF DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w PREPARED AT 25°C ON COOLING

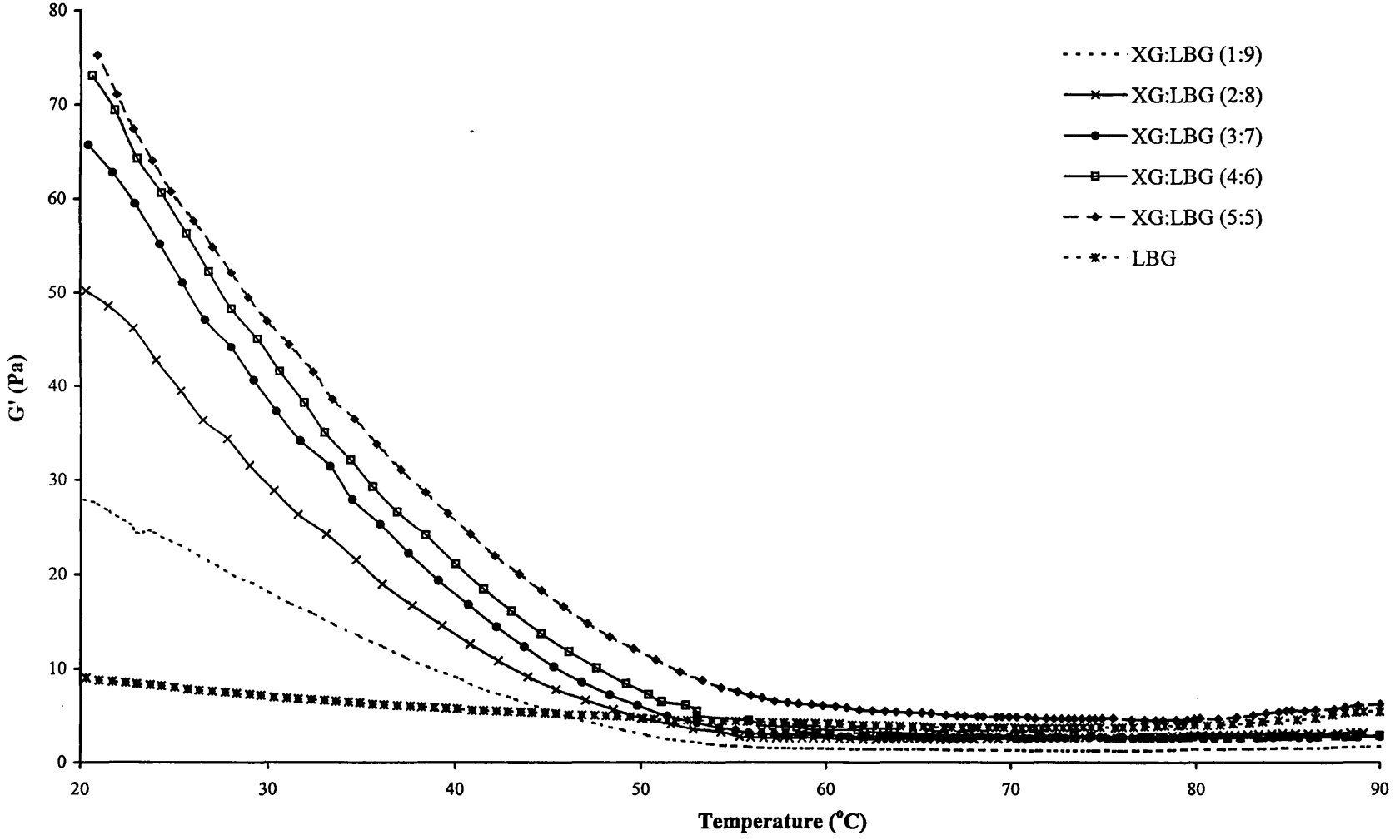


FIG. 4.12 : RHEOLOGICAL PROFILES OF DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1% w/w PREPARED AT 25°C ON HEATING

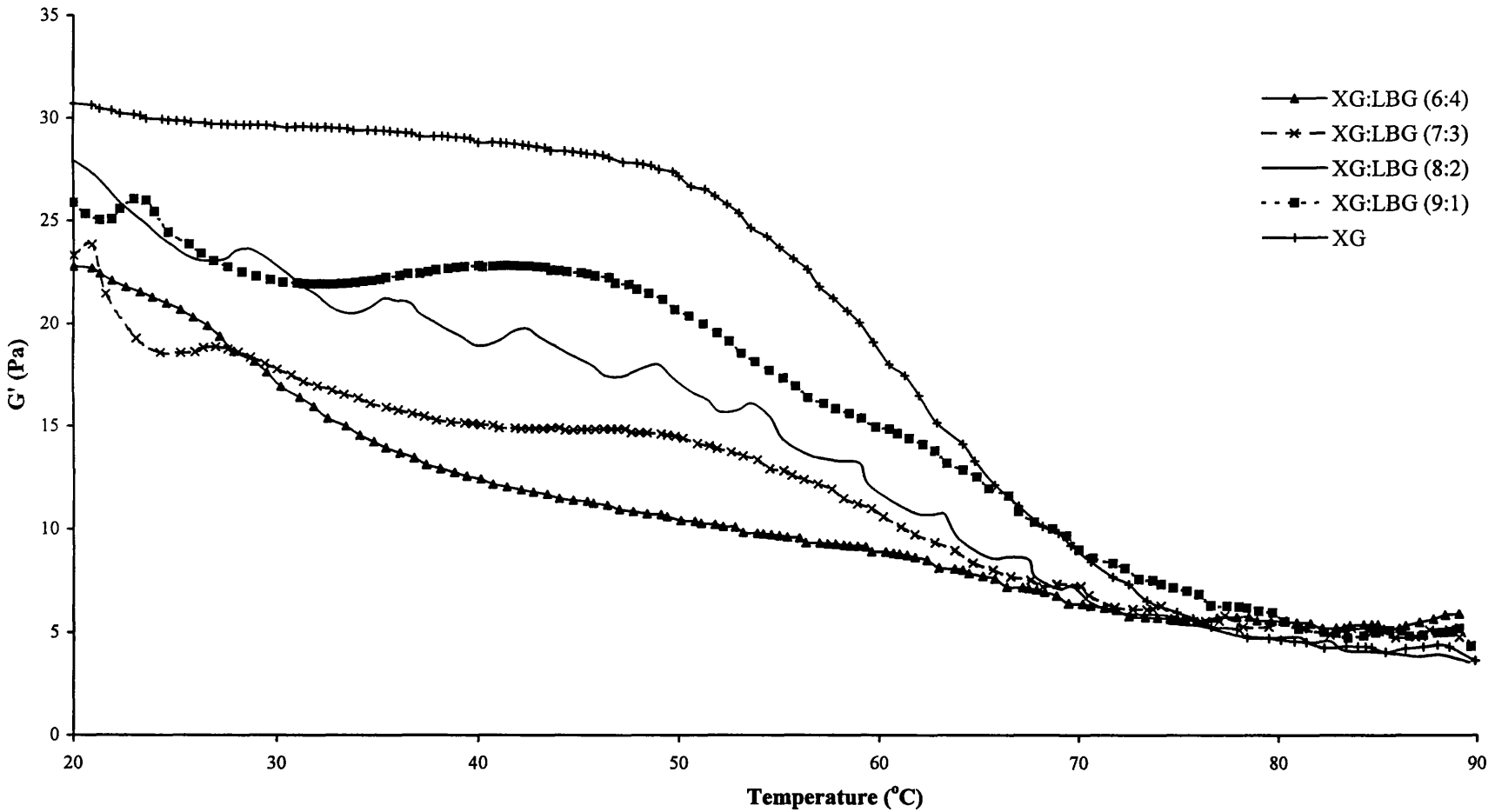


FIG. 4.13 : RHEOLOGICAL PROFILES OF DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1% w/w PREPARED AT 25°C ON COOLING

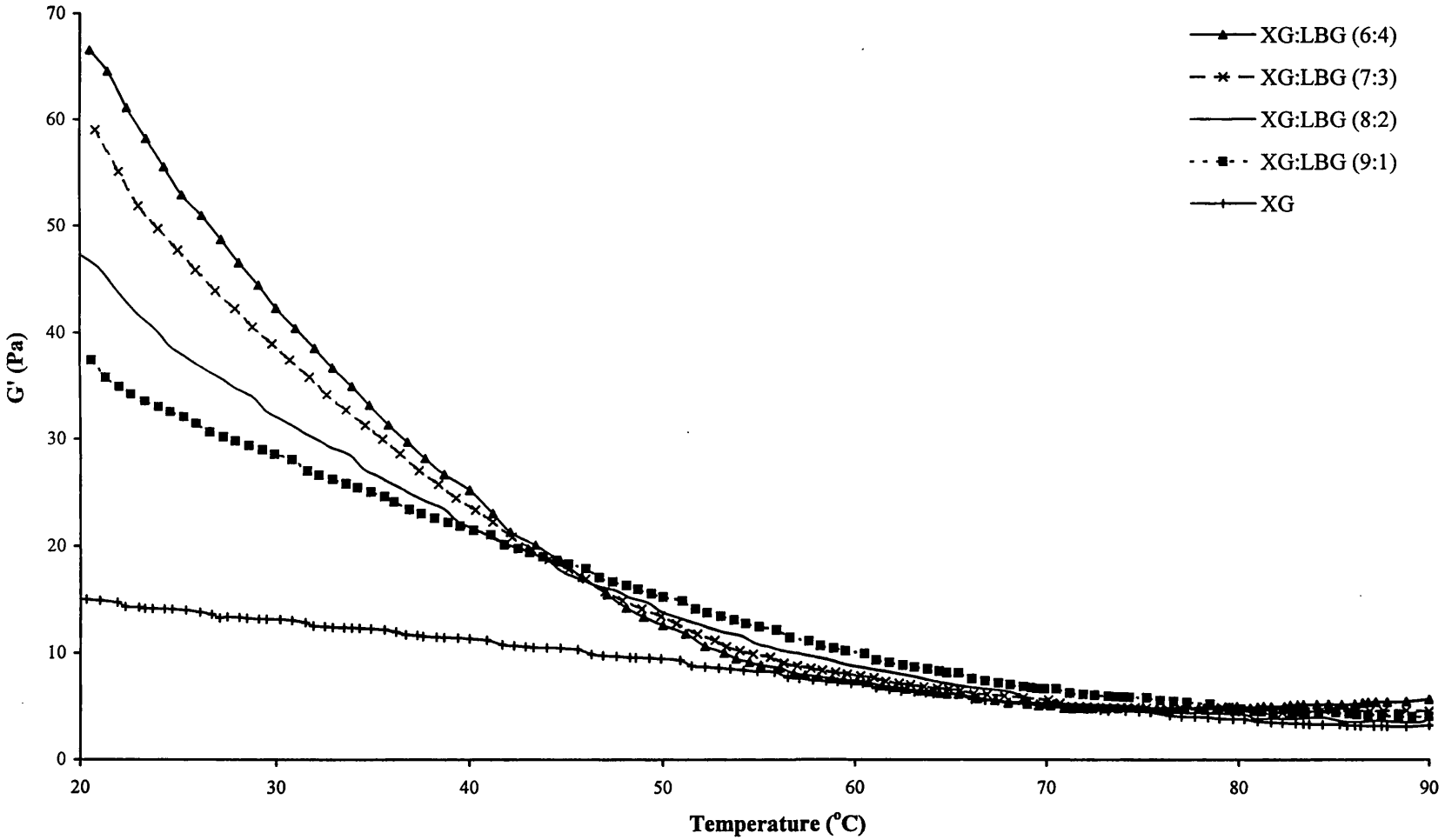


FIG. 4.14 : TEMPERATURE PROFILE FOR XANTHAN GUM 1%w/w PREPARED AT 25°C

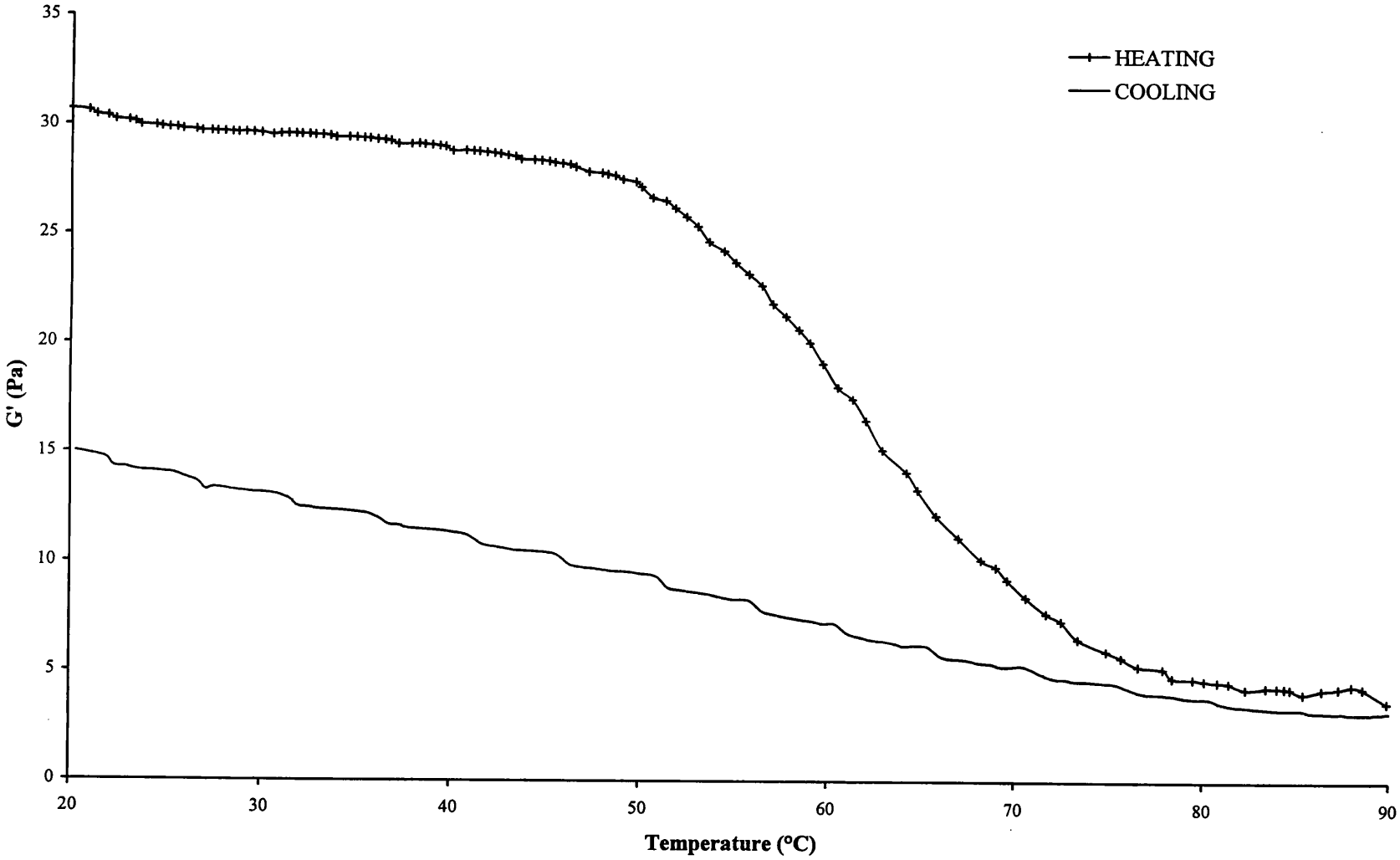


FIG. 4.15 : TEMPERATURE PROFILE FOR LOCUST BEAN GUM 1%w/w PREPARED AT 25°C

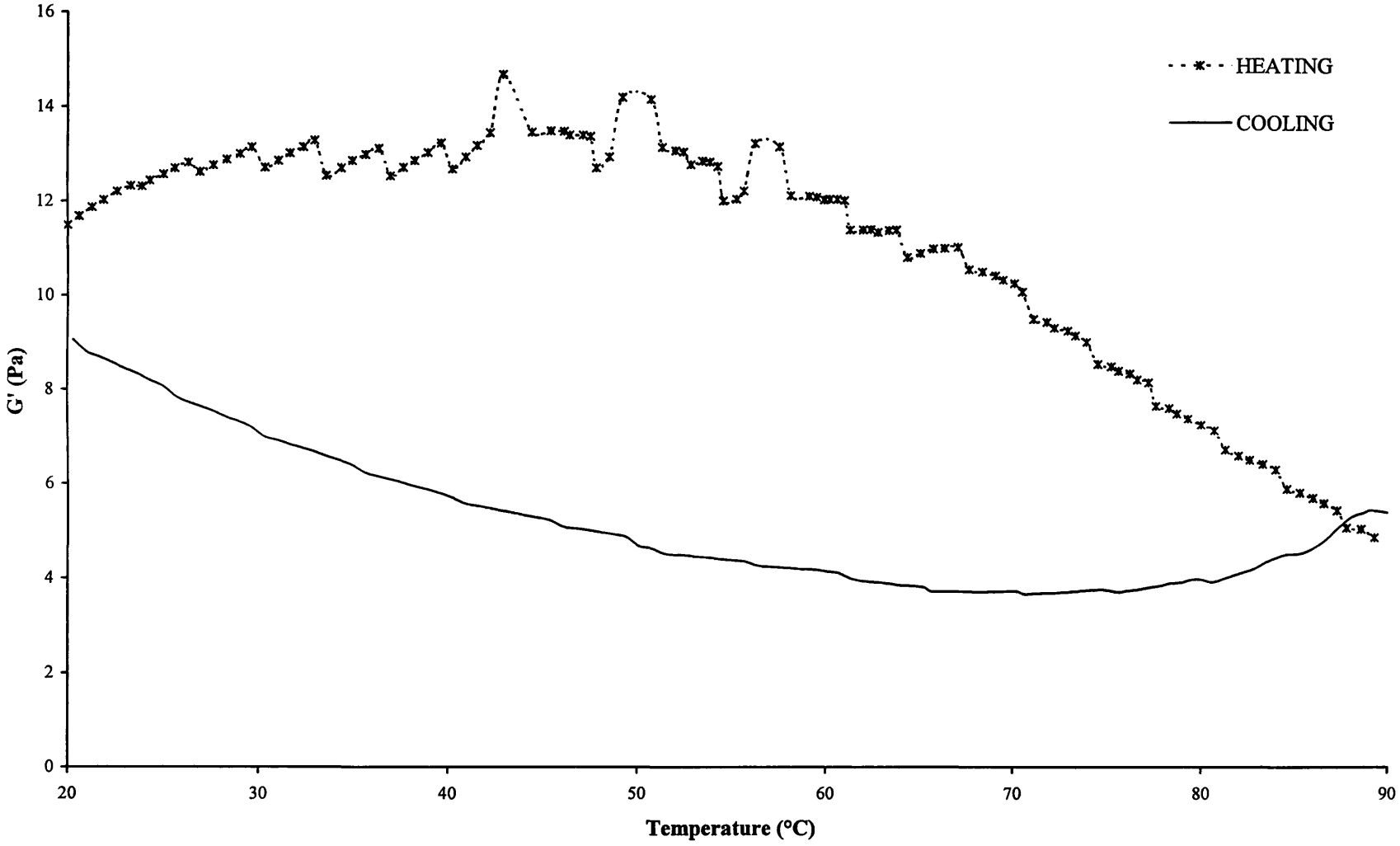


FIG. 4.16 : TEMPERATURE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (9:1) 1%w/w PREPARED AT 25°C

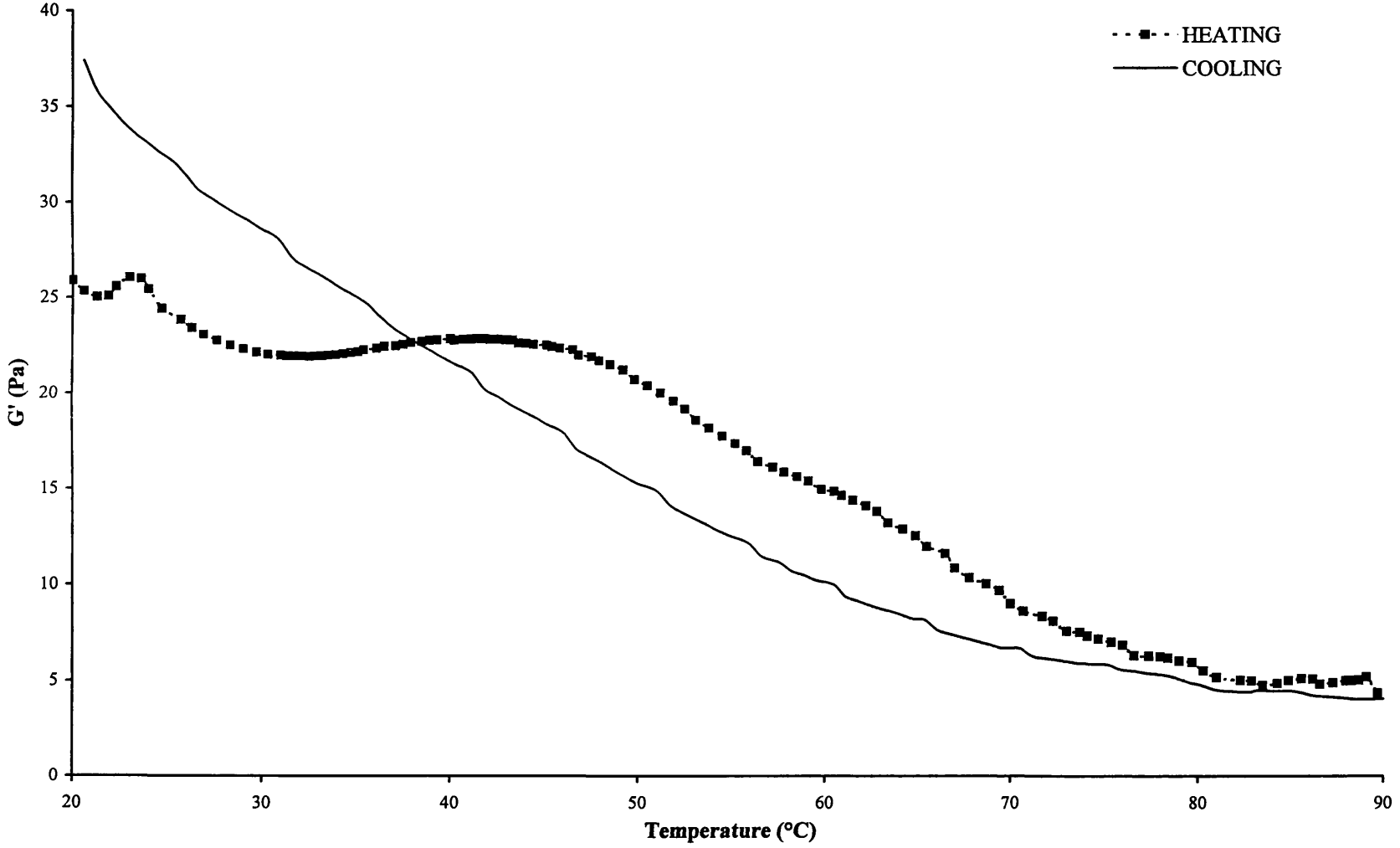
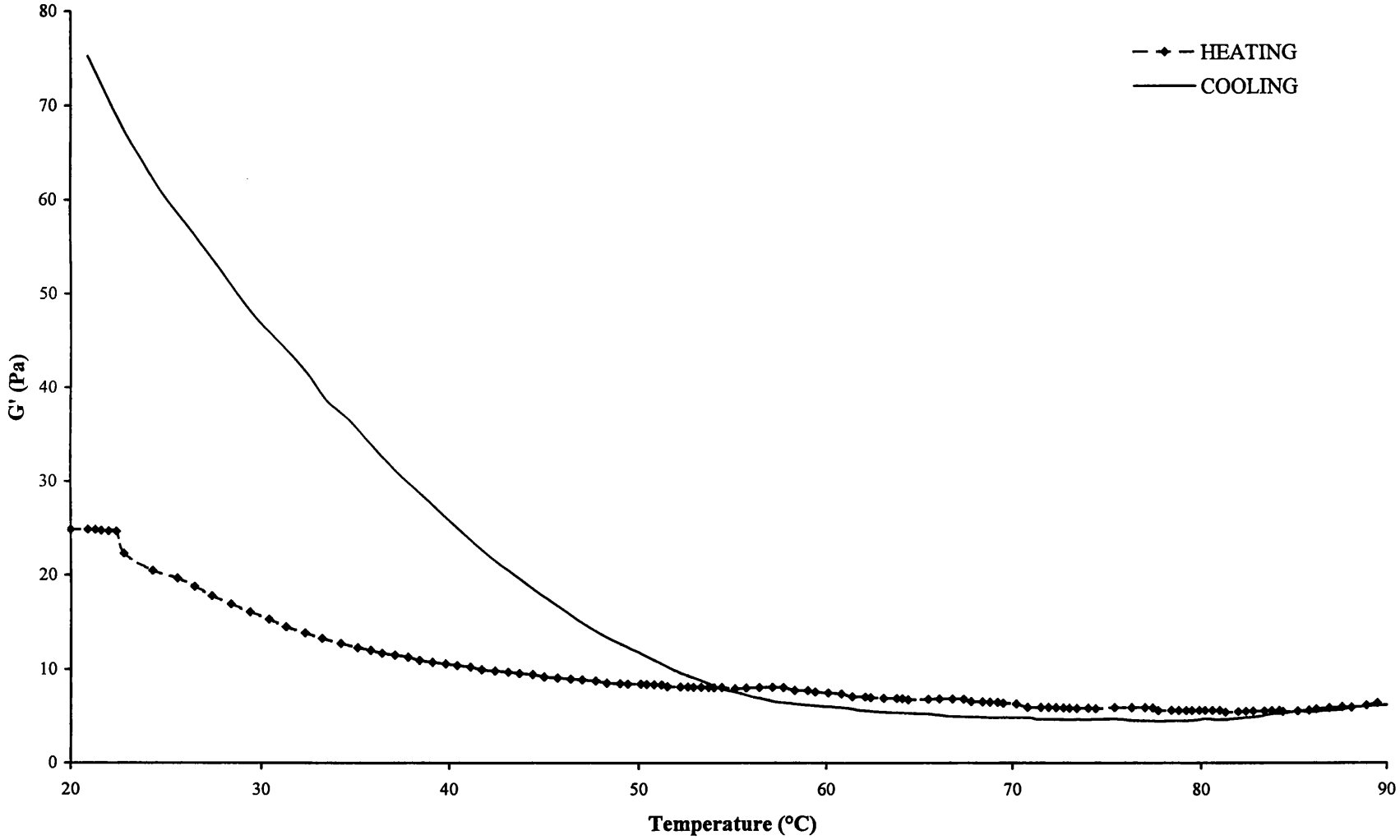


FIG. 4.17 : TEMPERATURE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w PREPARED AT 25°C



In addition, the heating and cooling profiles for xanthan gum 1%w/w prepared at 25°C (Figure 4.14) and 90°C (Figure 4.20) are similar with very similar storage moduli values which may indicate that the rheological behaviour of xanthan gum is not affected by temperature. These results are highlighted in Table 4.1.

The observations from Table 4.1 suggest that synergy between xanthan gum and locust bean gum is enhanced by heating and cooling, and may also indicate that the interaction and hence gelation process is not instantaneous.

TABLE 4.1 : STORAGE MODULI VALUES FOR XANTHAN GUM-LOCUST BEAN GUM MIXTURES 1%w/w PREPARED AT 25°C AND 90°C

	XANTHAN GUM:LOCUST BEAN GUM RATIO					
	1:9		5:5		XG	
	25°C	90°C	25°C	90°C	25°C	90°C
MEAN G′ (Pa) BEFORE HEATING	21.1	108.5	24.9	271.7	30.7	30.7
S.D	3.4	13.8	1.2	20.7	0.8	2.7
MEAN G′ (Pa) AFTER COOLING	28.0	28.7	75.3	172.5	15.0	26.1
S.D	1.4	2.2	3.0	8.0	1.2	0.6

FIG. 4.18 : TEMPERATURE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (1:9) 1%w/w PREPARED AT 90°C

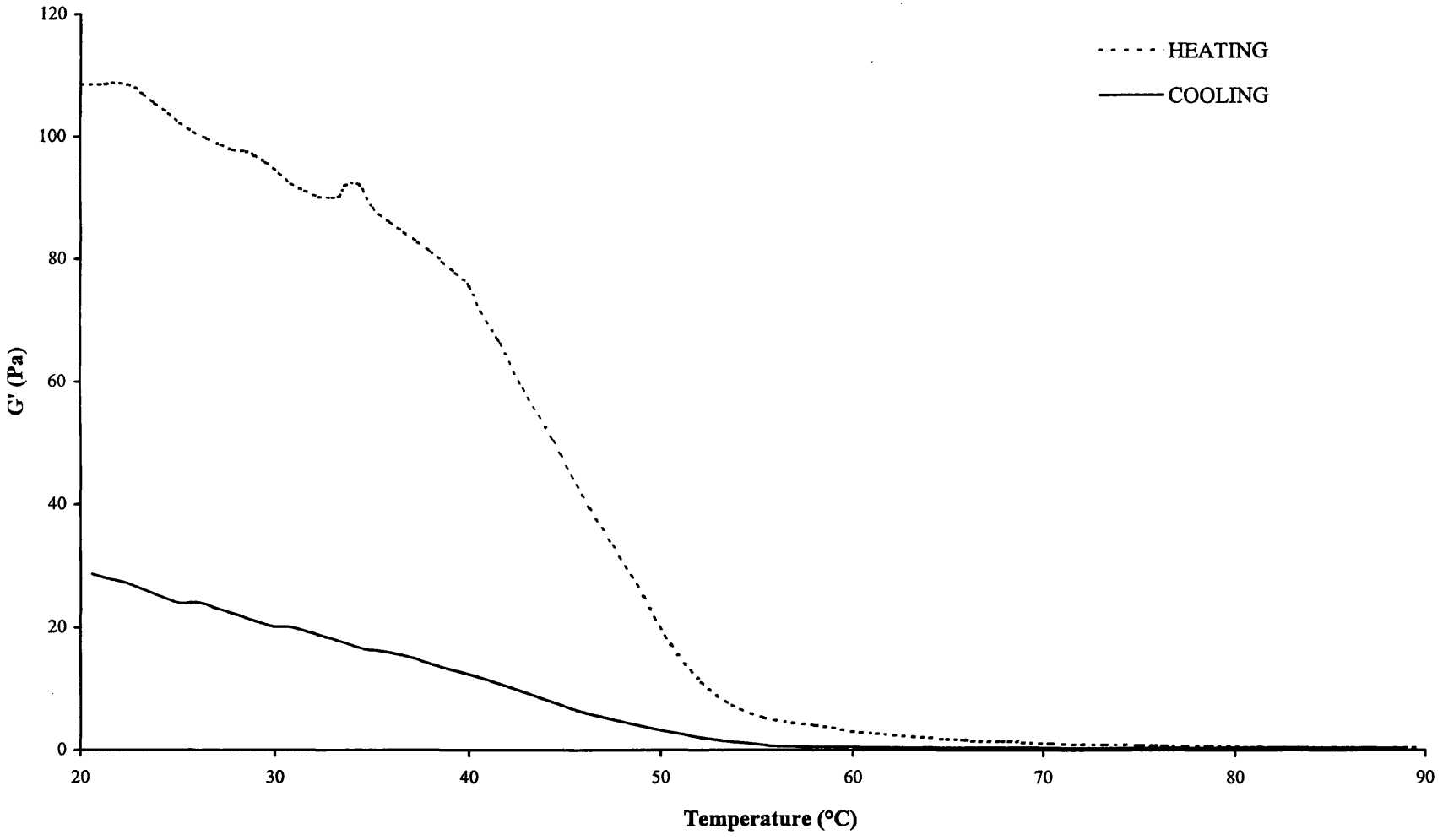


FIG. 4.19 : TEMPERATURE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w PREPARED AT 90°C

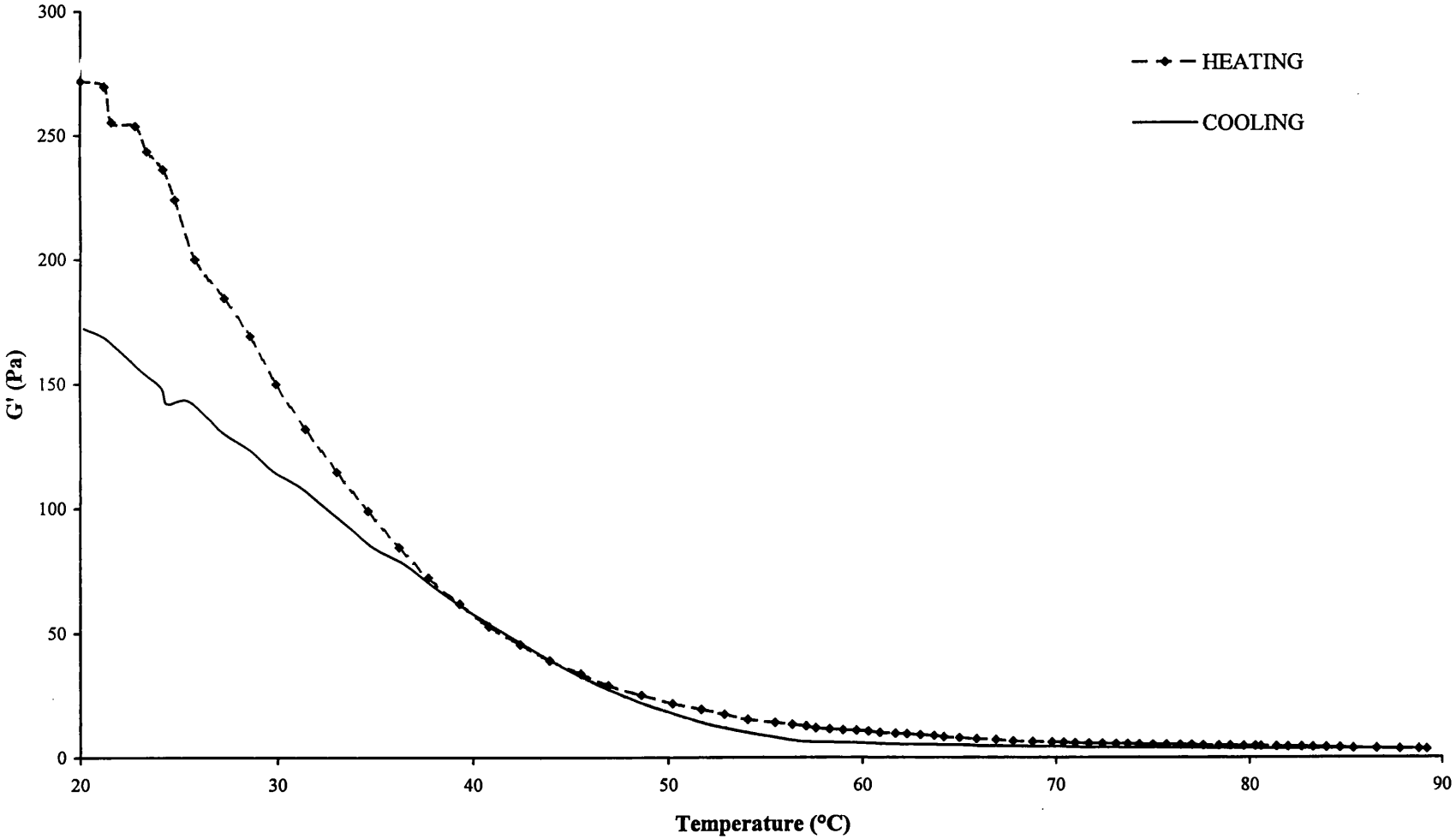
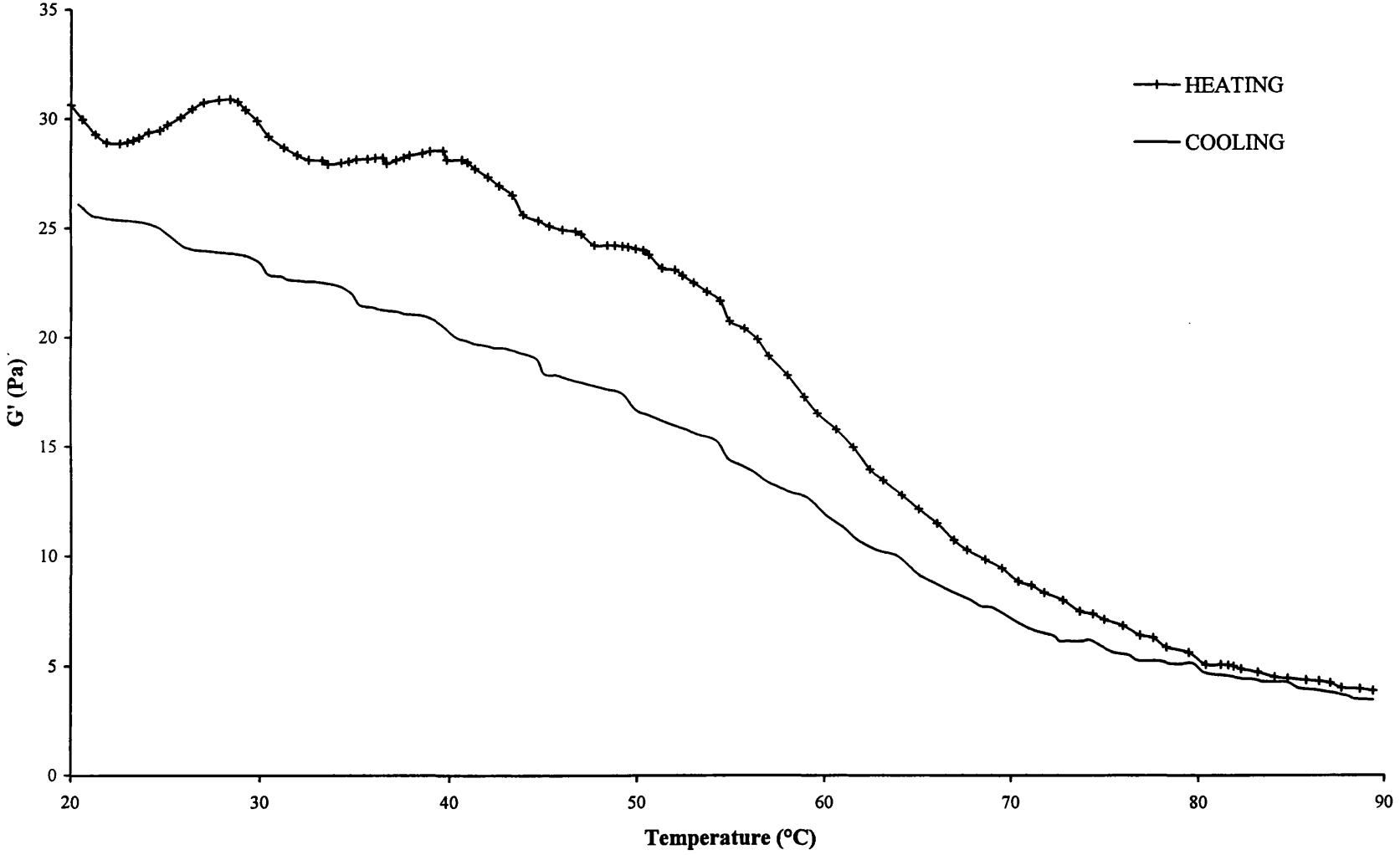


FIG. 4.20 : TEMPERATURE PROFILE FOR XANTHAN GUM 1%w/w PREPARED AT 90°C



4.4 THE INCLUSION OF ADDITIVES TO XANTHAN GUM-LOCUST BEAN GUM MIXES

It has been reported that the inclusion of salts and sugars can affect the rheological behaviour of xanthan gum-locust bean gum systems (Tako et al, 1984 and Watanabe et al, 1992 respectively). The addition of salts such as sodium chloride decrease viscoelastic measurements through ionic repulsions on charged trisaccharide chains on the xanthan molecules (Tako et al, 1984) or through aggregation of xanthan helices (Ross-Murphy et al, 1983), which indicates that the interaction between xanthan gum and locust bean gum depends on the presence of disordered xanthan gum.

Sucrose which is a sugar has been studied less extensively as an additive on xanthan gum-locust bean gum systems compared to sodium chloride as a salt additive. It has been found that the inclusion of sucrose to xanthan gum-locust bean gum hydrogels, increases the microscopic viscosity resulting in a decrease in drug release (Watanabe et al, 1992). Its actions on xanthan gum-locust bean gum mixes are not clearly understood and thus are investigated in the present work.

By studying the inclusion of these additives on the behaviour of xanthan gum-locust bean gum systems, ideas as to the mechanism for the interaction with respect to xanthan gum conformation can be made, with a means to developing pharmaceutical products which can be modified accordingly to control drug release through an understanding of their rheological characteristics.

4.4.1 THE EFFECT OF SUCROSE ON XANTHAN GUM-LOCUST BEAN GUM MIXTURES

4.4.1.1 Method

Temperature studies as described in Chapter 4.3.1.1 were performed on xanthan gum-locust bean gum (5:5) 1%w/w mixtures prepared in sucrose solutions ranging from 0.5%-15%w/w. The xanthan gum-locust bean gum (5:5) was chosen to investigate the effects of incorporating sucrose as additive, since this was the polysaccharide ratio at which maximum synergy was observed previously. The sucrose solutions and subsequent polymer mixes were prepared at 25°C as described in Chapter 2.2.2.

In addition, it was necessary to perform flow measurements on sucrose solutions 2%w/w, 8%w/w and 15%w/w, to ensure that the results observed for xanthan gum-locust bean gum mixes in sucrose solution, were not due to the effects of sucrose alone, i.e. to determine an interaction between polymer and sucrose.

Sucrose solutions (2%w/w, 8%w/w and 15%w/w) were prepared at 25°C according to the first part of the method explained in Chapter 2.2.2. Measurements were then performed on the AR-1000 rheometer using the flow package at 20°C and 50°C, and a steel 6cm cone and plate geometry with solvent trap attached. Samples were loaded using a spoon (one and a half spoons per run) and allowed to equilibrate to temperature (rheometer waits for correct temperature before performing experiments). The shear stress was then increased from 0.01-10Pa over 2min using stepped ramps. The controlled variable was set for shear stress, and measurements were repeated four times per sample using fresh solutions each time.

Unfortunately, measurements could not be performed at 90°C on the sucrose solutions due to problems with angular velocity and safety (i.e. the viscosities were too low to be measured in the limits of the rheometer).

4.4.1.2 Results

The heating profiles for xanthan gum-locust bean gum (5:5) mixes in different sucrose concentrations are shown in Figure 4.21. Apart from the curve for the polymer mixture in 15%w/w sucrose solution, all curves are similar to xanthan gum-locust bean gum (5:5) mixes in the absence of sucrose. The polymer mixture in 15%w/w sucrose solution shows an overall higher G′ on heating at all temperatures between 20°C and 90°C, compared to the other mixes containing a lower concentration of sucrose.

The corresponding cooling curves for xanthan gum-locust bean gum (5:5) mixes in different sucrose concentrations are shown in Figure 4.22. Not only is G′ greater after heating and cooling compared to before heating (Figure 4.21), but it is observed that the G′ increases with increasing sucrose concentration after heating and cooling from 90°C to 20°C. The most dramatic increase in G′ at 20°C after this heating and cooling process is for the polymer mixture in 15%w/w sucrose solution, in which there is almost

a 4-fold increase in G′ with the addition of 15%w/w sucrose (280Pa) compared to mixtures without any sucrose (75Pa). This difference is shown in Figure 4.23.

The flow curves for two of the sucrose solutions (2%w/w and 15%w/w) at 20°C and 50°C are shown in Figure 4.24. Although the viscosity is smaller for the lower concentration (2%w/w) and is also smaller at the higher temperature investigated (50°C) which would be expected, the increase in viscosity with increasing shear stress was not anticipated. This would imply that sucrose is shear thickening which is unlikely, since sucrose solutions at the concentrations investigated are very liquid resembling Newtonian samples. In support of this, viscosity values were very low at low shear stresses (in the region of 10^{-3}Pa.s) and it was observed during experiments that the spindle of the rheometer was moving faster as the shear stress was increased, such that the maximum angular velocity was reached very quickly which halts the experiments so as to protect the rheometer from mechanical damage.

The plots of normal force (N) in Figure 4.24, show that as the viscosity increases on the graph, the normal force decreases. This trend suggests that the apparent increase in viscosity with increasing shear stress can be accounted for by the low viscosities of the sucrose solutions being sucked up onto the cone which creates eddy currents resulting in turbulent flow. Thus, sucrose solutions 2%w/w, 8%w/w and 15%w/w have low viscosities and are not shear thickening. This finding indicates that the effects of sucrose on the polysaccharide systems observed are not due the viscosity effects of sucrose alone, and thus suggest that the addition of sucrose increases the strength of these systems.

Oscillatory and creep measurements on sucrose solutions were attempted to support the findings of sucrose with the polysaccharide mixes, but the sucrose solutions were too fluid to be measured by such techniques, which is why flow was chosen.

FIG. 4.21 : HEATING PROFILES FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w PREPARED IN SUCROSE SOLUTIONS AT 25°C

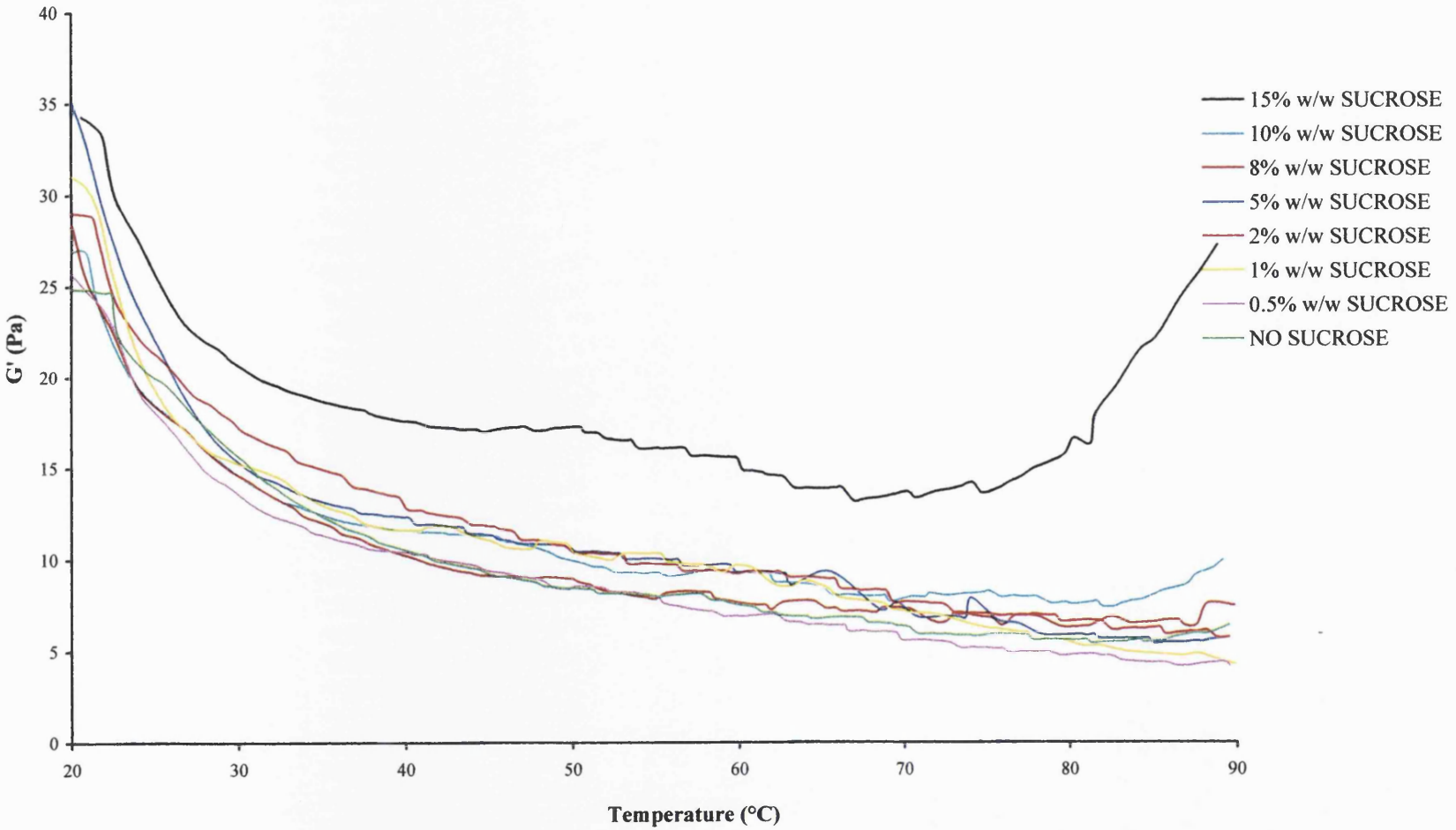


FIG. 4.22 : COOLING PROFILES FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w PREPARED IN SUCROSE SOLUTIONS AT 25°C

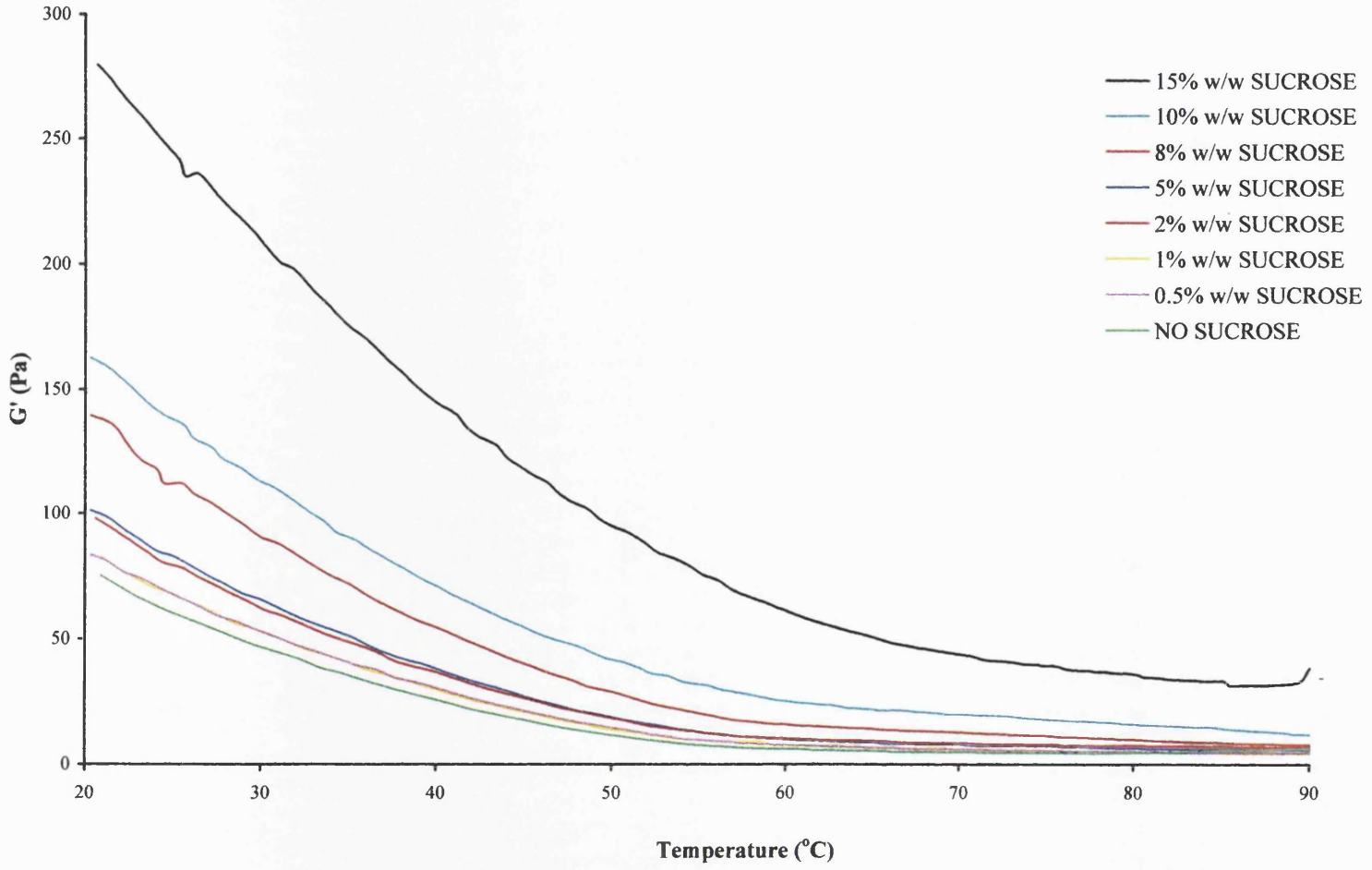


FIG. 4.23 : GRAPH TO COMPARE THE HEATING AND COOLING PROFILES OF XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w MIXES PREPARED AT 25°C IN THE ABSENCE AND PRESENCE OF SUCROSE

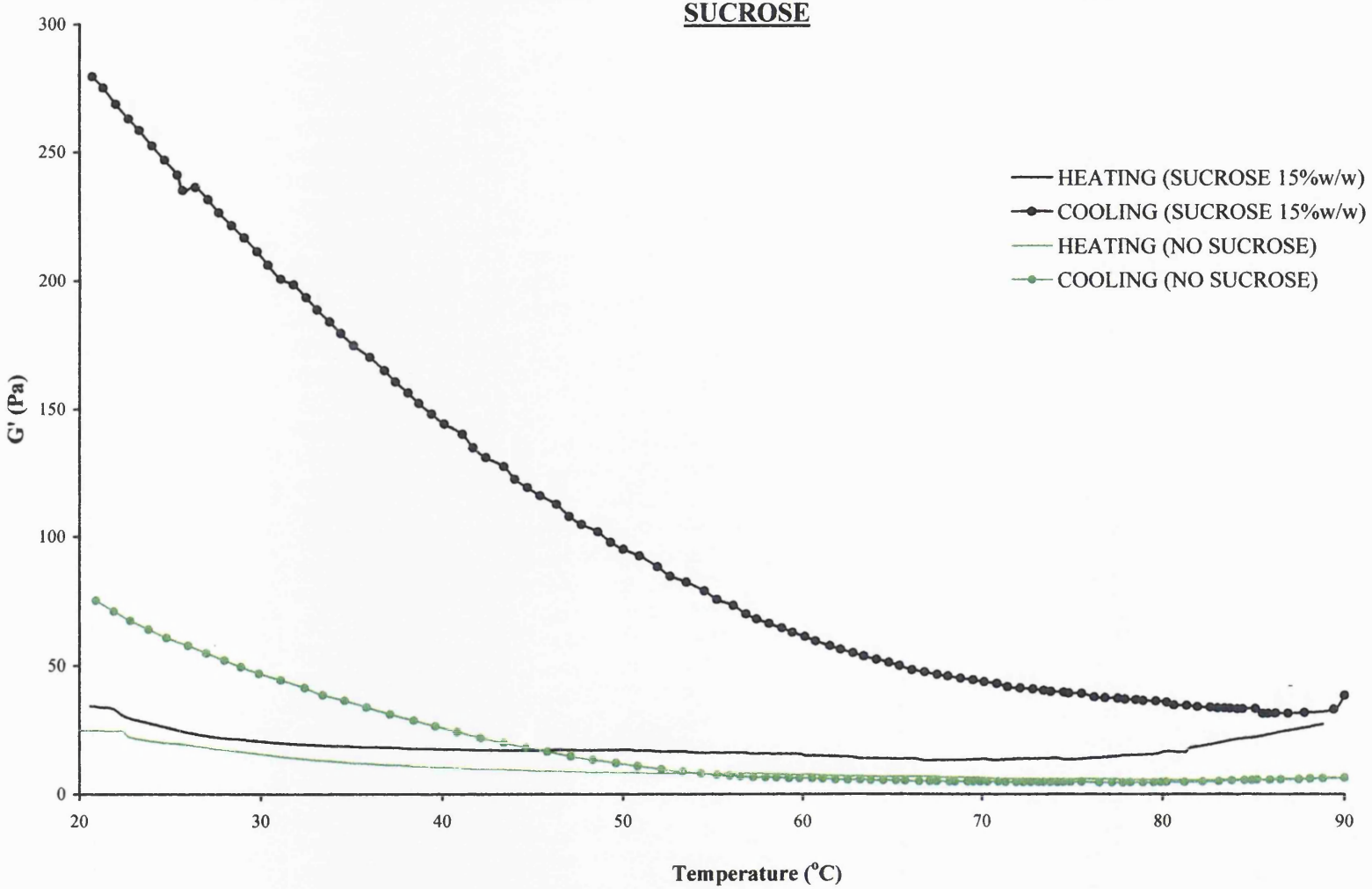
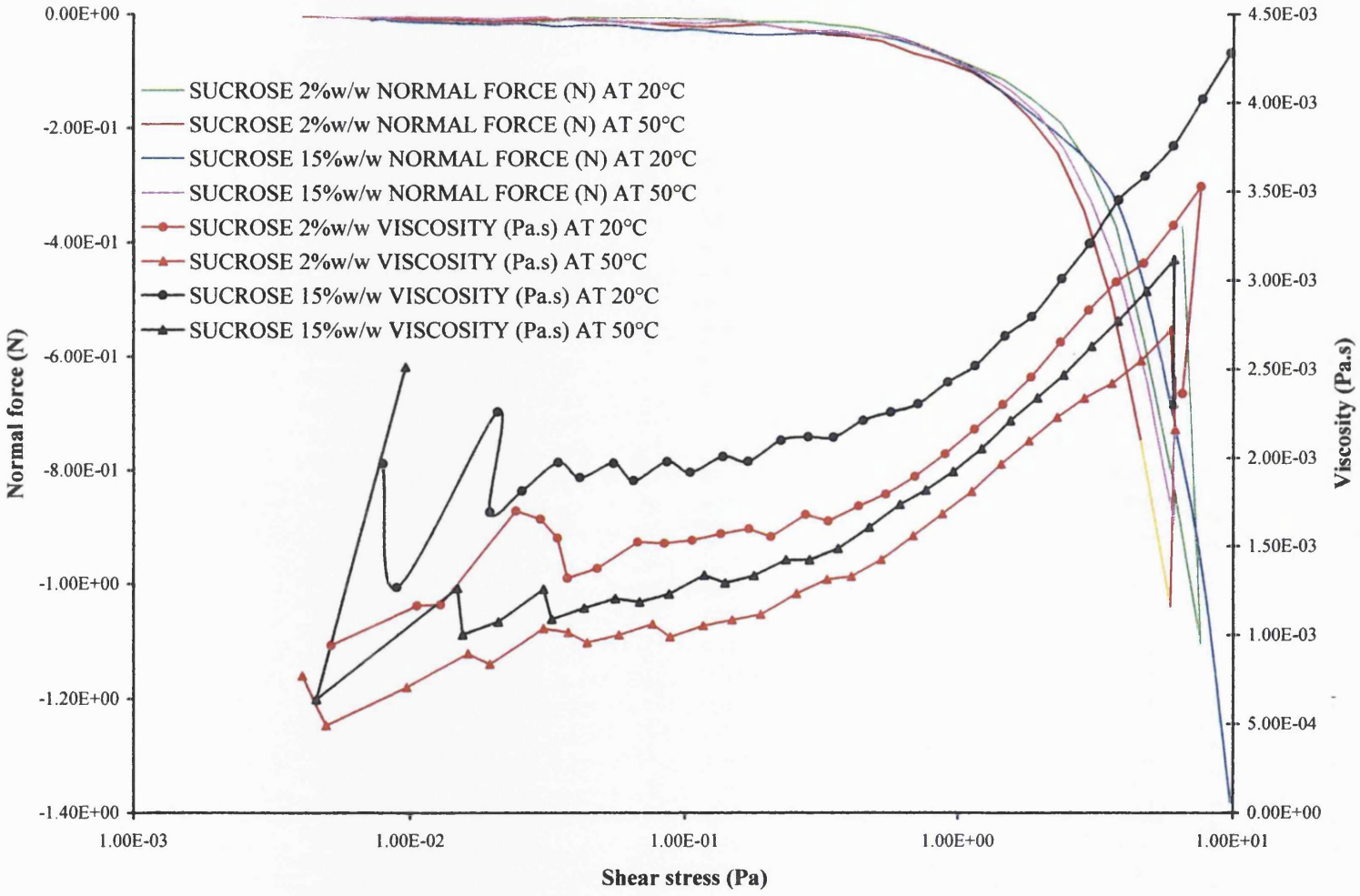


FIG. 4.24 : FLOW CURVES FOR SUCROSE SOLUTIONS



4.4.2 THE ADDITION OF SODIUM CHLORIDE TO XANTHAN GUM-LOCUST BEAN GUM MIXTURES

4.4.2.1 Method

The sodium chloride solutions (10mmol, 20mmol and 30mmol) and subsequent polymer mixes (xanthan gum-locust bean gum (5:5) 1%w/w) were prepared at 25°C as described in Chapter 2.2.3.

Temperature studies as detailed in Chapter 4.3.1.1 were performed on xanthan gum-locust bean gum (5:5) 1%w/w mixtures prepared in sodium chloride solutions. The displacement value used was changed from $5x10^{-4}$rad (Chapter 4.2.1) to $4x10^{-3}$rad to improve the waveforms for this set of experiments.

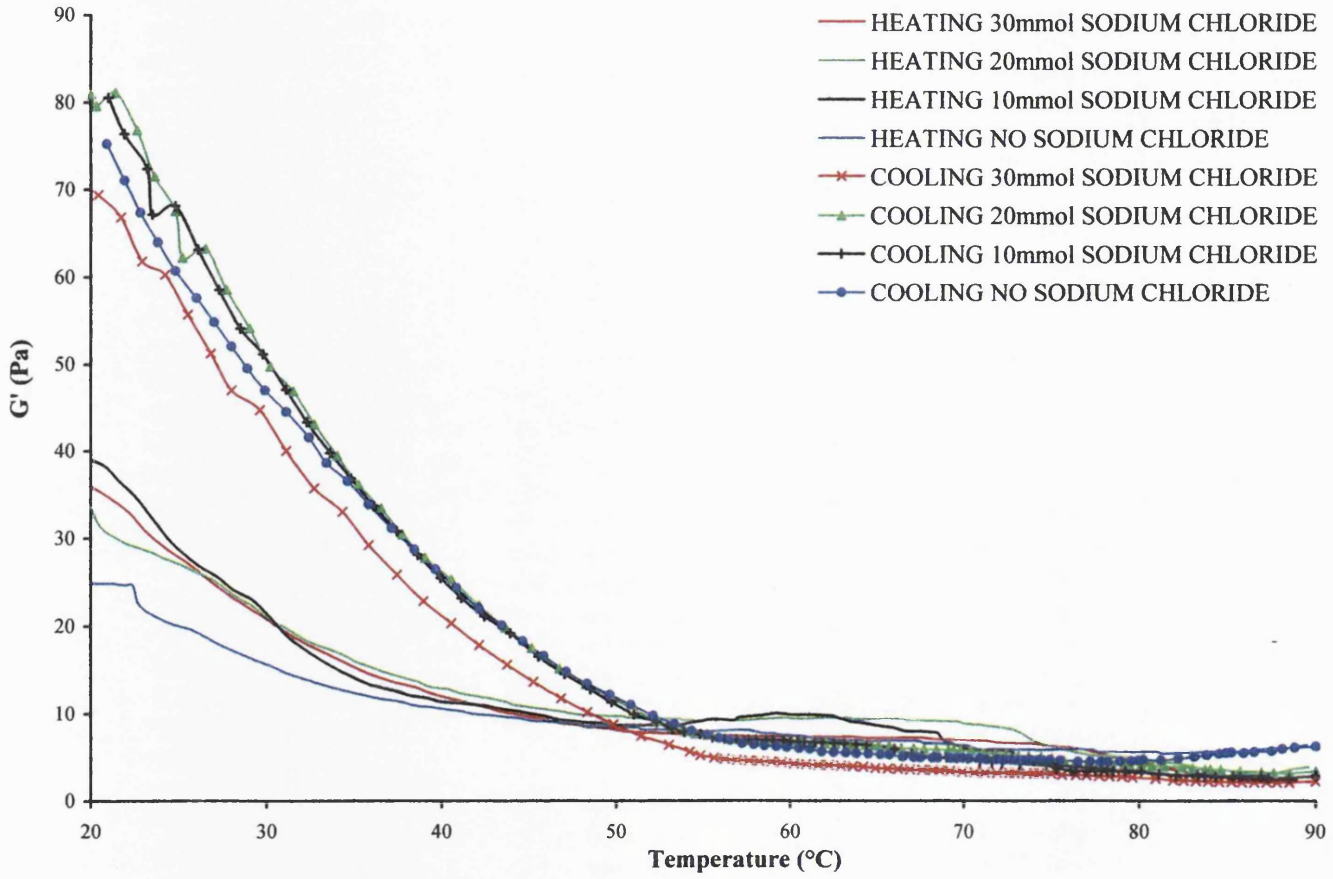
4.4.2.2 Results

The heating and cooling profiles for xanthan gum-locust bean gum (5:5) 1%w/w in the presence and absence of sodium chloride are shown in Figure 4.25, with G′ after heating and cooling being greater than before heating for all samples. There does not appear to be any marked changes in the G′ values for polymer mixes in the presence of sodium chloride before and after heating and cooling, compared to mixes in the absence of sodium chloride. Thus, the addition of sodium chloride (10mmol, 20mmol and 30mmol) does not alter the rheological behaviour of xanthan gum-locust bean gum (5:5) 1%w/w.

4.5 THE RHEOLOGICAL EFFECTS OF MIXES CONTAINING FRACTIONATED LOCUST BEAN GUM

It has been mentioned in Chapter 1.1.4.2 that locust bean gum is only partially soluble in water at low temperatures such as ambient. This may have an influence on interactions with other polysaccharides, hence xanthan gum in the present work.

FIG. 4.25 : RHEOLOGICAL PROFILES FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w PREPARED IN DIFFERENT SODIUM CHLORIDE CONCENTRATIONS AT 25°C ON HEATING AND COOLING



However, a fractionation process which separates locust bean gum into cold water and hot water soluble portions with low and high M:G ratios respectively, can increase the solubility (Gaisford et al, 1986). Thus in the present study, a fractionation process was used to separate locust bean gum into two different fractions (LBG35 – low M:G ratio and LBG80 – high M:G ratio) which were subsequently used to prepare xanthan gum-locust bean gum mixes in order to compare the rheological properties. This was an important consideration in the present work, since previous studies (Mannion et al, 1992) have involved heating locust bean gum to high temperatures followed by cooling to ambient prior to measurements at room temperature.

4.5.1 Method

The locust bean gum used in these temperature studies were fractionated into two different fractions namely LBG80 which has a high M:G ratio and LBG35 which has a low M:G ratio, according to the method described earlier in Chapter 3.4.1. These fractions were then subsequently mixed with xanthan gum to form 1%w/w solutions, as described in Chapter 2.2.1.

Temperature studies as described in Chapter 4.3.1.1 were performed on xanthan gum-locust bean gum (5:5) 1%w/w mixtures prepared at 25°C, in order to compare with the results for mixes containing unfractionated locust bean gum in Figure 4.17.

4.5.2 Results

The temperature profiles for xanthan gum-locust bean gum (5:5) 1%w/w mixes containing the low M:G ratio of locust bean gum (LBG35) are shown in Figure 4.26. Results are reproducible for both the heating and cooling measurements, and show that the G′ after heating and cooling is approximately two-fold smaller for mixes containing LBG35 compared to mixes containing unfractionated locust bean gum (37Pa and 75Pa respectively). The heating profiles for mixes containing both fractionated and unfractionated locust bean gum are similar, in which the G′ is 25Pa for mixes containing the unfractionated locust bean gum and 27Pa for mixes containing the fractionated form (LBG35) at 20°C before heating.

The temperature profiles for xanthan gum-locust bean gum (5:5) 1%w/w mixes containing the high M:G ratio of locust bean gum (LBG80) are shown in Figure 4.27. The cooling profiles for mixes containing the fractionated locust bean gum (LBG80) are not very reproducible, but tend to show that the G′ during cooling is higher than for mixes containing unfractionated locust bean gum, with G′ being higher at 20°C after the heating and cooling process (G′=75Pa for mixes with unfractionated locust bean gum and G′=99Pa-154Pa for mixes containing LBG80). In fact, the results at 20°C after heating and cooling for the different mixes can be summarised as follows;

G′ LBG80>G′ UNFRACTIONATED LBG>G′ LBG35
(99Pa-154Pa>75Pa>37Pa)

The heating profiles for mixes containing LBG80 are reproducible, and have a different shape to mixes containing LBG35 and unfractionated locust bean gum. It is observed that there is a maximum in G′ at approximately 60°C for mixes containing LBG80 on heating, which is not observed for the other mixes containing LBG35 and unfractionated locust bean gum.

These findings suggest that the synergy between xanthan gum and locust bean gum is reduced in the presence of locust bean gum with a low M:G ratio and increased in the presence of locust bean gum with a high M:G ratio after heating and cooling. This may indicate that the interaction between these two polysaccharides involves the unsubstituted parts (smooth regions) of the locust bean gum backbone, and that the solubility of locust bean gum is an important consideration in the interaction with xanthan gum.

4.6 DISCUSSION

The results from the frequency studies (Chapter 4.2.2), do not clearly ascertain if synergy occurs between xanthan gum and locust bean gum of various ratios at 25°C. This is because the highest G′ value observed was for xanthan gum 1%w/w, with both G′ and G″ decreasing as the xanthan gum-locust bean gum ratio decreased proportionally (Figure 4.9).

FIG. 4.26 : TEMPERATURE PROFILES FOR XANTHAN GUM-LOCUST BEAN GUM (LBG35) (5:5) 1%w/w PREPARED AT 25°C

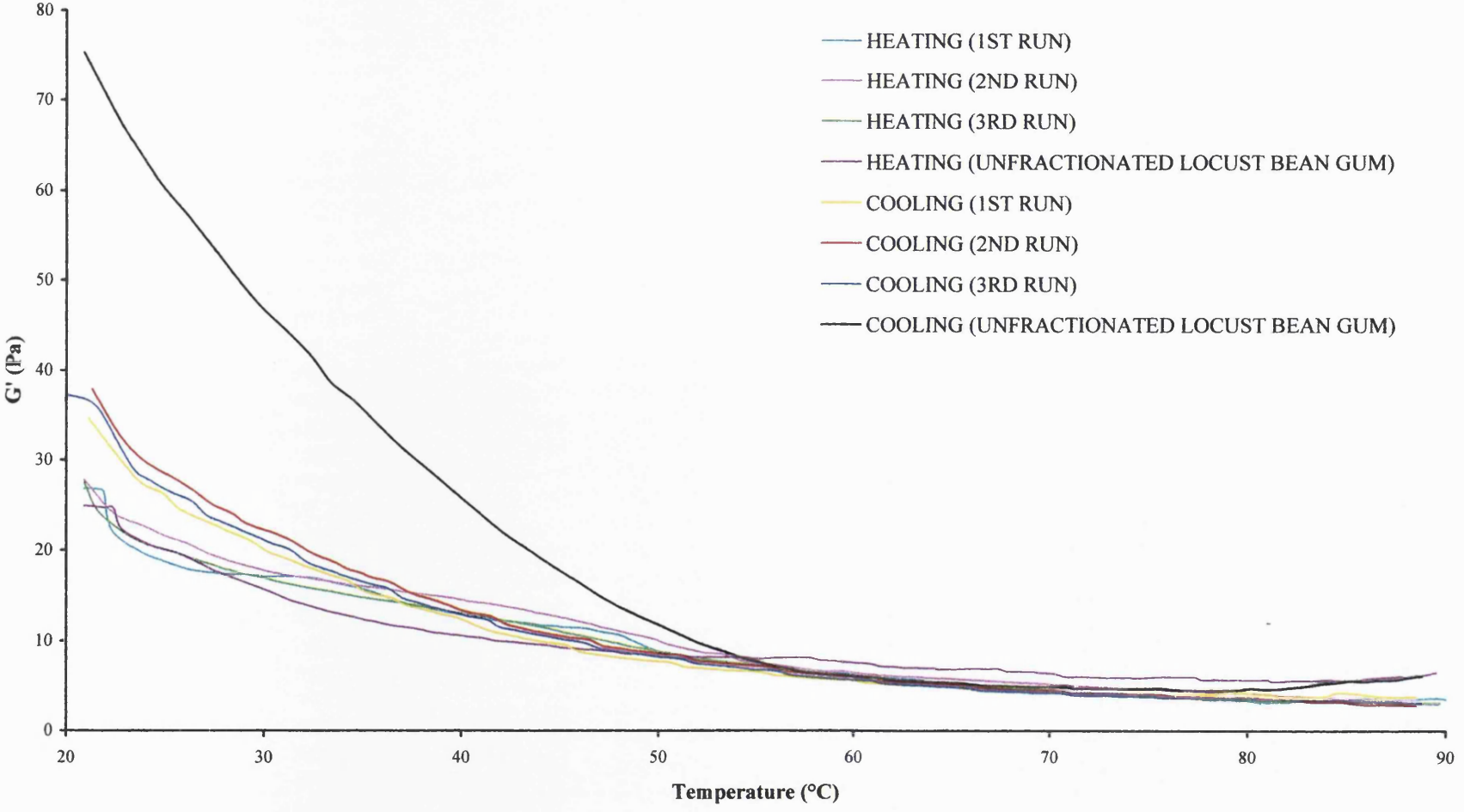
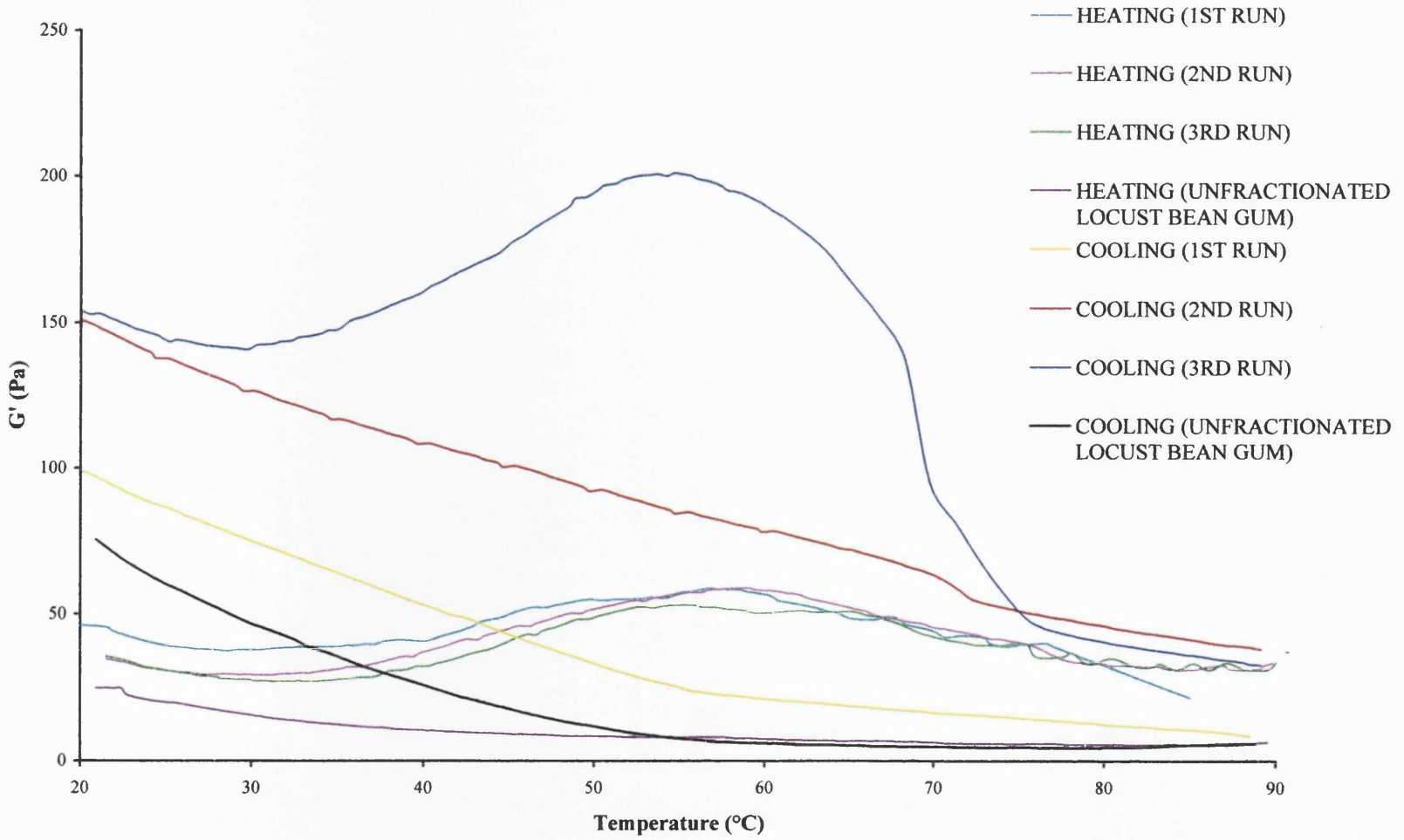


FIG. 4.27 : TEMPERATURE PROFILES FOR XANTHAN GUM-LOCUST BEAN GUM (LBG80) (5:5) 1%w/w PREPARED AT 25°C



Another explanation for this trend could be due to the partial solubility of locust bean gum at room temperature, thus preventing full interaction with xanthan gum, since it was observed in Figure 4.27 that the high M:G ratio locust bean gum fraction (LBG80 – hot water soluble) increased G′ after heating and cooling, suggesting that the interaction between xanthan gum and locust bean gum is enhanced by the unsubstituted regions of the locust bean gum backbone being present. This could also explain why the frequency scans for locust bean gum 1%w/w were not reproducible in this study. However, another possible reason for the poor reproducibility could simply be due to the low viscosity of locust bean gum. The small dependency of both moduli on frequency observed in Figure 4.9 suggests that the polysaccharide mixes are viscoelastic, but do not form true gels at 25°C since the moduli would be totally independent of frequency. The G′ is greater than G′′ in all cases, suggesting that the samples have more elastic than viscous properties, even for xanthan gum 1%w/w (i.e. tan δ is greater than 1).

On heating and cooling xanthan gum-locust bean gum mixes prepared at 25°C between 20°C and 90°C (Figures 4.10-4.13), the G′ value increased compared to mixes prepared and measured at 25°C. This indicates that the strength of the polysaccharide mixed systems are enhanced by heating and cooling between 20°C and 90°C. In addition, these observations were not observed for the individual polysaccharides (G′ virtually unchanged before and after heating and cooling), which suggests that synergy does occur between xanthan gum and locust bean gum after heating and cooling between 20°C and 90°C. Additionally, the observed cross over point between the heating and cooling curves for all ratios (Figures 4.16 and 4.17 as examples) may suggest that different structures are formed at the cross over point temperature which is approximately 50°C for the mixed systems. This temperature coincides with the transition temperature of xanthan gum (Williams et al, 1991), which may suggest that the disordered form of xanthan gum may be necessary for interactions with locust bean gum. No cross over point is observed for the individual polysaccharides (Figures 4.14 and 4.15) which suggests that any structural changes that may occur on heating and cooling are not permanent.

Maximum synergy was observed for the 5:5 ratio (i.e. 1:1) of xanthan gum-locust bean gum (Figure 4.11) which is supported by other workers, and may suggest that there is an

equal number of junction zones involved in the interaction (Kovacs, 1973, Williams et al, 1991).

The G′ measurements at 20°C before heating and cooling for mixes prepared at 90°C were considerably higher compared to mixes prepared at 25°C (see Table 4.1). This observation may indicate a time dependent gelation process which has been reported by Kovacs (1973), since the mixtures were left to cool for 24hrs after preparing at 90°C prior to the temperature studies. (i.e. oscillatory rheology studies measure the rheological properties of the mixes continuously with temperature i.e. at 2°C/min in the present work). Additionally, it was observed that the G′ after heating and cooling the mixed systems prepared at 90°C were lower than before heating followed by cooling. This may suggest that once the gel has been formed during the first heating and cooling cycle in the preparation method, repetitive heating followed by cooling on the rheometer does not further enhance the synergy or may even break down the gel structure, since G′ at 20°C after cooling decreased. Again this finding may reinforce the proposal that the disordered form of xanthan gum may be necessary for interactions with locust bean gum.

These observations for the mixed polysaccharide systems prepared at 90°C did not apply to xanthan gum 1%w/w prepared at 90°C, since the heating and cooling profiles and G′ values were similar as for xanthan gum 1%w/w prepared at 25°C. This observation could reflect the reversible xanthan gum helix melting and renaturation process (Morris et al, 1977), which would explain why no new structures were observed in the current work (i.e. no cross over point was observed in Figure 4.14).

The addition of sucrose increased the gel strength of xanthan gum-locust bean gum 5:5 mixes on heating and cooling, indicated by the increase in G′, which was greatest for the highest concentration of sucrose investigated i.e. 15%w/w (Figure 4.22). The reason for this is unknown, since only a small number of studies on xanthan gum-locust bean gum-sucrose interactions have been performed. However one study has mentioned that the addition of sucrose to xanthan gum-locust bean gum systems may favour polymer-polymer interactions compared to polymer-solvent interactions, due to solvent bonding to the sucrose molecules, which contributes to an overall increase in the viscosity of the system (Sudhakar et al, 1995).

The gel strength of xanthan gum-locust bean gum 5:5 mixes were independent of ionic strength, since there was no change in G′ with the inclusion of 10mmol, 20mmol or 30mmol sodium chloride (see Figure 4.25). These findings may suggest that once the interaction between the two polysaccharides is established, the sodium cations (Na^+) can not induce xanthan gum aggregation or stabilise the ordered helical form, since the gel strength was also independent of the salt concentration, and was the same as for polysaccharide mixtures in the absence of sodium chloride.

The findings for the polysaccharide mixtures containing fractionated locust bean gum (Figures 4.26 and 4.27) suggest that the interaction involves or is enhanced by the smooth regions of locust bean gum i.e. the unsubstituted mannan backbone, since the gel strength starting from the greatest was of the following order for locust bean gum type; LBG80>UNFRACTIONATED>LBG35. Similar results have been found by Dea and Morrison (1975), Mannion et al (1992) and Lundin & Hermansson (1995). In addition, the maxima at around 60°C on the heating profiles for xanthan gum-LBG80 mixtures (Figure 4.27) correspond closely to the transition temperature of xanthan gum, which again may indicate that the disordered form of xanthan gum is involved in the interaction with locust bean gum.

In summary of the findings from the present work, synergy between xanthan gum and locust bean gum may or may not occur at room temperature, but occurs after heating and cooling between 20°C and 90°C and is enhanced by the inclusion of sucrose and LBG80. These results suggest evidence to support that the interaction process involves the disordered form of xanthan gum and the unsubstituted regions of the locust bean gum backbone (Dea & Morrison, 1975, Dea et al, 1977 and Cheetham & Mashimba, 1988).

CHAPTER 5

CHAPTER 5 : TEXTURE ANALYSIS STUDIES ON XANTHAN GUM-LOCUST BEAN GUM MIXTURES

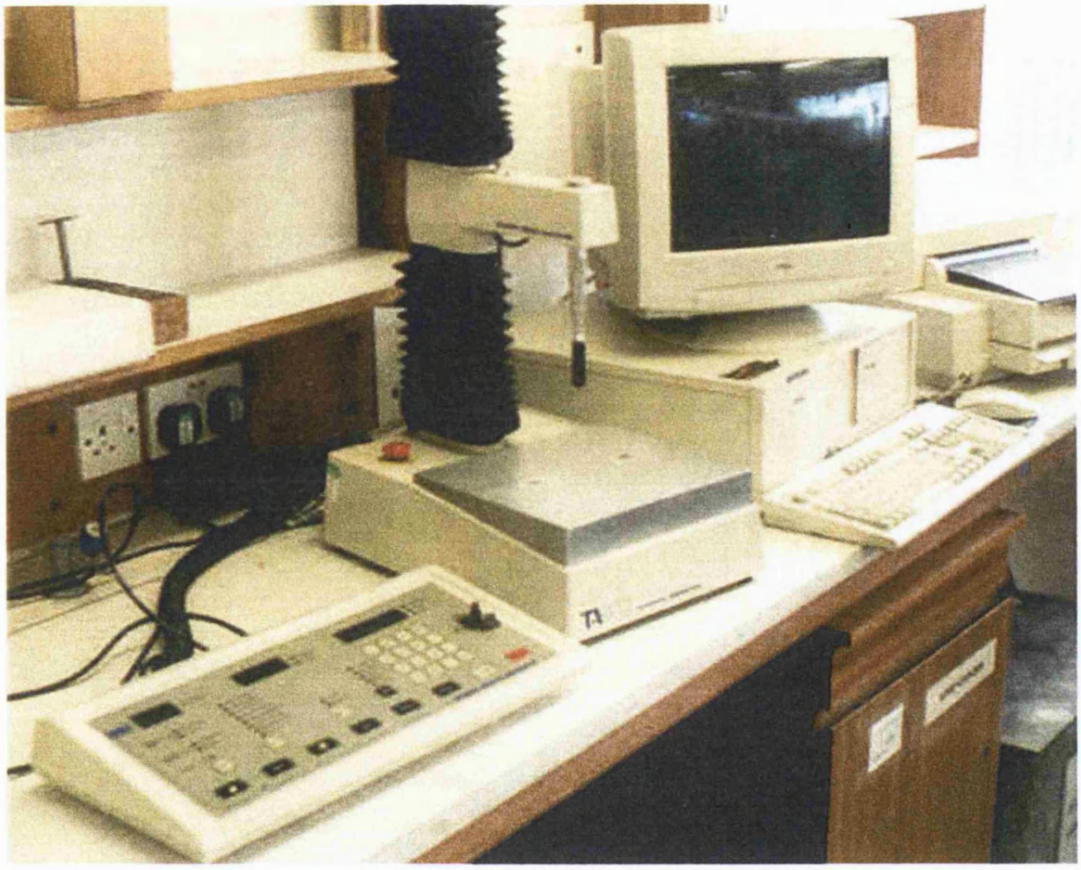
5.1 INTRODUCTION

Texture analysis is a simple and versatile means of characterising the physical properties of materials. Examples of products which have been analysed using this technique include foods, cosmetics, medical devices and pharmaceutical dosage forms.

The texture analyser otherwise known as a penetrometer is the instrument that measures the response of a sample to a compressive or tensile force. This can be achieved either by moving a probe into a sample and measuring the force, or applying a force and measuring the movement, both as a function of time. The instrument is manually calibrated before each set of experiments, and is linked to a computer so that data can be recorded and analysed. In the present project, texture analysis studies were performed using the Stable Micro Systems TA-XT2 as shown in Figure 5.1, to investigate the gel strengths of various xanthan gum-locust bean gum mixes. The instrument consists of a single column with a 5kg load cell. The probe speed has a range of between 0.01mm/sec to 10mm/sec.

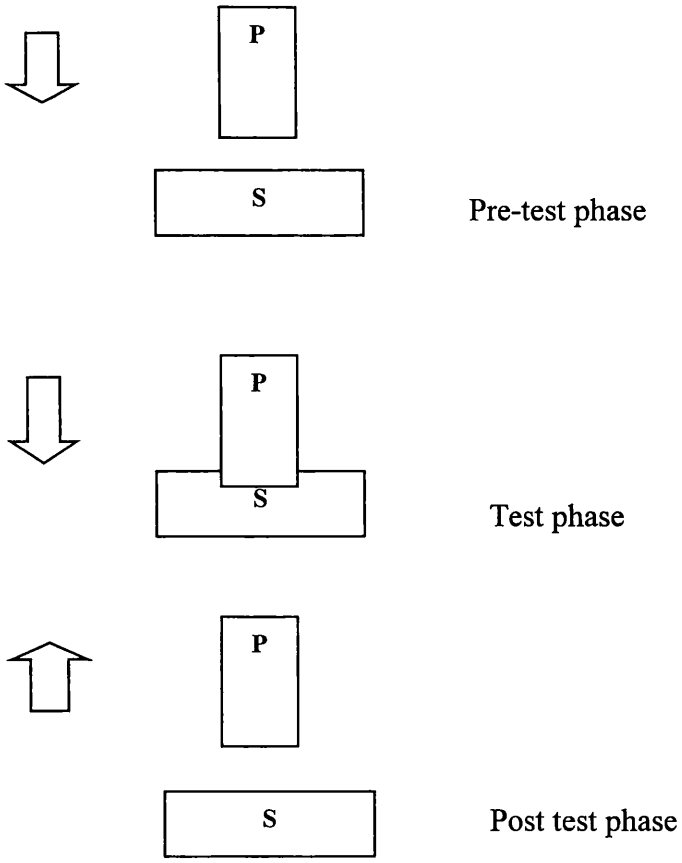
The experimental run consists of a pre-test phase, test phase and a post test phase. The pre-test phase is the speed of approach to the sample, so it is important that this speed is slow (up to 3mm/sec) otherwise air bubbles and fracture points will create artefacts in the data when it is collected during the test. Once the probe detects a force equal to the trigger force, the probe switches to the test phase which is when data is collected. The trigger force should be sufficiently high so that information relating to the sample properties are collected and not small forces unrelated to the samples properties such as vibrations. After the test has finished, the probe leaves the sample at the post test speed, which can be set at a high speed if information about the sample after the test is not required such as in a rupture test. This sequence of events is illustrated in Figure 5.2.

FIGURE 5.1 : THE STABLE MICRO SYSTEMS TA-XT2 INSTRUMENT



There are many test options available on the TA-XT2, and the one chosen for the present work was the return to start option, measuring force in compression. This test involves setting a distance to which the probe will move into the sample which then returns to the original start position at the post test speed. Since the sample being tested was of a larger surface area than the contact area of the probe being used, this is a penetration type test. The area under the curve in the positive region of force vs. distance graphs which is equivalent to the work required to penetrate the samples to a fixed depth, was used as an indicator of the gel strength in the present work. The type of probes which are suitable for this type of testing are the cylindrical probes, which have a flat end and range from 2mm-50mm in diameter.

FIGURE 5.2 : BASIC PRINCIPLE OF TEXTURE ANALYSIS



P = Probe

S = Sample

It is important to stress that all results from texture analysis are only empirical values and can only be used for comparing gel strengths in the present work. However, if the contact area of the probe is equal to or greater than the sample area which is applicable to self supporting gels, the results are dynamic.

Studies by Kovacs (1973), Williams et al (1991) and Craig et al (1997) have used texture analysis studies to investigate the gel strengths of xanthan gum-locust bean gum mixtures of varying polysaccharide ratio. Both Kovacs (1973) and Williams et al (1991) found similar results in that maximum gel strength occurred for the 1:1 ratio. This stoichiometric relationship may indicate that there are about an equal number of junction sites available on each polymer molecule. In contrast to these findings, Craig et al (1997) found that the maximum gel strength occurred at a xanthan gum-locust bean gum ratio of 1:9 for an unheated mixture, and approximately 3:7 for a mixture heated to 70°C-80°C followed by cooling to room temperature. These differences in results may

suggest that the choice of xanthan gum and locust bean gum and the preparation temperature can influence the physical properties of the mixes due to differences in the chemical characteristics.

Additional investigations by Williams et al (1991) showed that the addition of 0.04 mol/dm^3 sodium chloride to xanthan gum-locust bean gum mixes did not affect the maximum gel strength ratio of 1:1, suggesting that locust bean gum may interact with xanthan gum molecules in the same conformation in both environments.

Apart from Craig et al (1997), the effect of temperature on the gel strength of xanthan gum-locust bean gum mixes using texture analysis has not been properly investigated, neither have the effects of additives been extensively studied using this technique. Therefore, in the present work, these variables will be investigated which in combination with the other techniques used in the present study, may be a useful approach in studying the synergy between xanthan gum and locust bean gum.

5.2 FACTORS AFFECTING TEXTURE ANALYSIS STUDIES

5.2.1 INTRODUCTION

As previously mentioned in Chapter 5.1, texture analysis measurements are only empirical since the technique is sensitive to changes in variables such as the container size, force and probe diameter.

Various studies on different polysaccharide systems have been carried out to determine the factors which affect the gel strength measurements. For example, a study by Ferrari et al (1994) investigated some parameters that affect the texture analysis measurements using an apparatus with similar principles to the TA-XT2 penetrometer. The parameters which were investigated were; the sample holder shape (container), the lowering speed of the probe (force) and the test duration.

Ferrari et al (1994), found that the sample holder shape greatly affected the gel strength, in which a Petri dish (h=18mm, diameter=90mm) was compared to a beaker (h=80mm, diameter=50mm). The gel strength values were always greater for samples held within the Petri dish, which may be due to the different diameter/depth ratios of the sample in the two different containers, which gives rise to different shear and surface tension

forces being involved. In the current study, two different container sizes were used to compare the magnitude of the gel strength values for xanthan gum-locust bean gum mixtures.

The gel strength values of 6%w/w hydroxypropylmethylcellulose solution were found to be independent of the lowering speed of the probe since force vs. displacement curves were superimposable for the three lowering speeds investigated (Ferrari et al, 1994). This differs to a study by Jones et al (1997), in which it was found that the numerical values of hardness, compressibility and adhesiveness of bioadhesive oral gels including hydroxyethylcellulose (3%-5%w/w) were affected by the probe speed. The effect of different forces were investigated in the present work. However, the test duration has not been investigated in the present work, but it has been found in one study that this parameter is dependent upon the behaviour of the sample. For example, if the sample has a highly structured system as in the case of a firm gel such as carboxyvinyl polymer (2%w/w), then the gel strength value decreases as the duration of the test decreases. However, if the sample is a solution with some viscoelastic properties, as in the case of hydroxypropylmethylcellulose (6%w/w), then the gel strength is independent of the test duration because there is no system break point (Ferrari et al, 1994). In a further study, the effect of probe shape on the gel strength test has also been explored, in which it was found that a conical probe exerts a shear stress whereas a spherical probe exerts a compressive stress. A cylindrical probe was found to exert a large compressive force from the centre of the probe with a shear stress exerted at the edges of the probe, in which it was impossible to separate these two forces quantitatively (Ferrari et al, 1995). In the current study, cylindrical probes were employed.

In the present work, two different methods were employed for the texture analysis studies on xanthan gum-locust bean gum mixtures, in order to compare and investigate the influence of changing specific variables, such as the container size, probe size, trigger force and test speed on the gel strength values and profiles.

5.3 THE EFFECT OF THE PREPARATION TEMPERATURE OF XANTHAN GUM-LOCUST BEAN GUM RATIOS ON GEL STRENGTH

The effect of temperature on the gel strength of various xanthan gum-locust bean gum mixes were investigated using texture analysis, to compare with the findings from the rheological studies in Chapter 4. The synergy between xanthan gum and locust bean gum is affected by the preparation temperature (Zhan et al, 1993) which results in a change in the physical properties of the mixes, which can be characterised in terms of the strength of the system using the current technique.

5.3.1 Method 1

Xanthan gum-locust bean gum mixes 1%w/w solutions of different ratios were prepared at 25°C and at 90°C which were subsequently cooled back down to 25°C according to the method described in Chapter 2.2.1 . For each different sample, a total volume of 200ml solutions were mixed in 250ml glass beakers which were then subdivided into equal portions and transferred to 50ml plastic pots for texture analysis studies.

Texture analysis studies were performed using a TA-XT2 texture analyser (Stable Micro Systems) utilising Texture Expert Exceed, a Microsoft Windows^{TM} compatible software package. A 10mm diameter cylindrical probe was chosen, which penetrated the samples to a fixed depth of 7mm at a test speed of 0.2mm/sec using a return to start test measuring force in compression. A trigger force of 0.01N was set on the TA-XT2. The contact area between the probe and the sample was calculated to be 78.54mm^2. Data was collected at a rate of 50 points per second, and all measurements were performed at least four times on fresh samples at 25°C.

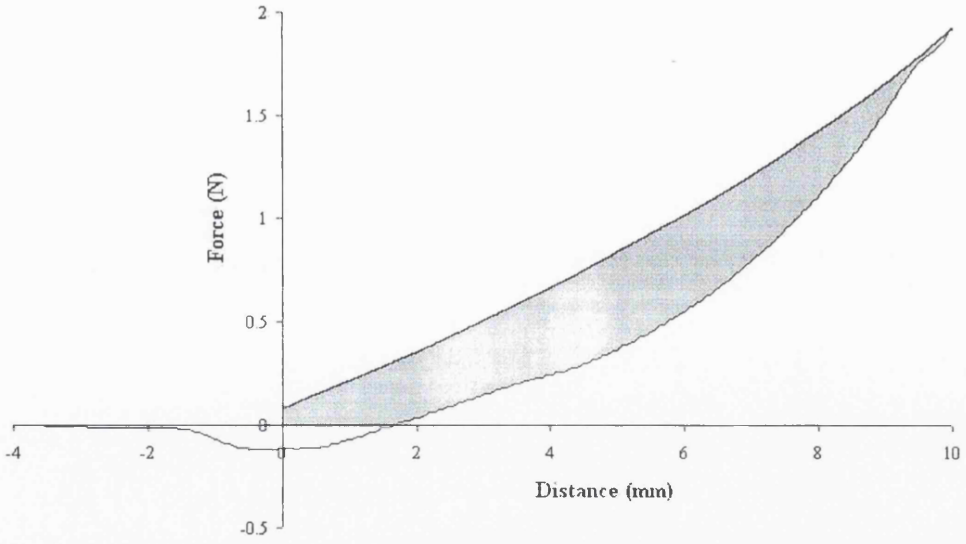
The gel strengths were calculated from the area under the curves (AUC) of force vs. distance graphs in the positive region, which represents the work required to penetrate the samples to a fixed depth. This is shown as the grey shaded area in Figure 5.3. The total AUC (shaded plus unshaded areas) in the positive region of the graph represents penetration and withdrawal, and was not chosen to represent gel strength, since

withdrawing the probe from the sample may create errors in measurements if some of the sample adheres to the probe on withdrawal.

5.3.2 Method 2

A second method was developed to look at the polysaccharide ratios at the two different preparation temperatures, to see if the shapes of the profiles could be improved (jagged lines could represent noise if the force is too low). In addition, this method was devised to investigate the influence of changing certain parameters on the gel strength values.

FIGURE 5.3 : A TYPICAL FORCE-DISTANCE PROFILE TO SHOW GEL STRENGTH ANALYSIS



The mixing procedure was identical to that described in Chapter 2.2.1, except that a total volume of 1L for each ratio was divided into equal portions prepared in four separate 250ml glass beakers. These were then equally transferred into four individual 250ml glass jars. Using this larger container size and a greater diameter cylindrical probe size of 35mm, the idea was to create a much larger surface area which would give rise to a better contact area between the probe and the sample (contact area = 926.11mm^2). This in turn would minimise any edge effects from the sample rising around the sides of the container.

The trigger force was increased to 0.1N to eliminate any vibrational forces, and the test speed was additionally increased to 1.0mm/sec. Since the sample volume was greater due to the larger container size, the distance to which the probe travelled was increased to 10mm. Data was collected at a rate of 200 points per second and the gel strengths were calculated in the same way as that described in Chapter 5.3.1.

5.3.3 Results

A typical texture analysis force vs. distance profile using Method 1 (Chapter 5.3.1) for a xanthan gum-locust bean gum mixture is shown in Figure 5.4. The force increases as the probe moves further into the sample due to the samples resistance which is dependent on the gel strength. The maximum peak force is a measure of the sample firmness and is not an indicator of the break point/rupture, since the heated and cooled gels were intact at the distance and trigger force set, whereas the room temperature prepared gels could not rupture since they were liquid in appearance. Beyond the maximum peak force, the force decreases which would be expected as the probe moves out of the sample and returns to the original start position. Thus, the area between the two curves (AUC = grey shaded area) in the positive region of the graph is a measure of the work required to penetrate the sample to a fixed depth, whereas the unshaded areas represent the resistance to withdrawal force. Therefore, the total area i.e. shaded area (AUC) plus unshaded areas is a measure of the total work involved to penetrate and leave the sample at a fixed depth. The Texture Expert Exceed software can run macros which enabled the AUC to be calculated which was used in this study as an indicator of gel strength for reasons explained in Chapter 5.3.1.

Figure 5.5 shows the gel strength values for the mixtures prepared at room temperature and the heated and cooled mixtures as a function of the polymer ratio. It is observed that maximum synergy occurs for the 1:1 ratio on heating and cooling between 90°C-25°C (990.0 ± 75.9μJ), in which there is a 6-fold increase in the gel strength compared to the unheated mixture (163.2 ± 12.0μJ). It is not clear from the texture analysis studies if there is a optimum ratio for maximum synergy for the unheated mixtures, and there does not appear to be a change in gel strength before and after heating and cooling the 1:9 xanthan gum-locust bean gum mixture. The texture analysis data and statistics for these results are shown in Table 5.1.

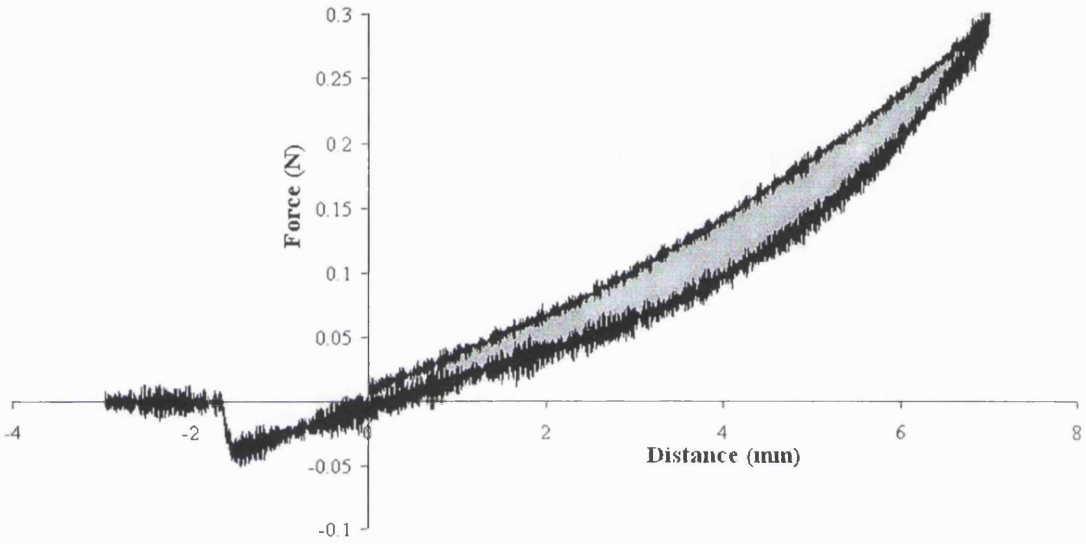
A typical texture analysis force vs. distance profile using Method 2 (Chapter 5.3.2) for a xanthan gum-locust bean gum mixture is shown in Figure 5.6. The principle of the test is the same as that described above. In comparison to the profile shown in Figure 5.4, the curve is much smoother which is probably due to the higher trigger force used in Method 2 compared to Method 1, and the AUC is comparatively larger.

Figure 5.7 shows the gel strength values for the mixtures prepared at room temperature and the heated and cooled mixtures as a function of the polymer ratio. Although the general trend in results are similar to those obtained using Method 1, the increase in gel strength after heating and cooling the 1:1 ratio is only 2.5 fold, and the profiles were less reproducible. Table 5.2 shows the texture analysis data and statistics.

5.4 TEMPERATURE EFFECTS ON THE OPTIMUM XANTHAN GUM-LOCUST BEAN GUM RATIO

The effect of different preparation temperatures on the maximum synergistic ratio of 1:1 xanthan gum-locust bean gum mixtures was investigated further using texture analysis, to determine if there is an optimum temperature at which maximum synergy occurs.

FIGURE 5.4 : A TYPICAL FORCE-DISTANCE GRAPH FOR XANTHAN GUM-LOCUST BEAN GUM (1:9) RATIO PREPARED AT 90°C USING METHOD 1



5.4.1 Method 1

Xanthan gum-locust bean gum 1:1 ratio 1%w/w mixes were prepared at different temperatures ranging from between 25°C to 90°C using the method described in Chapter 2.2.1. For each different sample, a total volume of 200ml solutions were mixed in 250ml beakers which were then subdivided into equal portions and transferred to 50ml plastic pots for texture analysis studies.

The texture analysis studies were performed on the TA-XT2 texture analyser (Stable Micro Systems) using the same test parameters and gel strength calculations as that described in Chapter 5.3.1.

FIG. 5.5 : TEXTURE ANALYSIS DATA AT 25°C BEFORE AND AFTER HEATING AND COOLING XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w (METHOD 1)

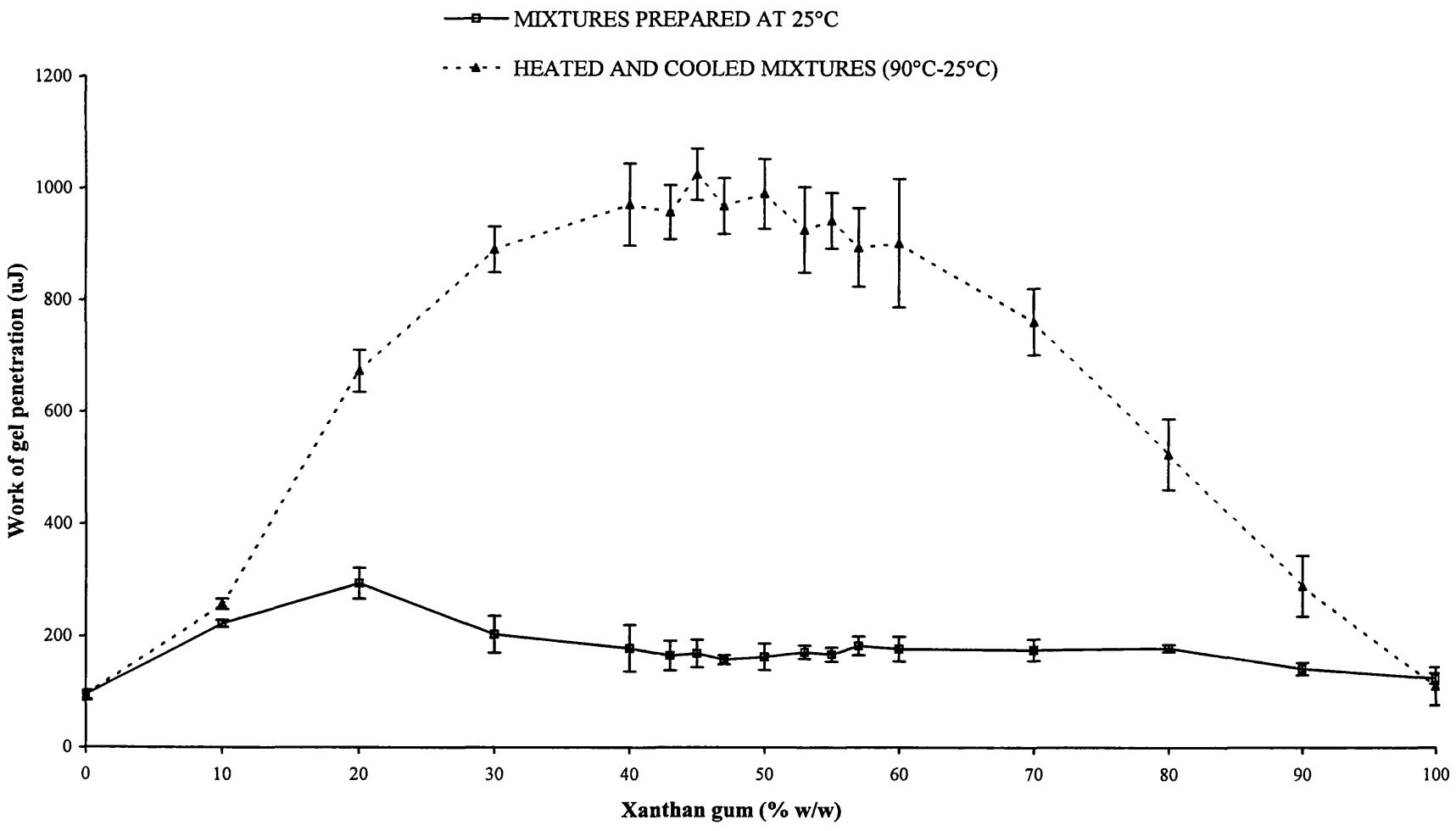
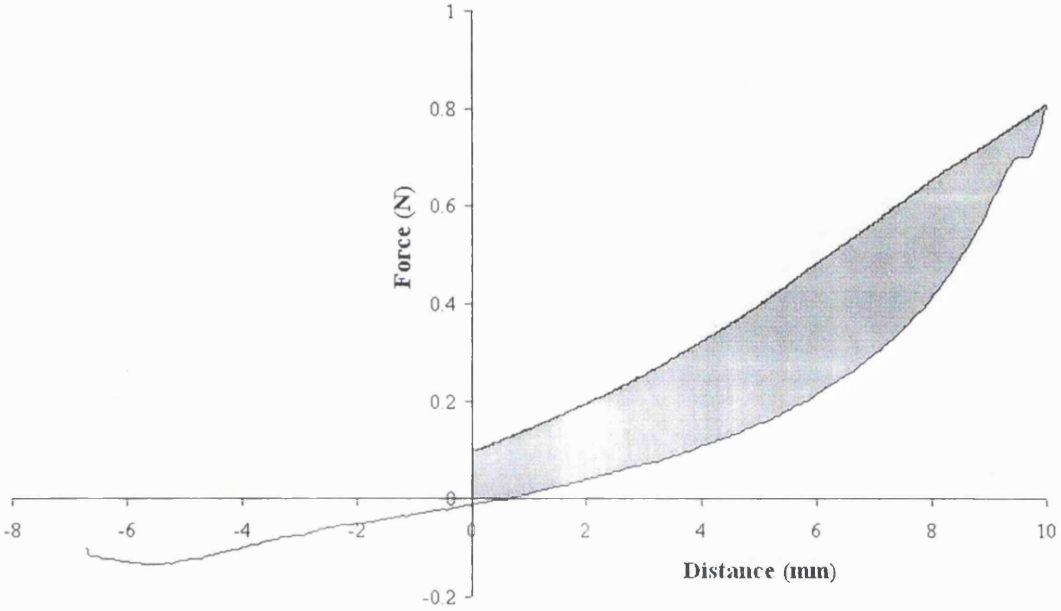


TABLE 5.1 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM MIXES PREPARED AT TWO DIFFERENT TEMPERATURES (METHOD 1)

	XANTHAN GUM-LOCUST BEAN GUM RATIO															
	0:1	**1:9**	**2:8**	**3:7**	**4:6**	**4.3:5.7**	**4.5:5.5**	**4.7:5.3**	**5:5**	**5.3:4.7**	**5.5:4.5**	**5.7:4.3**	**6:4**	**7:3**	**8:2**	**9:1**	**1:0**
MEAN AUC (μJ) (25°C)	94.1	222.2	294.0	203.5	178.6	165.3	169.3	158.2	163.2	171.7	167.3	183.0	177.4	175.4	178.8	141.5	124.2
± S.D.	6.3	27.5	32.9	41.9	26.6	24.7	8.3	24.0	12.0	12.4	16.8	22.1	19.4	6.2	11.1	9.8	7.7
COEFFECIENT OF VARIATION %	6.7	12.4	11.2	20.6	14.9	14.9	4.9	15.2	7.3	7.2	10.0	12.1	10.9	3.5	6.2	6.9	6.2
MEAN AUC (μJ) (90°C-25°C)	93.3	257.3	673.3	891.5	971.2	957.2	1024.5	968.2	990.0	925.9	942.2	894.5	902.2	761.5	524.6	289.8	110.4
± S.D.	9.1	37.4	40.9	73.1	47.8	45.8	49.7	62.2	75.9	49.4	70.3	114.3	59.5	63.3	54.2	34.4	9.3
COEFFECIENT OF VARIATION %	9.8	14.5	6.1	8.2	4.9	4.8	4.8	6.4	7.7	5.3	4.5	12.8	6.6	8.3	10.3	11.9	8.4

FIGURE 5.6 : A TYPICAL FORCE-DISTANCE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (1:9) RATIO PREPARED AT 25°C USING METHOD 2



5.4.2 Method 2

Xanthan gum-locust bean gum 1:1 ratio 1%w/w mixes were prepared at different temperatures ranging from 25°C to 90°C using the method described in Chapter 2.2.1. The total quantities of solutions involved, test parameters and gel strength calculations were identical to that described in Chapter 5.3.2. This method was performed as a comparative method to Method 1 described above.

FIG. 5.7 : TEXTURE ANALYSIS DATA AT 25°C BEFORE AND AFTER HEATING AND COOLING XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w (METHOD 2)

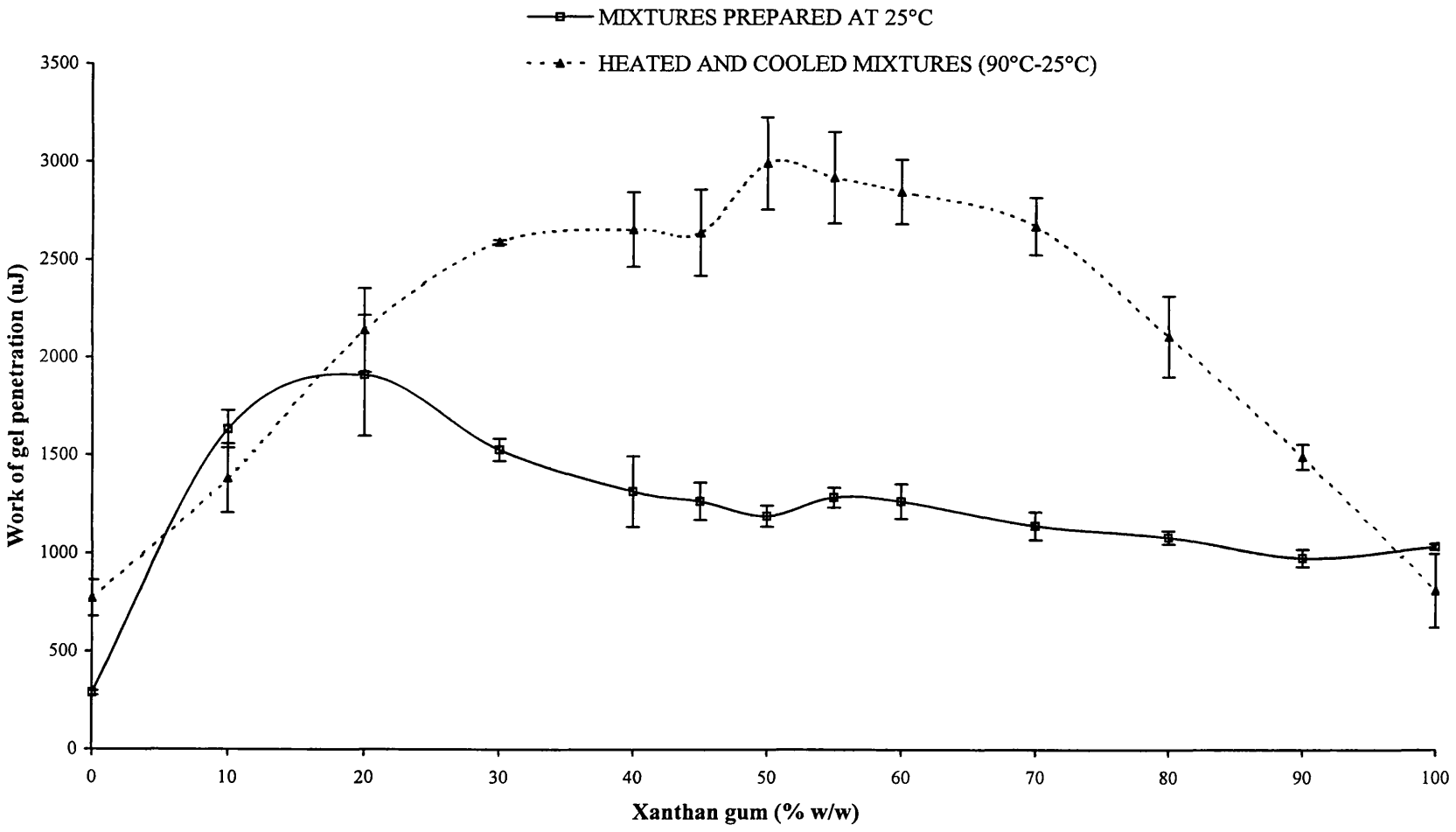


TABLE 5.2 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM RATIOS PREPARED AT TWO DIFFERENT TEMPERATURES (METHOD 2)

XANTHAN GUM-LOCUST BEAN GUM RATIO													
	0:1	**1:9**	**2:8**	**3:7**	**4:6**	**4.5:5.5**	**5:5**	**5.5:4.5**	**6:4**	**7:3**	**8:2**	**9:1**	**1:0**
MEAN AUC (μJ) (25°C)	289.0	1633.0	1907.0	1527.0	1315.0	1266.0	1191.0	1285.0	1266.0	1140.0	1082.0	977.0	1035.0
± S.D.	11.0	95.0	308.0	57.0	180.0	95.0	54.0	51.0	88.0	71.0	34.0	44.0	14.0
COEFFECIENT OF VARIATION %	3.9	5.8	16.2	3.7	13.7	7.5	4.5	4.0	7.0	6.2	3.1	4.5	1.4
MEAN AUC (μJ) (90°C-25°C)	771.0	1383.0	2137.0	2588.0	2652.0	2637.0	2991.0	2917.0	2845.0	2670.0	2107.0	1494.0	812.0
± S.D.	92.0	176.0	215	11.0	189.0	219.0	236.0	234.0	164.0	145.0	207.0	64.0	188.0
COEFFECIENT OF VARIATION %	11.9	12.7	10.1	0.4	7.1	8.3	7.9	8.0	5.8	5.4	9.8	4.3	23.2

5.4.3 Results

Texture analysis profiles from force vs. distance graphs for xanthan gum-locust bean gum 1:1 ratio mixes prepared at different temperatures are shown in Figures 5.8 and 5.10 for Method 1 and Method 2 respectively. The profiles produced using Method 1 (Chapter 5.4.1) are more reproducible but more jagged compared to those obtained using Method 2 (Chapter 5.4.2).

Figure 5.9 shows the gel strength values for mixtures prepared at different temperatures ranging from 25°C to 90°C using Method 1. The gel strength tends to increase as the preparation temperature increases, and between 50°C and 60°C there is a large jump in the gel strength value (355.0μJ at 50°C to 950.0μJ at 60°C). There after from 60°C to 90°C, there is no further increase in the gel strength.

The same set of results using Method 2 are shown in Figure 5.11. In comparison to the results obtained using Method 1, there is no clear discontinuity in the graph between 50°C and 60°C. Although the gel strength values also increase as the preparation temperature increases, there does not appear to be a temperature at which the gel strength plateau's at the preparation temperature range investigated (25°C-90°C). Tables 5.3 and 5.4 show the texture analysis data and statistics for the results using Method 1 and Method 2 respectively.

5.5 THE INCLUSION OF ADDITIVES TO XANTHAN GUM-LOCUST BEAN GUM MIXTURES

5.5.1 EFFECTS OF SUCROSE

The effects of sucrose on xanthan gum-locust bean gum mixes were investigated using texture analysis studies, since it was found from Chapter 4.4.1.2 that the addition of sucrose greatly enhanced the strength of the polysaccharide system 1:1 ratio. Since texture analysis studies measure the physical characteristics of materials, this makes it an ideal comparative technique to use with oscillatory rheology.

5.5.1.1 Method

Sucrose solutions ranging from 0.5%w/w to 15%w/w were prepared as described in Chapter 2.2.2. The sucrose solutions were used as vehicle to prepare xanthan gum-locust bean gum (1:1) 1%w/w solutions at 90°C, according to the method described in Chapter 2.2.1.

Since it has been shown throughout Chapter 5 that results are only empirical, the method described in Chapter 5.3.1 was chosen for the texture analysis studies, as this method is a more sensitive test.

FIGURE 5.8 : A TYPICAL FORCE-DISTANCE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (1:1) RATIO PREPARED AT 50°C USING METHOD 1

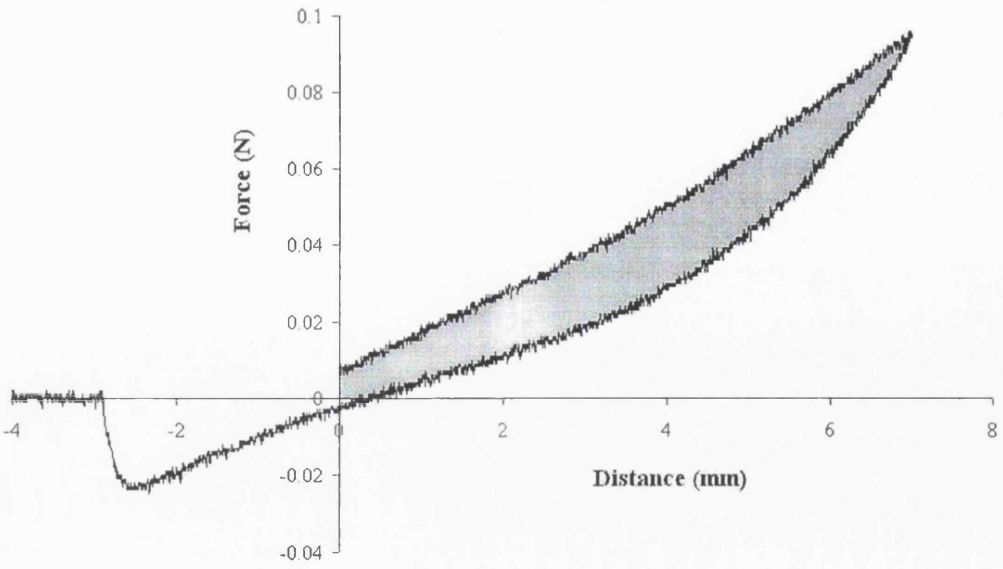


FIG. 5.9 : TEXTURE ANALYSIS DATA AT 25°C OF 1% w/w XANTHAN GUM-LOCUST BEAN GUM 1:1 RATIO MIXES PREPARED AT DIFFERENT MIXING TEMPERATURES (METHOD 1).

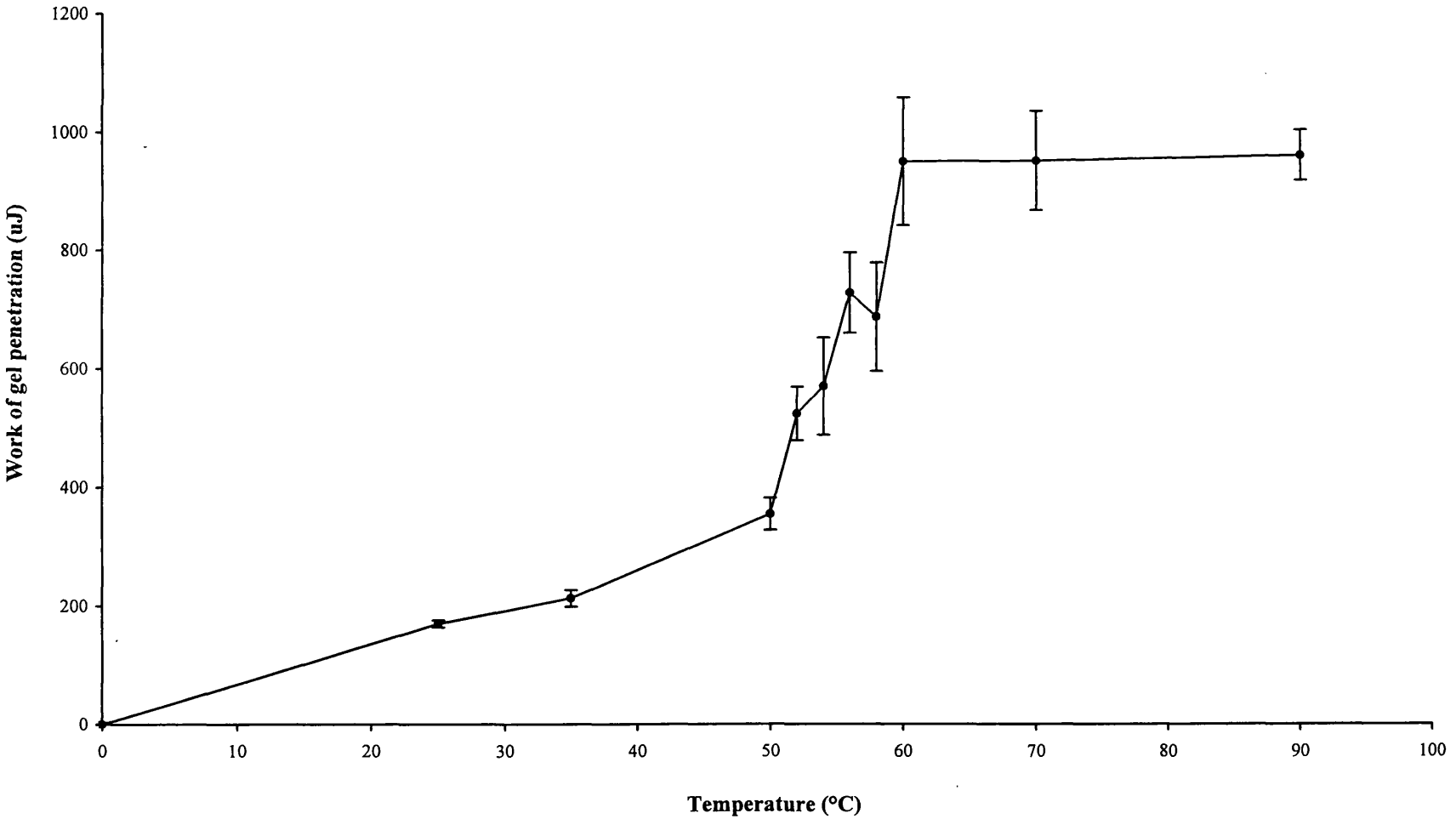
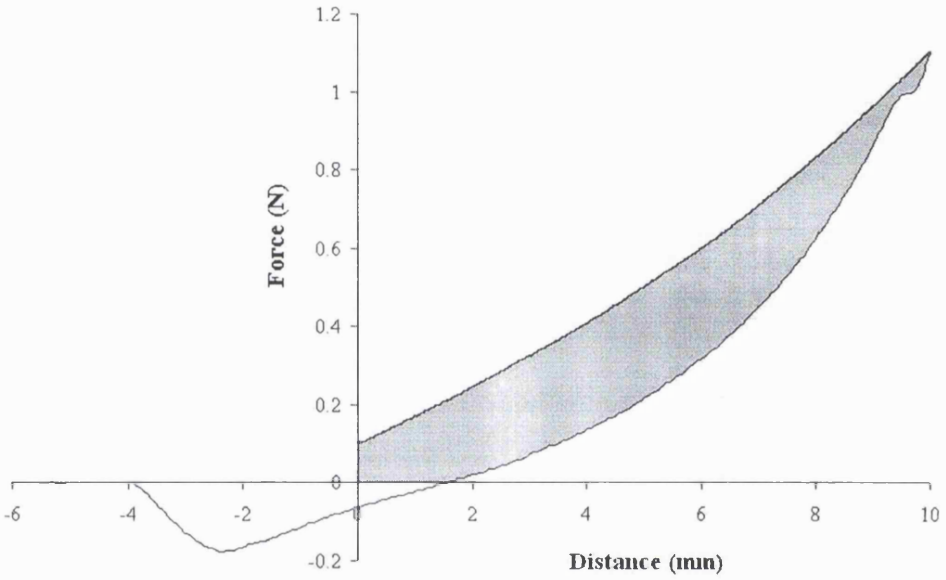


TABLE 5.3 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM 1:1 MIXES PREPARED AT DIFFERENT TEMPERATURES (METHOD 1)

	TEMPERATURE (°C)									
	25	**35**	**50**	**52**	**54**	**56**	**58**	**60**	**70**	**90**
MEAN AUC (μJ)	170.0	213.0	355.0	524.0	570.0	728.0	687.0	950.0	951.0	960.0
± S.D.	6.0	14.0	27.0	45.0	82.0	68.0	92.0	108.0	84.0	43.0.
CV %	3.6	6.7	7.7	8.5	14.5	9.4	13.4	11.4	8.8	4.7

FIGURE 5.10 : A TYPICAL FORCE-DISTANCE PROFILE FOR XANTHAN GUM-LOCUST BEAN GUM (1:1) RATIO PREPARED AT 50°C USING METHOD 2



5.5.1.2 Results

The findings from Figure 5.12 do not show any trend in gel strength vs. sucrose concentration. The profile does not form a smooth curve, but the values average out close to the gel strength value obtained in the absence of sucrose (compare 921.6μJ i.e. average value with sucrose, to 915.0μJ without sucrose from Table 5.5 and 960.0μJ without sucrose from Table 5.3, for a xanthan gum-locust bean gum (1:1) mixture prepared at 90°C). The data and statistics displayed in Table 5.5 show good reproducibility (coefficient of variation not greater than. 7.0%).

FIG. 5.11 : TEXTURE ANALYSIS DATA AT 25°C OF 1% w/w XANTHAN GUM-LOCUST BEAN GUM 1:1 RATIO MIXES PREPARED AT DIFFERENT MIXING TEMPERATURES (METHOD 2)

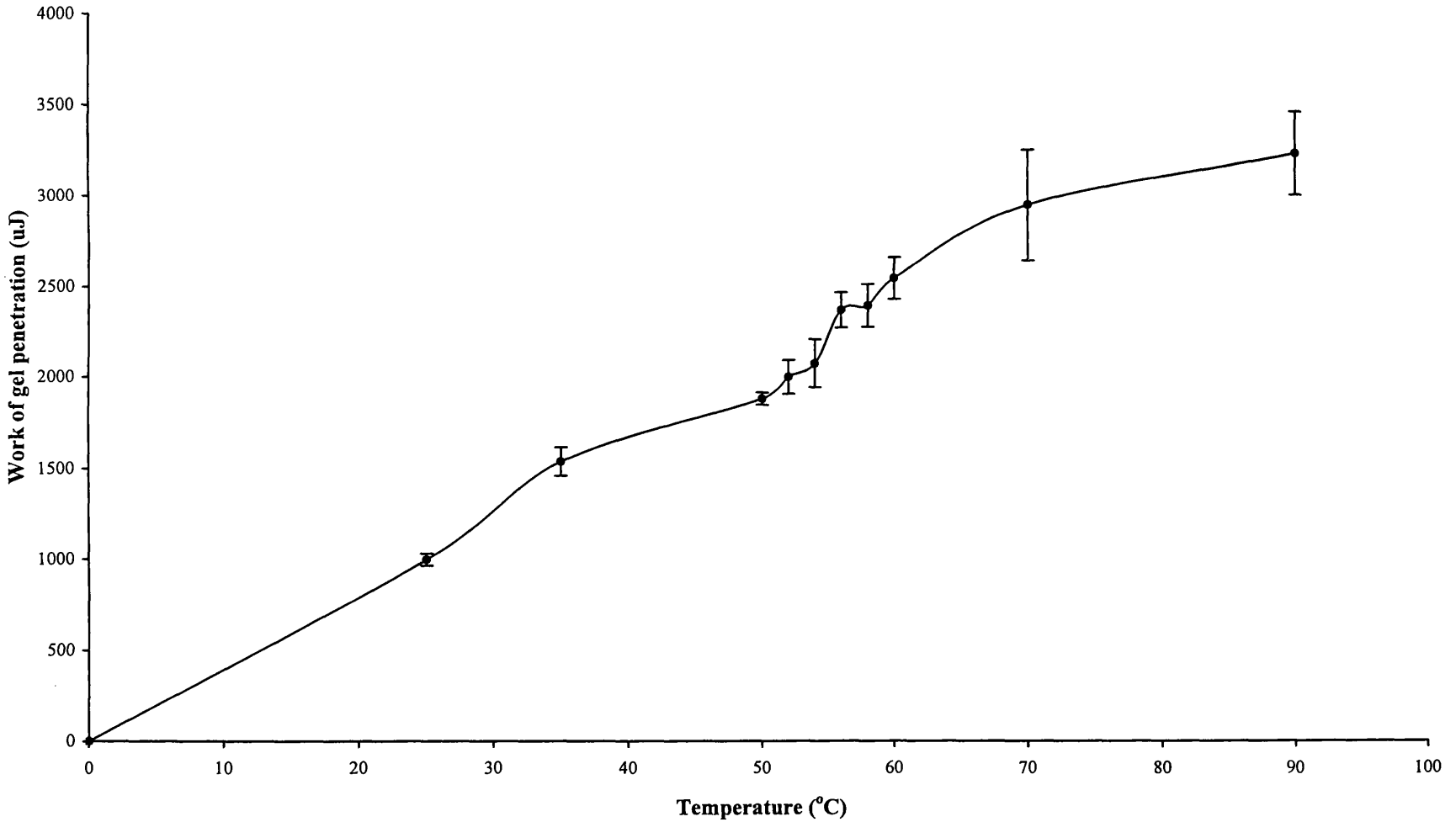


TABLE 5.4 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM 1:1 MIXES PREPARED AT DIFFERENT TEMPERATURES (METHOD 2)

	TEMPERATURE (°C)									
	25	**35**	**50**	**52**	**54**	**56**	**58**	**60**	**70**	**90**
MEAN AUC (μJ)	997.0	1538.0	1879.0	1999.0	2073.0	2368.0	2393.0	2543.0	2945.0	3226.0
± S.D.	33.0	78.0	34.0	93.0	132.0	97.0	118.0	114.0	305.0	229.0
CV %	3.3	5.1	1.8	4.6	6.4	4.1	5.0	4.5	10.3	7.1

FIG. 5.12 : TEXTURE ANALYSIS DATA AT 25°C OF XANTHAN GUM-LOCUST BEAN GUM MIXES (1:1) RATIO 1%w/w PREPARED IN DIFFERENT CONCENTRATIONS OF SUCROSE SOLUTIONS AT 90°C

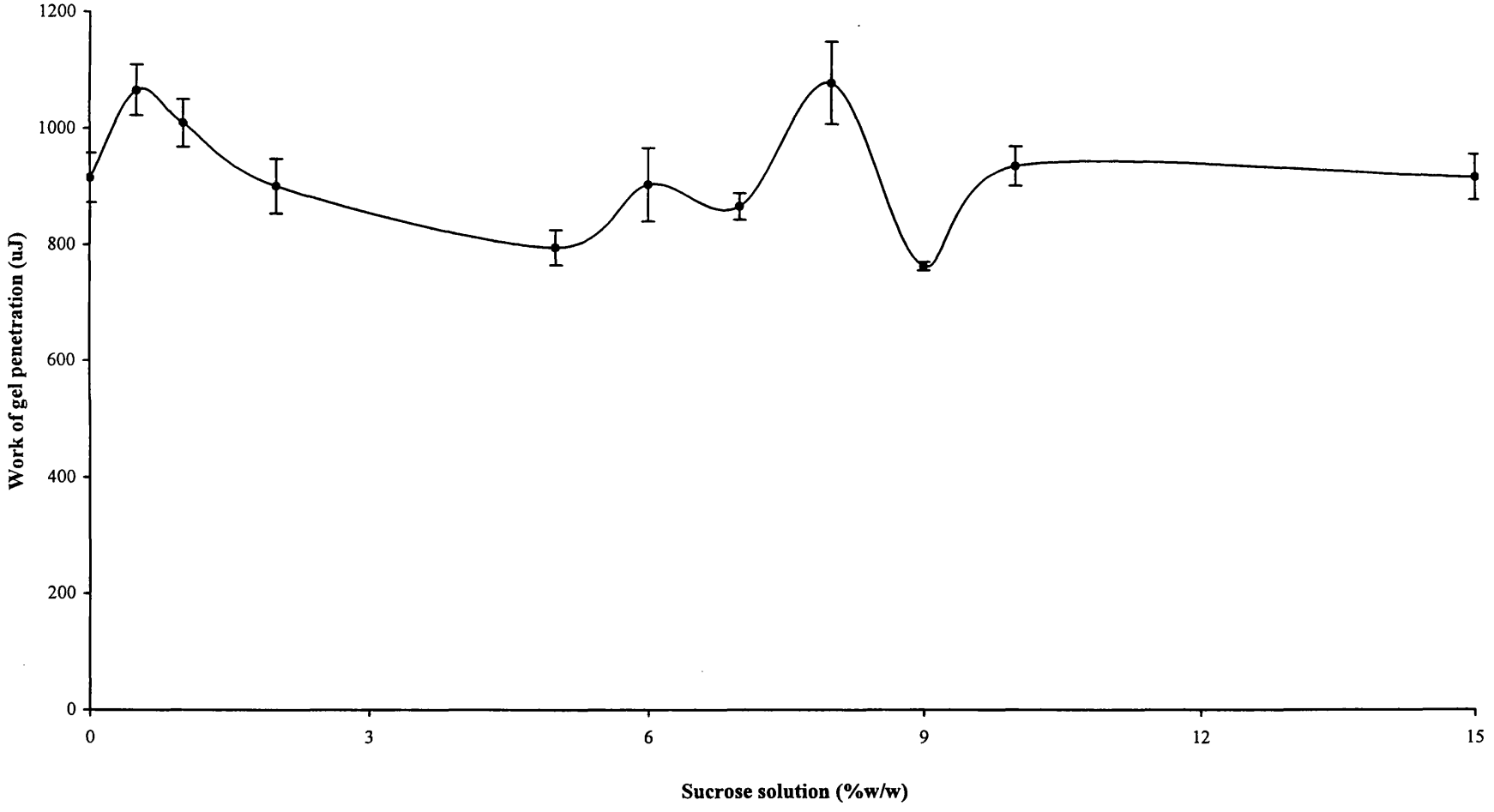


TABLE 5.5 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM MIXES (1:1) RATIO 1%w/w PREPARED AT 90°C (METHOD 1)

	SUCROSE CONCENTRATION (%w/w)										
	0	**0.5**	**1**	**2**	**5**	**6**	**7**	**8**	**9**	**10**	**15**
MEAN AUC (μJ)	915.0 960.0*	1066.0	1009.0	900.0	794.0	902.0	864.0	1077.0	762.0	934.0	915.0
± S.D.	43.0 43.0*	44.0	41.0	47.0	30.0	63.0	23.0	71.0	7.0	34.0	39.0
CV %	4.7 4.7*	4.2	4.1	5.3	3.8	7.0	2.6	6.6	0.9	3.7	4.3

*= Data from Table 5.3

5.5.2 THE INFLUENCE OF SODIUM CHLORIDE ON THE GEL STRENGTH OF XANTHAN GUM-LOCUST BEAN GUM MIXES

Although it was observed in Chapter 4.4.2.2 that the inclusion of sodium chloride did not affect the rheological behaviour of xanthan gum-locust bean gum (1:1) mixes, the current technique was necessary in order to conclude or contradict these findings.

5.5.2.1 Method

Sodium chloride solutions of concentrations 10mmol, 20mmol and 30mmol were prepared as described in Chapter 2.2.3. These solutions were used as vehicle to prepare xanthan gum-locust bean gum (1:1) 1%w/w solutions at 90°C, according to the method described in Chapter 2.2.1. The method described in Chapter 5.3.1 was performed for the texture analysis studies.

5.5.2.2 Results

The results are shown in Figure 5.13, and suggest that the gel strengths of the mixes are independent of the addition of sodium chloride at the concentrations investigated. Table 5.6 shows the data and statistics which indicate good reproducibility from the low standard deviations and coefficient of variation values (less than 5.2%).

5.6 DISCUSSION

The results are in agreement with the findings from the oscillatory experiments in that maximum synergy occurs for the 1:1 ratio of xanthan gum-locust bean gum mixes after heating and cooling between 90°C-25°C (Figures 5.5 and 5.7). This ratio at which maximum synergy is observed is also supported by the texture analysis studies performed by Kovacs (1973) and Williams et al (1991).

FIG. 5.13 : TEXTURE ANALYSIS DATA AT 25°C OF XANTHAN GUM-LOCUST BEAN GUM MIXES (1:1) RATIO 1%w/w PREPARED IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE SOLUTIONS AT 90°C

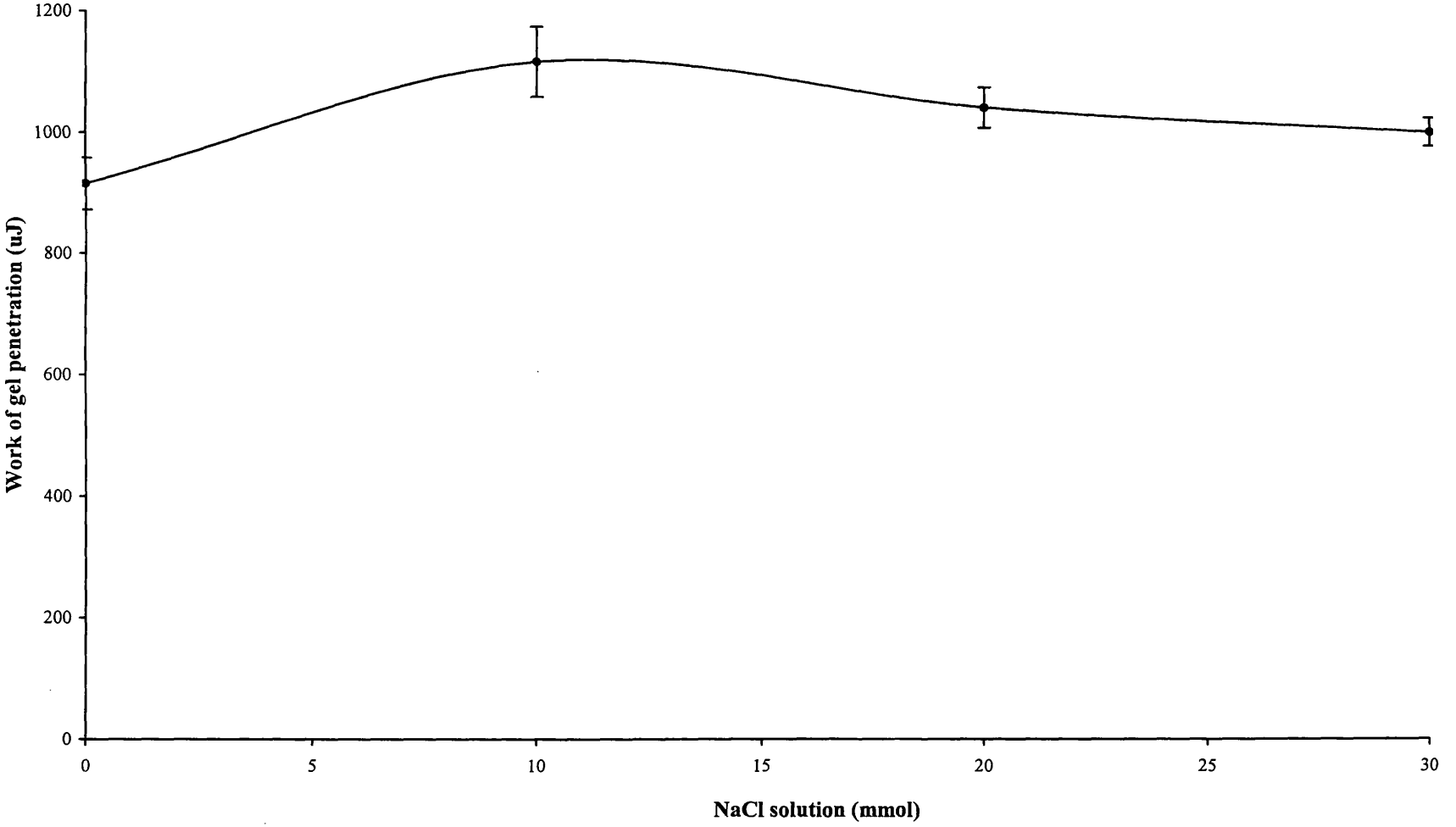


TABLE 5.6 : MEAN GEL STRENGTH VALUES OF XANTHAN GUM-LOCUST BEAN GUM MIXES (1:1) RATIO 1%w/w PREPARED AT 90°C (METHOD 1)

SODIUM CHLORIDE CONCENTRATION (MMOL)				
	0	**10**	**20**	**30**
MEAN AUC (μJ)	920.0 960.0*	1116.0	1040.0	998.0
± S.D.	44.0 43.0*	58.0	33.0	23.0
COEFFECIENT OF VARIATION %	4.9 4.7*	5.2	3.2	2.3

* = Data from Table 5.3

In addition, the changes in the gel strength values with polysaccharide ratio for mixes prepared at room temperature are less marked compared to the gel strength values of the heated and cooled mixes. This makes the results more difficult to assess in terms of whether synergy occurs between the two polysaccharides without heating and cooling, since the gel strength values of xanthan gum-locust bean gum mixes are similar to the gel strength values of xanthan gum. In addition, if synergy does occur between these two polysaccharides at room temperature, then the ratio at which maximum synergy is observed is for the 2:8 ratio. A change in the ratio for maximum synergy after heating and cooling was also observed by Craig et al (1997). The gel strength of the 1:9 xanthan gum-locust bean gum ratio prepared at 25°C compared to that prepared at 90°C and subsequently cooled back down to room temperature remains virtually unchanged. Such findings may suggest that there is insufficient xanthan gum available to interact with locust bean gum.

The general trend in gel strength with polysaccharide ratio as a function of temperature using the two different methods produced similar results (Figures 5.9 and 5.11). However, Method 2 (see Figure 5.11) was a less sensitive test, since the increase in gel strength after heating and cooling was less marked compared to the results obtained using Method 1 (Figure 5.9). Also, the profiles were less reproducible using Method 2 although the curves were smoother compared to those obtained using Method 1. However, since these tests give empirical values which are only useful for comparing the gel strengths, any errors involved are consistent such that they cancel out in the gel strength analysis.

The results observed for xanthan gum-locust bean gum (1:1) mixes (optimum ratio for maximum synergy) prepared at different temperatures, suggest that the disordered coil conformer of xanthan gum may be necessary for the interaction with locust bean gum. This is because the discontinuities observed from the profiles take place between 50°C and 60°C which has been reported to be the transition temperature range for xanthan gum in water (Cuvelier & Launay, 1986b and Williams et al, 1991). In order to support this theory, the transition temperature of xanthan gum will be determined using high sensitivity differential scanning calorimetry (HSDSC) and synchrotron circular dichroism in Chapter 6. In addition, the results suggest that perhaps the transition temperature of xanthan gum is the optimum temperature at which maximum synergy occurs, since there was no further increase in the gel strength after 60°C (Figure 5.9).

Again, this would support that the disordered form of xanthan gum may be necessary or enhances the interaction with locust bean gum. The difference in results from using Method 1 compared to Method 2, indicate that the latter method is less sensitive, since the discontinuity in gel strength between 50°C and 60°C was less marked. However, the standard deviations and coefficient of variations obtained using Method 2 are smaller compared to those obtained using Method 1 (compare Tables 5.3 and 5.4). Again, it must be borne in mind that results are only empirical suitable for comparing gel strengths.

The inclusion of additives namely sucrose and sodium chloride to xanthan gum-locust bean gum mixes (1:1) ratio prepared at 90°C do not affect the gel strengths of the polysaccharide systems in the present study (Figures 5.12 and 5.13 respectively). These findings for the mixtures in the presence of sucrose do not support the findings from the oscillatory rheology studies in Chapter 4.4.1.2, in which it was found that the addition of sucrose increased the gel strength (G′ increased as the concentration of sucrose increased; see Figure 4.22). Since texture analysis detects physical changes rather than changes/interactions at a molecular level, this technique may not be sensitive enough to detect differences between samples unless their consistencies and textural properties are largely different from each other. Therefore this technique was useful for investigating the effect of temperature on the mixes, because the samples showed large differences in their physical appearance. On the other hand, oscillatory rheology can detect structural changes at the molecular level which makes this technique more sensitive, suitable for looking at small differences in structure.

The findings for mixes in the presence of sodium chloride (Figure 5.13) do support the results from the oscillatory rheology work in Chapter 4.4.2.2 (Figure 4.25) i.e. the gel strength of xanthan gum-locust bean gum (1:1) mixes are independent of the addition of sodium chloride (10, 20, 30mmol). This finding has been discovered by some workers for example Williams et al (1991), and may suggest that xanthan gum molecules are in the same conformation in both environments if they interact with the locust bean gum molecules. Alternatively, this finding could further support the idea that xanthan gum interacts with locust bean gum in the disordered form, which is reduced in the presence of sodium chloride by stabilisation of the ordered helical form. However the subsequent decrease in gel strength which would be expected was not observed in the present study,

may be because the mixtures were prepared at 90°C, in which heating increases the presence of the disordered form, thus cancelling out the effects of sodium chloride.

In summary from the present work, the preparation temperature of the mixed polysaccharide systems greatly affects the gel strength of the final mixes, in which a preparation temperature of between 50°C and 60°C seems to have maximum effect. Temperatures above this temperature range do not further enhance the gel strength of the xanthan gum-locust bean gum (1:1) system, which was the ratio at which maximum synergy was observed. The results also tend to suggest that the disordered form of xanthan gum may be involved in the interaction with locust bean gum. Although certain additives such as salt can influence the conformational behaviour of xanthan gum (Morris et al, 1977), it may not necessarily result in a difference in gel strength. Additionally, the sensitivity and basic principles of the techniques chosen to study these polysaccharide systems are an important consideration, for example, oscillatory rheology is good for looking at changes at the molecular level which may be small in terms of the physical properties, such was the case for looking at the effects of sucrose. Whereas, texture analysis studies are good for looking at large changes in the physical properties and comparing the strengths of the systems.

In addition, the parameters chosen for the methods used in the present work namely the container size, probe size and forces, do influence the gel strength measurements and the reproducibility and sensitivity of the test. In the current study, the gel strength measurements were always of a greater magnitude when the larger container (250ml glass jar), force and probe were used. This is analogous to the study by Ferrari et al (1994).

CHAPTER 6

CHAPTER 6 : CHARACTERISATION OF CONFORMATIONAL AND TRANSITIONAL BEHAVIOUR USING HIGH SENSITIVITY DIFFERENTIAL SCANNING CALORIMETRY AND SYNCHROTRON CIRCULAR DICHROISM

6.1 PRINCIPLES AND APPLICATIONS OF HIGH SENSITIVITY DIFFERENTIAL SCANNING CALORIMETRY (HSDSC)

High sensitivity differential scanning calorimetry (HSDSC) is a thermal analysis technique, which measures heatflow to and from a sample as the sample is subjected to a temperature program. In essence, it consists of monitoring a chemical or physical event within a system against time or temperature. Its general principles are based on conventional differential scanning calorimetry (DSC).

There are two types of DSC which are used in practice namely power-compensation DSC and heat-flux DSC. In the first type of DSC, the sample and reference are maintained at the same temperature such that the temperature difference is close to zero, by using individual heating elements. The electrical power input needed to maintain equal temperatures is measured as shown in Equation 6.1.

$$\Delta P = d(\Delta Q/dt) \qquad \text{Equation 6.1}$$

Where:

ΔP = power difference

$d(\Delta Q/dt)$ = rate of heat change

In the second type of DSC, the heat differential (ΔT) between the sample and reference is monitored. The sample and reference are heated from the same source, and the signal is converted to ΔP (i.e. heatflow – mW) as shown in Equation 6.2, using the calorimetric sensitivity described in Equation 6.3. The calorimetric sensitivity is determined from calibration of the instrument with a known standard, in which its transition temperature or heat of fusion is well established such as pure indium 99.999%. The area under the peak is used to determine the calorimetric sensitivity. For HSDSC, the heat-flux principle is applied.

$$\Delta Q = (T_s - T_r)/R_T \qquad \text{Equation 6.2}$$

Where:

ΔQ = heat difference (J)

T_s = sample temperature (K)

T_r = reference temperature (K)

R_T = thermal resistance of the cell (kJ^{-1})

Thermal events which are detected by DSC can be endothermic, exothermic or a change in the heat capacity. An endothermic reaction represents the heat absorbed by the sample for example during melting, whereas an exothermic reaction is due to heat lost from the system such as during a crystallisation process. A heat capacity change is the energy required to raise the temperature of a sample by 1K, and in fact represents the baseline if the heat capacities of the sample and reference are identical or the pans/vessels are empty.

The peak area of an enthalpic reaction is related to an enthalpic change as described in Equation 6.3. The equation is only valid if a system contains a given sample type and a heating rate. The end of an enthalpic event occurs when the baseline is re-established. Quantitative information is provided from the enthalpic peaks. For example, the area under the peak is proportional to the heat evolved or absorbed by the sample, whereas the height of the peak is proportional to the rate of reaction.

$$A = K'm(-\Delta H) \qquad \text{Equation 6.3}$$

Where:

A = peak area

K' = calorimetric sensitivity

m = sample mass

$-\Delta H$ = enthalpy change

Differences in baseline values before and after enthalpic events are due to changes in the heat capacity which accompany changes in the properties of the samples. This can create errors in terms of over or under estimating the peak area in relation to calculating the enthalpic values for the reaction.

Peak resolution can be improved in a number of ways. For example, thermal events which occur over similar temperature ranges can be improved by using low heating rates. This is because the first thermal process will be complete before the second thermal process commences, thus the two processes are highly resolved. However, the consequence of using a low heating rate is that the sensitivity decreases, thus small thermal events may go undetected. On the other hand, high heating rates cause low resolution due to temperature re-equilibration following the first thermal process will not have occurred before the start of the second thermal process. However, the sensitivity is improved by using a higher heating/scanning rate. In addition, the sample size is also an important consideration in DSC, since large sample sizes exhibit poor heat transfer which prolongs the temperature lag of the sample with respect to the reference, thus resulting in poor resolution (Craig et al, 1996).

HSDSC is a relatively new technique and overcomes some of the problems faced using conventional DSC. Firstly, the technique is more sensitive than conventional DSC by 10-fold, which enables small thermal events to be detected which may go unnoticed using conventional DSC. The instrument itself is slightly different to the conventional DSC instrument, in that HSDSC uses stainless steel vessels which hold the sample and reference material (solids and liquids) as opposed to pans which can only hold solids and need to be sealed when using conventional DSC instruments. These vessels have a fixed volume of $1cm^3$ which makes loading easier and reduces any weight variation. In addition, they incorporate an o-ring made of viton which is inert and seals the vessel, analogous to hermetically sealing the pans in conventional DSC, so that the sample and reference are contained within a closed environment. A diagram of the stainless steel vessel is shown in Figure 6.1. The vessels are protected from the environment by a gold calorimetric block which provides good temperature homogeneity, low background noise (less than $0.2\mu W$) and thus a stable baseline which are important features for obtaining accurate results. The transducers which surround the vessels ensure that the temperature inside the vessels are identical to the calorimetric block, thus providing ultra high sensitivity. The vessels are indirectly heated and cooled via the controller with a circulating non-freezing liquid, namely undecane, between –20°C and 120°C. An internal view of the instrument is illustrated in Figure 6.2. Since the cooling source is internal, this reduces outside temperature fluctuations so small thermal events can be accurately detected. The detection limit is thus high i.e. a calorimetric signal below one microwatt will be recorded. The heating rates are low, typically between 0.001°C/min

to 1.2°C/min which produces high resolution as temperature equilibration will be fully complete.

FIGURE 6.1 : DIAGRAM OF THE STAINLESS STEEL VESSEL USED IN HSDSC STUDIES

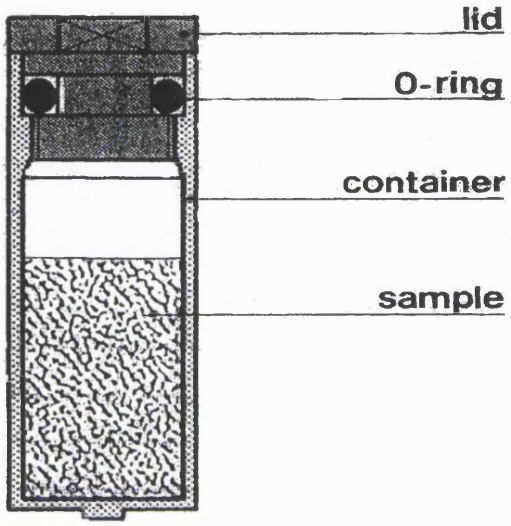
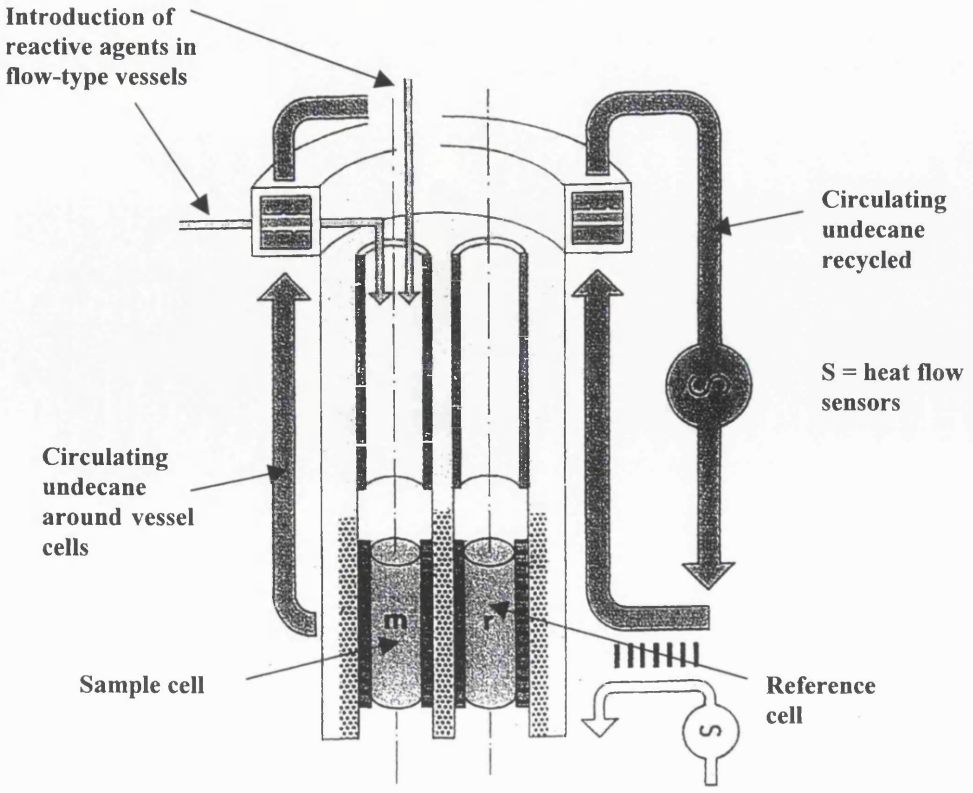


FIGURE 6.2 : INTERNAL VIEW OF THE HSDSC CALORIMETRIC BLOCK



The response times are shorter and thermal equilibration is faster after an enthalpic event has taken place using HSDSC compared to conventional DSC.

There have been a limited number of studies which have used HSDSC to investigate the conformation of xanthan gum during the interaction with locust bean gum (Williams et al, 1991, Craig et al, 1997 and Bresolin et al, 1998). In other studies, this technique has been used to investigate the effects of salt on the thermally induced conformational transition of xanthan gum in solution (Norton et al, 1984 and Kitamura et al, 1991) and the conformational stability of a polytetramer xanthan gum sample with locust bean gum and konjac mannan (Foster & Morris, 1994b).

Thus, HSDSC has been employed in the present study to investigate the transition temperature of xanthan gum and its conformational behaviour with locust bean gum in the presence and absence of certain additives, in order to gain an insight into the mechanism for the interaction, with respect to the findings from the oscillatory rheology work (Chapter 4) and the texture analysis data (Chapter 5). This technique may provide evidence as to whether the disordered form of xanthan gum is a prerequisite for interaction with locust bean gum, which has been suggested from the findings in Chapters 4 and 5.

6.2 CONFORMATION AND TRANSITIONAL BEHAVIOUR WITH VARIATION OF THE XANTHAN GUM-LOCUST BEAN GUM RATIO

The current study was used to investigate the conformational and transitional behaviour of various xanthan gum-locust bean gum mixes with respect to temperature. It is known that xanthan gum undergoes a reversible transition on heating and cooling from an ordered helix at low temperatures to a disordered coil at high temperatures (Morris et al, 1977). Thus, the individual polysaccharides were also studied to determine if one or both of them are responsible for the thermal events taking place within the mixed systems, as a means to determining the conformations of the polysaccharides involved in the interacted products.

6.2.1 Method

Xanthan gum-locust bean gum mixes 1%w/w of various ratios were prepared at 25°C as described in Chapter 2.2.1. A DSC III (Setaram in Caluire, France) equipped with 1cm^3 closed batch vessels were used to hold the sample and reference where the reference vessel contained deionised water. It was important that for each experiment, that the weights of both sample and reference materials were as close as possible (±0.001%). Additionally, it was important that the volume of sample and reference were below the thread inside the vessel to ensure that heat transfer was identical, and that no material was trapped between the threads on the vessel and the lid. Disposable o-rings made of viton which can withstand high temperatures and do not react with the sample were used to form tight seals between the lids and vessels, such that the heating and cooling cycle takes place within a closed environment. Once the sample and reference had both equilibrated to 25°C, the vessels were heated and cooled between 25°C and 95°C at 1K/min (i.e. 0.0037°C/min) (1 cycle). A minimum of three runs on fresh samples were performed for each system. The data were collected and transferred by ASCII format for analysis using Excel.

6.2.2 Results

The results for different ratios of xanthan gum-locust bean gum mixes subjected to one heating cycle between 25°C-95°C are shown in Figure 6.3. The profiles for locust bean gum and xanthan gum-locust bean gum (1:9) ratio are similar, forming a curve which resembles the baseline profile. In contrast, the profiles for xanthan gum and the remaining xanthan gum-locust bean gum ratios all exhibit one broad endotherm ranging from approximately 45°C-75°C. Similar temperature ranges have been reported for xanthan gum and some xanthan gum-locust bean gum mixtures, and is thought to be attributed to the xanthan gum helix to coil transition (Williams et al, 1991 and Craig et al, 1997). The precise midpoint of the Tm and enthalpies can not be accurately calculated for the mixtures in the present study, since the peaks are too broad hence a temperature range is used for quantitative analysis.

The corresponding cooling curves for the different ratios of xanthan gum-locust bean gum mixes subjected to cooling between 95°C-25°C are illustrated in Figure 6.4. Again, no peaks are identified for locust bean gum and xanthan gum-locust bean gum

(1:9) ratio, however one broad exotherm is observed for xanthan gum and the other xanthan gum-locust bean gum ratios at the same temperature range as was observed for the heated mixes (i.e. 45°C-75°C). This 'mirror image' effect is illustrated in Figures 6.5(a) and 6.5(b), in which the peaks are more pronounced when a scanning rate of 1K/min (i.e. 0.0037°C/min) (Figure 6.5(a)) was used compared to 2K/min (i.e. 0.0073°C/min) (Figure 6.5(b)). In addition, although the reproducibility of the HSDSC traces were not always good for all three runs, the reproducibility of the peaks in general at the temperature ranges stated were good, as illustrated in Figure 6.6 (see repetitive temperature cycling studies in Chapter 6.7 for improvement of reproducibility).

6.3 THE EFFECTS OF SODIUM CHLORIDE ON THE TRANSITION OF XANTHAN GUM

It has been well reported that the addition of sodium chloride shifts the transition of xanthan gum to higher temperatures, through screening of electrostatic repulsions between side chains which stabilises the ordered helix (Holzwarth & Ogletree, 1979). In this chapter, the effects of sodium chloride of different concentrations on the transitional behaviour of xanthan gum was studied.

6.3.1 Method

Xanthan gum mixes 1%w/w were prepared at 25°C in sodium chloride solutions (10mM, 20mM and 30mM) according to the method described in Chapter 2.2.3. The HSDSC protocol used was identical to the method described in the previous section (Chapter 6.2.1).

6.3.2 Results

The addition of sodium chloride to xanthan gum 1%w/w solutions shifts the endothermic peaks to higher Tm values on heating, as can be shown in Figure 6.7. The shift in Tm appears to be dependent on the concentration of sodium chloride, with higher Tm values occurring as the concentration of sodium chloride is increased. In addition, the peaks observed are less broad compared to xanthan gum without sodium chloride, and appear to narrow as the concentration of sodium chloride is increased.

FIG. 6.3 : HSDSC PROFILES COMPARING DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w ON HEATING (25°C-95°C)

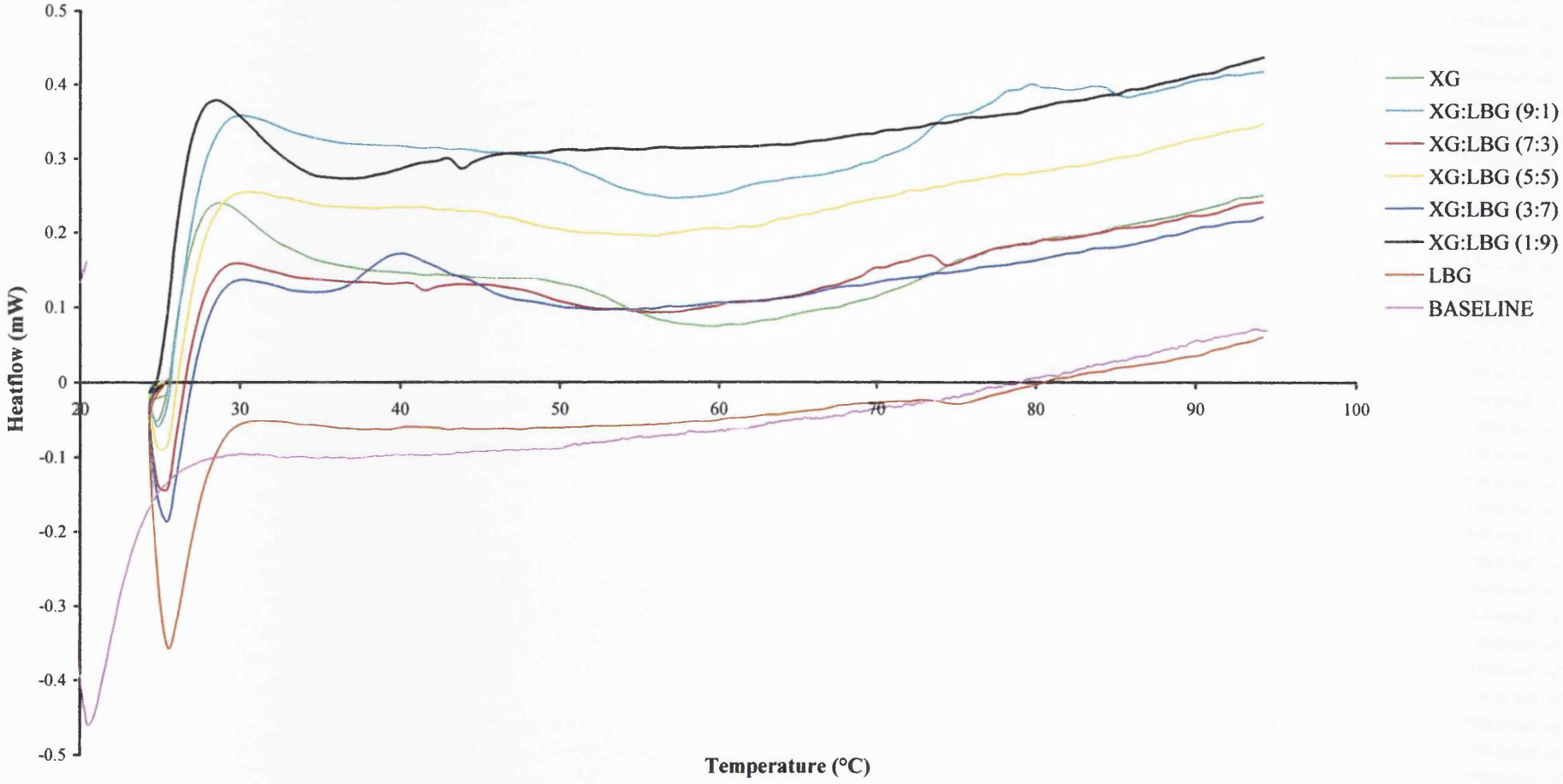


FIG. 6.4 : HSDSC PROFILES COMPARING DIFFERENT RATIOS OF XANTHAN GUM-LOCUST BEAN GUM MIXES 1%w/w ON COOLING (95°C-25°C)

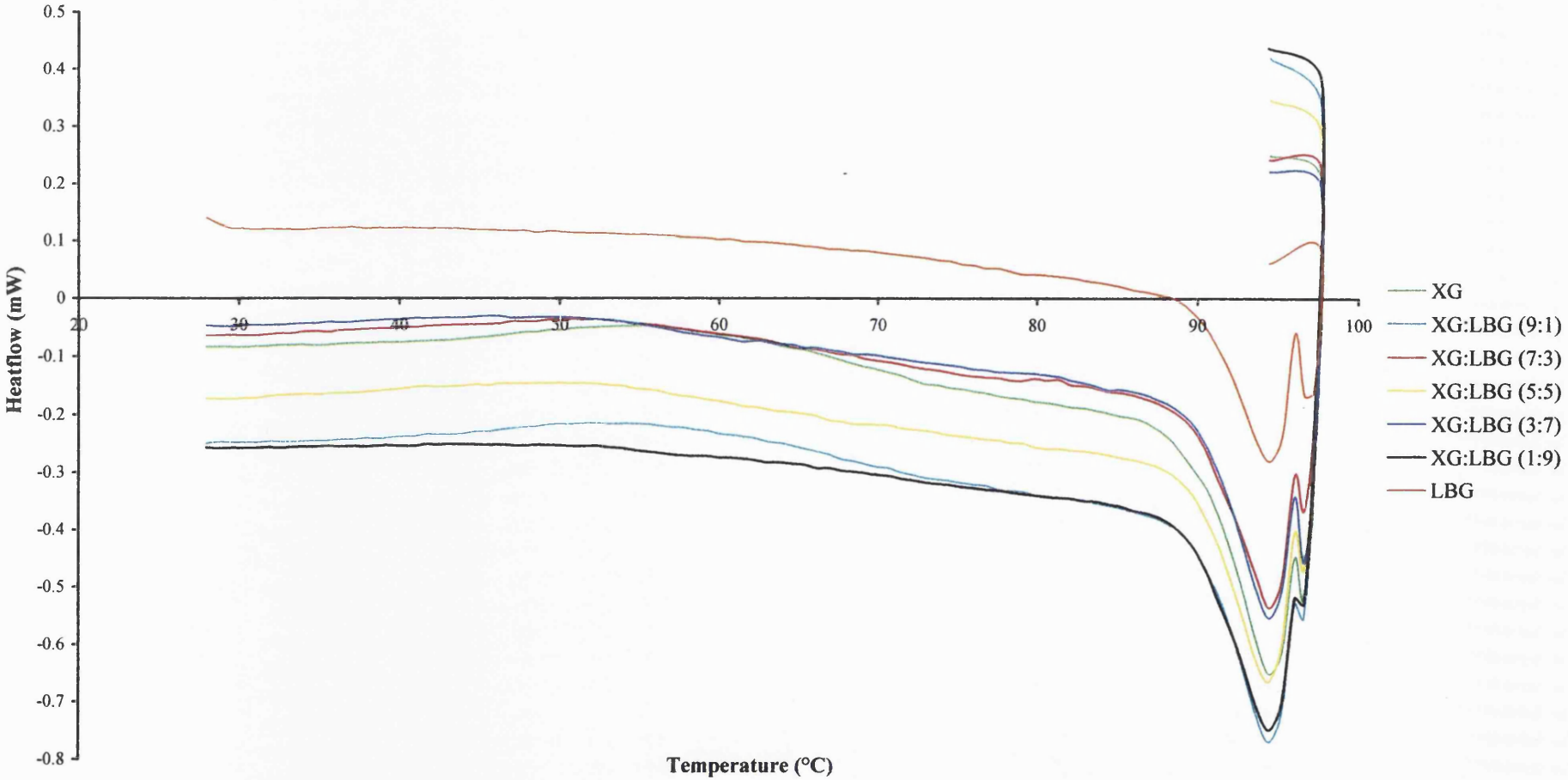


FIG. 6.5(a) : HSDSC PROFILE OF XANTHAN GUM 1%w/w ON HEATING AND COOLING BETWEEN 25°C-95°C AT 1K/MIN OR 0.0037°C/MIN

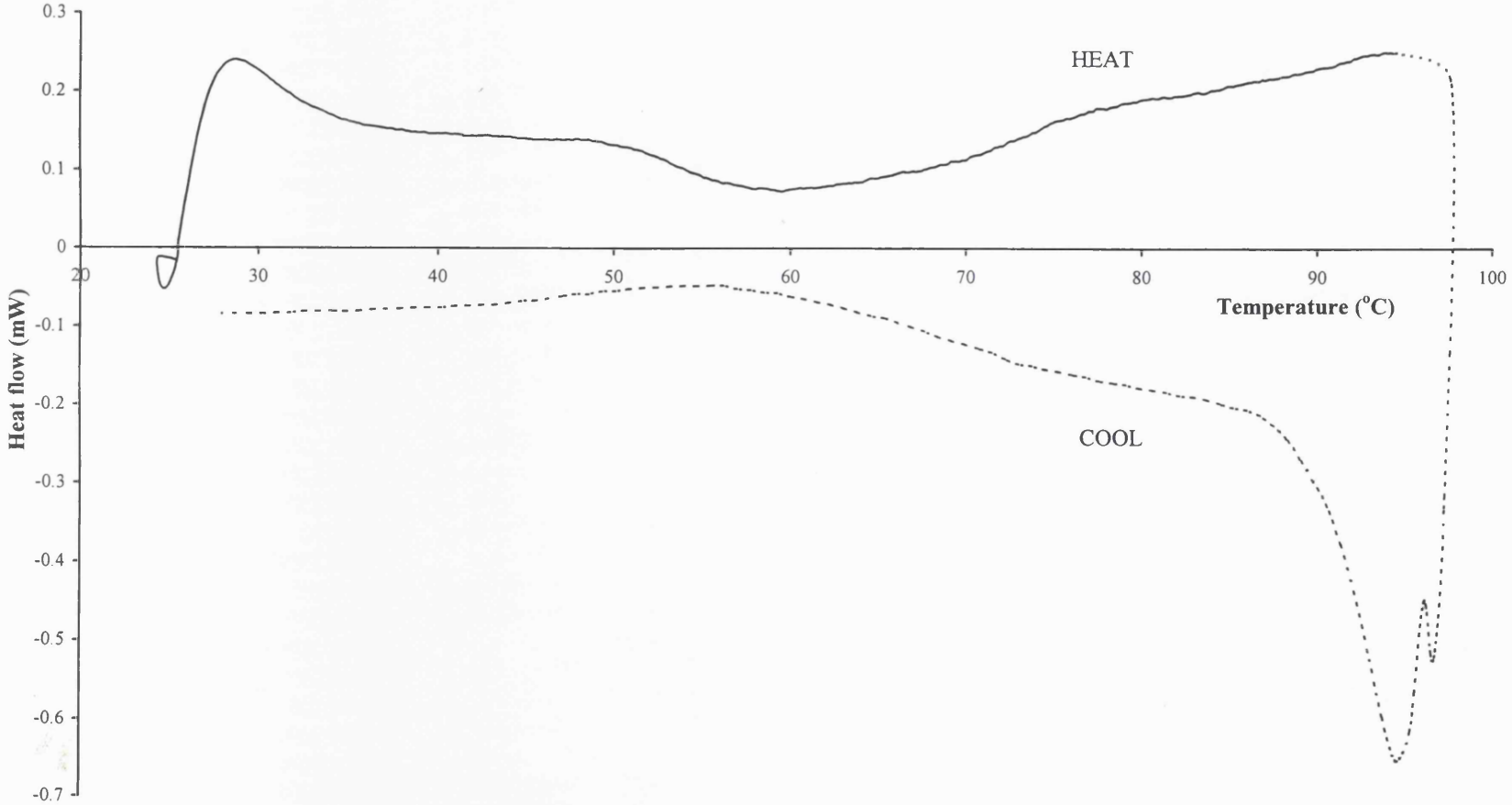


FIG. 6.5(b) : HSDSC PROFILE OF XANTHAN GUM 1%w/w ON HEATING AND COOLING BETWEEN 25°C-95°C AT 2K/MIN OR 0.0073°C/MIN

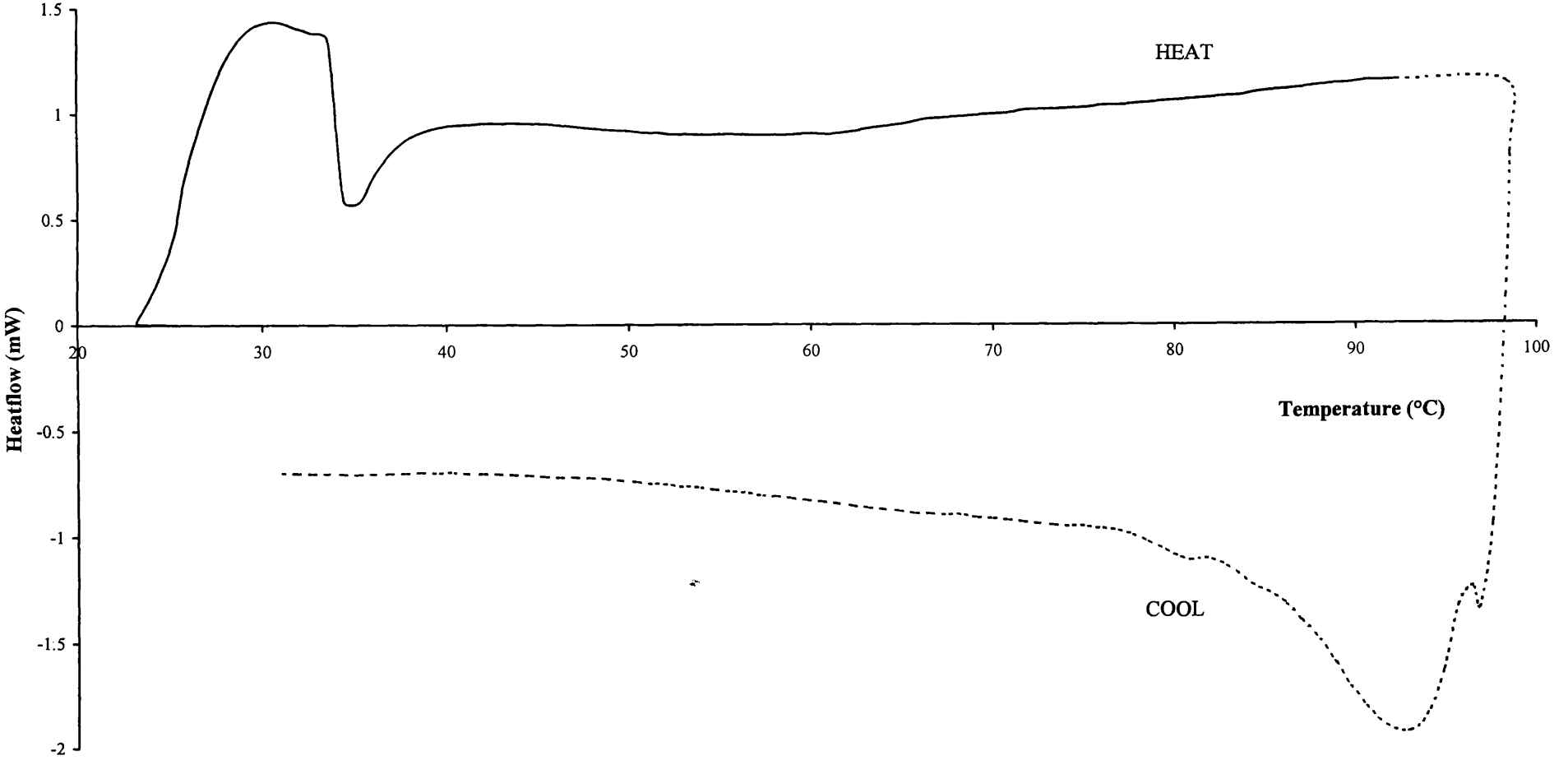
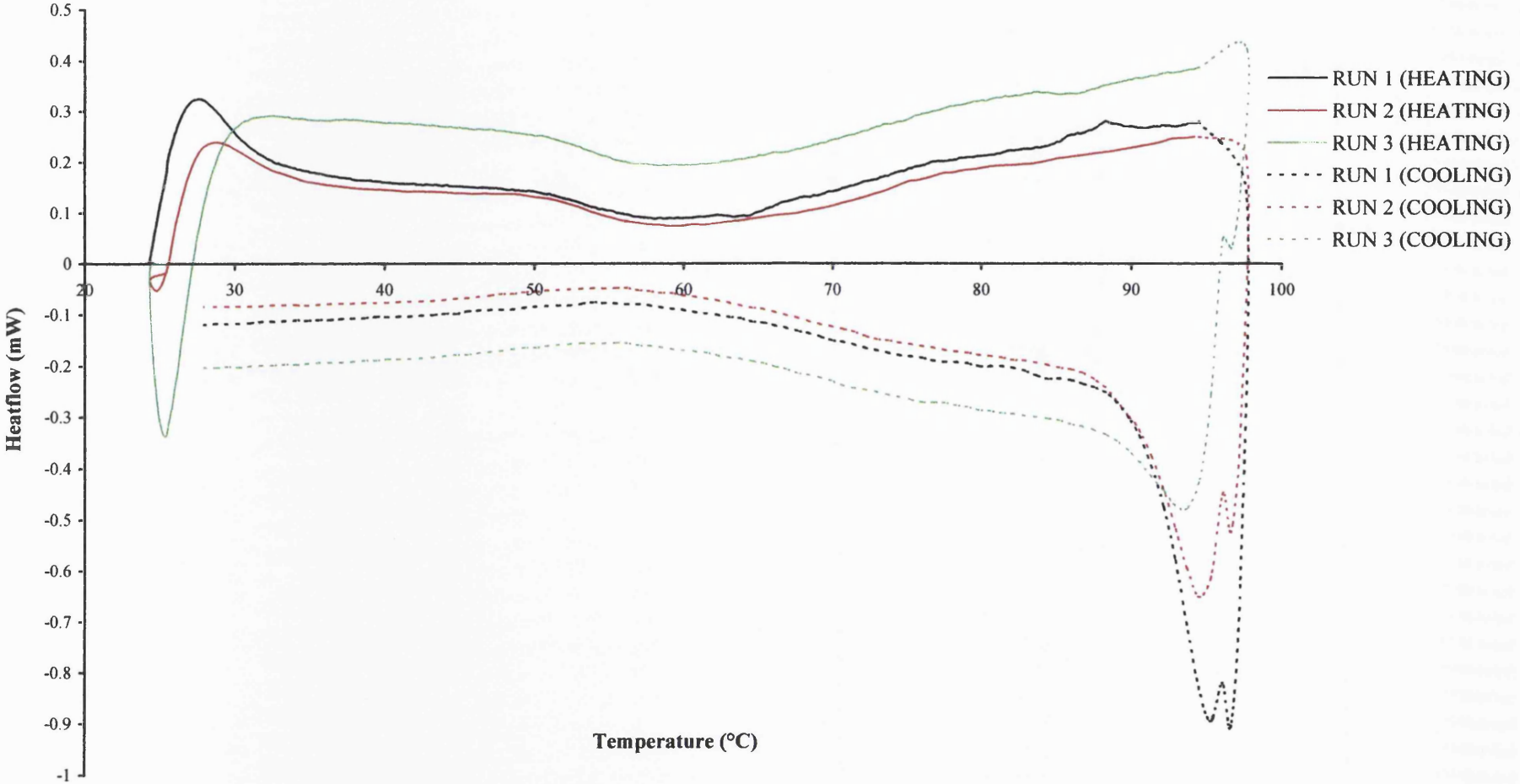


FIG. 6.6 : AN EXAMPLE OF PEAK REPRODUCIBILITY FROM THREE RUNS FOR XANTHAN GUM 1%w/w



The corresponding cooling curves for xanthan gum with and without sodium chloride in Figure 6.7 show the 'mirror image' peaks (exothermic) of the heating profiles which are also less broad compared to the peak for xanthan gum in the absence of additive. The temperature ranges shown in Figures 6.7 are the approximate Tm values observed for the HSDSC profiles of xanthan gum in different solutions.

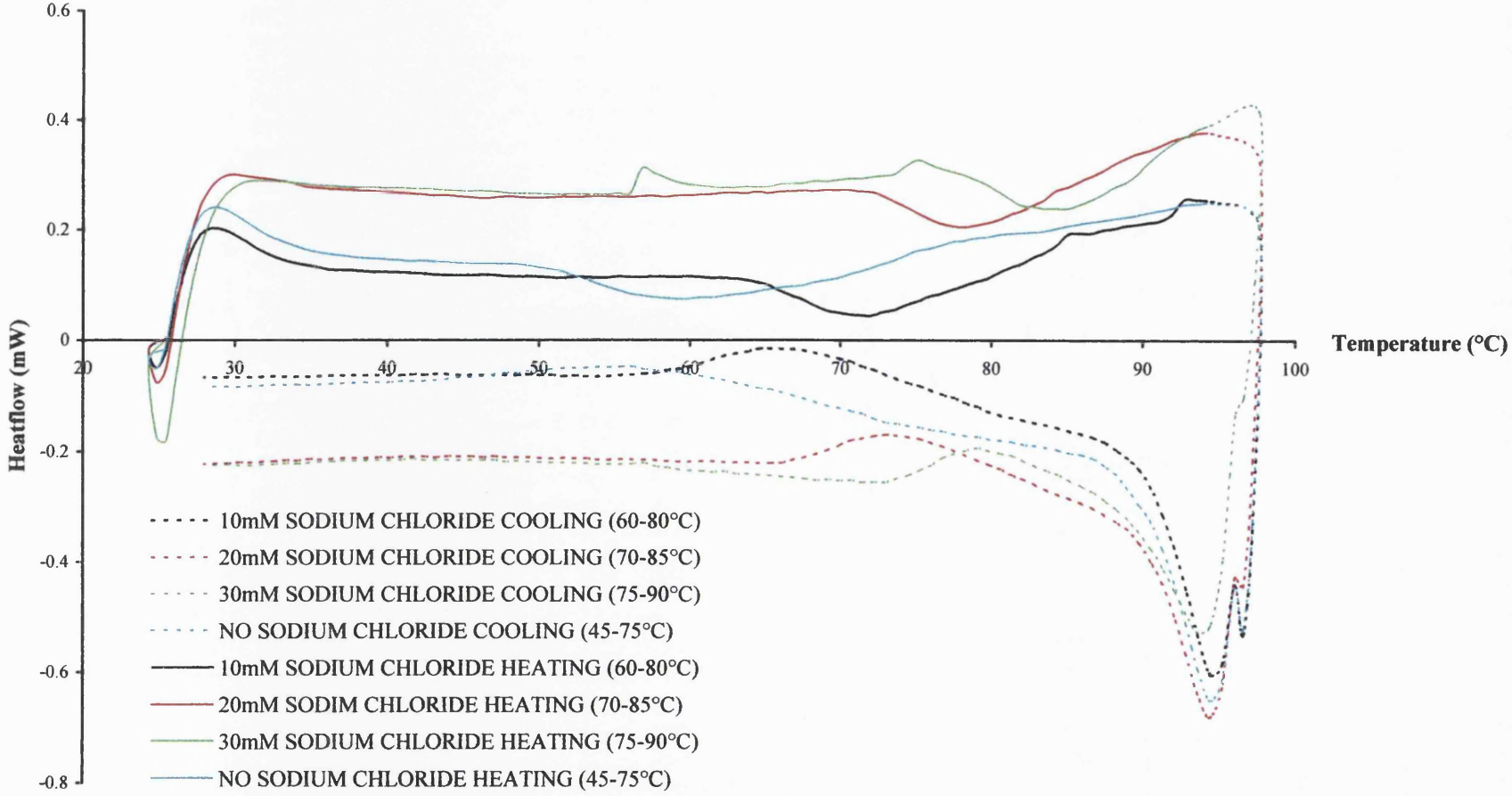
6.4 THE INFLUENCE OF SODIUM CHLORIDE ON XANTHAN GUM-LOCUST BEAN GUM MIXES

This study was undertaken in order to compare the effects of sodium chloride on the conformation and transitional behaviour of xanthan gum during the interaction with locust bean gum with xanthan gum alone. By performing this study, information regarding the conformation of xanthan gum during the interaction may be gained, since Williams et al (1991) discovered that the xanthan gum ordered helix interacts with locust bean gum both in the presence and absence of salt, since gelation occurred below the transition temperature in both environments.

6.4.1 Method

Xanthan gum-locust bean gum (5:5) mixes 1%w/w were prepared at 25°C in sodium chloride solutions (10mM, 20mM and 30mM) according to the method described in Chapter 2.2.3. Since the results from Chapter 6.2.2 displayed the same transition temperature (i.e. the Tm for xanthan gum) regardless of the xanthan gum-locust bean gum ratio, the xanthan gum-locust bean gum ratio (5:5) was chosen for the following study, since it has been shown from Chapters 4 and 5 to be the optimum ratio at which maximum synergy occurs. The HSDSC protocol used was identical to the method described in Chapter 6.2.1.

FIG. 6.7 : HSDSC PROFILES OF XANTHAN GUM 1%w/w IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE SOLUTIONS ON HEATING AND COOLING (25°C-95°C)



6.4.2 Results

The addition of sodium chloride to xanthan gum-locust bean gum (5:5) 1%w/w mixes on heating shown in Figure 6.8, has the same effect as that observed in Figure 6.7 for sodium chloride with xanthan gum 1%w/w, i.e. the endothermic peaks occur at the same temperature range as that for xanthan gum 1%w/w alone, although the peaks are broader. Similar results are seen for the corresponding cooling curves in Figure 6.8, in that exothermic peaks occur at the same temperature range as for the endothermic peaks observed on heating, with the peaks being broader compared to those observed for xanthan gum 1%w/w in sodium chloride solutions on cooling, shown in Figure 6.7. The temperature ranges displayed in Figures 6.8 are the approximate Tm values observed for the HSDSC profiles of xanthan gum-locust bean gum (5:5) ratio in different sodium chloride solutions.

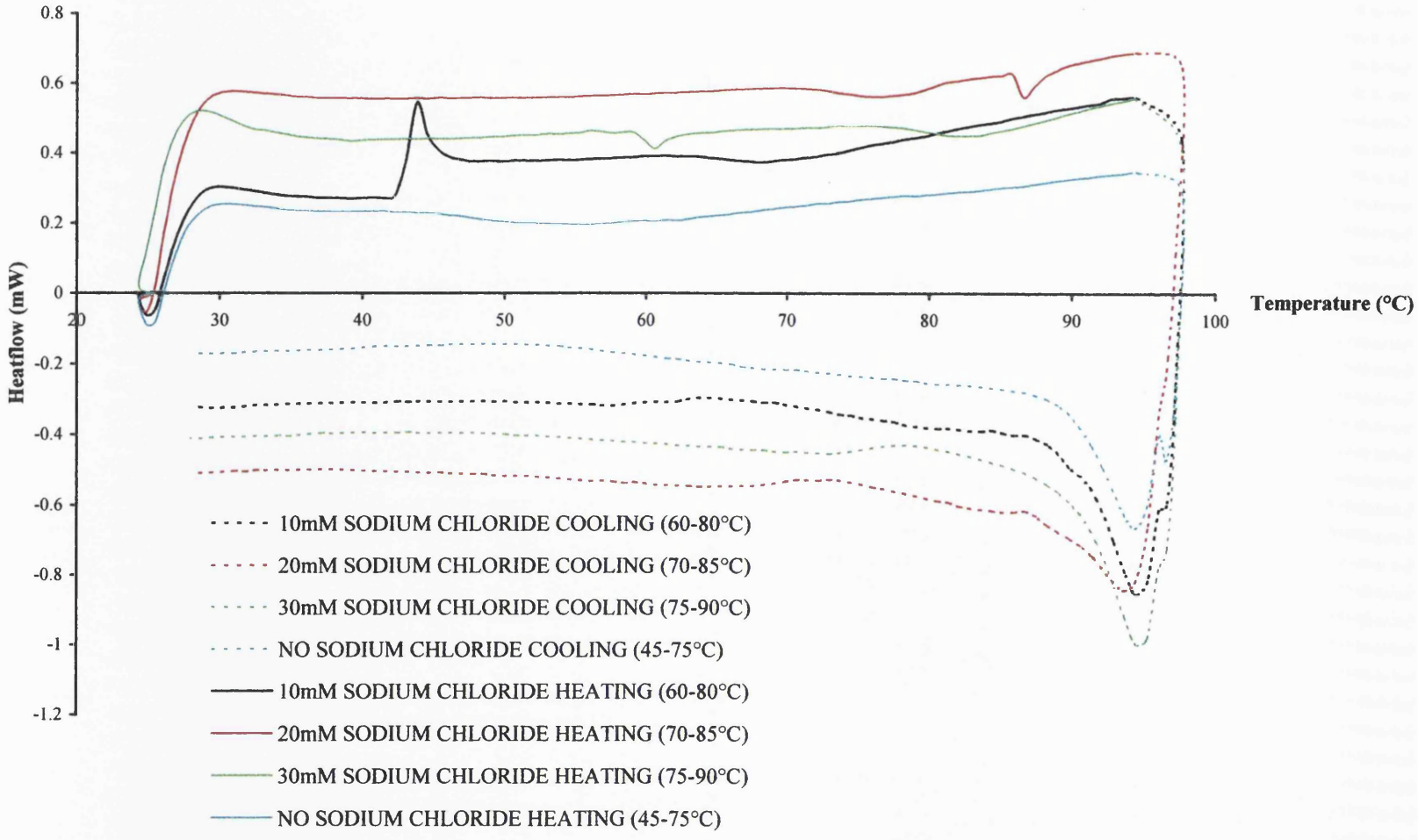
6.5 THE ADDITION OF SUCROSE AND THE EFFECTS ON XANTHAN GUM BEHAVIOUR

It was observed from Chapter 4.4.1.2 that the addition of sucrose increased the gel strength of xanthan gum-locust bean gum (5:5) 1%w/w mixtures. Therefore, the present work was conducted in order to investigate whether or not the enhanced synergy between the two polysaccharides, is due to an effect of sucrose on xanthan gum conformation, since it has been described by Sudhakar et al (1995) that the addition of sucrose increases polymer-polymer interactions. If this is the case, this would possibly result in an increase in helix-helix aggregation which would increase the Tm value. Consequently, this would reduce or prevent interactions with locust bean gum if the disordered form of xanthan gum is a prerequisite for the synergy between the two polysaccharides.

6.5.1 Method

Xanthan gum mixes 1%w/w were prepared at 25°C in various sucrose solutions (1%, 8% and 15%) according to the method described in Chapter 2.2.2. The HSDSC protocol described in Chapter 6.2.1 was used for this study.

FIG. 6.8 : HSDSC PROFILES OF XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE SOLUTIONS ON HEATING AND COOLING (25°C-95°C)



6.5.2 Results

The HSDSC profiles of xanthan gum 1%w/w in the presence of sucrose (1%, 8% and 15%) on heating and cooling are split into two graphs, namely Figure 6.9 for the addition of 1% sucrose and no sucrose and Figure 6.10 for the addition of 8% and 15% sucrose, in order to compare the results on similar scales. It is possible to detect an endothermic and exothermic peak on heating and cooling respectively for xanthan gum in 1%w/w sucrose solution at 45°C-75°C (Figure 6.9), which coincides with the transition temperature of xanthan gum 1%w/w in the absence of sucrose. However, no peaks were observed for xanthan gum in the presence of higher concentrations of sucrose as shown in Figure 6.10.

6.6 INVESTIGATION OF SUCROSE EFFECTS ON XANTHAN GUM–LOCUST BEAN GUM MIXES

The current work was necessary in order to compare the effects of sucrose on xanthan gum with the sucrose effects on a mixture of xanthan gum-locust bean gum, in order to find out more about the interaction process. In addition, this study was performed as a means to obtaining a possible explanation for the increase in gel strength with increasing sucrose concentration, as observed from oscillatory rheology measurements in Chapter 4.4.1.2.

6.6.1 Method

Xanthan gum-locust bean gum (5:5) mixes 1%w/w were prepared at 25°C in sucrose solutions using the method described in Chapter 2.2.2. This ratio was chosen for the same reasons as detailed in Chapter 6.4.1 i.e. the Tm for xanthan gum remains unchanged regardless of the xanthan gum-locust bean gum ratio. The HSDSC method used is described in Chapter 6.2.1.

FIG. 6.9 : HSDSC PROFILES OF XANTHAN GUM 1%w/w IN 1%w/w SUCROSE SOLUTION ON HEATING AND COOLING (25°C-95°C)

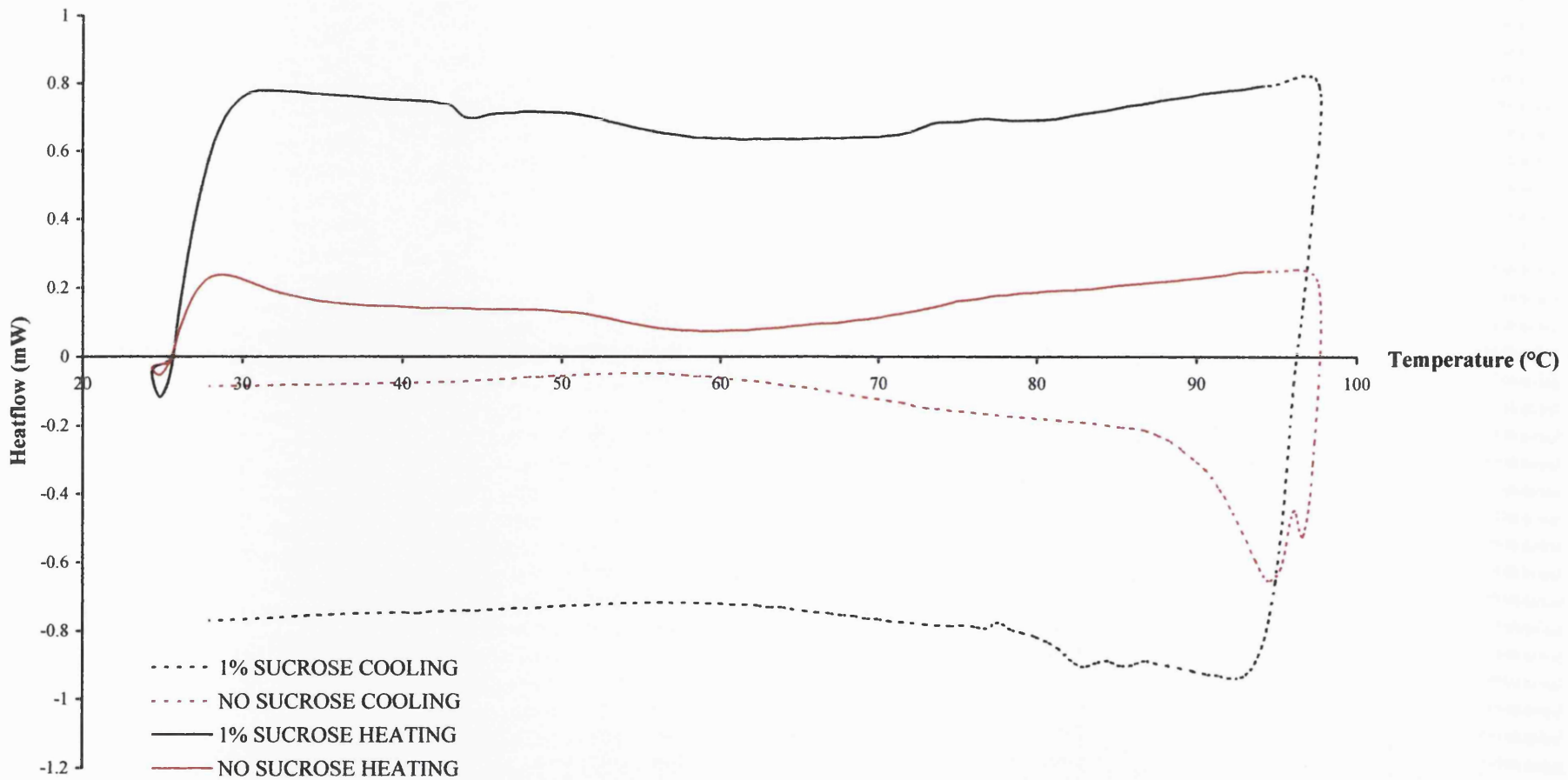
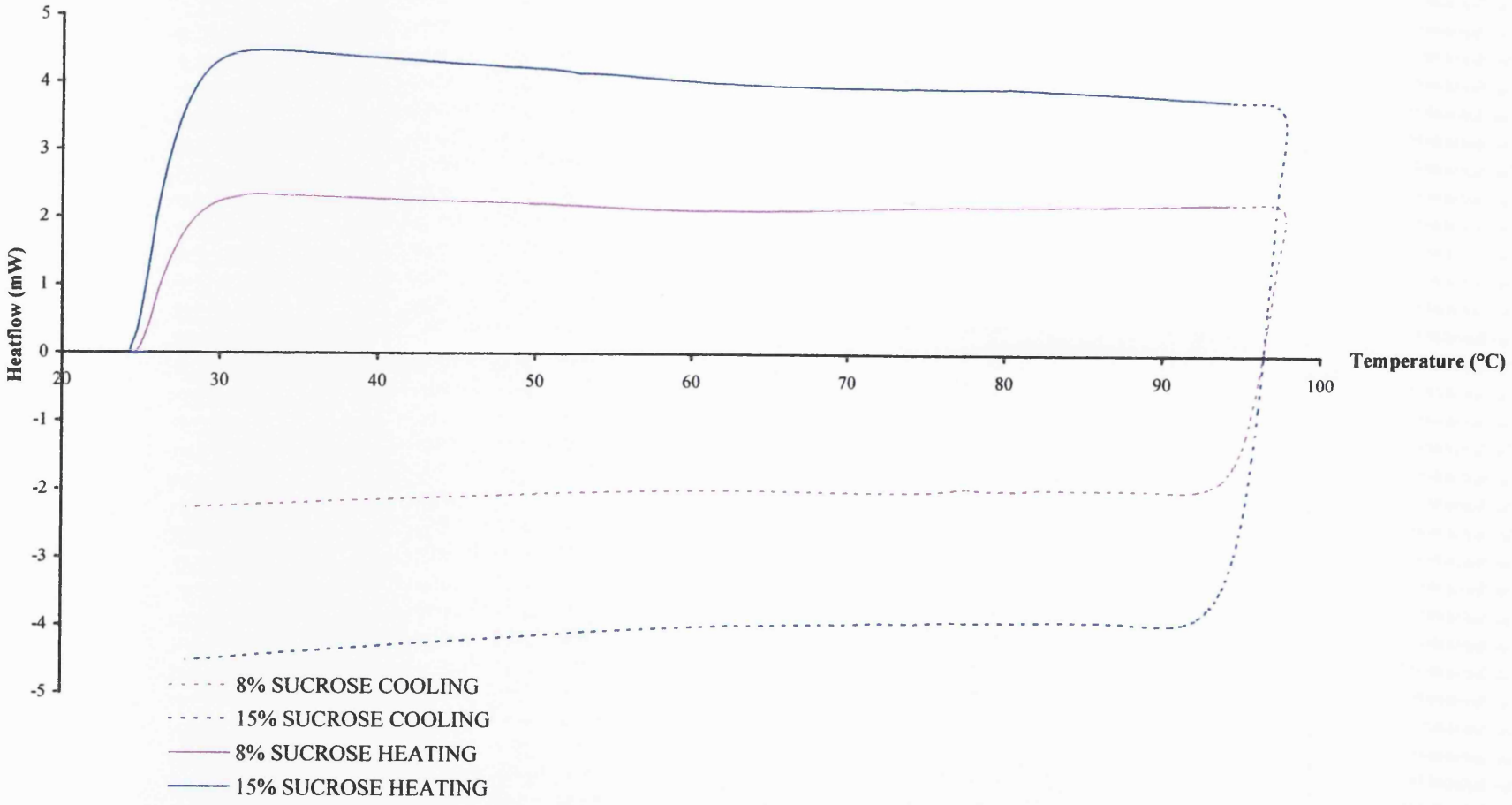


FIG. 6.10 : HSDSC PROFILES OF XANTHAN GUM 1%w/w IN DIFFERENT SUCROSE SOLUTIONS ON HEATING AND COOLING (25°C-95°C)



6.6.2 Results

Figures 6.11 and 6.12 represent HSDSC profiles for xanthan gum-locust bean gum (5:5) 1%w/w in the presence of sucrose on heating and cooling for the addition of 0-2%w/w sucrose and 5-15%w/w sucrose respectively. The HSDSC profiles were split according to the sucrose concentration, in order to compare graphs on similar scales and for clarity purposes. It is observed from the graphs that below a sucrose concentration of about 2%w/w (Figure 6.11), an endothermic and exothermic peak on heating and cooling respectively are observed, in which the transition temperature range coincides with the transition temperature of xanthan gum 1%w/w (i.e. no sucrose). In contrast, no peaks are observed on heating and cooling mixes in sucrose concentrations above about 2%w/w, as shown in Figure 6.12.

6.7 REPETITIVE TEMPERATURE CYCLING EFFECTS ON XANTHAN GUM-LOCUST BEAN GUM (5:5) IN 0.5%w/w SUCROSE SOLUTIONS

This study was performed to investigate the effect of temperature cycling on mixed polysaccharide systems, since it has been found by Lundin & Hermansson (1995) that the rheological properties of the mixed systems are independent of temperature cycling. In addition, this study was performed to see if the overall reproducibility and definition of peaks could be improved, since the first heating and cooling cycle should reduce any inhomogeneity problems in the mixing process and fill the cell volume, thus improving thermal conduct in subsequent heating and cooling cycles. Since peak definition and reproducibility was lowest for the systems in sucrose solutions, a xanthan gum-locust bean gum (5:5) ratio in 0.5%w/w sucrose solution total polymer concentration of 1%w/w was chosen for this study.

FIG. 6.11 : HSDSC PROFILES OF XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w IN DIFFERENT SUCROSE SOLUTIONS ON HEATING AND COOLING (25°C-95°C)

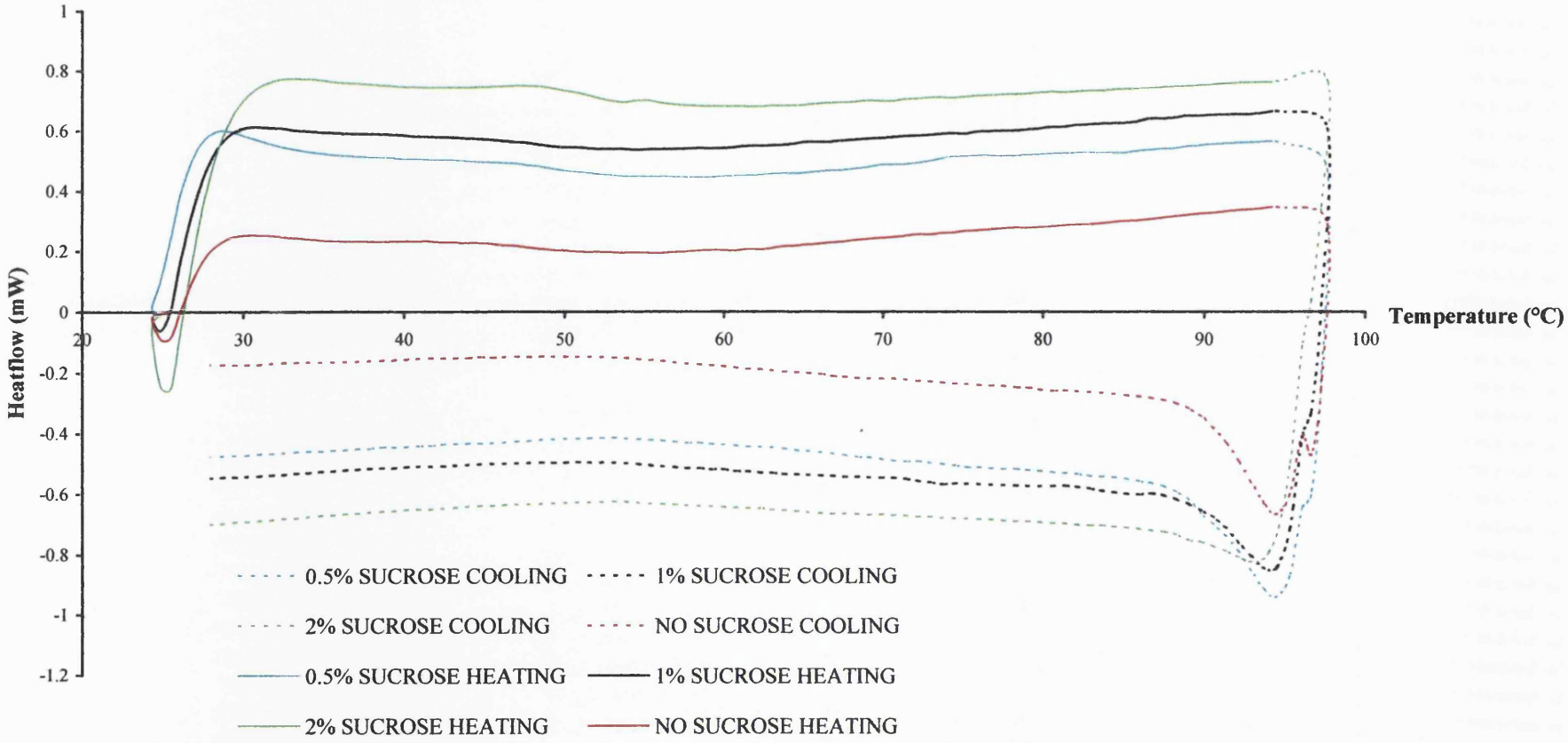
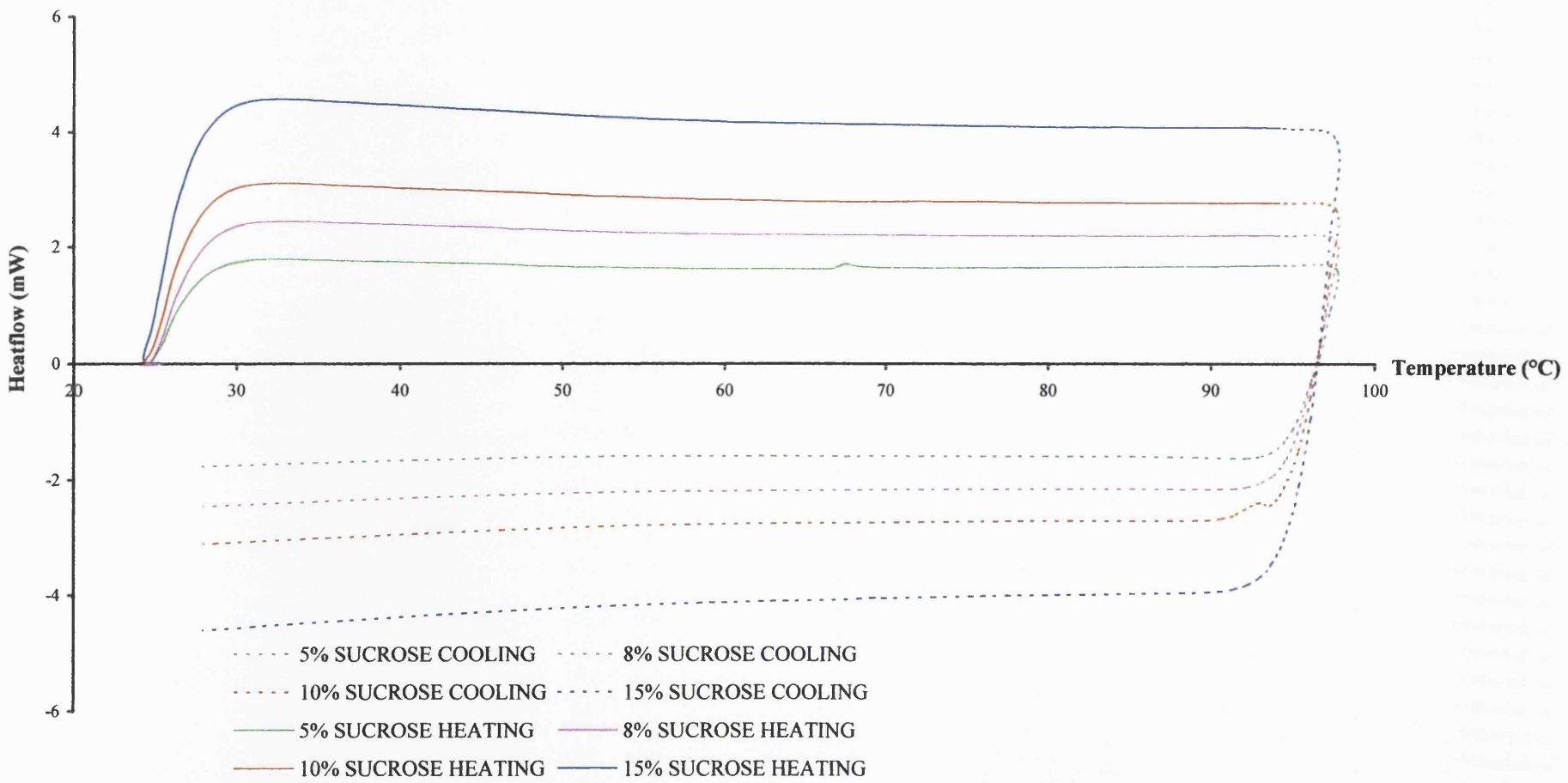


FIG. 6.12 : HSDSC PROFILES OF XANTHAN GUM-LOCUST BEAN GUM (5:5) 1%w/w IN DIFFERENT SUCROSE SOLUTIONS ON HEATING AND COOLING (25°C-95°C)



6.7.1 Method

Xanthan gum-locust bean gum (5:5) mixes 1%w/w were prepared in 0.5%w/w sucrose solution as described in Chapter 2.2.2. The same protocol as that described in Chapter 6.2.1 was used for the HSDSC experiments, except that mixtures were heated and cooled between 25°C and 95°C twice in succession (i.e. 2 cycles).

6.7.2 Results

The results shown in Figure 6.13 display two broad endotherms (1st and 2nd heating cycles) and two broad exotherms (1st and 2nd cooling cycles) all occurring at 45°C-75°C. These results are similar for the same system subjected to one cycle in Chapter 6.6.2.

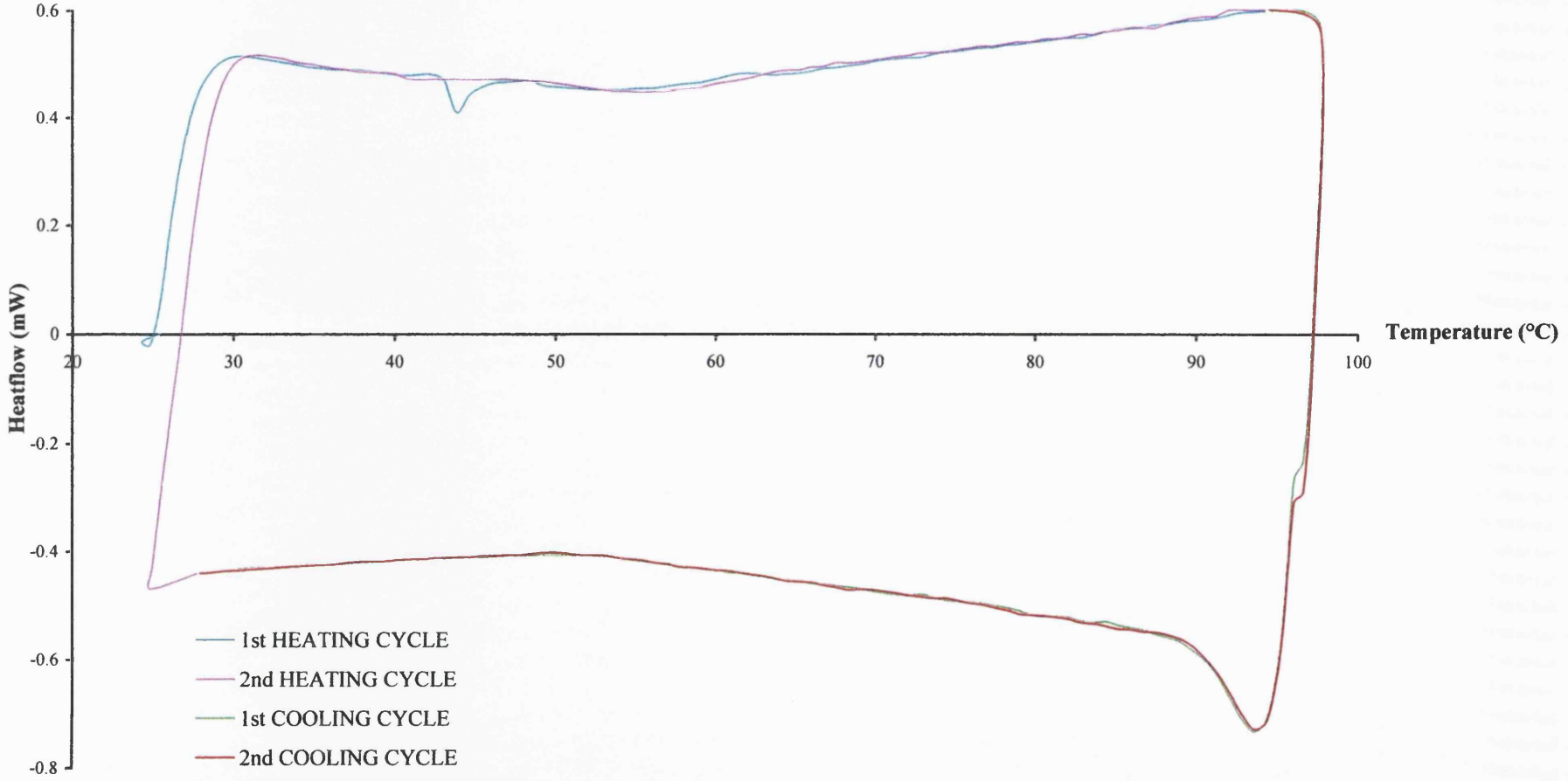
6.8 THE INFLUENCE OF FRACTIONATED LOCUST BEAN GUM ON XANTHAN GUM-LOCUST BEAN GUM MIXES

The effects of fractionated locust bean gum in xanthan gum-locust bean gum mixes were investigated using HSDSC, to see if there were any differences in the temperature range for the peaks (if any) compared to the profiles containing unfractionated locust bean gum in the mixed systems. This study was carried out in order to relate to the rheological findings in Chapter 4.5.2, in which differences in the extent of the interaction were observed for the different fractions, i.e. the interaction or gel strength of the mixed system was reduced in the presence of a low M:G ratio fraction (LBG35).

6.8.1 Method

Locust bean gum was fractionated into two samples namely LBG35 and LBG80 which contained different mannose:galactose (M:G) ratios using the method described in Chapter 3.4.1. It is observed from Table 3.4 (Chapter 3.4.2) that LBG35 contained an average M:G ratio of 3.21 or 2.98, whereas LBG80 contained an average M:G ratio of 4.70 or 4.34 depending on the method of analysis used (i.e. the first ratios described were obtained using the acid hydrolysis method whereas the second ratios described were obtained using the H^1 NMR method).

FIG. 6.13 : HSDSC PROFILES OF XANTHAN GUM-LOCUST BEAN GUM (5:5) IN 0.5%w/w SUCROSE SOLUTION ON CYCLING BETWEEN 25°C-95°C



Xanthan gum-locust bean gum (5:5) mixes 1%w/w were prepared at 25°C as described in Chapter 2.2.1 using fractionated locust bean gum samples in replace of the unfractionated locust bean gum used in previous experiments. The HSDSC method performed is described in Chapter 6.2.1.

6.8.2 Results

The heating and cooling HSDSC profiles for xanthan gum-LBG35 (5:5) 1%w/w and xanthan gum-LBG80 (5:5) 1%w/w, are shown in Figures 6.14 and 6.15 respectively. In both cases, the heating profiles for mixtures containing the fractionated locust bean gum are erratic and not reproducible, as can be seen from the spikes in the traces. Due to the small yields obtained for the locust bean gum fractions which involves a long, complex procedure, further experiments to try to improve the reproducibility of the profiles were not possible. However, it is possible to observe reproducible broad exotherms on cooling for both mixtures containing different locust bean gum fractions. The temperature range at which the exotherms are detected occur between 40°C-70°C.

6.9 DISCUSSION

It has been observed from the HSDSC studies, that an endotherm on heating (Figure 6.3) and an exotherm on cooling (Figure 6.4) both between 45°C-75°C, occurs for xanthan gum and xanthan gum-locust bean gum mixes, which represents the transition temperature of xanthan gum. Williams et al (1991) and Craig et al (1997) have also reported this temperature range using HSDSC, which is thought to be attributed to the reversible helix to coil transition as described by Morris et al (1977). This would explain why the endotherm and exotherm occur at the same temperature range, hence the mirror image effect on the heating and corresponding cooling profiles (Figure 6.5(a)). Additionally, this finding can be supported by the oscillatory rheology results in Chapter 4, in which no new structures were observed for xanthan gum 1%w/w (Figure 4.14). Since all ratios for the mixed systems showed this mirror image effect on heating and cooling between 45°C and 75°C, this may suggest that the disordered form of xanthan gum may be necessary to interact with locust bean gum initially, although the ordered conformer of xanthan gum may be retained in the post interactive state on cooling. This idea has been suggested by Copetti et al (1997).

FIG. 6.14 : HSDSC PROFILES OF XANTHAN GUM-LBG35 (5:5) 1%w/w ON HEATING AND COOLING (3 RUNS) BETWEEN 25°C-95°C

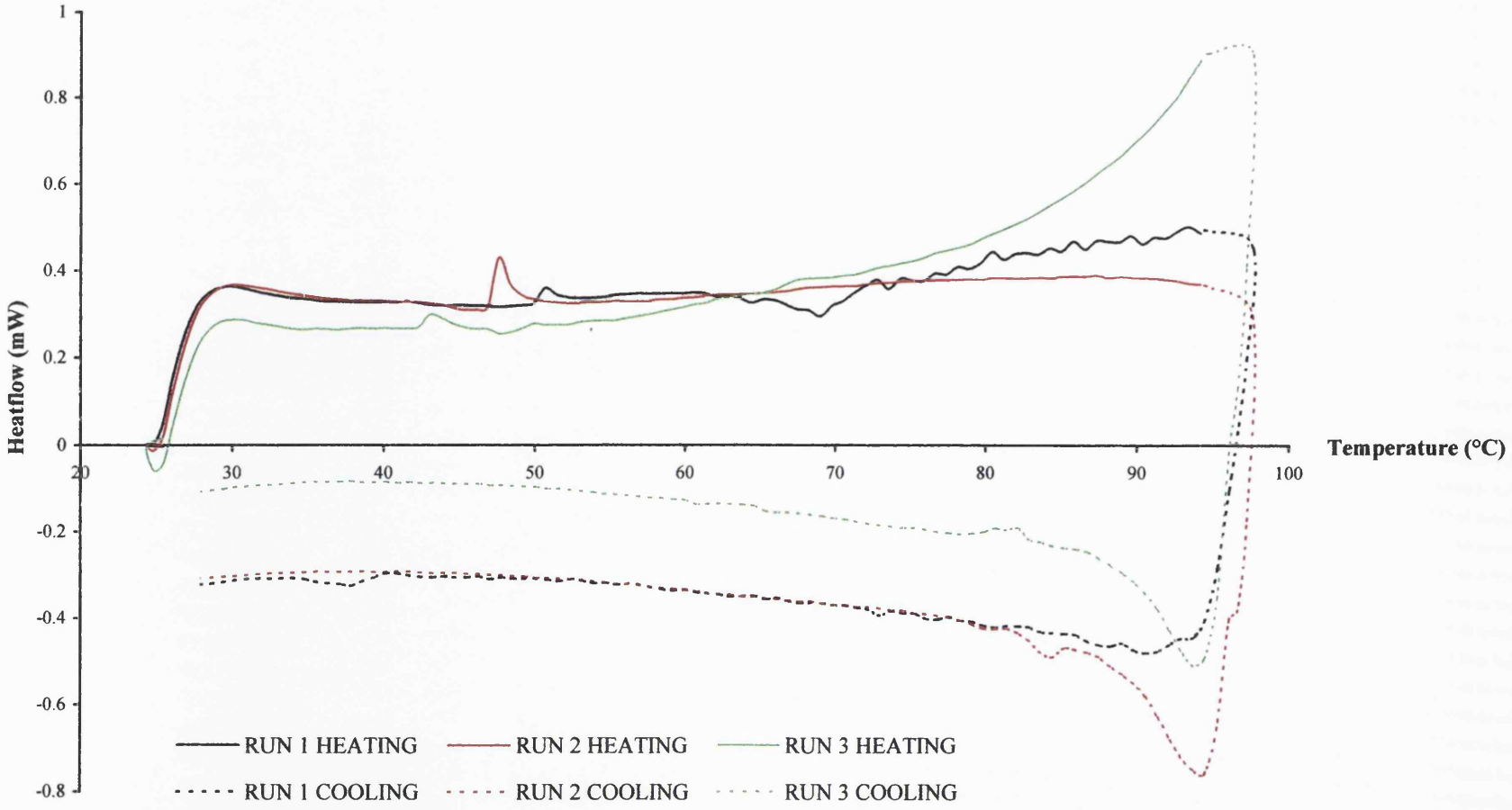
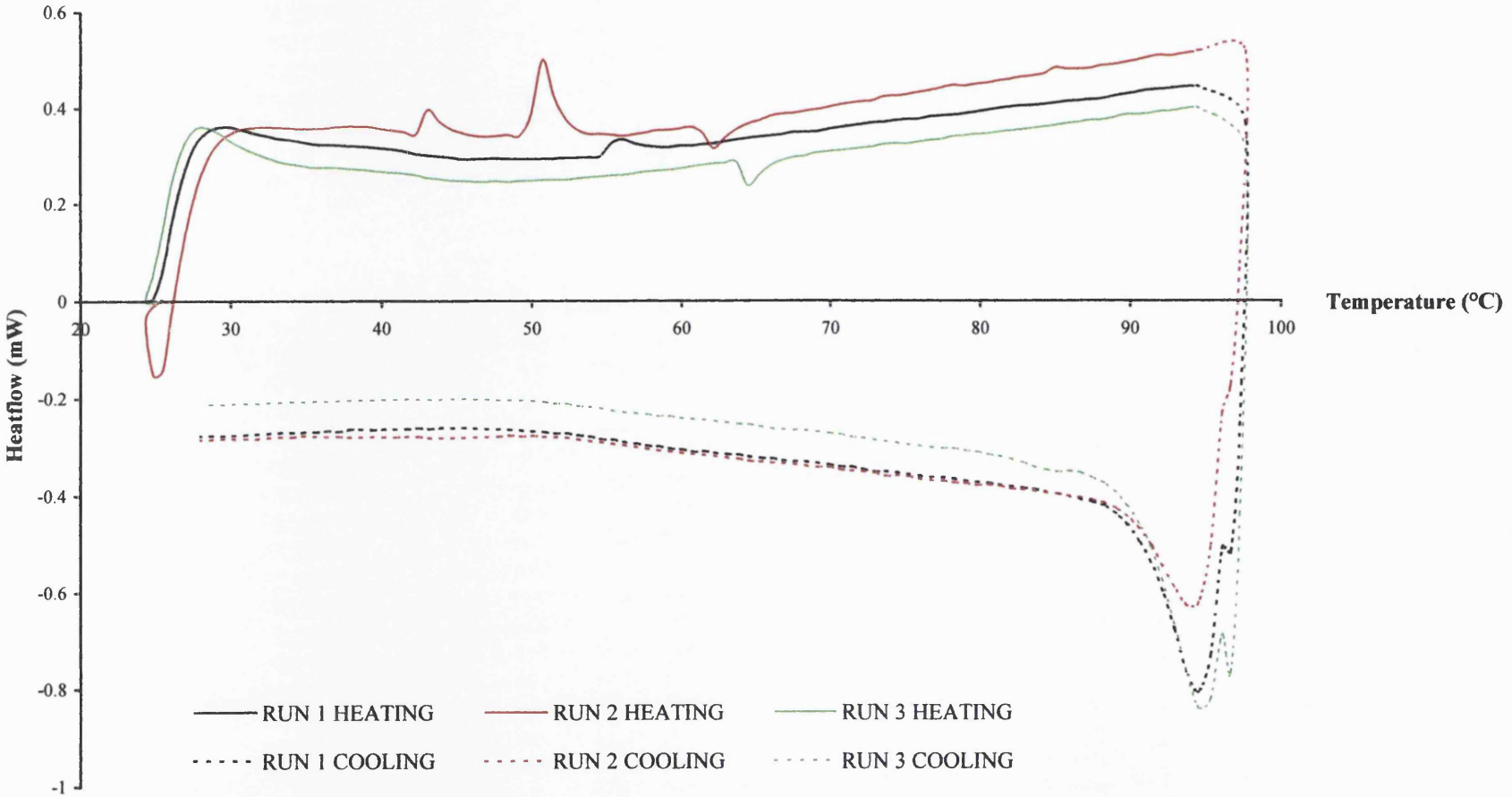


FIG. 6.15 : HSDSC PROFILES OF XANTHAN GUM-LBG80 (5:5) 1%w/w ON HEATING AND COOLING (3 RUNS) BETWEEN 25°C-95°C



This finding can be further supported by the oscillatory rheology and texture analysis studies in Chapters 4.3.1.2 and 5.4.3 respectively, since the oscillatory rheology results showed a cross over point between the heated and cooled profiles of xanthan gum-locust bean gum mixes at around 50°C in most cases (see Figure 4.17 as an example). In relation to the texture analysis studies, discontinuities were observed between 50°C-60°C for xanthan gum-locust bean gum (5:5) ratios prepared at temperatures between 25°C and 90°C (Figure 5.9).

Since locust bean gum 1%w/w did not show any peaks on heating or cooling (see Figures 6.3 and 6.4), this polysaccharide has no transitions as expected, and further supports that the peaks observed for xanthan gum-locust bean gum mixtures are due to the properties of xanthan gum. In addition, the results suggest that by adding locust bean gum to xanthan gum, the Tm of xanthan gum is unaffected since the peaks observed on heating and cooling always occur at the same temperature range i.e. between 45°C and 75°C. A possible explanation as to why a transition was not clearly detected for the xanthan gum-locust bean gum (1:9) ratio, may suggest that xanthan gum exists in an equilibrium between its two conformational states. This can be supported by the texture analysis data in which it was observed that there was no change in gel strength after heating and cooling this ratio. Another explanation could be that there is simply not enough xanthan gum available to interact with locust bean gum or with itself if it is extensively dispersed, hence the profile's resemblance to locust bean gum 1%w/w.

The second thermal history of xanthan gum-locust bean gum (5:5) mixes in 0.5%w/w sucrose has not been found to necessarily improve the peak profiles or reproducibility in the present work (see Figure 6.13), which may suggest that the systems prepared are homogenous. In addition, since the results are similar to those observed for the same mixture subjected to one heating and cooling cycle, the second cycle does not appear to change the structure or behaviour of either polysaccharide, and further supports the reversible helix melting and renaturation of xanthan gum with heating and cooling respectively.

The endotherms and exotherms observed in the present work are very broad, which makes the precise midpoint of the transition difficult to determine in terms of defining the baseline. Hence accurate enthalpies could not be calculated, which is why a

temperature range was chosen to represent the peaks. In addition, the use of a higher scanning rate of 2K/min (0.0073°C/min) broadens the peaks, which is due to less time available for completion of thermal processes, hence lower resolution. Reproducibility was also harder to achieve using the higher scanning rate of 2K/min (0.0073°C/min), which is probably due to the greater sensitivity as a consequence (see Figure 6.5(b)).

The addition of sodium chloride to xanthan gum shifts the Tm to higher temperatures which is concentration dependent in terms of sodium chloride (Figure 6.7). These results are in agreement with the literature, in which the observed shift in Tm can be explained by the salt screening effect of the xanthan gum side chains. This results in a decrease in charge-charge repulsions between pyruvate groups, which thus stabilises the ordered helix conformer of xanthan gum (Holzwarth & Ogletree, 1979 and Williams et al, 1991). Since the pyruvate groups are screened by salt which have a destabilising effect on the xanthan gum helix, this can account for the broad peaks observed if the pyruvate population is heterogeneous. Thus, the observed sharpening of the peaks may be due to a more homogenous population of pyruvate groups due to the addition of salt (Holzwarth & Ogletree, 1979). Another explanation for the broad peaks observed in the absence of salt could be due to differences in the free energy for the two conformational states of xanthan gum, i.e. at low temperatures the helix form exists because it has the lowest free energy, whereas increasing the temperature results in the disordered form which is more stable at higher temperatures. Thus, on heating and cooling xanthan gum, the helix and disordered forms exist in an equilibrium, such that overall the peaks observed are very broad. However, on adding sodium chloride, the helix form becomes more stable (lowest free energy) which consequently sharpens the peaks on heating and cooling and thus increases the Tm, since a greater energy input is needed to melt the helix to the disordered form.

Similar results are observed for xanthan gum-locust bean gum (5:5) mixes in the presence of sodium chloride compared to xanthan gum with sodium chloride, with shifts in the Tm values occurring at the same temperature ranges for the different sodium chloride concentrations (Figure 6.8). These observations can be explained by the screening effect of the xanthan side chains by salt or differences in free energy of the xanthan gum conformations as described above. However, the peaks observed are much broader on heating and cooling compared to xanthan gum in the presence of salt. A possible explanation for the increased broadening of the peaks for xanthan gum-

locust bean gum (5:5) 1%w/w mixes in the presence of salt compared to xanthan gum 1%w/w with salt present, could be due to a reduction in the quantity of xanthan gum available. Since it has been observed and mentioned that locust bean gum does not undergo a transition, this could explain why the peaks become less sharp. In relation to the results from the oscillatory rheology experiments (Chapter 4.4.2.2) in which the gel strength was independent of sodium chloride, the HSDSC findings could still support that the disordered form of xanthan gum is necessary for interaction with locust bean gum, since the Tm values for xanthan gum in the presence of sodium chloride are still within the temperature range of the oscillatory rheology experiments (20°C-90°C).

The effects of sucrose on the xanthan gum transition, seem to either diminish or shift the Tm to higher temperatures or may be lower temperatures (compare Figures 6.9 and 6.10). The latter idea could not be tested, since temperatures greater than 100°C are beyond the scope of the HSDSC instrument when taking into account stable baselines, and problems such as air bubbles on sample and reference boiling could arise since these systems are aqueous. Similarly at low temperatures (testing below 0°C), problems with sample and reference freezing will occur. A possible explanation for these results are at present unknown. Similar findings were observed for the addition of sucrose to xanthan gum-locust bean gum (5:5) 1%w/w compared to xanthan gum 1%w/w in the presence of sucrose, i.e. sucrose either diminishes the transition of xanthan gum or shifts the Tm to higher or possibly lower temperatures (compare Figures 6.11 and 6.12). In relation to the findings from the oscillatory rheology experiments in Chapter 4.4.1.2, it was found that the addition of sucrose increased the gel strength of the mixes on heating and cooling (Figure 4.22), which can be supported by Sudhakar et al (1995), in which it has been suggested that polymer-polymer interactions are enhanced by sucrose which increases the overall viscosity of the system. In light of the HSDSC results, since the Tm is diminished, this could suggest that the ordered helix molecules of xanthan are prevented from becoming disordered by the sucrose molecules on heating. This could lead to self-aggregation of the xanthan helix molecules if sucrose enhances polymer-polymer interactions. However, this idea contradicts the findings so far which may suggest that the disordered form of xanthan gum may be necessary or enhances interactions with locust bean gum.

The effect of using fractionated locust bean gum samples with xanthan gum to produce mixed polysaccharide systems, suggest that the mechanism for the interaction is

unchanged, since the temperature range at which the exotherms are detected (40°C-70°C) in Figures 6.14 and 6.15 coincides with the Tm of xanthan gum (Figure 6.5(a)). Since fractionated locust bean gum like the unfractionated form is not known to undergo a transition nor affect the transition of xanthan gum, it is likely that the exotherms observed are due to xanthan gum undergoing a conformational change with temperature. These results confirm the hypothesis for the increased gel strength and decreased gel strengths observed for mixes containing LBG80 and LBG35 respectively in Chapter 4.5.2, in which it was suggested that the differences in gel strength were due to differences in the M:G ratios of locust bean gum, i.e. the unsubstituted regions of the backbone interact with xanthan gum.

The reproducibility of the endothermic peaks in general are poor for the mixtures containing fractionated locust bean gum (LBG35 - Figure 6.14 and LBG80 - Figure 6.15). A possible explanation for the poor reproducibility observed could be due to inhomogeneities in the mixtures, although the M:G ratio results of fractionated locust bean gum from Chapter 3.4.2 (Table 3.4) using both techniques were reproducible and consistent. However this does not explain the good reproducibility of the exothermic peaks observed for the corresponding cooling curves, unless the heating process prior to cooling dissolves the locust bean gum and thus improves the homogeneity of the system.

6.10 BASIC THEORY AND USES OF SYNCHROTRON CIRCULAR DICHROISM IN RELATION TO POLYSACCHARIDES

Circular dichroism (CD) is a non-destructive spectroscopic technique, which measures the absorption of polarised light as a function of wavelength usually within the UV region. It is useful in the determination of secondary structure, conformation and interactions, additionally requiring only small amounts of sample and is ideal for looking at molecules in solution. Moreover, accurate measurements can be obtained when monitoring samples in changing environments such as with temperature, salt or pH. For these reasons, this technique has been exploited for detecting the conformation of xanthan gum with respect to temperature (Morris et al, 1977 and Dea et al, 1977), in which Dea et al (1977) used CD on xanthan gum, since the carboxyl groups and related chromophores are diagnostic of xanthan gum conformation and interaction. A discontinuity at around 60°C was observed on heating, indicative of an order-disorder

transition confirmed by optical rotation measurements. In addition, CD has been used to look at xanthan gum in different salt concentrations and ionic strengths (Milas & Rinaudo, 1979, Dentini et al, 1984 and Bresolin et al, 1998) and has been used to study the aggregation behaviour of xanthan gum (Meyer et al, 1993) plus the interaction between xanthan gum with locust bean gum (Bresolin et al, 1998).

Circular dichroism is a measure of the difference in the absorption of left and right circularly polarized light of an absorbing sample with two different components. This can be expressed using Beer's law in Equation 6.4.

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R = (A_L - A_R)/cl = \Delta A/cl \qquad \text{Equation 6.4}$$

Where:

$\Delta\varepsilon$ = differential molar extinction coefficient

ΔA = differential absorbance between A_L and A_R

c = concentration

A_L = left circularly polarized light

A_R = right circularly polarized light

l = path length

In order for CD to be a applied to a system, the molecules present have to be optically active, which in turn requires that the molecules are not superimposable on their mirror image, a phenomenon otherwise known as chirality. The introduction of an optically active sample into the instrument results in a preferential absorption during one of the polarization periods, which in turn causes variation in the intensity of the transmitted light during the cycle. This variation in light intensity is directly related to the circular dichroism of the sample at a specified wavelength. Detection for a series of different wavelengths leads to the formation of a full CD spectrum. It has been shown in Equation 6.4 that there are two different absorptions for an optically active sample, one for left and one for right circularly polarized light i.e. $A_L = \log_{10}(I_0/I_L)$ and $A_R = \log_{10}(I_0/I_R)$. Since I_0 which represents the initial light intensity upon entering the absorbing sample will always be equal, the index of L and R of I_{0L} and I_{0R} can be dropped. Upon entering the sample, the light intensity will be changed due to sample absorbance, oscillating between left and right circular polarization. The difference in absorbancies is recorded directly as shown in Equation 6.5 which is equal to $\Delta\varepsilon = \Delta A/cl$ in Equation 6.4. Since I_0 does not appear in the final equation, the instruments are of the single beam type, as a reference beam is not required.

$$\Delta A = A_L - A_R = \log_{10}(I_0/I_L) - \log_{10}(I_0/I_R) = \log_{10}(I_R/I_L) \qquad \text{Equation 6.5}$$

The concentration of sample used is very important in CD measurements, since the more concentrated the system, the longer will be the path length and hence the greater will be the CD signal (see Equation 6.4). However, a balance is necessary since as a consequence of using a higher concentration of sample, the greater will be the absorption which results in a lower light throughput and thus increased noise. On the other hand, the greater the light striking the detector (less absorption by the sample) registered by a low voltage on the instrument, the lower the noise (Drake, 1994).

Xanthan gum displays chirality due to the α and β D-mannose residues, which will absorbe left and right hand circular polarised light slightly differently. The difference in left and right handed absorbance (ε_L-ε_R) is very small (usually in the range of 0.0001) which corresponds to an ellipticity of a few 1/100th of a degree. Ellipticity of polarised light arises since the light absorbed by these two components are of different amplitude and phase, and thus the polarised light is non-linear. The absorbance difference of the two components is related to the molecular ellipticity, and is expressed as follows in Equation 6.6.

$$[\theta]=(\varepsilon_L-\varepsilon_R)3300 \qquad \text{Equation 6.6}$$

Where: θ=molecular ellipticity

ε_L and ε_R=molar absorptivity terms for the two components

From a CD spectrum, information regarding an event such as a transition can be correlated to the structure of a sample which can be obtained from the sign and the magnitude of the CD band or peak. This involves theoretical calculations or more simply comparison of the CD of the sample with a sample having an established CD spectrum (Drake, 1994).

The main use of CD has been in protein research, as a means to determining secondary structure and interactions. This is because proteins have a simple set of structural motifs such as α helix and β sheets. However, carbohydrates are more complex having no simple set of structural motifs, often existing as disordered coils (extended or collapsed) or helices in solution which applies to xanthan gum and locust bean gum. The conformation of a carbohydrate can be determined by its multidimensional

potential energy surface in which theoretical models are currently under study (French & Brady, 1990). These energy surfaces are usually expressed as functions of the linkage dihedral angles which can be used as frameworks to provide information for describing helical and disordered conformations of carbohydrates even in the absence of simple structural motifs. In some cases where backbone linkage can not be extracted from experimental data, the CD spectra can be used empirically to provide information (Stevens, 1996). This type of analysis is common to gel forming polysaccharides such as xanthan gum-locust bean gum mixes. In addition, CD is sensitive to the order-disorder transition of some bacterial polysaccharides which is applicable to xanthan gum, in which some workers have used CD to investigate this property of xanthan gum (Morris et al, 1977 and Dentini et al, 1984). Stevens (1996) has suggested that disorder which is reflected in CD variations may reflect a loss in side chain ordering, since branching is common to bacterial polysaccharides, in which xanthan gum has trisaccharide side chains attached to its backbone (Morris et al, 1977). Again, CD is used empirically in these cases as a probe to extracting information on various aspects of conformation, even if the absolute conformation can not be determined.

In terms of locust bean gum, a study by Buffington et al (1980) used CD and showed that galactomannans display a dependence on the M:G ratio, since a negative band at 149nm and a positive band at 169nm increased in intensity systematically with decreasing galactose content. In particular it was found for locust bean gum (low galactose levels – 19%), the CD intensity was high due to conformational restriction of galactose by chain packing, which cancel out the mannose backbone contributions. Analysis by linkage CD contribution was not possible due to lack of data for methyl β-D-mannopyranoside.

Synchrotron circular dichroism (SCD) is a relatively novel technique with only one facility available in the UK at Daresbury laboratory. The difference between SCD and normal CD is the light source used. With SCD, a synchrotron radiation source is used, which is a powerful source of vacuum ultraviolet radiation used for extended wavelengths ($\lambda \leq \sim$ 100nm). This light source is of high intensity and short wavelengths. It is derived from rings of magnets with electrons moving at the speed of light. Energy is lost from these electrons as photons, and a small source of radiation comes off at different angles in the spectroscopic region of X-rays to infrared. The advantage of using this radiation source with CD, is that samples can be analysed at

shorter wavelengths, which is particularly useful when looking at carbohydrates, since most of the structural changes for most carbohydrates occur in the shorter wavelength region. To produce synchrotron radiation, a charged particle must pass through a magnetic field (Winick, 1994), and the direction of the field and velocity of the charged particle are usually exactly perpendicular (not parallel). The force on the charged particle from the magnetic field bends the trajectory of the particle to produce a radial or 'centripetal' acceleration which emits electromagnetic radiation according to Maxwell's equations. For charged particles moving much slower than the speed of light, radiation is emitted in a dipole pattern with finite intensity in all directions except along the radial direction of the accelerating particle. However for SCD, charged particles move at the speed of light, in which the emitted light appears within a narrow cone centred along the direction of the instantaneous velocity of the particle. The resulting radiation is of a fan shaped pattern. Synchrotrons were initially introduced to overcome the limitations of previous accelerators, in which the change in rest mass of a particle as its velocity approaches the speed of light prevents further acceleration. Thus, by increasing the strength of the bending magnets, the charged particles can be obtained in a stable orbit in synchrony with the increasing mass of particles. In order to do this efficiently, storage rings were introduced which inject and accelerate charged particles to the desired energy level. Storage rings can maintain these particles in a stable orbit, ranging from a few hours to a few days. In storage rings, the circulating beam is composed of electrons or positrons as their light weight produces rapid accelerations, hence more synchrotron radiation at shorter wavelengths compared to heavier particles of the same energy. Additionally, the strength of the magnetic field also determines the spectrum of synchrotron radiation, which is produced from the bending magnets and inserted magnets between the bending magnets (wigglers and undulators) which maintain the particles in a closed path circulating around the storage ring. These magnets generate different types of radiation ranging from infrared to x-rays which influence the CD experiments (Sutherland, 1996). In particular, synchrotron radiation is useful in the UV and x-ray spectral regions where conventional sources are of lower intensity.

Two important properties for CD measurements are broad spectral distribution and high intensity. In the present work, synchrotron radiation was used at wavelengths greater than 100nm which are really extensions of conventional CD providing similar information on conformations, although the use of SCD has a greater performance over conventional CD, since the radiation source is more intense. Other advantages of using

synchrotron radiation include shorter data collection time and high quality time-resolved data.

In the current work, SCD was used to determine the conformational behaviour of xanthan gum with respect to temperature, since SCD has not been performed on this particular polysaccharide before. Thus, additional information about the xanthan gum structures may be detected which may have gone unnoticed in previous studies such as Dea et al (1977) using conventional CD. In addition, this technique was also used to determine interactions and any structural changes between xanthan gum and locust bean gum with respect to temperature.

6.11 SCD ANALYSIS OF XANTHAN GUM AND LOCUST BEAN GUM MIXES

This study was performed to investigate the conformational behaviour of the two individual polysaccharides in solution at different temperatures i.e. the non-interacted system, in order to compare to the conformational states of the polysaccharides in the interacted product when the two polymers are mixed together.

6.11.1 Method

Xanthan gum 1%w/w and locust bean gum 1%w/w were prepared at 25°C using the method described in Chapter 2.2.1. The CD spectra were obtained using the circular dichroism facility on station 3.1 of the synchrotron radiation source at CLRC Daresbury Laboratory. This station is designed to operate in the 30 to 500nm wavelength range depending on the grating installed. The switching between left and right polarised light occurs at a frequency of 50 kHz. All parts of the beam path not in vacuum are purged with dry nitrogen gas to eliminate absorption by oxygen.

The instrument was firstly calibrated using (+)-10-camphorsulphonic acid (CSA) 10mg/ml, which has a well known CD spectrum with a negative band at 192nm and a positive band at 290.5nm. The experiment was performed in 0.1mm quartz cells, which gave a CD spectrum containing a minima at 190nm and a maxima at 290nm. This CD spectra can be viewed in Appendix 3. The value for the minima at 190nm was determined (-0.428×10^6) which has no units. This value is divided by $4.9 M^{-1} cm^{-1}$ which

is the known $\Delta\varepsilon$ value for the negative band at 192nm. This then provides the conversion factor of 87346.939 for scaling the CD spectra of the samples to units of ellipticity on the y-axis.

Samples were carefully loaded so as to avoid air bubbles onto 0.1mm quartz cells (Grays cells) using a pipette to eject one drop of sample. These cells were the 165 series which are water jacketed so as to allow temperature control using a circulating water bath for the higher temperature studies. However, for the room temperature studies, the 124 series cells were used since these cells are ideal for looking at very viscous samples.

The temperatures investigated were 25°C, 55°C and 90°C for the polysaccharide mixes. Experiments were performed in triplicate for each sample, with a CD spectrum of water (blank) carried out in between samples which were subtracted from the sample spectra to obtain accurate measurements. The wavelength region investigated was initially between 160nm to 300nm to ensure that any changes within the samples would not go undetected. Since no changes were occurring until the 175nm region, the scanning region was then changed to 175nm to 300nm to save experimental time.

6.11.2 Results

The CD spectrum for xanthan gum 1%w/w at 25°C is displayed in Figure 6.16, which shows a maximum just below 180nm and a broad minimum at around 225nm. In contrast, the CD spectrum for locust bean gum 1%w/w is displayed in Figure 6.17, which exhibits a maximum at a shorter wavelength compared to xanthan gum, followed by a very broad minimum which occupies most of the wavelength region investigated. Obviously the differences between the two CD spectra suggest that the structures of the two polysaccharides are very different to one another. The position of the peaks on the CD spectrum for xanthan gum may suggest a helical structure, whereas the shape of the CD spectrum for locust bean gum may indicate a disordered coil.

On heating xanthan gum 1%w/w, the CD spectrum is very different to unheated xanthan gum 1%w/w prepared at 25°C. This can be shown in Figure 6.18 which shows the CD spectra for xanthan gum 1%w/w after heating and cooling between 25°C-55°C and 25°C-90°C compared to an unheated solution prepared at 25°C. Both spectra change

for the heated and cooled xanthan gum compared to an unheated solution, but the changes seem to be greater on heating from 25°C-90°C compared to heating from 25°C-55°C. In addition, the sign of the peak at short wavelength changes from a positive peak at 25°C and 55°C to a negative peak at 90°C, and any changes do not seem to be reversed on cooling, particularly for the polysaccharide at 90°C since the cooled spectra in Figure 6.18 are different to xanthan gum 1%w/w at 25°C (i.e. no heating). In contrast, the spectra for locust bean gum 1%w/w on heating and cooling between 25°C-55°C and 25°C-90°C shown in Figure 6.19 remains unchanged compared to locust bean gum 1%w/w prepared at 25°C (unheated).

6.12 AN INVESTGATION INTO XANTHAN GUM-LOCUST BEAN GUM (5:5 RATIO) STRUCTURE AND INTERACTIONS USING SCD

The present study was used to explore the synergy between xanthan gum and locust bean gum in relation to polysaccharide structure and conformational changes if any. SCD was used as a complimentary technique with the HSDSC studies, to investigate the mechanism for the interaction with regards to determining xanthan gum structure and behaviour at different temperatures in the mixed polysaccharide systems.

6.12.1 Method

Xanthan gum-locust bean gum (5:5) ratio 1%w/w mixes were prepared at 25°C using the method described in Chapter 2.2.1. The SCD calibration and experimental methods used were identical to that explained in Chapter 6.11.1. The mixed polysaccharide systems were subjected to the same experimental temperatures i.e. 25°C, 55°C and 90°C, in order to compare the spectra to the spectra obtained for the individual polysaccharide mixes in Chapter 6.11.2.

6.12.2 Results

The xanthan gum-locust bean gum (5:5) ratio 1%w/w mixture at 25°C shows the maximum at the same wavelength observed for xanthan gum 1%w/w, except that the minimum is suppressed (see Figure 6.20). If this is compared to the added spectra of

the individual polysaccharides which show no structural changes due to no interactions between the two polysaccharides (same graph), the spectra are different, which reflects changes in structure after mixing the two polysaccharides together.

The data displayed in the CD spectra for xanthan gum-locust bean gum (5:5) ratio 1%w/w mixtures heated and cooled between 25°C-55°C and 25°C-90°C shown in Figure 6.21, provide strong evidence that structural changes occur on mixing the two polysaccharides together. This is because, the CD spectrum for xanthan gum-locust bean gum (5:5) ratio 1%w/w mixtures heated to 55°C compared to the mixture prepared at 25°C are very different in appearance, with the maximum observed at the short wavelength for the mixture prepared at 25°C changing to a minimum for the mixture heated to 55°C. On cooling the mixture at 55°C to 25°C, the spectrum in the 220nm-300nm region reverses to that observed for the unheated mixture at 25°C, except that the minimum observed in the short wavelength region remains. Similar changes are observed after heating and cooling mixtures to 90°C, except that the cooled spectrum is completely different to the spectrum for unheated mixture at 25°C. In addition, a positive to negative change in the peak at short wavelengths (180nm) is observed for the mixture at 55°C but not for the added spectra of the individual polysaccharides at the same temperature as observed in Figure 6.22. Likewise, similar changes are observed for the mixture at 90°C, but to a lesser extent (see Figure 6.23).

The mixture for xanthan gum-locust bean gum (5:5) ratio heated to 55°C has a similar spectrum to xanthan gum 1%w/w heated to 90°C (Figure 6.24), although the minimum is deeper for xanthan gum alone. This may indicate that most of the changes observed for the mixed system are due to conformational changes of xanthan gum and not locust bean gum which remains unchanged on heating, since the CD spectra are virtually identical regardless of the temperature (Figure 6.19).

6.13 DISCUSSION

The CD spectrum for xanthan gum 1%w/w at 25°C suggests a helical structure, indicated by the position of the maximum and minimum peaks on the spectrum (Figure 6.16). These values for the helix structure are similarly in agreement with the findings from the CD studies by Milas & Rinaudo (1979) and Dentini et al (1984). In contrast,

FIG. 6.16 : CD SPECTRUM FOR XANTHAN GUM 1%w/w AT 25°C

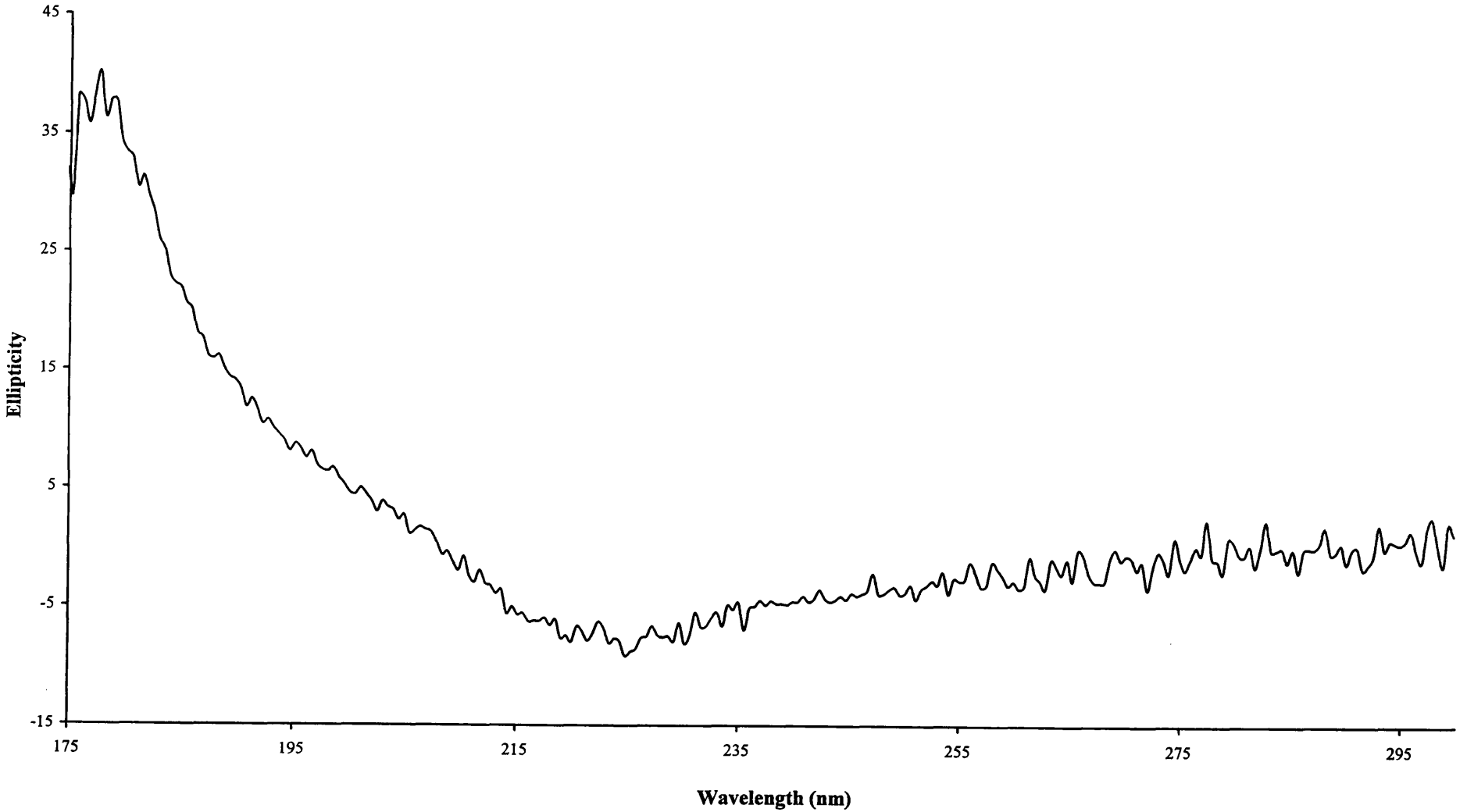


FIG. 6.17 : CD SPECTUM FOR LOCUST BEAN GUM 1%w/w AT 25°C

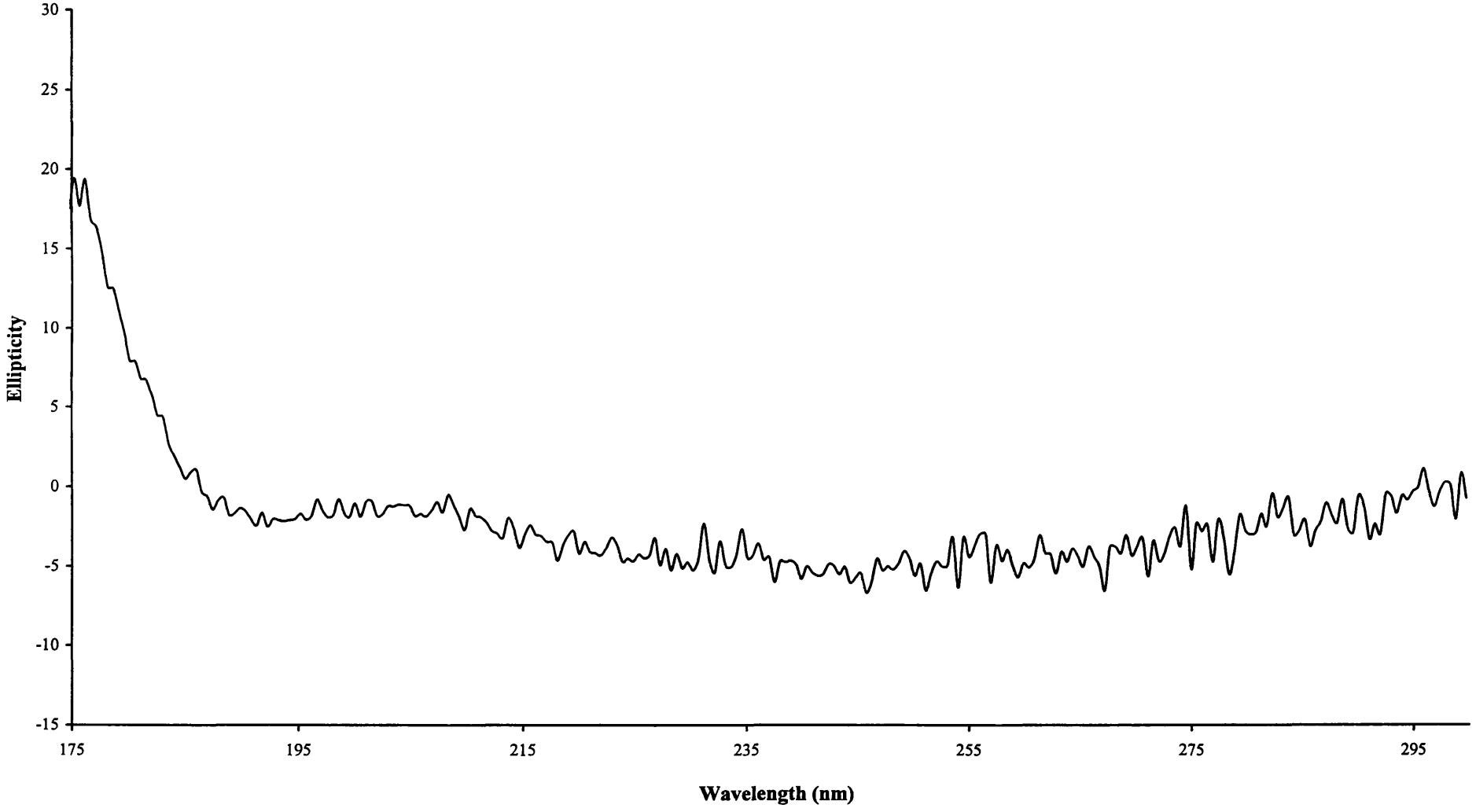


FIG. 6.18 : CD SPECTRA FOR XANTHAN GUM 1%w/w AT DIFFERENT TEMPERATURES

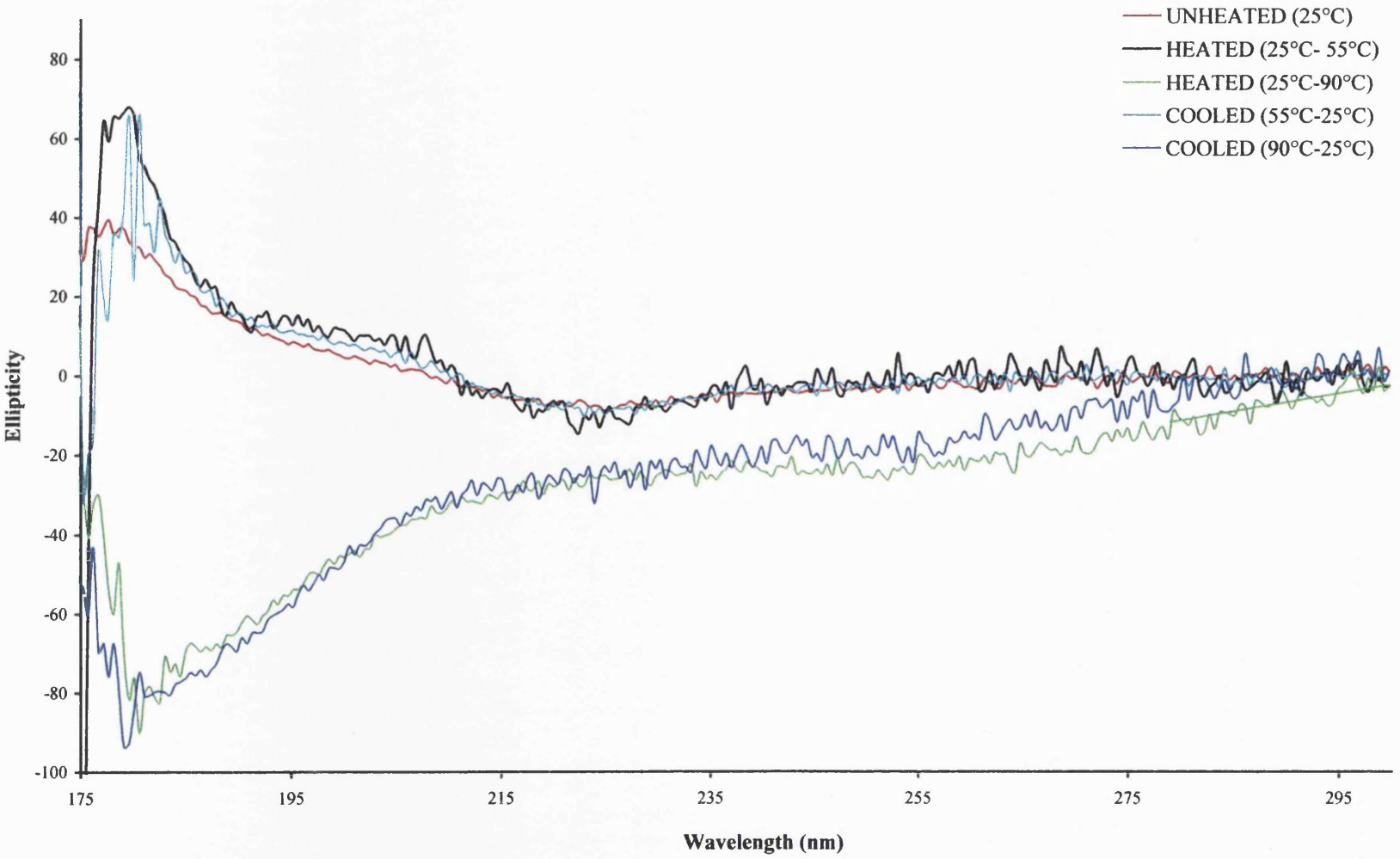


FIG. 6.19 : CD SPECTRA FOR LOCUST BEAN GUM 1%w/w AT DIFFERENT TEMPERATURES

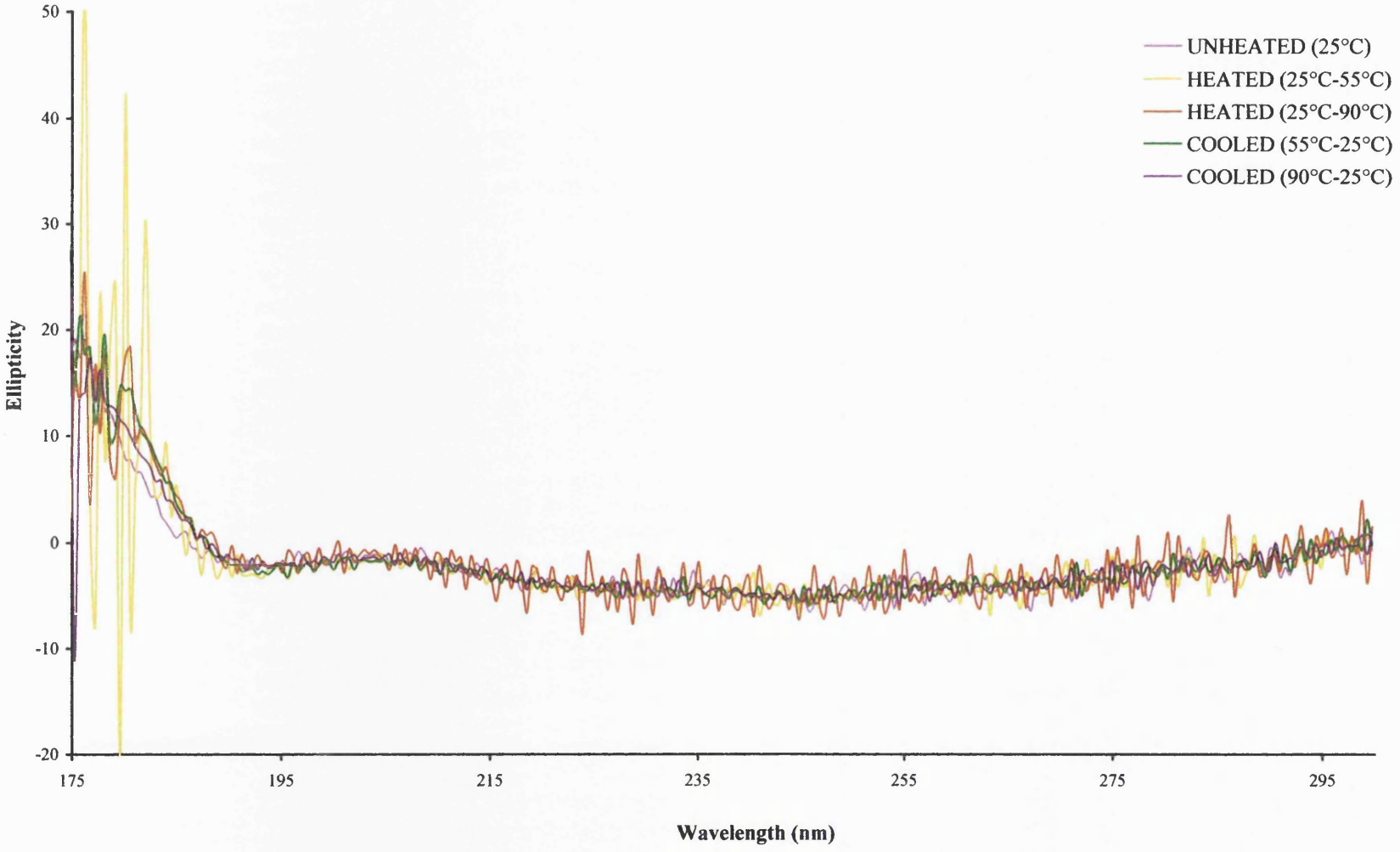


FIG. 6.20 : CD SPECTRA FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) RATIO 1%w/w MIXTURES AND XANTHAN GUM 1%w/w AT 25°C

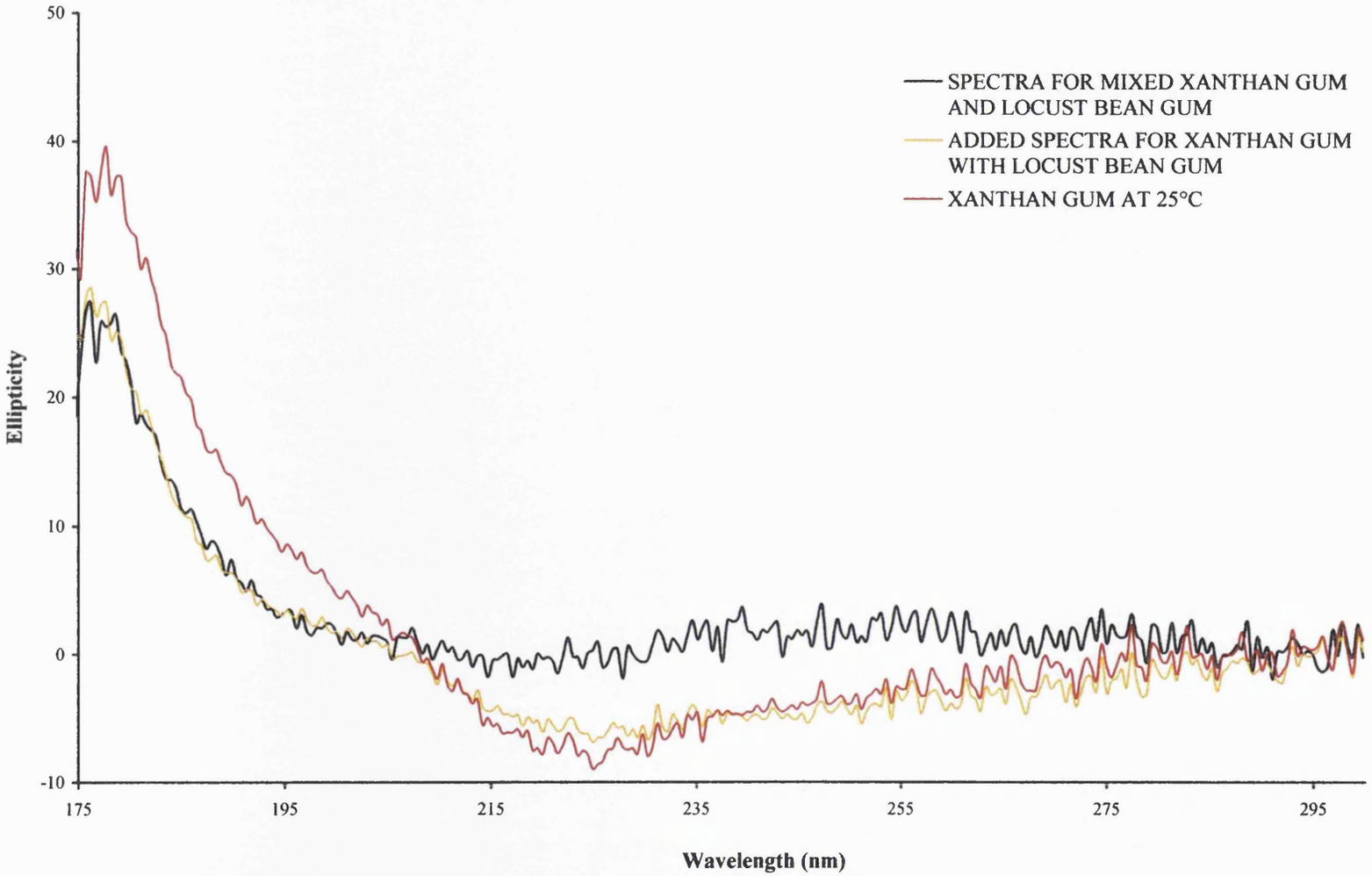


FIG. 6.21 : CD SPECTRA COMPARING XANTHAN GUM-LOCUST BEAN GUM (5:5) RATIO 1%w/w MIXTURES AT DIFFERENT TEMPERATURES

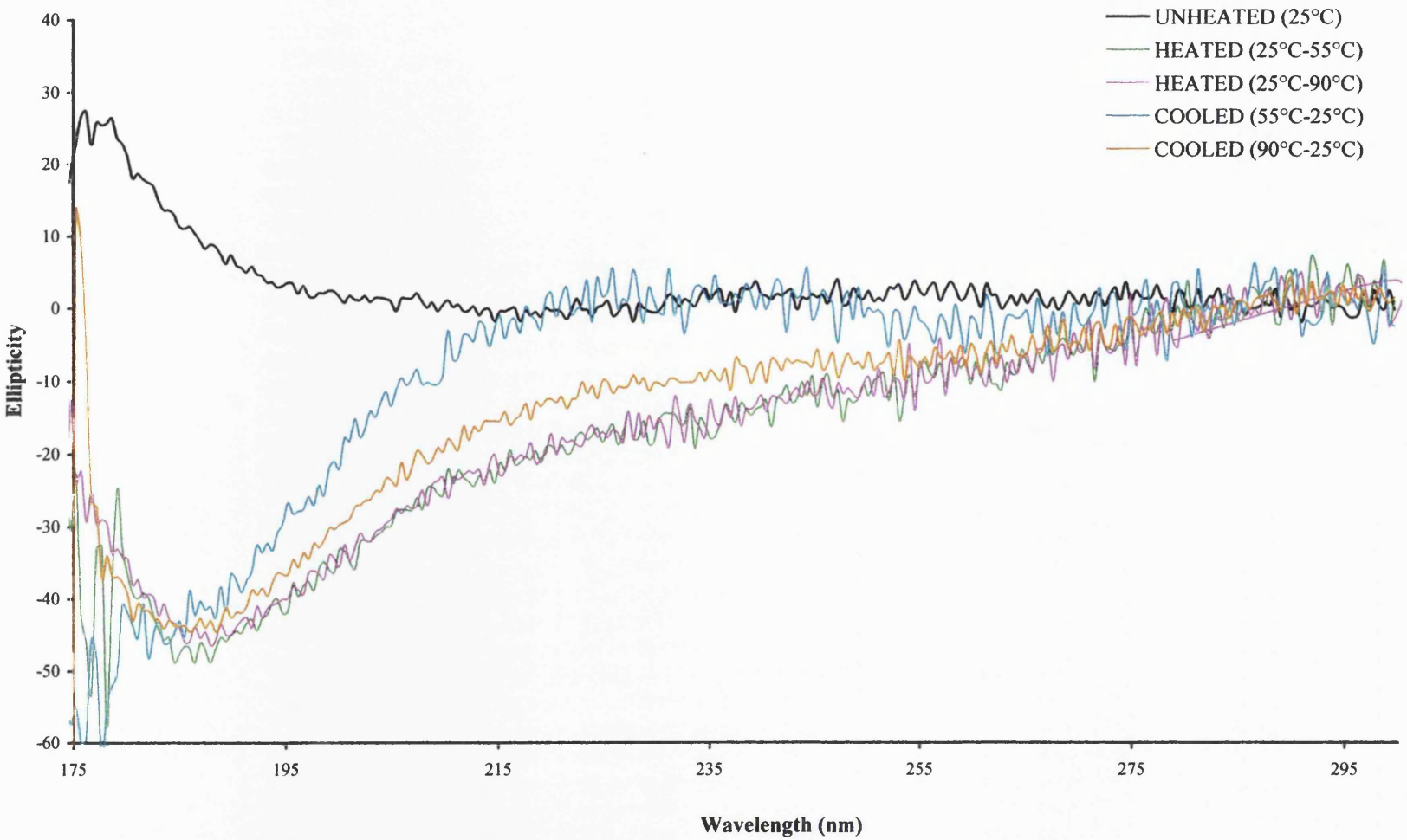


FIG. 6.22 : CD SPECTRA FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) RATIO 1%w/w MIXTURES AT 55°C

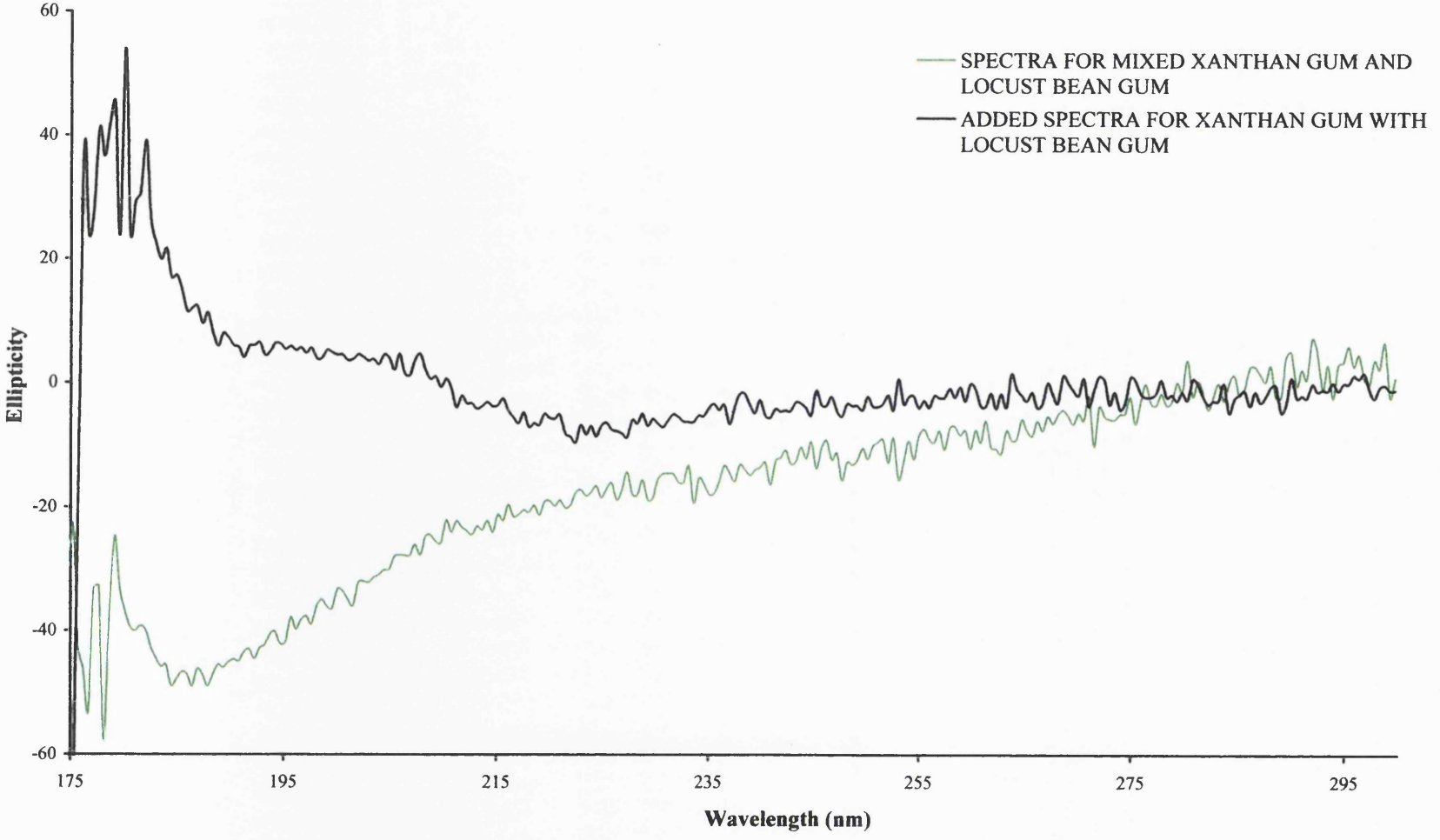


FIG. 6.23 : CD SPECTRA FOR XANTHAN GUM-LOCUST BEAN GUM (5:5) RATIO 1%w/w MIXTURES AT 90°C

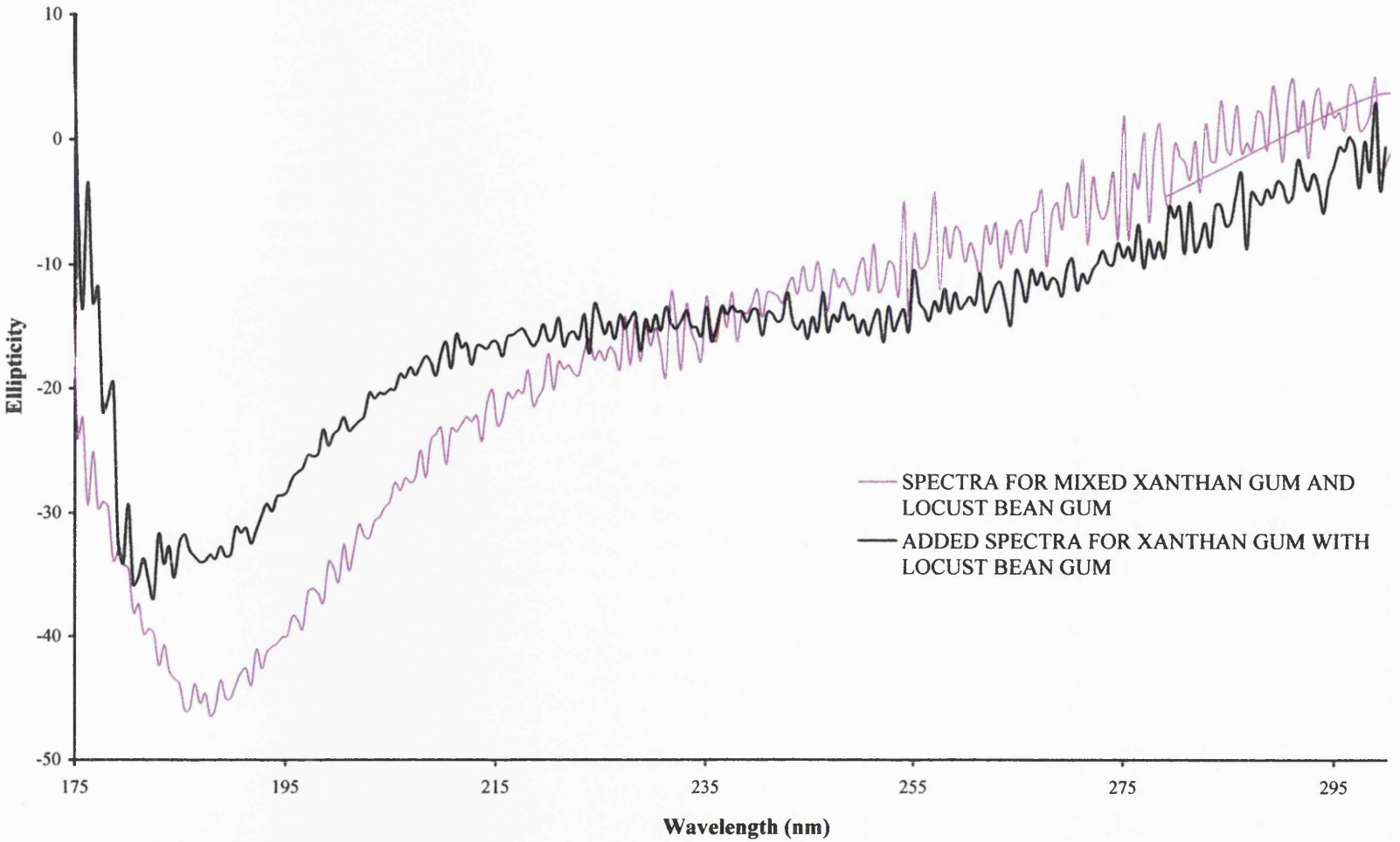
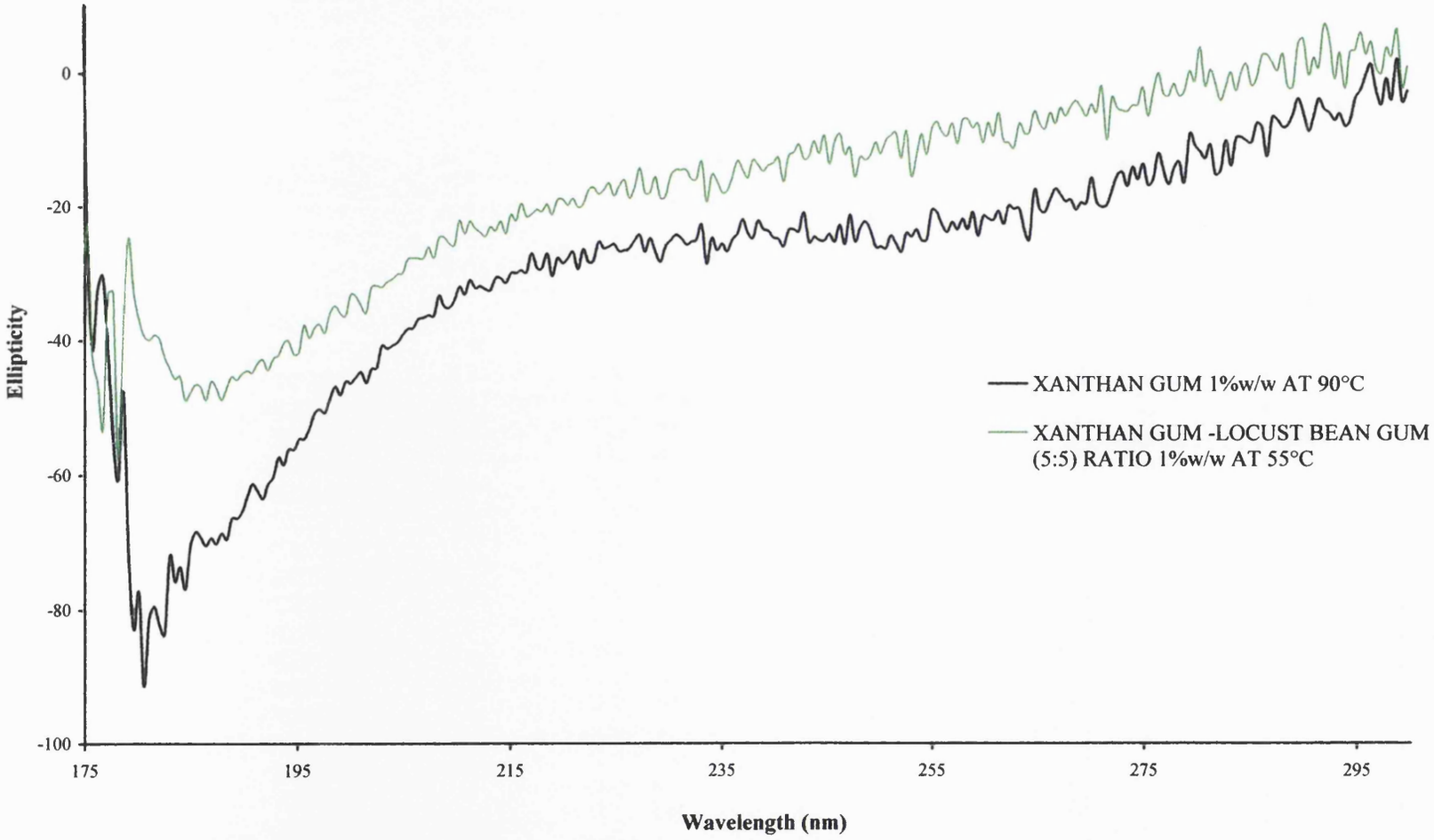


FIG. 6.24 : CD SPECTRA COMPARING XANTHAN GUM 1%w/w AT 90°C WITH XANTHAN GUM-LOCUST BEAN GUM (5:5) RATIO 1%w/w AT 55°C



the breadth of the minimum on the spectrum for locust bean gum 1%w/w (Figure 6.17) suggests a disordered structure. Both findings are in agreement with the literature.

On heating xanthan gum 1%w/w, the spectrum at 90°C reflects strong structural changes, since the spectrum is very different to xanthan gum 1%w/w at 25°C, in which a negative band at around 180nm with a slow return to zero is observed for xanthan gum 1%w/w heated to 90°C (Figure 6.18). In protein CD analysis, this observation is characteristic of a random coil, however it can not be assumed that this is certainly the case for xanthan gum since it is a polysaccharide, not a protein. Similar changes are observed for heating xanthan gum to 55°C, although these changes appear to be much smaller in comparison to heating the system to 90°C (i.e. the spectrum for xanthan gum at 55°C still bears some resemblences to the spectrum for xanthan gum 1%w/w at 25°C).

The differences in the signs of the peaks at 55°C (positive) and 90°C (negative) at the short wavelength region may indicate a change in the handedness of the helix, which is an indication of a change in direction of helix 'twist'. In addition, the cooled spectrum for xanthan gum 1%w/w at 90°C is similar to the spectrum prior to cooling, which indicates that the structural change is not reversible. These changes are also observed at 55°C but to a smaller extent. This contradicts the reversible nature of the helix to coil transition of xanthan gum found from the earlier findings in the present work (Chapter 4 – Return to approximate original G′ value on heating and cooling xanthan gum and Chapter 6 – 'mirror image' of peaks on heating and cooling xanthan gum) and the literature (Morris et al, 1977). However, perhaps the reversibility in the transition with temperature was not observed in the present study, because the samples in the cooled states were not left long enough for the structures to recover to the original structures before the CD measurements were take. This may also explain why the changes observed in contrast to the unheated mixtures at 25°C are smaller for xanthan gum 1%w/w at 55°C compared to at 90°C. In comparison, the spectra for locust bean gum 1%w/w are almost identical at all temperatures investigated (25°C, 55°C and 90°C), which suggests that locust bean gum shows no structure change with temperature (Figure 6.19). This is an expected finding, since locust bean gum is not known to undergo a transitional change in conformation with temperature.

The findings for xanthan gum-locust bean gum (5:5) ratio 1%w/w mixtures at different temperatures (25°C (Figure 6.20), 55°C (Figure 6.22) and 90°C (Figure 6.23) when compared to the added spectra of the individual polysaccharides (i.e. no interaction) strongly suggest that an interaction occurs between these two polysaccharides at all temperatures studied, since the spectra are different, suggesting that the structure of one or both of the polysaccharides changes. From the results (Figure 6.24), it is likely that the changes which occur in the mixture are due to conformational changes of xanthan gum, since it has been determined that locust bean gum does not change structure with temperature. The interaction appears to have most effect at 55°C, since the spectrum for the mixed polysaccharides compared to the added spectrum of the individual polysaccharides at this temperature are most different, in which there is a positive to negative change in the peak at short wavelength (Figure 6.22). From the results, it can be suggested that a transition occurs above 55°C and below 90°C. The exact transition temperature can not be determined from these experiments since there are not enough data points in order to do this. However, the results do correlate with the findings from HSDSC experiments in the present work in Chapter 6.2.2 (Tm = 45°C-75°C) and texture analysis studies (Chapter 5.4.3) which showed that above 60°C, there was no further increase in the gel strength of xanthan gum-locust bean gum (5:5) ratio 1%w/w mixtures, probably due to maximum synergy occurring. This may suggest that the disordered form of xanthan gum is necessary for the interaction or increases the extent of the interaction with locust bean gum, which is most abundant at the Tm value. In addition, the findings suggest that the xanthan gum structure changes when it is in the mixture, but not very much on its own at 55°C (compare xanthan gum 1%w/w at 55°C to at 25°C in Figure 6.18). This finding is supported by Bresolin et al (1998).

On cooling the mixtures from 55°C and 90°C, the original structures are not restored (i.e. the cooled spectra are different to xanthan gum-locust bean gum (5:5) ratio 1%w/w at 25°C in Figure 6.21). However, there may be a partial reversal to the original structure for the mixture cooled from 55°C, indicated by the similarity in spectra between the mixture at 25°C and the mixture at 55°C in the 200nm-300nm wavelength region, although the spectra are very different in the short wavelength region. However this is not the case for mixtures at 90°C, in which the spectrum is different to the mixture at 25°C at all wavelengths studied, indicating that the changes are permanent.

CHAPTER 7

CHAPTER 7 : WET GRANULATION AND TABLETTING STUDIES

7.1 INTRODUCTION

The synergy between xanthan gum and locust bean gum has been exploited pharmaceutically for controlled release properties, since the synergistic product resulting in a gel can be used to retard drug release. The TIMERx system is one such pharmaceutical product as discussed in detail in Chapter 1.2.6, in which a 1:1 polysaccharide ratio gives an optimum slow release granulation (Baichwal et al, 1991). The system is produced by a wet granulation process, since it was discovered that an aqueous environment was necessary in order for locust bean gum to cross link xanthan gum to form the matrix.

A similar product which utilises the xanthan gum-locust bean gum synergy is Buccastem (Sugden, 1986) which is also discussed in Chapter 1.2.6. During the invention of this buccal dosage form, it was found that by using sucrose as the filler led to an improvement in the gelling and disintegration properties of the tablet. The effect of sucrose on xanthan gum-locust bean gum interactions has not been extensively studied pharmaceutically, so the reasons for these effects are not clear.

In the present work, it has been observed that a synergy between xanthan gum and locust bean gum occurs in aqueous solution, which is affected by temperature, the polysaccharide ratio and additives (sodium chloride and sucrose). Consolidating the results has shown that the interaction between xanthan gum and locust bean gum may occur at room temperature, although the gels formed are much weaker compared to heating followed by cooling the systems between 90°C and 20°C (from oscillatory rheology and texture analysis experiments). However, SCD strongly supports that the interaction does occur at room temperature but has most effect at 55°C in which new structures are formed. In addition, texture analysis studies has clearly shown that maximum gel strength occurs at 60°C, in which a 1:1 polysaccharide ratio is the optimum ratio at which maximum synergy is observed.

HSDSC identified that 55°C to 60°C lies well within the transition temperature of xanthan gum, suggesting that the disordered form of xanthan gum may be necessary or enhances the interaction with locust bean gum. It was also observed that the presence of

sucrose greatly increased the gel strength of xanthan gum-locust bean gum mixes, as shown from the oscillatory rheology experiments.

In the current work, the main objective of the tabletting studies is to utilise the findings from the different techniques applied so far, to produce pharmaceutical applications based on the xanthan gum-locust bean gum synergy. A wet granulation method was chosen in accordance with that employed for Buccastem and TIMERx. The method devised was a modification of that used for Buccastem. Different variables were investigated to try to control the drug retarding properties of these matrix systems, since different drugs require different dosing times. The variables investigated were the drying temperature of the granules, since it has been found throughout the present work that temperature greatly affects the synergy and hence gel strength of these mixed polysaccharide systems. The xanthan gum-locust bean gum concentration was also investigated, since it is usual that the more gum present, the more likely drug will be retarded, although this may cause problems if excess swelling occurs. The filler type (lactose or sucrose) was also studied, since it has been observed that sucrose increases the gel strength and may diminish or possibly shift the Tm of xanthan gum (from oscillatory rheology and HSDSC studies respectively) and was the preferred choice of filler for Buccastem. The wet granulation method was also modified to gain an insight into the mechanism for the interaction, and as a means to regulating the controlled release properties of the matrices, since the controlled release properties of the TIMERx system can be modified by varying the ratio of drug:gum concentration which also depends on the solubility of the drug. Finally, kinetic determinations for drug release with respect to the order of the reaction and the rate constant were investigated, since it has been found from the TIMERx system that drug release is zero-order, indicating that drug is released at a constant rate independent of the initial concentration.

7.1.1 Formulation

The granule batch sizes were 500g in all cases, except for granules prepared according to method 2 detailed in Chapter 7.1.2.2 due to experimental impracticability (250g and 200g batch sizes for granules produced from polysaccharide powders recovered from 1%w/w mixtures prepared at 35°C and 90°C respectively).

Below is the formulation chosen for the tabletting studies, which is based on the formulation for Buccastem. Details of the materials chosen and batch numbers are described in Chapter 2.

FORMULATION

1. Polysaccharide gums - Xanthan gum : locust bean gum (1:1 ratio)
2. Binder (5% of total granule batch size) – Polyvinylpyrrolidone (PVP K30)
3. Model drug (5% of total granule batch size) – Theophylline
4. Lubricant (0.5% of granule batch size after sieving) – Magnesium stearate
5. Glidant (1% of granule batch size after sieving) – Talc
6. Filler – Sucrose/lactose

Since different variables were investigated in the present work, a summary of the formulation codes relating to these variables is included in this section (Table 7.1), which will become apparent later on in Chapter 7.2 (results).

TABLE 7.1 : FORMULATION CODES

CODE	% DRUG	% GUM	*FILLER TYPE	% FLUID ADDED	DRYING T°C	DRYING TIME (MIN)
CAL01	0	5	S	5	35	55
CAL02	5	5	S	5	35	55
CAL03	5	5	S	5	90	15
CAL04	5	5	S	5	55	30
CAL05	5	20	S	6	35	65
CAL06	5	20	S	6	55	35
CAL07	5	20	S	6	90	20
CAL08	5	10	S	5.6	35	75
CAL09	5	10	S	5.6	55	35
CAL10	5	10	S	5.6	90	15
CAL11	5	20	L	14	35	210
CAL12	5	20	L	14	55	80
CAL13	5	20	L	14	90	30
CAL14	5	20	S:L	9	35	112
CAL15	5	20	S:L	9	55	51
CAL16	5	20	S:L	9	90	23
CAL17	5	0	S	5	55	41
CAL18	5	20	L	$14F_1$	35	225
CAL19	5	20	L	$14F_2$	35	225
CAL20	5	$20R_1$	L	16	35	180
CAL21	5	$20R_2$	L	17	35	185

* S=Sucrose L=Lactose S:L=Sucrose:Lactose 1:1Ratio

F_1=XG:LBG 1:1 ratio at 35°C as granulating fluid F_2=XG:LBG 1:1 ratio at 90°C as granulating fluid

R_1=Recovered powder (1%w/w XG:LBG 1:1 ratio solution at 35°C)

R_2=Recovered powder (1%w/w XG:LBG 1:1 ratio solution at 90°C)

7.1.2 Wet granulation methods

7.1.2.1 Method 1 : Modification of the standard method for Buccastem

The method used to formulate tablet dosage forms was a wet granulation method similar to that used for Buccastem. A wet granulation process was chosen over direct compression, since an aqueous environment is thought to be necessary in order for the polysaccharides to interact (Baichwal et al, 1991) and was the preferred method of choice for Buccastem (Sugden et al, 1986). The method used to produce the granules consisted of two steps namely 1. Dry blending and 2. Granulating which are detailed as follows. Formulations based on this method were CAL01-CAL17.

1. Dry blending

All powders in the formulation detailed in Chapter 7.1.1 were weighed, followed by dry powder blending (except for magnesium stearate and talc) in a Turbula mixer type T2C, Glenn Creston Ltd U.K. for 5 minutes to ensure powder homogeneity before the granulating stage.

2. Granulating

The dry blended powders were transferred to a Magimixer cuisine system 3000, France for further powder blending (2 minutes) followed by massing. After dry blending for 2 minutes, a 20ml syringe was used to slowly drip deionised water via the inlet whilst the powders were still being mixed. The volume of granulating fluid used depended upon achieving the desired granule texture, which in turn varied according to the formulation (see formulation codes in Table 7.1). After the desired granule texture was achieved, the granules were mixed for a further 3 minutes to ensure granule homogeneity.

7.1.2.2 Method 2 : Adaptation to Method 1 – The use of recovered polysaccharide powder from 1%w/w polysaccharide solutions prepared at 35°C and 90°C

This method was employed to investigate the effects of forming the interaction between xanthan gum and locust bean gum in 1%w/w aqueous solution, prior to the granulation stage with the excipients in Chapter 7.1.2.1. Two different mixing temperatures were chosen for comparison, one below the Tm of xanthan gum (35°C) and one above the Tm of xanthan gum (90°C). The 1%w/w solutions were dried to constant weight and the powder form was recovered by milling as described below. This powder was then

used in the formulation (Chapter 7.1.1) which was processed into granules (batch size 250g for granules prepared using recovered polysaccharide powder from a 1%w/w mixture prepared at 35°C and 200g for granules prepared using recovered polysaccharide powder from a 1%w/w mixture prepared at 90°C) as described in Chapter 7.1.2.1 (dry blending and granulating). Formulations based on this method were CAL20 and CAL21.

1. Preparation of xanthan gum-locust bean gum (1:1 ratio) mixtures 1%w/w

Polysaccharide powders were dry blended in equal quantities (120g each) in a Turbula mixer type T2C, Glenn Creston Ltd U.K. for 15 minutes to ensure uniform mixing. The powders were subsequently used to make 1%w/w solutions in 0.15%w/w methylparaben solution, mixed at either 35°C or 90°C using a water bath for 30 minutes (mechanical stirring at 1150rpm). Since approximately 50g of recovered polysaccharide powder was required for each formulation (20% gum concentration; 250g and 200g granule batch sizes), the total volume required for each 1%w/w polysaccharide solution was 5000ml. Therefore 6000ml of a 1%w/w polysaccharide solution was made for each formulation to allow for excess. To ensure that the mixing process was adequate, the total volume of 6000ml of methylparaben solution 0.15%w/w, was divided equally into four portions (1500ml divided into 4x2000ml beakers) before slowly adding 15g of polysaccharide powder to each beaker with mixing at the designated temperature. Note that methylparaben was added as an antimicrobial preservative, since it is most effective against moulds and yeasts which may precipitate during long drying periods at 35°C, which were necessary in order to recover the polysaccharide powders (see Chapter 7.1.3). Since methylparaben is poorly soluble, the solutions were sonicated in a 60°C water bath until all powder had dissolved in the deionised water, using two separate volumetric flasks (5000ml and 1000ml). A concentration of 0.15%w/w was chosen for antimicrobial activity in accordance with that described in the Pharmaceutical Codex, (1994).

After 30 minutes of mixing, the polysaccharide solutions were poured equally into three pre-weighed empty trays, which were covered with aluminium foil and left for 24 hours at 25°C before drying to constant weight at 35°C (see Chapter 7.1.3.2 for details).

2. Drying 1%w/w polysaccharide solutions

This protocol was necessary as part of the powder recovery process, and is described in Chapter 7.1.3.2.

3. Milling

Milling was necessary for the final stages of polysaccharide powder recovery, so that the powder could be incorporated into the formulation described in Chapter 7.1.1. Since the material after drying was very elastic and rubbery, a Retsch autocentrifugal mill type ZM1, Glen Creston U.K. was used with a 0.5mm mesh size attached, in which the material was passed through. The yields obtained were 48.9g and 38.4g for powders recovered from 1%w/w solutions prepared at 35°C and 90°C respectively.

4. Dry blending

This method was the same as that detailed in Chapter 7.1.2.1.

5. Granulating

The method employed is described in Chapter 7.1.2.1.

7.1.2.3 Method 3 : Adaptation to Method 1 – Replacing deionised water as the granulating fluid with a solution of xanthan gum-locust bean gum (1:1 ratio) 1%w/w, prepared at 35°C and 90°C

This method was used to study the behaviour of the interacted gums in the granulating fluid (aqueous system) with the non-interacted gums in the dry state in the formulation as shown in Chapter 7.1.1. Formulations based on this method were CAL18 and CAL19.

1. Preparation of the granulating fluid

Two separate 1%w/w solutions of xanthan gum-locust bean gum 1:1 ratio were prepared at 35°C and 90°C as described in Chapter 2.2.1 (total volume of 200ml), except that the solutions were not left at 8°C for 24 hrs prior to adding to the dry blended powders.

2. Dry blending

This method was the same as that detailed in Chapter 7.1.2.1.

3. Granulating

The method employed is described in Chapter 7.1.2.1, except that xanthan gum-locust bean gum (1:1 ratio) 1%w/w replaced deionised water as the granulating fluid. These solutions were immediately added after mixing at the designated temperatures to the dry powders. This was to ensure that the solutions would not have time to cool and solidify, particularly in the case for the solution prepared at 90°C, in which it has been shown from previous chapters that these mixes form strong gels on cooling.

7.1.3 Drying protocols

7.1.3.1 Granule drying

The granules were dried after wet granulating to 1% to 3% moisture content retained, as in accordance with Buccastem. This could be calculated from knowing the volume of granulating fluid used in the wet granulation stage (see Table 7.1 for details).

Granule batch sizes of 500g were divided into approximately two equal portions and transferred to two pre-weighed pyrex trays (1) to allow efficient drying. Since the batch sizes for granules produced using the wet granulation method described in Chapter 7.1.2.2 were 250g and 200g, it was not necessary to divide the granules equally into two trays to ensure efficient drying. The granules were spread out evenly and thinly in the trays and re-weighed (2). The trays were placed in a cross flow circulating air oven on two different shelves at the designated drying temperature (35°C, 55°C or 90°C) shown in Table 7.1. Since the oven used was fan assisted, the heat distribution throughout the oven was equal.

The trays containing the granules were taken out at different time intervals depending on the drying temperature, which were covered and allowed to cool for 10 minutes at room temperature before re-weighing (3). The granules were turned and replaced in the oven for a further time period. This procedure was repeated until 1% to 3% moisture content was retained, which could be determined by subtracting the mass of the tray from the final mass of the tray plus granules i.e. (3)-(1).

To ensure that the 1% to 3% moisture content retained was due to the granulating fluid remaining in the granules, loss on drying determinations were performed as described in Chapter 7.1.3.3.

7.1.3.2 Recovery of polysaccharide powders by drying from 1%w/w solutions

A drying process was necessary for the wet granulation method 2 described in Chapter 7.1.2.2, in order to recover the dry material which could be milled to obtain powders for subsequent granulation.

The 1%w/w polysaccharide solutions were each divided into three pre-weighed stainless steel trays and weighed. These trays were then placed in a cross flow circulating air oven at 35°C and taken out at different time intervals for re-weighing, after leaving the material covered for 10 minutes to allow for cooling before determining the percentage water loss. This process was repeated until constant weight was achieved (sixteen days). However, in the case for the 1%w/w polysaccharide solution prepared at 90°C, the drying process was very slow, so after drying in the cross flow circulating oven for sixteen days, the material was transferred into a vacuum oven to drive off the remaining water. Since there was a lot of material to dry, the gel was cut into small pieces and separated into two halves in which each half was approximately divided equally and transferred to two pre-weighed pyrex trays. The trays were placed in a pre-calibrated vacuum oven (Laboratory thermal equipment Qualivac) consisting of a JAVAC Model DDC 75 vacuum pump at 35°C/10mmHg. The trays were taken out at different time intervals for re-weighing, after leaving the material covered for 10 minutes to allow for cooling before determining the masses. This process was repeated until constant weight was achieved (two days). As constant weight was approaching, the materials were transferred to lighter trays to obtain more accurate weightings.

7.1.3.3 Loss on drying determinations

This protocol was necessary to ensure that the granules formed containing 1-3% moisture content after drying were due to the granulating fluid. Representative formulations were chosen for this study namely CAL01, CAL03, CAL06, CAL11, CAL17, CAL19 and CAL20 (see Table 7.1 for formulation details). Determinations were carried out in duplicate for each sample.

Fourteen tins with lids were cleaned, labelled and dried in a vacuum oven (Haraeus series 500) for 60 minutes at 60°C/26mmHg. Each tin and lid were removed from the oven after 60 minutes and allowed to cool for 10 minutes before weighing accurately

using a four-place balance. Approximately 2g of sample weighed accurately was added to each tin and spread evenly, followed by re-weighing with the lid replaced. Each tin was placed in the vacuum oven at 60°C/26mmHg without a lid for 4 hours (same determination used for Buccastem). The samples were then removed after 4 hours, allowed to cool before re-weighing with the lids replaced, to prevent moisture content changes. The samples were put back in the oven for 1 hour more and the same process was repeated, to ensure constant weight was achieved. The percentage weight changes were calculated after 5 hours of drying under vacuum as follows (Equation 7.1);

Percentage weight change = [Change in sample weight/initial sample weight]100

Equation 7.1

7.1.4 Granule preparation for tabletting

The dried granules were sieved to establish a uniform particle size distribution for subsequent tabletting. The granules were gently hand-sieved using an 850μm brass sieve with steel meshing (non-analytical) to ensure that the granules remained intact. Granules with particle sizes of 850μm and below were retained for tabletting. These yields were higher for granules containing lactose as filler compared to using sucrose, since it was more difficult to form granules of the same texture for the former. Particle size analysis was necessary to determine these distributions which were performed using a Malvern Master Sizer S, as large variations in particle size could affect the flow properties during tabletting and the drug release rates during dissolution studies. The Malvern technique measures particle size in relation to light scattering from these particles, since large particles will scatter light at smaller angles compared to small particles. The principles of the instrument measure the sphere of an equivalent volume which is the diameter of a sphere which would produce the same scattering intensity as the particle.

The addition of magnesium stearate (0.5%=1.5g) and talc (1%=3g) were calculated for a granule batch size of 300g, which were mixed with the granules (295.5g) in a Turbula mixer type T2C, Glenn Creston Ltd U.K. for 3 minutes before tabletting.

7.1.5 The tabletting process

An F3 tablet press, Manesty machines Ltd, Liverpool was used to automatically produce tablets (200mg±5%) from the final granules. Bevel edged shape punches of 7mm diameter were used to manufacture 50-100 tablets of between 4.7-10.5kp hardness (non-B.P standards), using a CT40 tablet strength tester, I. Holland Ltd, Long Eaton U.K. initially during tablet manufacture. In many of the formulations, it was necessary to check the tablet weight and hardness at different stages during the tabletting process after the initial adjustments. This was because the physical properties of the granules tended to change during tablet manufacture, accounting for the wide range of hardness values in particular (see Table 7.4).

An Erweka Multi check PhS002 was used to determine the average tablet weight, hardness, diameter and thickness after tabletting from 10 tablets for each formulation.

7.1.6 Dissolution studies

The dissolution tests were based on USP method II, using manual sampling over automated, because the filters needed frequent replacing due to blockage by the gums from tablet disintegration. The test consisted of six measurements per formulation (i.e. six dissolution vessels - Caleva model 7ST) using a PU8700 series Phillips spectrophotometer for U.V drug detection.

Deionised water (500ml) maintained at 37°C was chosen as the dissolution medium, in which tablets were dropped and adhered to the bottom of the vessels. Paddles were rotated at 100rpm, and 2ml samples were taken out of the dissolution vessels at different time intervals and replaced with 2ml deionised water (sink conditions maintained) using 5ml syringes. The samples were filtered through 0.2μm acrodisks into 1cm U.V cells, and placed in the spectrophotometer at 272nm using U.V absorbance for drug detection. Manual sampling was continued until all six tablets had disintegrated and the U.V absorbance readings were constant with time.

7.1.6.1 Calibration curve

A calibration curve for theophylline was a pre-requisite, in order to ensure that the drug would be detected from the disintegrating tablets at all stages by U.V during the dissolution tests.

A 0.03mg/ml theophylline solution was scanned in the spectrophotometer to find the wavelength at which absorbance occurred, which was 272nm in agreement with the literature (Clarkes - Isolation and identification of drugs, 1986). Since each tablet contained 10mg theophylline which needed to be detected in 500ml of dissolution medium (deionised water) at all stages during the dissolution test, a series of different concentrations of theophylline solutions were prepared from a stock solution of 100mg/500ml (0.02%w/w) ranging from 0.03mg/ml to 0.002mg/ml. These solutions were assayed in the U.V spectrophotometer at 272nm, and the average value was taken from three absorbance readings per solution. These values were used to construct a calibration curve, and ranged from 0.110 to 1.668 absorbance units, which is a suitable range for drug to be detected at all stages during the dissolution tests. In addition, dissolution runs were carried out on tablets containing no drug (CAL01 – see Table 7.1) to ensure that none of the excipients absorbe at 272nm which would interfere with the drug assay.

7.1.6.2 Granule homogeneity

A granule homogeneity test was necessary to ensure that each tablet contained 10mg theophylline which should be detected in the dissolution tests.

Five tablets of the same formulation (CAL02 – see Table 7.1) were crushed and mixed in a pestle and mortar. A sample of 200mg was weighed out and dissolved in 500ml of deionised water (made up to volume in a volumetric flask). A sample of the solution was then filtered immediately into a 1cm U.V cell and assayed at 272nm in the spectrophotometer.

To ensure that the sieving process had not led to theophylline segregating, granules (197mg) above and below particle sizes of 850μm without lubricant (1mg) and glidant

(2mg) were dissolved in 500ml deionised water (volumetric flask) separately, followed by filtering into a 1cm U.V cell and assaying at 272nm in the spectrophotometer.

7.2 RESULTS

7.2.1 Conformity of methods employed

7.2.1.1 Loss on drying determination of granules

The percentage water content (%wc) in Table 7.2 is satisfactory to suggest that the moisture content retained in the granules after granule drying is due to the granulating fluid used in the wet granulation stage. This is because the average %wc values fall within 1%-3% which is the range for moisture content retention after granule drying (see Chapter 7.1.3.1), except for CAL17 (0.60%) and CAL19 (3.51%), although these values are still close to the desired range for moisture content retention.

7.2.1.2 Particle size analysis of granules

From the results in Table 7.3, there is some variation in the particle size distributions of the granules for the different formulations, since the derived parameters D(v, 0.5), D(v, 0.1) and D(v, 0.9) vary greatly within each formulation. The derived parameters are a measure of the average values at the 50%, 10% and 90% ranges. Thus D(v, 0.5) is the mass median diameter, and is the value at which 50% of the sample lies above and 50% lies below this value. On the other hand, D(v, 0.1) is the size at which 10% of the sample lies below this value and D(v, 0.9) is the size at which 90% of the sample lies below this value. In all cases, although there is some variation in particle size, the D(v, 0.9) is less than 850μm (sieve size) and the variations do not seem to influence the reproducibility of the dissolution profiles (see Chapter 7.2.2). Note that the results for xanthan gum are in conformity with the certificate of analysis (Chapter 2, Table 1).

7.2.1.3 Physical properties of the tablets (weight, thickness, hardness and diameter)

The results in Table 7.4 show the average (mean) and the standard deviation (relative) for tablet weight, thickness, hardness and diameter, with the nominal values at the top of the table. The tablet weights are between 192.5mg and 209.8mg, the thickness is between 3.71mm and 4.18mm, the hardness range is between 4.7kp and 10.5kp and the diameters are between 6.77mm and 7.08mm.

TABLE 7.2 : LOSS ON DRYING DETERMINATIONS AFTER 5 HOURS (60°C/26mmHg)

CODE	WEIGHT (GRAMS)					
	TIN AND LID	INITIAL SAMPLE	TIN+LID+ SAMPLE	FINAL SAMPLE	WATER CONTENT (%WC)	MEAN % WC
(1) CAL01	29.4145	2.0942	31.4844	2.0699	1.16	1.18
(2) CAL01	29.5207	2.0923	31.5878	2.0671	1.20	
(1) CAL03	29.5428	2.0906	31.6103	2.0675	1.10	1.15
(2) CAL03	29.3507	2.0616	31.3878	2.0371	1.19	
(1) CAL06	29.2878	2.0310	31.2559	1.9681	3.10	3.03
(2) CAL06	29.3708	2.0414	31.3517	1.9809	2.96	
(1) CAL11	29.4798	2.0872	31.5052	2.0254	2.96	2.80
(2) CAL11	29.6909	2.0311	31.6683	1.9774	2.64	
(1) CAL17	29.5181	2.0798	31.5861	2.0680	0.57	0.60
(2) CAL17	29.2977	2.0777	31.3626	2.0649	0.62	
(1) CAL19	29.5284	2.0845	31.5396	2.0112	3.52	3.51
(2) CAL19	29.7666	2.0869	31.7804	2.0138	3.50	
(1) CAL20	29.4669	2.0205	31.4445	1.9776	2.12	2.16
(2) CAL20	29.4577	2.0225	31.4357	1.9780	2.20	

TABLE 7.3 : PARTICLE SIZE ANALYSIS OF GRANULES

CODE	DERIVED PARAMETERS (μm)		
	D(v, 0.1)	D(v, 0.5)	D(v, 0.9)
XANTHAN GUM	64.11	124.62	209.67
LOCUST BEAN GUM	26.63	125.96	235.99
CAL01	99.21	241.08	499.85
CAL03	117.14	341.79	647.30
CAL06	213.72	544.38	790.68
CAL11	149.97	436.27	724.88
CAL17	204.62	413.60	680.12
CAL18	118.01	282.28	599.52
CAL19	104.14	241.01	508.94
CAL20	88.80	235.91	550.49
CAL21	86.70	203.44	455.65

These are all non-pharmacopoeial tests. The tablet hardness was very difficult to standardise for each formulation and was adjusted several times because the granule properties tended to change with time. This could have been due to the automated run compacts the powders to a greater extent compared to manual running which was initially used to obtain the correct parameters, although this does not account for such large differences which were observed. Another explanation could be that the flow properties were unsatisfactory which would lead to variability in die filling and hence tablet hardness, although a granulation process was used which improves powder flow. Differences in the particle size distribution is another likely reason, since the particle size analysis results from Table 7.3 support variation. However the variability in tablet hardness did not affect the reproducibility of the dissolution results.

The remaining tablet physical properties were satisfactory and easy to control, as can be seen from the small standard deviations in Table 7.4.

TABLE 7.4 : PHYSICAL PROPERTIES OF THE TABLETS

	WEIGHT (200mg)		THICKNESS (3.83mm)		HARDNESS (8.0kp)		DIAMETER (7.00mm)	
CODE	MEAN (mg)	S.D (rel) (%)	MEAN (mm)	S.D (rel) (%)	MEAN (kp)	S.D (rel) (%)	MEAN (mm)	S.D (rel) (%)
CAL01	204.6	2.00	3.83	0.77	10.5	15.09	7.03	0.17
CAL02	198.0	2.17	3.83	0.71	9.0	16.91	7.02	0.10
CAL03	198.8	2.35	3.96	1.22	5.0	36.92	7.05	0.19
CAL04	200.2	1.18	3.87	0.28	7.0	10.32	6.99	0.11
CAL05	209.4	2.92	4.10	1.18	8.0	18.32	7.03	0.09
CAL06	195.3	2.11	3.71	0.91	6.9	15.9	7.03	0.17
CAL07	207.9	2.71	3.89	1.45	7.0	15.15	7.06	0.15
CAL08	205.9	0.83	4.00	0.18	7.7	7.52	7.03	0.10
CAL09	208.2	1.18	3.93	0.45	8.5	7.52	7.04	0.20
CAL10	202.2	1.43	3.94	0.40	5.5	10.72	7.04	0.10
CAL11	196.5	4.05	3.82	2.15	4.7	29.03	6.77	1.18
CAL12	199.8	0.50	3.87	0.17	5.9	5.50	7.07	0.80
CAL13	209.8	0.95	4.04	0.42	6.4	6.89	7.08	1.04
CAL14	203.6	2.88	3.96	1.10	7.3	22.18	7.06	0.25
CAL15	201.7	2.12	3.96	0.58	5.2	18.81	7.05	0.24
CAL16	200.3	2.46	3.93	1.63	5.9	17.79	7.05	0.13
CAL17	202.9	0.75	3.88	0.34	6.2	10.28	7.03	0.14
CAL18	192.5	1.19	3.85	0.41	6.1	7.29	6.98	0.10
CAL19	200.7	0.37	3.81	0.28	7.8	9.39	6.95	0.15
CAL20	205.2	0.47	4.18	0.14	6.6	6.57	6.98	0.11
CAL21	200.6	1.14	4.04	0.31	6.9	11.67	6.96	0.12

7.2.1.4 Calibration curve for theophylline

The calibration curve for theophylline in Figure 7.1 shows a linear relationship between absorbance and concentration, in accordance with Beer-Lambert's law. This curve confirms that the maximum amount of drug that should be released from a tablet (10mg) will be detected at 272nm in the dissolution medium (500ml) during dissolution testing for drug release (i.e. 0.02mg/ml), in addition to detecting concentrations above and below this value. The equation of the curve in Figure 7.1 was necessary in order to calculate the percentage drug release according to Equation 7.2 for the dissolution results.

Equation of the curve in Figure 7.1:

$$x = (y - 0.0025/55.809)500$$

Where:

X = concentration (mg/500ml)

Y = absorbance units

Thus:

$$\% \text{ Drug release} = (x/10)100 \qquad \text{Equation 7.2}$$

7.2.1.5 Granule homogeneity results

The results from Table 7.5 suggest that some segregation during sieving may have occurred, which would account for the absorbance value for the 200mg sample from the five crushed tablets being 6.9% lower than expected. However, the granules used for tabletting were less than 850μm which only showed a 10.4% decrease in drug content. Thus, these amounts are small and should not affect the dissolution studies. Therefore, the tablets were assumed to have a uniform drug content of 10mg.

FIG. 7.1 : CALIBRATION CURVE FOR THEOPHYLLINE

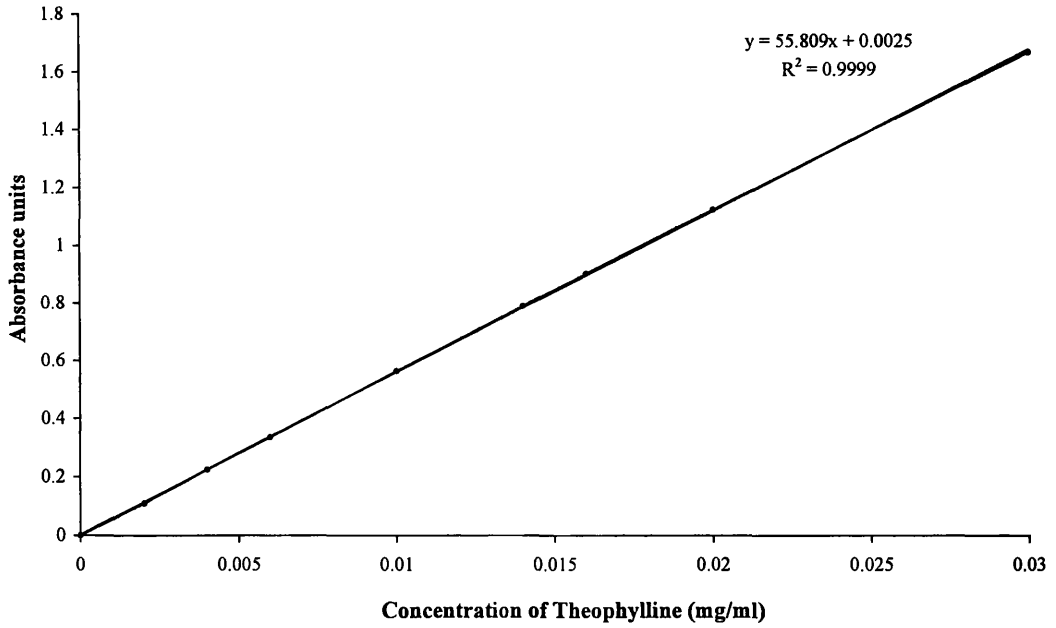


TABLE 7.5 : RESULTS TABLE FOR GRANULE HOMOGENEITY TESTS

TEST (CAL02)	(1) ACTUAL % DRUG DETECTED	(2) THEORETICAL % DRUG IN A 200mg TABLET	% DRUG RECOVERED
200mg FROM FIVE CRUSHED TABLETS	93.1	100	6.9% DECREASE
GRANULES<850μm	89.6	100	10.4% DECREASE
GRANULES>850μm	133.6	100	33.6% INCREASE

7.2.2 Investigation of Variables

7.2.2.1 The effect of drying temperature

It is observed from Figure 7.2 that granule drying temperatures of 35°C, 55°C and 90°C do not affect the drug release profiles of tablets containing 5% gums and sucrose as filler. In addition, Figure 7.2 shows the dissolution profile for tablets containing no drug (CAL01) to support that the formulation excipients do not interfere and absorbe at the same wavelength as the drug theophylline.

FIG. 7.2 : DISSOLUTION PROFILES TO SHOW THE EFFECT OF DRYING TEMPERATURE ON TABLETS CONTAINING 5% GUMS AND SUCROSE AS FILLER

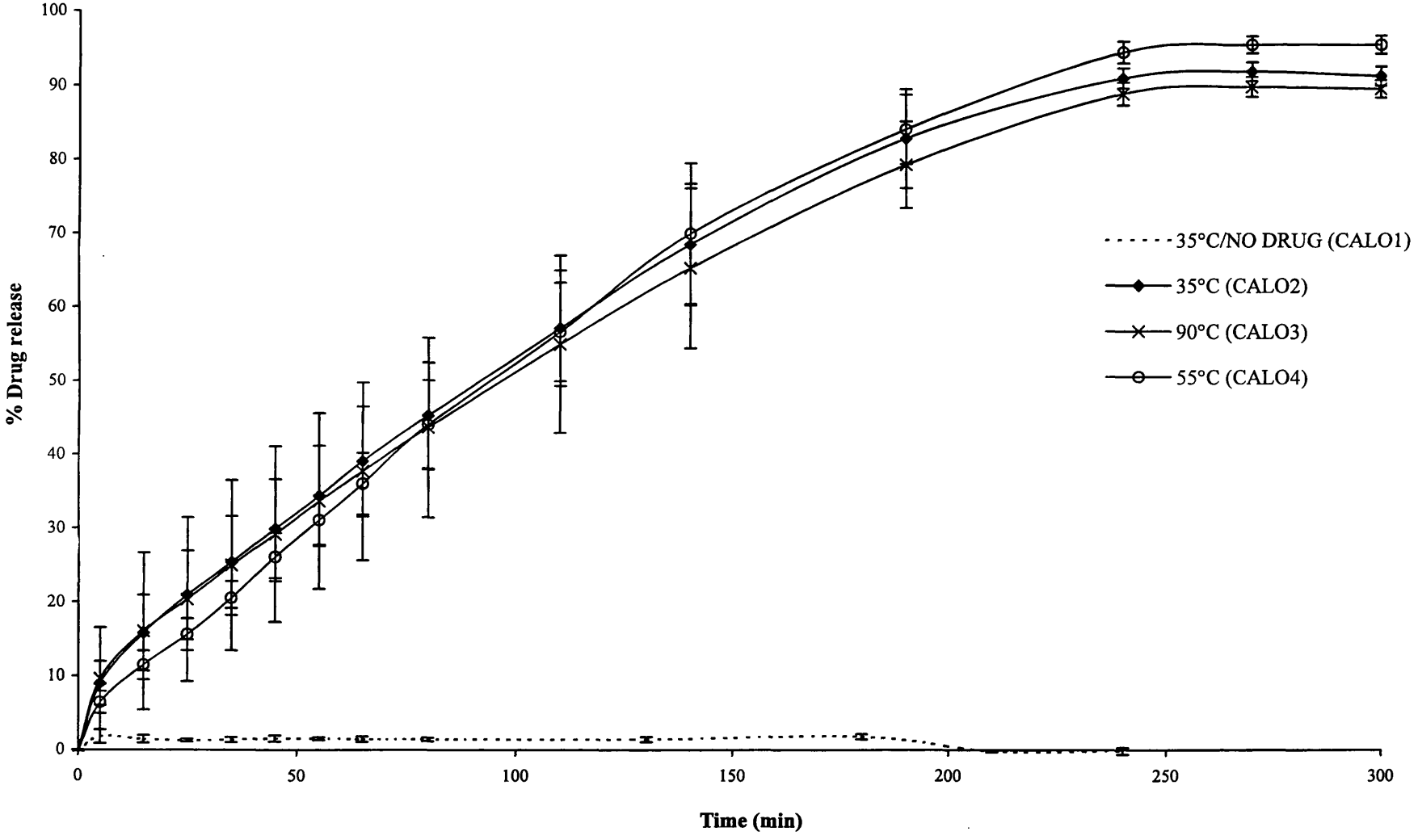


FIG. 7.3 : DISSOLUTION PROFILES TO SHOW THE EFFECT OF DRYING TEMPERATURE ON TABLETS CONTAINING 20% GUMS AND SUCROSE AS FILLER

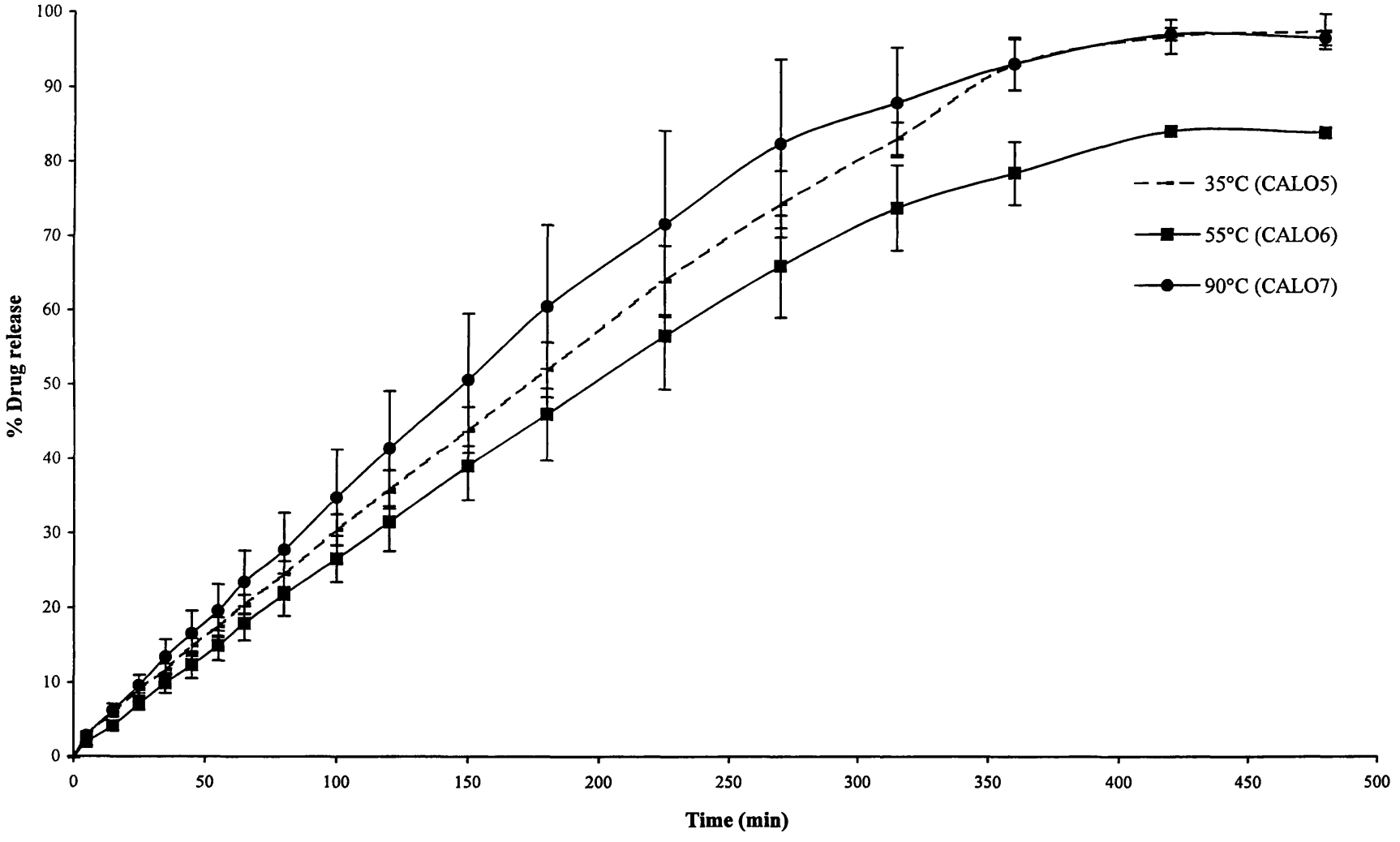
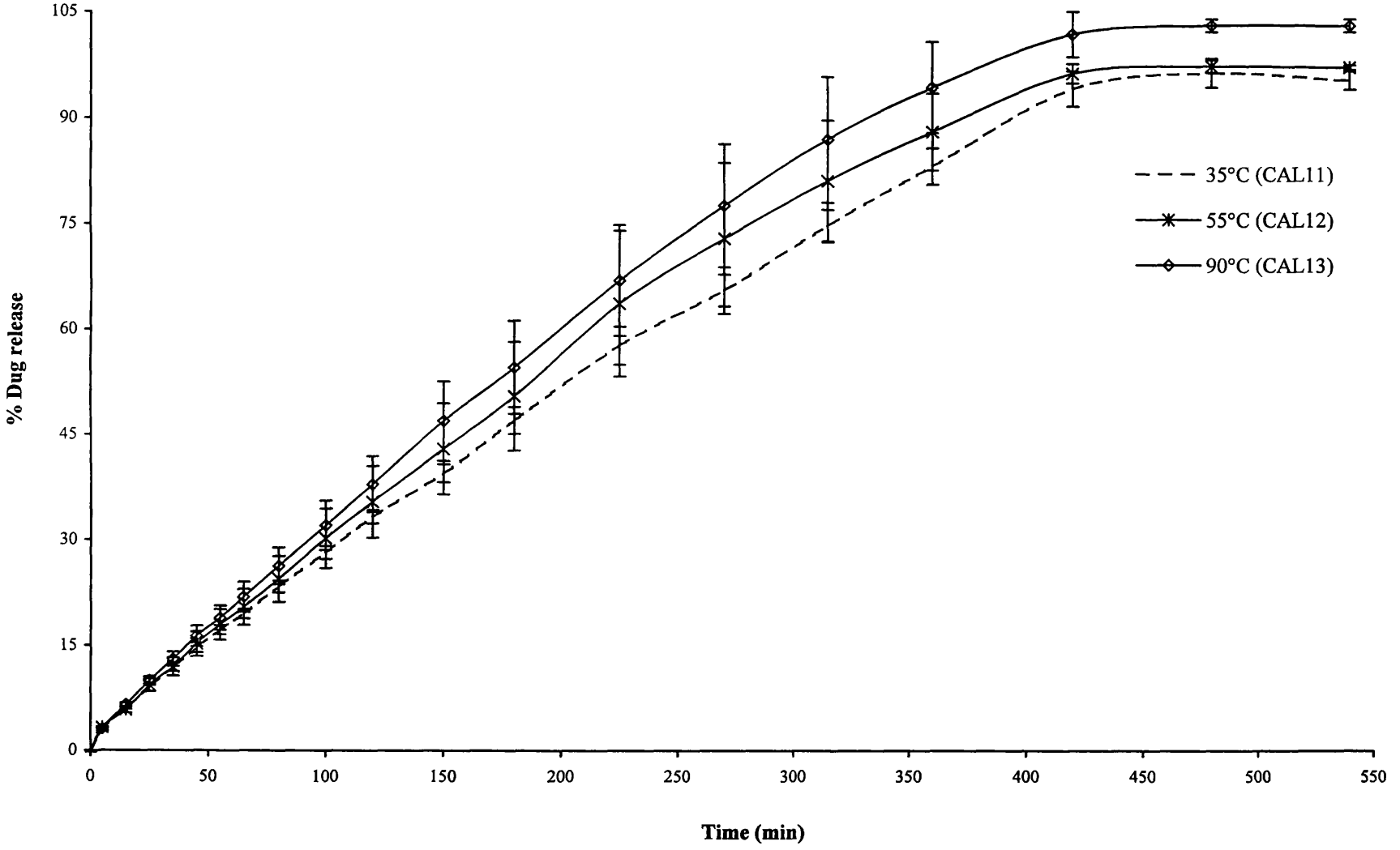


FIG. 7.4 : DISSOLUTION PROFILES TO SHOW THE EFFECT OF DRYING TEMPERATURE ON TABLETS CONTAINING 20% GUMS AND LACTOSE AS FILLER



To ensure that these similarities in release profiles at the different granule drying temperatures were not due to the small concentration of gums used (5%), the effect of drying temperature on formulations containing 20% gums were investigated (Figure 7.3). It is observed from Figure 7.3 that perhaps there are some differences in drug release depending on the drying temperature, although any changes are small. The slowest release of drug from the tablets are for granules dried at 55°C followed by granules dried at 35°C, with the fastest release occurring for granules dried at 90°C. Additionally, it is observed in Figure 7.2 and 7.3 that not all drug is released from the tablets when the drug release plateaux are reached, particularly so for formulation CAL06 (84% drug released). This may be because of granule segregation effects before or during tabletting.

Figure 7.4 shows the effect of granule drying temperature on drug release from tablets containing lactose as filler. It is difficult to ascertain from Figure 7.4 if the granule drying temperature has any effect on drug release from tablets containing lactose as filler. It is possible that drug release is slowest for granules dried at 35°C and fastest for granules dried at 90°C. If these differences are apparent, then the results differ to tablets containing sucrose as filler, in that release was slowest for granules dried at 55°C. In addition, it is observed from the graph that maximum drug release is not 100%.

7.2.2.2 The effect of xanthan gum-locust bean gum concentration

The results in Figure 7.5 suggest that incorporating xanthan gum-locust bean gum 1:1 ratio in the formulations does retard drug release, since it is observed from the graph that the addition of gums retards drug release, in which increasing the gum concentration from 5% to 20% decreases drug release by almost two-fold (i.e. time to reach maximum % drug release is approximately 4 hours and 7 hours for tablets containing 5% and 20% gums respectively). Whereas, drug is released almost immediately at the start of the dissolution test, with all drug being released after approximately 30 minutes for tablets containing no gums. The error bars have not been included in this graph for clarity, but can be observed in Figures 7.2 and 7.3.

7.2.2.3 The filler type (sucrose and lactose)

The filler type does not appear to greatly affect the release profiles or retardation properties of the tablets (Figure 7.6). Each profile has the same characteristic shape, exhibiting controlled release properties, with approximately the same amount of drug released at similar time points. The error bars have not been included in this graph for clarity, but can be observed in Figures 7.3 and 7.4.

7.2.2.4 The effect of varying the wet granulation method

The results for drug release with respect to the wet granulation method used are displayed in Figure's 7.7, 7.8 and 7.9. In Figure 7.7, dissolution profiles for tablets prepared by method 1 (modification of the standard method for Buccastem) are compared to dissolution profiles for tablets prepared by method 2 (the use of recovered polysaccharide powder from 1%w/w solutions prepared at 35°C and 90°C). The drug release properties of the formulations are very different, i.e. drug is released almost instantaneously from tablets produced using method 2 (recovered powder at 90°C), in which all drug is released within 35 minutes. Although a slow release profile is observed for tablets produced using method 2 (recovered powder at 35°C), drug is released much faster compared to release from tablets prepared using method 1. In addition, maximum drug release is greater than 100% for tablets produced by method 2, which is probably due to burst effects giving rise to high concentration zones which are thus detected by UV during sampling.

In comparison, Figure 7.8 shows the dissolution profiles for tablets prepared using method 1 compared to method 3. The release profiles are very similar and do not seem to be affected by using a solution of the mixed polysaccharide system as the granulating fluid. However, the volumes of granulating fluid used were very small in order to achieve the desired granule texture in comparison to the total granule weight. The dissolution profiles for tablets produced using all three methods are illustrated for comparative purposes in Figure 7.9, notice that error bars have not been included here for clarity, but are included in Figures 7.7 and 7.8 for the different formulations.

FIG. 7.5 : DISSOLUTION PROFILES TO SHOW THE EFFECT OF GUM CONCENTRATION ON DRUG RELEASE

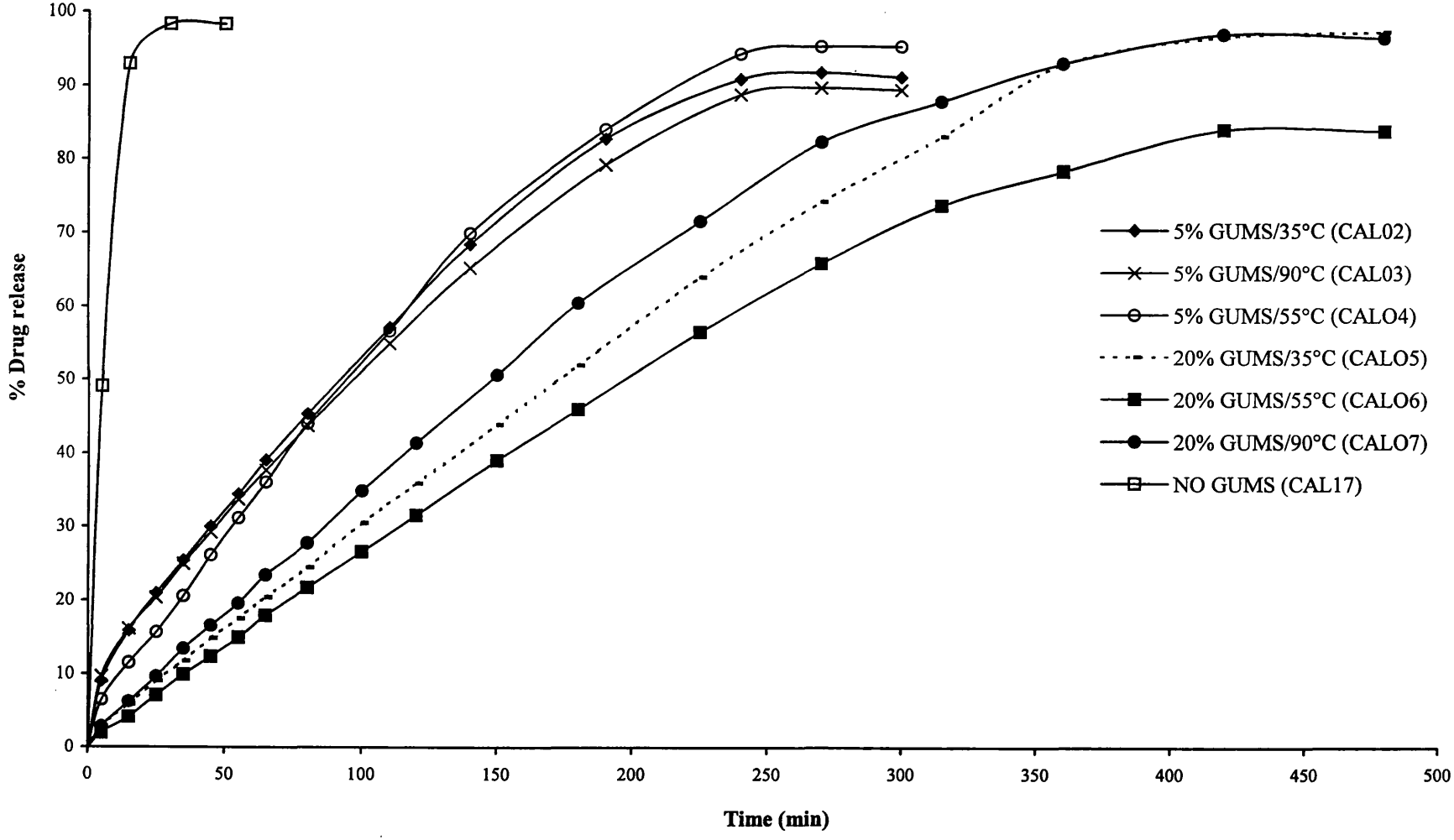


FIG. 7.6 : DISSOLUTION PROFILES TO COMPARE THE FILLER TYPE USED IN THE FORMULATIONS

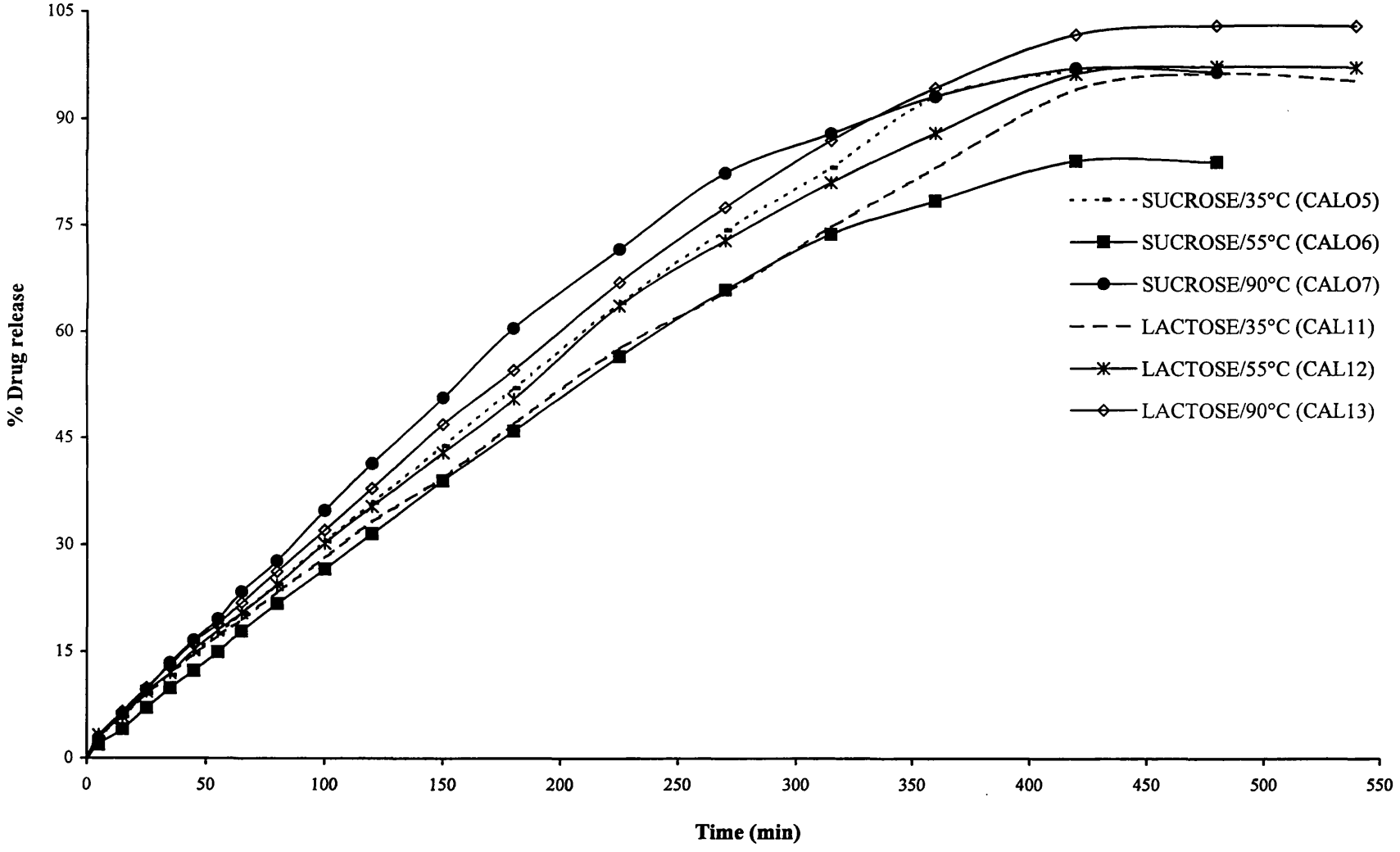


FIG. 7.7 : DISSOLUTION PROFILES COMPARING DRUG RELEASE FROM TABLETS PREPARED FROM METHOD 1 AND METHOD 2

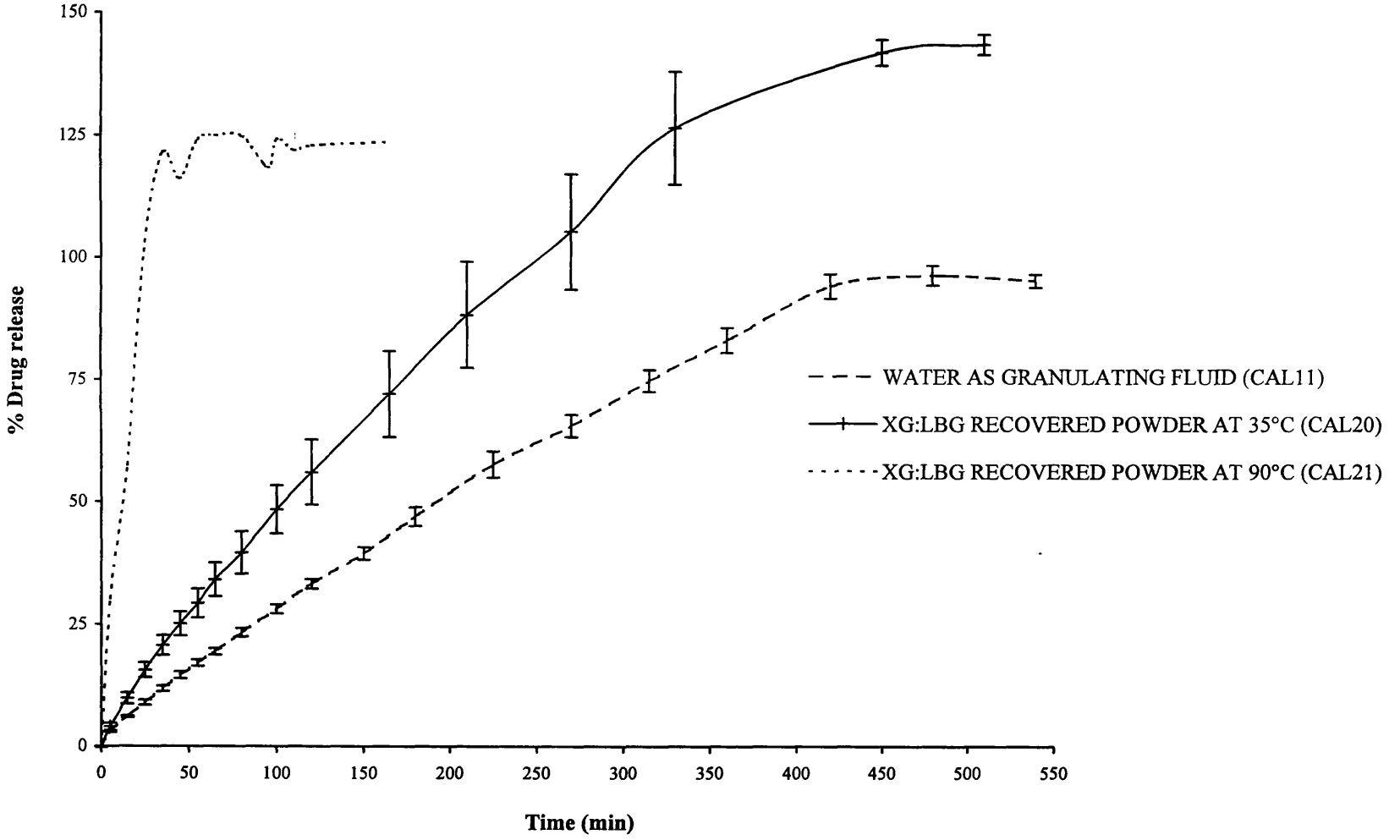


FIG. 7.8 : DISSOLUTION PROFILES COMPARING DRUG RELEASE FROM TABLETS PREPARED FROM METHOD 1 AND METHOD 3

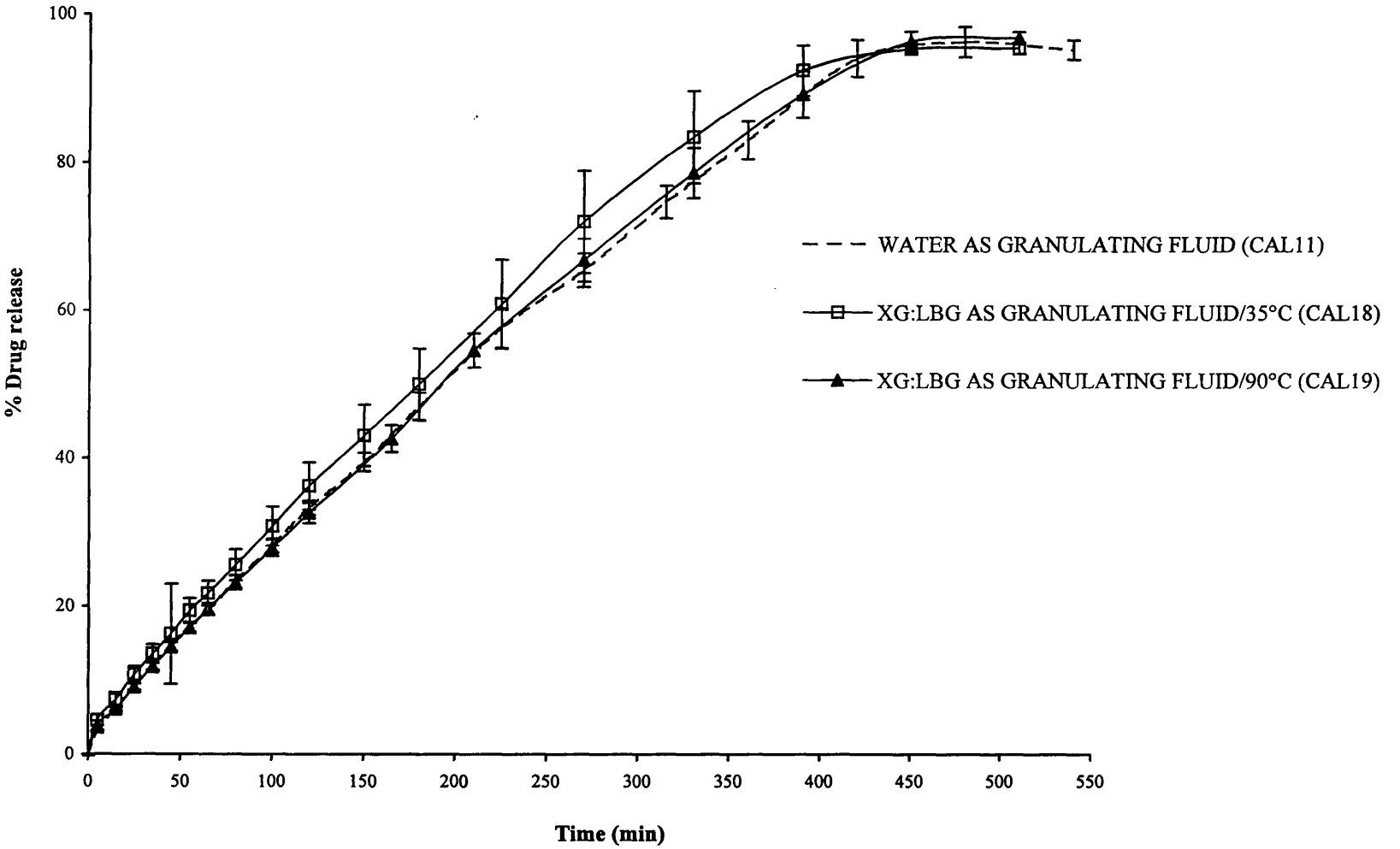
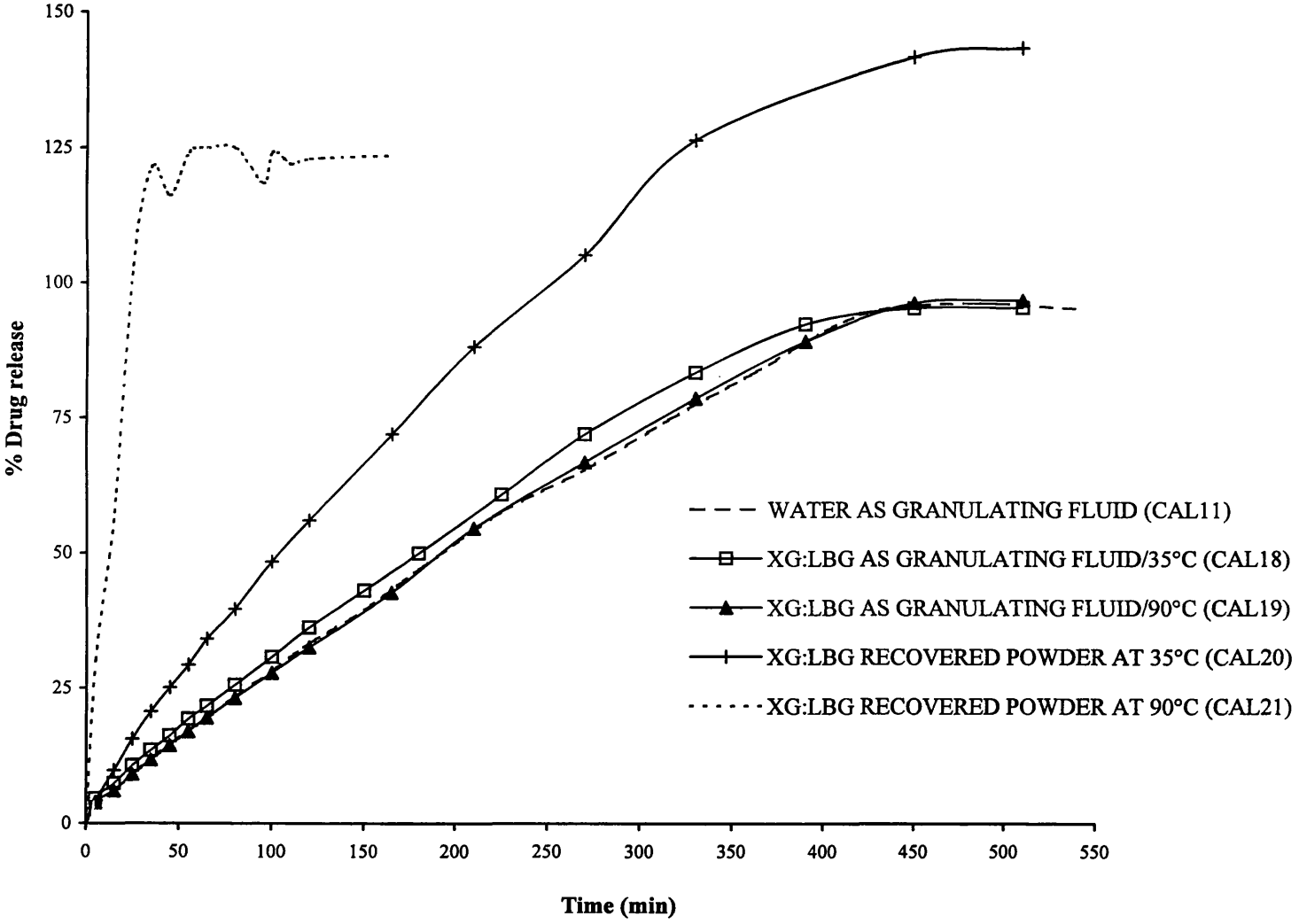


FIG. 7.9 : DISSOLUTION PROFILES COMPARING DRUG RELEASE FROM TABLETS PREPARED BY DIFFERENT WET GRANULATION METHODS (METHOD 1, 2 AND 3)



7.2.3 Kinetic determinations

7.2.3.1 Order of reaction

Drug release from a tablet can be classified by the order of the reaction. If the concentration of a reactant A is denoted by [A], then the rate of reaction can be expressed as the rate of change of the reactant concentration with time i.e. –d[A]/dt. Thus, the differential equation expressing rate as a function of the concentration of each of the species which affect the rate is called the general rate equation, as expressed in Equation 7.3.

$$-d[A]/dt = k[A]^2 [B] \qquad \text{Equation 7.3}$$

In Equation 7.3, the reaction is described as second-order with respect to A and first-order with respect to B giving the overall order of reaction as third-order. The proportionality constant is k which represents the rate constant.

Zero-order reactions occur at a constant rate which are independent of the concentrations of the reactants. Thus, the integrated rate equation is described in Equation 7.4;

$$x = kt \qquad \text{Equation 7.4}$$

Where:

x = concentration released at

time t

k = rate constant

First-order reactions differ to zero-order reactions in that the rate is dependent on one concentration term. The integrated rate equation is expressed in Equation 7.5;

$$\log a/a-x = kt/2.303 \qquad \text{Equation 7.5}$$

Where:

a = initial concentration

x = concentration released at time t

k = rate constant

a-x = concentration remaining in tablet

The order of a reaction can be determined graphically by comparing the linear equations for the various orders of reaction until a straight line plot is obtained. Thus in the present study, graphs of (a-x) against time (zero-order plot) and log (a-x) against time (first-order plot) were plotted and the linearities were compared. Two examples are shown in Figures 7.10 and 7.11. The order of the reaction was determined from the graph giving the greatest linearity (determined by R; measure of good fit). Thus the closer R is to 1, the greater is the linearity which was used to determine the order of the reaction. These results are displayed in Table 7.6, in which it is observed that R is above 0.9 for all formulations except for CAL21, in which the order for the reaction was undetermined (i.e. neither zero-order or first-order). The R values obtained from the zero-order and first-order plots are very close for the formulations, which makes it difficult to differentiate the order of reaction.

7.2.3.2 Rate constants

The rate constant is a proportionality constant of a reaction at a given temperature. In the present study, it provides information about the rate of drug release between different formulations. The rate constants are calculated from the gradients of the zero-order and first-order plots. Thus, for zero-order reactions, k = gradient, and for first-order reactions, k = -2.303xgradient. The rate constants for each formulation are displayed in Table 7.6. It was not possible to determine the rate constant for CAL21, since the plots were non-linear as shown by R=0.690 for the zero-order plot.

FIG. 7.10 : FIRST-ORDER PLOT FOR FORMULATION CAL02

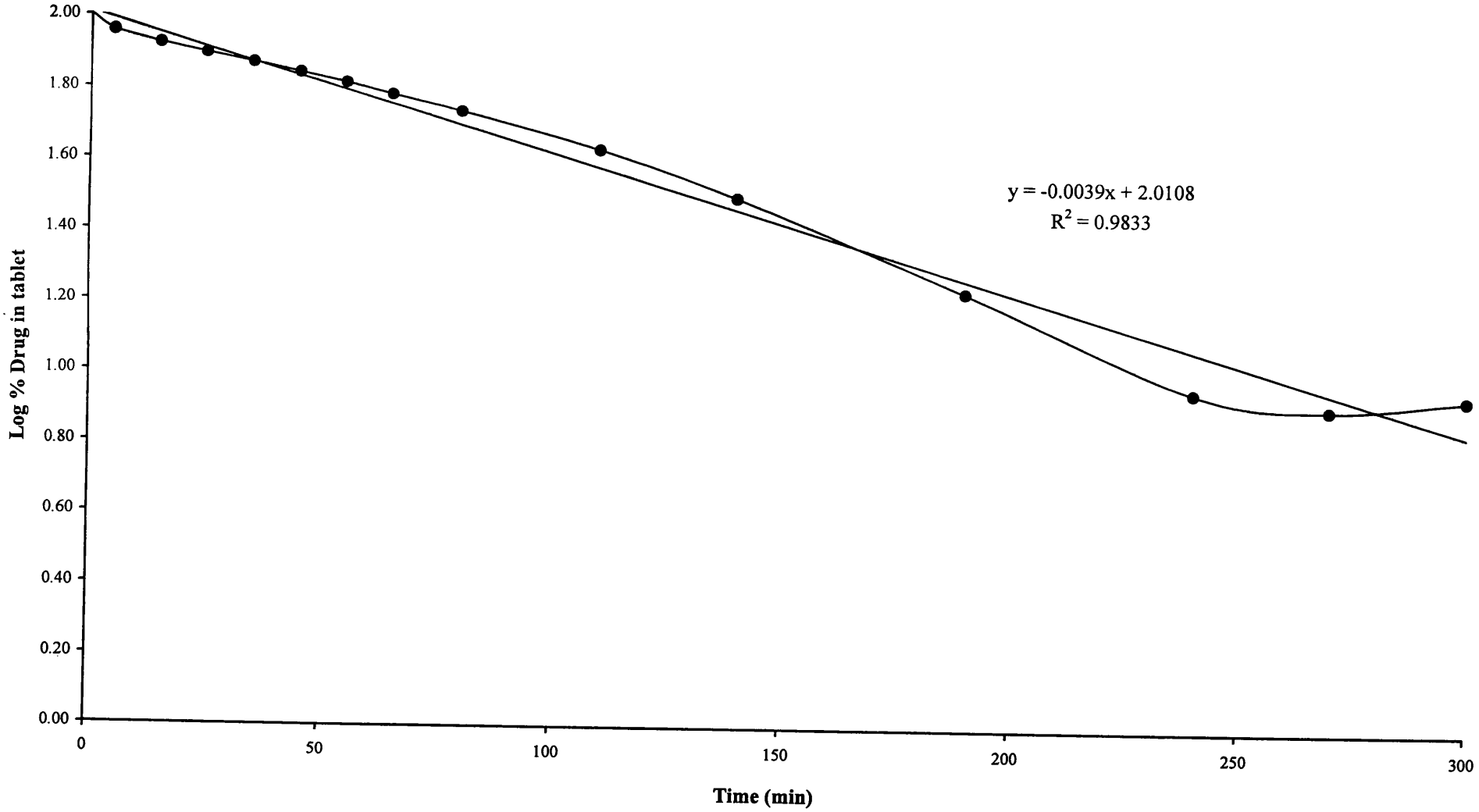


FIG. 7.11 : ZERO-ORDER PLOT FOR FORMULATION CAL19

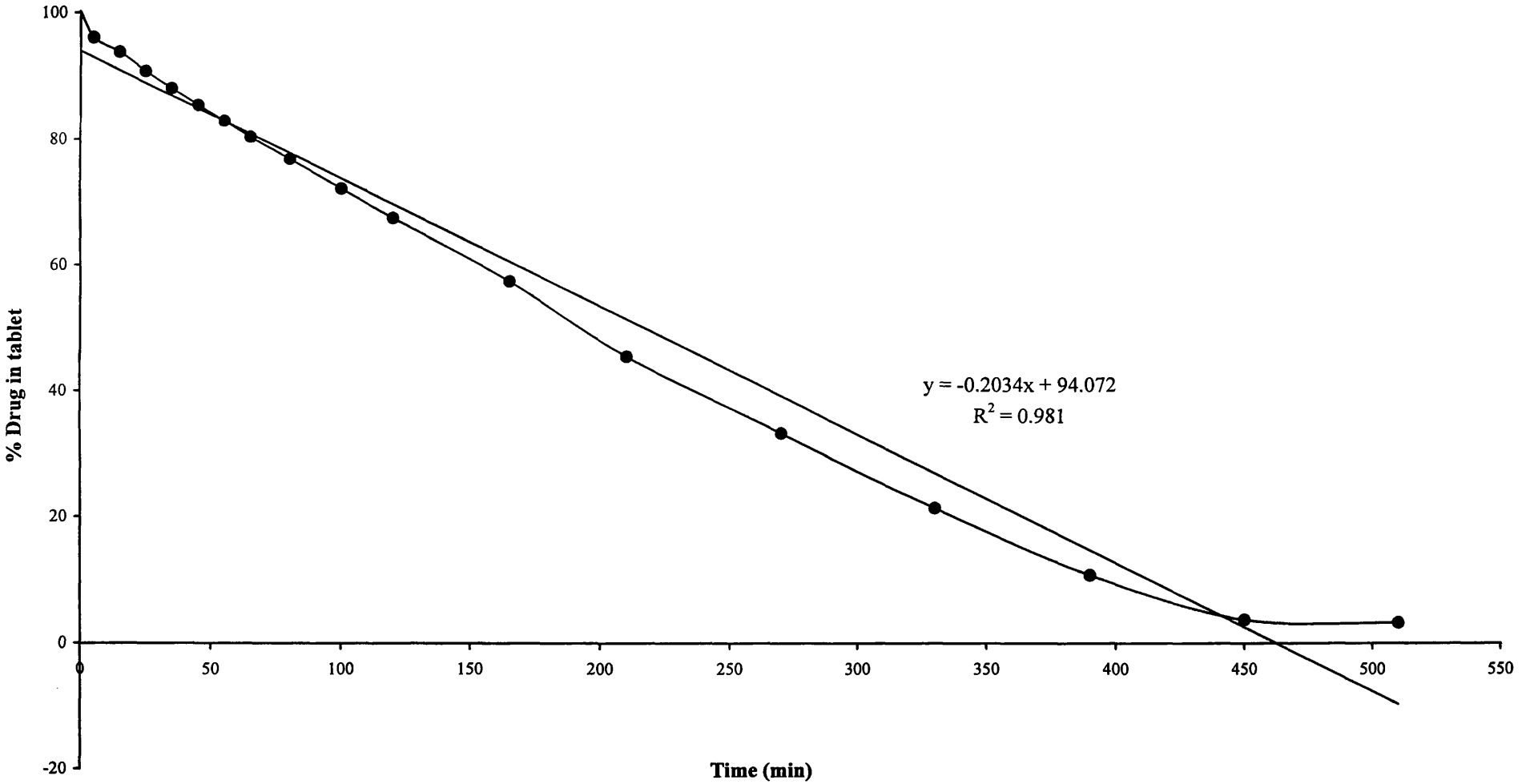


TABLE 7.6 : RESULTS TABLE FOR KINETIC DETERMINATIONS

CODE	k FROM ZERO-ORDER PLOT (%MIN^{-1})	R FROM ZERO-ORDER PLOT (MIN^{-1})	k FROM FIRST-ORDER PLOT (%MIN^{-1})	R FROM FIRST-ORDER PLOT (MIN^{-1})	ORDER OF REACTION
CAL02	-0.305	0.967	0.009	0.992	FIRST
CAL03	-0.296	0.970	0.008	0.993	FIRST
CAL04	-0.333	0.973	0.011	0.989	FIRST
CAL05	-0.225	0.985	0.007	0.972	ZERO
CAL06	-0.196	0.982	0.004	0.994	FIRST
CAL07	-0.226	0.970	0.008	0.986	FIRST
CAL11	-0.195	0.985	0.006	0.970	ZERO
CAL12	-0.201	0.977	0.007	0.977	ZERO
CAL13	-0.213	0.976	0.009	0.964	ZERO
CAL18	-0.203	0.982	0.006	0.982	ZERO
CAL19	-0.203	0.991	0.006	0.969	ZERO
CAL20	-0.299	0.978	0.011	0.947	ZERO
CAL21	-0.581	0.691	-	-	?

7.3 DISCUSSION

From the results, the granule drying temperature does not affect the drug release properties of tablets containing 5% gums (Figure 7.2), since graphically there are no differences and the rate constants (for first-order plots) are similar (see Table 7.6). However, there appears to be some differences in drug release from tablets containing 20% gums with respect to granule drying temperature from the graph (Figure 7.3), although the rate constants are again quite close in value suggesting that drug release is independent of the drying temperature. It is possible that a granule drying temperature of 55°C may slow drug release since the rate constant is slightly smaller compared to

drying temperatures at 35°C and 90°C (k = 0.004 for CAL06 (55°C) compared to k = 0.007 for CAL05 (35°C) and k = 0.008 for CAL07 (90°C)). If this difference from Table 7.6 is significant, this would support the SCD results and texture analysis findings, in which the interaction between xanthan gum and locust bean gum has most effect at 55°C (SCD results) and maximum gel strength occurs at around 50°C-60°C with no further increase at higher temperatures (texture analysis findings). This temperature (55°C) coincides with the Tm of xanthan gum (HSDSC data) suggesting that the interaction is either enhanced of depends on the disordered form of xanthan gum. However, if drug release is independent of the drying temperature, these findings contradict the results from the temperature studies using oscillatory rheology and texture analysis, which showed that heating and cooling above ambient increased the gel strength of the systems. However, these temperature studies were performed in aqueous i.e. using solutions of xanthan gum-locust bean gum mixes (1%w/w) mixed at different temperatures. This is different to the granule drying temperature, in which only a small volume of water (5-17% of total granule weight) was added to the polysaccharides at room temperature before drying the granules at different temperatures.

In relation to these findings, the different wet granulation methods employed suggest that an aqueous environment is necessary for the polysaccharides to interact, which in turn depend on the temperature of mixing the polysaccharide solutions. This can be shown from the results in Figure 7.7 using wet granulation method 2 (recovered polysaccharide powder from 1%w/w polysaccharide solutions prepared at 35°C and 90°C) which suggests that once the interaction between xanthan gum and locust bean gum is established in aqueous (without drug and excipients), the interacted product can not interact with the drug and excipients, which is why the retarding properties of the matrix system are reduced. In addition, the results suggest that the interaction between xanthan gum and locust bean gum is greatly enhanced or depends on xanthan gum present in the disordered form (polysaccharide solution prepared at 90°C, i.e. above the Tm of xanthan gum), because the dissolution profile for these tablets (CAL21) suggests that the system behaves as a disintegrant (i.e. drug is released instantaneously; k = -0.581). Whereas, for the dissolution profile for tablets (CAL20) produced using method 2 (polysaccharide solution prepared at 35°C, i.e. below the Tm of xanthan gum), a controlled release profile is still observed although drug is released much faster (k = -0.299) compared to the dissolution profile for tablets (CAL11) produced using

method 1 (non-recovered polysaccharide powder; k = -0.195). This finding suggests that perhaps the interaction between xanthan gum in the ordered helix conformation and locust bean gum is reduced (thus room for interaction with the excipients). The dissolution profiles for tablets produced by method 3 (xanthan gum-locust bean gum 1%w/w prepared at 35°C and 90°C used as the granulating fluid) compared to method 1 (water used as the granulating fluid) again suggest the importance of interactions between xanthan gum and locust bean gum occurring within an aqueous environment (i.e. only 14% of total granule weight), because the release profiles and rate constants (k = -0.195 to -0.203) are very similar when comparing the two different methods. (see Figure 7.8) These similarities in dissolution profiles and rate constants could also highlight the importance of time dependent gelation, since xanthan gum-locust bean gum mixes 1%w/w were added to the dry powders straight after mixing at the designated temperatures in method 3, whereas the polysaccharide mixes 1%w/w were left to hydrate at room temperature for 24 hours after mixing in method 2 prior to powder recovery.

The addition of sucrose as the choice of filler improved the texture of the desired granules which required less granulating fluid compared to using lactose as filler. However, in terms of the type of filler used (lactose or sucrose), there were no differences in the drug release profiles of the tablets (see Figure 7.6). Thus, the results in the present work do not support the findings for Buccastem, that sucrose improves the tablet gelling and disintegrating properties.

From the dissolution studies, tablets containing gums exhibit a controlled release profile over approximately 4 to 7 hours depending on the gum concentration (Figure 7.5). This is due to an increase in gum concentration increases the number of chains available for cross-linking, which in turn increases the gel strength of the matrix which results in retardation of drug (Baichwal et al, 1991). From the kinetic studies in the present work (Table 7.6), it was difficult to ascertain whether these controlled release profiles were zero-order or first-order since the R values were very close for the two different plots. This could suggest that the reaction is a fractional order. The order of the reaction could not be determined for formulation CAL21 since the zero-order plot was non-linear and a first-order plot could not be determined since maximum release was greater than 100%, because this formulation behaved as a disintegrant.

Overall from the kinetic results (Table 7.6), it appears that the order of the reaction is first-order for tablets containing sucrose as filler and zero-order for tablets containing lactose as filler. Baichwal et al (1991) determined drug release to be zero-order from these systems in which sucrose, lactose, dextrose or a mixture of these excipients were used in the dosage forms.

Due to the conclusions from the findings and time constraints, it was not necessary to test the tablets having intermediate variables namely CAL08-CAL10 (10% gum concentration) and CAL14-CAL16 (sucrose:lactose 1:1 ratio) using dissolution studies.

CHAPTER 8

CHAPTER 8 : CONCLUSIONS

The overall objective of the project was to investigate the mechanism for the interaction between xanthan gum and locust bean gum, with respect to xanthan gum structure in particular. Hence, HSDSC and SCD were employed to study the conformational and transitional behaviour of the polysaccharides. Different factors contribute to the xanthan gum conformation and/or extent of the interaction such as temperature, additives (sodium chloride and sucrose), polysaccharide ratio and the M:G ratio of locust bean gum, which were the variables investigated in the present work. This was necessary in order to explore and develop pharmaceutical applications based on this synergy, with the aim to manipulating and controlling drug release through different formulation methods. Although the performance of the TIMERx system which is based on the xanthan gum-locust bean gum synergy can be regulated by the concentration and ratio of polysaccharides used, this may be unsuitable in cases where the amount of gel swelling needs to be minimised such as in buccal delivery or formulations requiring drugs with a high therapeutic index. Therefore if the formulation can be optimised by the methods employed, this would overcome some of the problems that may be encountered with the present TIMERx system. In addition, the effects of sucrose on these systems used pharmaceutically are quite novel, and an explanation is at present unknown. By investigating the influences of this additive in the current study, information about the interaction between the two polysaccharides may be gained and/or sucrose may prove a useful excipient for controlling drug release.

The basic characterisation of the polysaccharides with regards to the Na^+ and Ca^{2+} cations and acetate and pyruvate content for xanthan gum and the M:G ratio for locust bean gum were important, in order to understand and establish the chemical properties of the initial starting materials, which is not always considered in the literature. This is because such factors are known to affect the structure and hence interactive properties of the polysaccharides. It has also been observed that differences in the cationic, acetate and pyruvate content compared to the literature may suggest that the chemical properties of xanthan gum depend on the supplier, batch, fermentation and experimental process, in addition to the technique used for analysis. However, in relation to the fractionation process of locust bean gum, there was good agreement in the M:G ratios using the two different techniques, although the H^1 NMR is a much quicker method

involving simple sample preparation for quantitative analysis compared to the acid hydrolysis method.

Oscillatory rheology and texture analysis studies were employed in the present work as a means to determining the strength of the different polysaccharide systems i.e. measures the physical properties of the mixtures, although oscillatory rheology can in addition detect changes at the molecular level such as interactions. Additionally, texture analysis has not been widely applied to the systems used in the present study and proved complimentary. It was difficult to ascertain whether a synergy between the two polysaccharides occurs at room temperature, as results were non-conclusive from both techniques used. For example, it was observed in the oscillatory rheology studies that G′ was greatest for xanthan gum 1%w/w, with G′>G′′ for all mixtures in which both moduli decreased as the xanthan gum-locust bean gum ratio decreased. However, both moduli were only slightly dependent on frequency indicative of gel like behaviour. These uncertainties may be due to the techniques used are not sensitive enough to detect synergy at room temperature if the gels are very weak, since SCD measurements employed in the present work for the first time, confirm synergy between xanthan gum and locust bean gum at 25°C. Another possible reason may be due to the partial solubility of locust bean gum at low temperatures as discussed in Chapter 4.

Both oscillatory rheology and texture analysis studies showed that heating and cooling the mixed polysaccharide systems between 20°C-90°C greatly increased the strength of the mixtures compared to mixtures prepared at room temperature, due to interactions occurring between the two polysaccharides or enhanced synergy if synergy occurs at room temperature. These observations were not observed for the individual polysaccharide mixtures. In addition, both techniques showed that maximum synergy occurs for the 1:1 ratio after heating and cooling, in which a discontinuity observed at a temperature range of 50°C-60°C is the optimum temperature, since no further increase in gel strength was observed for preparation temperatures above this temperature range (texture analysis data). In addition, a cross over point between the heating and cooling profiles were observed at about 50°C for the mixed polysaccharide systems but not for the individual polysaccharide mixtures in the oscillatory rheology studies, indicative of new structures being formed for the mixed polysaccharide systems. The HSDSC and SCD results support that these temperatures fall within the temperature range for the Tm of xanthan gum, suggesting that the disordered form of xanthan gum is involved and

enhances the interaction with locust bean gum. For example, the SCD results showed that the interaction has most effect at 55°C, which corresponds with the Tm range of xanthan gum (45°C-75°C from HSDSC studies). Since G′ remains unchanged and no new structures are formed after heating and cooling xanthan gum between 20°C-90°C, these results reflect the reversible behaviour of the helix to coil transition with temperature, again supported by the mirror image heating and cooling profiles observed in the HSDSC studies. Further evidence is provided from the SCD spectrum for xanthan gum, which strongly suggests that the helical form exists at 25°C, with structural changes occurring on heating possibly to a random coil conformer. The Tm for xanthan gum from the HSDSC studies is also observed for the mixed polysaccharide systems at all ratios studied except for the 1:9 xanthan gum-locust bean gum ratio. Since the Tm at 45°C-75°C is reversible on heating followed by cooling for the mixed polysaccharide systems, this may suggest that although the interaction involves or is enhanced by the presence of disordered xanthan gum, the xanthan gum helix molecules may be retained in the post interactive product. The peaks at 45°C-75°C were not clearly observed for the 1:9 xanthan gum-locust bean gum ratio, which may suggest that a certain amount of xanthan gum is needed in order to interact with locust bean gum. In conjunction with the texture analysis studies, the gel strength for this ratio was unchanged before and after heating and cooling between 25°C-90°C, which may further support the importance of the concentration of xanthan gum molecules available, necessary to evoke a synergy with locust bean gum molecules.

The findings from the oscillatory work also suggest that the gelation process is time dependent for xanthan gum-locust bean gum mixes, since G′ was much greater after cooling from 90°C for 24hrs prior to measurements compared to mixtures heated and cooled via the rheometer at 2°C/min (1 cycle). Synergy was not enhanced on a second heating and cooling cycle, suggesting that once the synergy is established it is irreversible. In support of this, SCD studies showed that the changes in structure of the interacted product are permanent and in addition, the repetitive temperature cycling studies using HSDSC showed that the Tm of xanthan gum was independent of a second temperature cycle.

It has been determined from oscillatory rheology studies that the M:G ratio of locust bean gum affects the extent of the interaction with xanthan gum, in which increasing the M:G ratio enhances the synergy with xanthan gum, indicative that the unsubstituted

smooth regions of the backbone interact with xanthan gum. The maxima observed on the heating profiles at 60°C for mixes containing LBG80 may be linked to the conformation of xanthan gum during the interaction, which may further support that the disordered form of xanthan gum is significant.

The gel strength of xanthan gum-locust bean gum mixes are independent of sodium chloride, since it was observed both from the oscillatory rheology and texture analysis studies, that the gel strength remained unchanged compared to mixes in the absence of sodium chloride. From the HSDSC studies, the addition of sodium chloride shifts the Tm of xanthan gum to higher temperatures due to increased stabilisation of the ordered helix conformer. In relation to these three techniques used, overall these findings may suggest that either xanthan gum can interact with locust bean gum in both the disordered and ordered form, or xanthan gum molecules are in the same conformation in both environments with locust bean gum. Another possibility could be that the heating and cooling process counteracts the stabilisation of the xanthan gum helix by the Na^+ cations, such that xanthan gum still interacts with locust bean gum in the disordered form, hence no change observed in gel strength.

It was observed from the oscillatory rheology studies that the incorporation of sucrose to xanthan gum-locust bean gum mixes increased the gel strength of the systems which was concentration dependent, thus the addition of 15%w/w sucrose (maximum concentration studied) increased G′ considerably after heating and cooling compared to mixes without sucrose present. However, these results were not observed in the texture analysis studies, in which the gel strengths were virtually unchanged in the presence of sucrose. These differences may highlight contrasts in the sensitivities of the two techniques used and in relation to what is being measured i.e. texture analysis purely measures bulk physical differences/properties, whereas oscillatory rheology is a more sensitive test which also detects changes at the molecular level which may be more subtle. HSDSC studies were employed in conjunction with the oscillatory rheology studies to investigate the effects of sucrose on xanthan gum conformation. The results are at present unexplainable, in which the peaks previously observed in the absence of sucrose were either diminished or shifted. Perhaps the effects of sucrose are not on xanthan gum structure directly but either influence locust bean gum behaviour or the aqueous environment. Further work is required to investigate the effects of sucrose on these polysaccharide systems.

The Tm of xanthan gum is not influenced by the presence of locust bean gum (fractionated and unfractionated), since the mirror image effect of the heating and cooling profiles (endotherms and exotherms respectively) from the HSDSC studies were still observed at the same temperature range (45°C-75°C). Thus, xanthan gum structure is independent of locust bean gum. In further support of this, the SCD results strongly suggest that the synergy between the two polysaccharides is due to changes within xanthan gum structure and not locust bean gum.

The development of pharmaceutical applications of xanthan gum-locust bean gum systems were investigated in Chapter 7. The findings from the present project were used to investigate factors which may enhance the performance or control the release properties of these systems, which were developed into tablets based on a wet granulation process. Thus, the variables investigated were the granule drying temperature, filler type (sucrose and lactose), gum concentration and from these findings changes in the wet granulation method were exploited.

Drug release was not affected by the granule drying temperature, although drug release was possibly slightly slower from tablets consisting of granules dried at 55°C (Tm = 45°C-75°C from HSDSC studies). If this result is significant, then this would support that the disordered of xanthan gum increases the extent of the interaction with locust bean gum, as determined from the texture analysis and SCD studies discussed earlier. In relation to the TIMERx system, increasing the gum concentration increases the retardation properties of the tablets and hence decreases drug release as anticipated.

The release profiles were independent of the filler type which contradicts the findings from the oscillatory experiments with the addition of sucrose. Since the gel strength was increased with the addition of sucrose to the mixed polysaccharide system from the oscillatory rheology studies, it would be expected that the tablets would retard drug to a greater extent and thus reduce drug release in the dissolution medium when the tablets swell and gel in aqueous. From these findings, it was thought that the effects of sucrose on the behaviour of these systems and hence drug release were not observed, due to the initial wet granulation method employed. Since the oscillatory rheology experiments were performed on 1%w/w xanthan gum-locust bean gum systems in an aqueous environment, the wet granulation method was exploited as a means to modifying the behaviour of these systems and thus controlling drug release. The different wet

granulation methods employed suggest that once the interaction is established which depends on an aqueous environment, the system can not interact with other substances (i.e. drug and excipients). In addition, the different wet granulation methods used highlight that the interaction between xanthan gum and locust bean gum can occur with xanthan gum both in the disordered and ordered forms. However, the interactions are greatest when the disordered xanthan gum molecules are available. These findings may suggest that drug release can be controlled other than by altering the ratio and concentration of drug and gum, which may cause problems in terms of excess swelling and high tablet weights which can be undesirable or impractical.

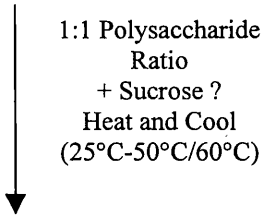
The kinetic results indicate that zero-order release is possible to achieve from these systems which may depend on the filler incorporated, which is desirable in terms of patient needs for most drugs. This is because the advantage of zero-order drug release is that the amount of drug delivered per unit time is constant and can be determined.

This project has shown that synergy occurs between xanthan gum and locust bean gum at room temperature and after heating and cooling between 20°C and 90°C (i.e. at all temperatures studied). The interaction between the two polysaccharides can occur for both xanthan gum conformations, but is enhanced when the disordered form is present which results in stronger gels. The disordered form of xanthan gum can be increased by heating (Tm = 45°C-75°C) and is decreased by sodium chloride and no heating. At room temperature, locust bean gum interacts with xanthan gum in the helix conformation, but the resulting gels are much weaker. In addition, the gelation process in general is time dependent.

Maximum synergy is observed for the 1:1 xanthan gum-locust bean gum ratio after heating and cooling between 20°C-90°C and possibly the 1:4 xanthan gum-locust bean gum ratio for mixtures prepared at room temperature. The optimum temperature at which this maximum synergy is observed is between 50°C-60°C in which the gel strength is independent of temperatures above this temperature range. The gel strength is increased by the addition of sucrose and heating followed by cooling, but is unaffected by the presence of sodium chloride. In addition, the gel strength is enhanced by locust bean gum fractions containing a high M:G ratio (LBG80) suggesting that the unsubstituted regions of the backbone interact with xanthan gum.

In summary, interactions between xanthan gum and locust bean gum are maximised under the following set of conditions;

Xanthan gum disordered conformer + Locust bean gum high M:G ratio (LBG80)



Maximum Synergy

In addition, it has been shown that these mixed polysaccharide systems exhibit controlled release characteristics which have the potential to be used for pharmaceutical applications, in which this study has suggested ways in which their behaviour and hence drug release properties may be regulated and controlled. The prime factor in controlling the release properties resides in understanding the interaction mechanisms between the two polysaccharides, which in turn depend upon an aqueous environment. Since the extent of the gelation process is temperature dependent, chronotherapeutic approaches may be a future possibility with these polysaccharide systems. In addition, these polysaccharides are natural, biodegradable with FDA approval which attracts patient acceptance.

The techniques chosen for the present study are complimentary, and provide an insight into the mechanisms for the interaction between the two polysaccharides, with a view to developing pharmaceutical uses. By measuring the physical properties of the systems (oscillatory rheology, texture analysis studies) and the chemical properties (polysaccharide characterisation, HSDSC, SCD), useful information regarding these systems can be obtained.

The research in the current project would benefit further work in exploring and developing granulation methods in an aqueous environment at different temperatures, which incorporate drug and excipients before interactions between xanthan gum and locust bean gum are established. The choice of other fillers similar to sucrose (e.g. dextrose) should also be investigated to determine if the kinetics depend on the filler type. In addition, follow on studies incorporating fractionated locust bean gum, sucrose and sodium chloride would complete the picture for the SCD studies, which provided

useful information regarding the structures and changes involved during the interactions. Finally, further work is a pre-requisite for determining the effects of sucrose on these polysaccharide systems, in order to explain the findings observed in the present project. SCD, microscopy methods and creep studies may provide useful tools in finding the answers.

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APPENDICES

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APPENDIX 1

CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

FES MEASUREMENTS FOR KNOWN STANDARD SOLUTIONS OF Ca^{2+}

Ca^{2+} ppm	Emission 1	Emission 2	Emission 3	Average Emission
0	0	0	0	0
0.2	0.197	0.202	0.203	0.201
0.4	0.415	0.417	0.415	0.416
0.6	0.630	0.633	0.625	0.629
0.8	0.822	0.822	0.818	0.821
1.0	1.001	0.996	0.990	0.996

FES MEASUREMENTS FOR KNOWN STANDARD SOLUTIONS OF Na^{+}

Na^{+} ppm	Emission 1	Emission 2	Emission 3	Average Emission
0	0	0	0	0
0.1	0.178	0.178	0.180	0.179
0.2	0.239	0.243	0.241	0.241
0.4	0.455	0.459	0.454	0.456
0.6	0.706	0.708	0.712	0.709
0.8	0.942	0.938	0.939	0.940
1.0	1.008	1.046	1.017	1.024

FES MEASUREMENTS OF Ca^{2+} IONS FOR XANTHAN GUM 1%w/w SOLUTION

Xanthan gum 500ppm	Emission 1	Emission 2	Emission 3	Average Emission
	0.468	0.476	0.472	0.472

FES MEASUREMENTS OF Na^{+} IONS FOR XANTHAN GUM 1%w/w SOLUTION

Xanthan gum 5ppm	Emission 1	Emission 2	Emission 3	Average Emission
	0.223	0.222	0.222	0.222

Calculations:

Calcium ions (Ca^{2+})

Xanthan gum 500ppm → 0.472 average emission units.

From FES measurements of known standard solutions of Ca^{2+} used to construct calibration curve (Figure 3.3) ;

0.472 average emission units ≡ Ca^{2+} 0.5ppm.

Thus, xanthan gum 500ppm contains Ca^{2+} 0.5ppm.

Since ;

500ppm ≡ 0.05%

A xanthan gum 1% w/w solution contains Ca^{2+} 10ppm.

And:

A xanthan gum 1% w/w solution contains Ca^{2+} 0.001% w/w

Sodium ions (Na^{+})

Xanthan gum 5ppm → 0.222 average emission units.

From FES measurements of known standard solutions of Na^{+} used to construct calibration curve (Figure 3.4) ;

0.222 average emission units ≡ Na^{+} 0.2ppm.

Thus, xanthan gum 5ppm contains Na^{+} 0.2ppm.

Since ;

5ppm ≡ 0.0005%

A xanthan gum 1% w/w solution contains Na^{+} 342ppm.

And ;

A xanthan gum 1% w/w solution contains Na^{+} 0.03% w/w

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RECKITT & COLMAN

Confidential

Pharmaceutical R&D
Reckitt & Colman Products
Dansom Lane
Hull
HU8 7DS

Prototype Test Method
Technology Research

Region:	PHARMACEUTICAL	Laboratory: UK
Authorisation Status:	Part-Authorised	
Reference number:	22492	Issue:
Method Descriptor:	Determination of Fibre Content via the Englyst Procedure CG059B	
Alternative Description:	Englyst Procedure	

Project: Fibre Analysis

Title: The Englyst Procedure for Measuring Total and Insoluble Fibre Content.

Introduction: The Englyst procedure defines fibre as Non-Starch Polysaccharides $(NSP)_4$ and determines the concentration of in samples as a percentage of their weight. Initially the sample must be homogenous and representative of the whole. Total and insoluble fibre are measured separately. The procedure removes any starch, fat and free sugars before hydrolysing the polysaccharides. The resulting sugars are reduced and acetylated before analysis by GC. Uronic acid levels are measured by UV spectroscopy.

Health & Safety:

ACETONE: Highly inflammable. Dispense in a spark free fume cupboard and avoid sources of ignition.

DIMETHYL SULPHOXIDE: Avoid contact with eyes and skin, harmful if swallowed.

GLACIAL ACETIC ACID: Inflammable. Causes severe burns. Avoid contact with skin and eyes.

ABSOLUTE ETHANOL: Highly inflammable. Dispense in a spark free fume cupboard and avoid sources of ignition.

CONC. SULPHURIC ACID: Highly corrosive, causes severe burns. Avoid contact with skin and eyes.

12M SULPHURIC ACID: Corrosive, causes burns. Avoid contact with skin and eyes.

OCTAN-2-OL: Irritant. Avoid contact with skin and eyes.

AMMONIUM HYDROXIDE (conc. and 12M): Avoid contact with eyes and skin. Irritating to respiratory system. Do not breathe vapour, always dispense in a fume cupboard.

SODIUM BOROHYDRIDE: Inflammable. Irritant. Avoid contact with skin and eyes.

1-METHYLIMIDAZOLE: Inflammable and corrosive, causes burns. Dispense in a spark free fume cupboard and avoid sources of ignition. Avoid contact with skin and eyes.

7.5M POTASSIUM HYDROXIDE: Causes burns. Avoid contact with skin and eyes.

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CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

3,5 - DIMETHYLPHENOL: Toxic. Avoid contact with skin and eyes.

Instrumentation:

GC: Hewlett Packard HP 5890 or 6890 GC with HP7673 autosampler (or other suitable GC with autosampler)
CENTRIFUGE: MSE Coolspin II with 0.25 radius rotor (or other suitable centrifuge capable of providing accelerations of 1900g)
UV SPECTROMETER: ATI/UNICAM UV4 Spectrometer (or other suitable UV Spectrometer capable of measuring and recording absorbencies at 400nm and 450nm).

Reagents:

ACETONE

DIMETHYL SULPHOXIDE

50% SATURATED BENZOIC ACID: Prepare a saturated solution by dissolving 7g of benzoic acid in 250ml of boiling water. Pour the solution into 1.5 litres of water in a 2 litre volumetric flask. Mix well and allow to cool to room temperature. Dilute to 2 litres with water and allow to stand for 24 hours. Filter if necessary and dilute 1:1 v/v with water before use.

GLACIAL ACETIC ACID

1M CALCIUM CHLORIDE

SODIUM ACETATE BUFFER (0.1M, pH 5.2): Dissolve sodium acetate anhydrous (8.2g) or trihydrate (13.6g) in 50% saturated benzoic acid and dilute to 1 litre. Adjust to pH 5.2 with glacial acetic acid, and add 4ml/litre of 1M calcium chloride solution.

ALPHA-AMYLASE SOLUTION: Mix 90μl of alpha-amylase suspension (Sigma A-4268) with 1ml of sodium acetate buffer to give a 2150 units/ml solution. Prepare immediately before use.

PULLULANASE SOLUTION: Mix 10μl of pullulanase suspension (Sigma P-5420) with 1ml of sodium acetate buffer solution to give a 1unit/ml solution. Prepare immediately before use.

ABSOLUTE ETHANOL

85% ETHANOL: Dilute absolute ethanol with water.

SODIUM PHOSPHATE BUFFER: Dissolve 28.4g anhydrous disodium hydrogen orthophosphate (or molar equivalent of hydrated) in water and make up to 1 litre with water to give a 0.2M solution. Adjust to pH7 using 0.2M sodium dihydrogen orthophosphate solution (made up with 24g anhydrous or 27.6g of the monohydrated salt in 1 litre of water).

CONCENTRATED SULPHURIC ACID

12M SULPHURIC ACID: Place approximately 70ml of water in a 250ml volumetric flask and place in an ice-water bath in a fume cupboard. (Ensure that the ice-water remains at 0°C during preparation). Slowly, about 10ml at a time, add 160ml concentrated sulphuric acid, leaving time between additions for the solution to cool. Leave the solution to cool to room temperature and make up to 250ml with water.

2M SULPHURIC ACID: Dilute 12M sulphuric acid 1:5 with water, with cooling.

EXTERNAL STANDARD SOLUTION: Dissolve approximately 0.5mg/ml (recorded accurately) of each of rhamnose, arabinose, xylose, mannose, galactose and glucose in 2M sulphuric acid. Prepare a second solution containing approximately 0.25mg/ml (recorded accurately) of the six sugars as described above.

ALLOSE INTERNAL STANDARD SOLUTION: Dissolve 1mg/ml (recorded accurately) of allose (dried to constant weight under reduced pressure over phosphorous-V-oxide) in

APPENDIX 2

CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

50% saturated benzoic acid.

12M AMMONIUM HYDROXIDE: Dilute 62ml of concentrated ammonia solution to 100ml with water in a fume cupboard.

OCTAN-2-OL

SODIUM BOROHYDRIDE/AMMONIUM HYDROXIDE SOLUTION: Weigh out 200mg of sodium borohydride and dissolve in 1ml of 12M ammonium hydroxide and 3ml water. Prepare immediately before use.

1-METHYLIMIDAZOLE

ACETIC ANHYDRIDE

BROMOPHENOL BLUE SOLUTION: Dissolve 0.4g of bromophenol blue in a litre of water.

7.5M POTASSIUM HYDROXIDE: Dissolve 420g of potassium hydroxide pellets in water, cool and dilute to 1 litre.

SODIUM CHLORIDE/BORIC ACID SOLUTION: Dissolve 2g of sodium chloride and 3g of boric acid in 100ml water.

3,5- DIMETHYLPHENOL SOLUTION: Dissolve 0.1g of 3,5- dimethylphenol in 100ml of glacial acetic acid.

GLUCURONIC ACID

Chromatography:

Column:	Supelco SP2380 capillary column. (30m x 0.25mm ID)	
Carrier:	Helium at column head pressure of 17psi at 250°	
Temperatures:	Oven	250°C
	Injector	260°C
	Detector	280°C
Split ratio:	4.4:1 (3ml/minute)	
Injection volume:	3 to 5 μl	
Run time:	10 to 20 minutes	

Procedure:

Notes:

1. BREAKS IN THE PROCEDURE: The procedure may be interrupted at the following points:

   a) Dry residue from stage 2.3 may be stored for several weeks

   b) Hydrolysates from stage 3 may be stored at 5°C for 24 hours before alditol acetate derivitisation and for several weeks before uronic acid determination.

   c) Acidified solutions from stage 4.1 may be stored at room temperature for 2 to 3 days before starting stage 4.2

   d) The alditol acetate derivatives from stage 4.2 may be stored at 5°C in autosampler vials for up to a week before GC analysis.

2. CENTRIFUGATION: The accelerations in "g" are followed by spin rates in brackets which apply when using a rotor of radius 0.25m on the MSE Coolspin II.

3. EVAPORATING ACETONE: Care must be taken to ensure that the sample does not bump excessively and adhere to the tube walls. Gentle stirring and a gradual increase in the temperature of the water bath up to 80°C should prevent excessive bumping. Reducing the amount of sand in the sample will reduce bumping, though may leave a more aggregated sample after centrifugation.

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CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

Standard Preparation and Calibration:

1. NEUTRAL SUGARS: For the purposes of determining response factors and as a check for gas chromatography and acetylation stages, at least two external standard solutions should be made up as described in the reagents section.

   At the beginning of stage 4.1 in the procedure, instead of taking 3ml of hydrolysate, take 6ml of external standard solution and proceed through stage 4.1 adding twice the stated volume of each reagent. At the start of stage 4.2 pipette two 0.5ml aliquots from each standard solution sample into different 8-dram vials, and then proceed as usual.

   Use calculation 1 in the calculations section to determine response factors for each of the six neutral sugars.

2. URONIC ACIDS: For calibration of the uronic acid determination make up five solutions of glucuronic acid in water: 20, 30, 50, 80 and 100 μg/ml. Take 0.3ml aliquots of each solution in duplicate and analyse according to Section 8 of the method.

   Subtract the absorbance at 400nm from the absorbance at 450nm and plot a graph of this against concentration of uronic acid. The gradient and y-intercept of this graph are then used in calculating the uronic acid concentration in the samples. (See calculation 3 in the calculation section.)

Sample Preparation

1. PREPARATION:

   Samples should be homogenous and representative of the whole. Any milling should ensure that fibre in the sample is not degraded e.g. by excessive heat.

   Weigh out duplicate aliquots of each sample so that each portion contains no more than 50mg of NSP and no more than 200mg of dry material. (500mg for soft drinks.) Place these samples in 50ml glass screw-top centrifuge tubes with a small PTFE coated magnetic follower and approximately 0.5ml acid rinsed sand (to help prevent aggregation of the sample during centrifugation).

   With liquid samples it may be necessary to freeze-dry a quantity of the sample and then weigh portions from the dry residue.

2. FAT EXTRACTION:

   This is not required if the samples are dry and do not contain more than 5% fat, e.g. ispaghula.

   Add approximately **40ml of acetone** to the sample and stir for 30 minutes. Centrifuge the tubes for 10 minutes at 1900g (2610 r.p.m.). Remove as much as possible of the supernatant by aspiration, ensuring that the residue is not disturbed. Place the tubes in a water bath at 80°C and stir gently until the residue is completely dry.

3. DISPERSION OF STARCH:

   Add **2ml of dimethyl sulphoxide** to the dry sample and mix carefully, ensuring that none of the sample adheres to the tube walls, and that all the sample is wetted. Place the tubes in a boiling water bath for 30 minutes, cover and stir continuously. (A mucilage may prevent the motion of the magnetic follower, but this will not interfere with the procedure).

   Pre-equilibrate sufficient sodium acetate buffer at 50°C. (8ml per tube required.)

4. ENZYMATIC HYDROLYSIS OF STARCH:

Add, without cooling, **8ml of sodium acetate buffer** to each tube. Mix, ensuring that none of the sample adheres to the test-tube wall. Cool the samples and the sodium acetate buffer to below 35°C, then prepare and add **0.5ml alpha-amylase solution** and **0.1ml pullulanase solution**. (Do not mix the solutions beforehand).

Incubate the tubes at 42°C (±2°C) for 16 to 18 hours, mixing after the first hour. Again, ensure that none of the sample adheres to the tube walls.

5. EXTRACTION AND WASHING OF THE RESIDUE:

5.1 TO ANALYSE FOR TOTAL FIBRE ONLY:

Remove the tubes and cool to room temperature. Add approximately **40ml absolute ethanol**, and mix by inversion. Place in ice-water for 30 minutes.

Centrifuge at 1900g (2610 r.p.m.) for 10 minutes. Remove the tubes and aspirate as much of the supernatent as possible without disturbing the residue. If the supernatent is cloudy at any stage after centrifugation sonicate the tubes for 30 minutes and re-centrifuge at 1900g (2610 r.p.m.).

Add approximately **10ml 85% ethanol**, vortex mix and then add a further **40ml of 85% ethanol**. Mix by inversion and then stir for at least 5 minutes to form a suspension of the residue. Centrifuge at 1900g (2610 r.p.m.) for 10 minutes. Remove the tubes and aspirate as much of the supernatent as possible without disturbing the residue. *Go to stage 5.3.*

5.2 TO ANALYSE FOR INSOLUBLE FIBRE ONLY:

5.2.1. - If suspension material is suspected, i.e. samples have not been irradiated.

Remove the tubes and add **40ml sodium phosphate buffer**. Place the capped tubes in a boiling water bath for 30 minutes, cover and stir continuously. Remove and cool in water at room temperature, then centrifuge for 10 minutes at 500g (1300rpm) to separate the gelatinous suspension material from the pelleted insoluble material. Using a large bulb pastette, remove the supernatant with the suspension material and place into a separate centrifuge tube. Mark the tubes clearly - one containing the insoluble material and one containing the suspension material.

Centrifuge the suspension tubes at 1900g (2610 r.p.m.) for 10 minutes, and ensure that the supernatent is clear before continuing. (If not, sonicate and re-centrifuge as described in 7.2.1A.) Remove as much as possible of the supernatent by aspiration without disturbing the residue. From this point onwards, follow the same procedure for the insoluble and suspension tubes.

Add approximately **10ml water**, vortex mix and then **fill the tubes to approximately 50ml with water**. Mix by inversion, and stir for at least 5 minutes to form a suspension of the residue. Centrifuge at 1900g (2610 r.p.m.) for 10 minutes and remove as much as possible of the clear supernatent by aspiration without disturbing the residue. *Go to stage 5.3.*

5.2.2. - If suspension material is not expected, i.e. samples known to be irradiated.

Remove the tubes and add **40ml sodium phosphate buffer**. Place the capped tubes in a boiling water bath for 30 minutes, cover and stir continuously. Remove and cool in water at room temperature for 10 minutes, then centrifuge at 1900g (2610 r.p.m.) for 10 minutes, and ensure that the supernatent is clear before continuing. (If not sonicate and re-centrifuge as described in 7.2.1A.) Remove as much as possible of the supernatent by aspiration without disturbing the residue.

Add approximately **10ml water**, vortex mix and then **fill the tubes to**

approximately 50ml with water. Mix by inversion, and stir for at least 5 minutes to form a suspension of the residue. Centrifuge at 1900g (2610 r.p.m.) for 10 minutes and remove as much as possible of the clear supernatent by aspiration without disturbing the residue. *Go to stage 7.2.2.*

5.3. ALL SAMPLES:

Add approximately **10ml absolute ethanol**, vortex mix and then **fill the tubes to approximately 50ml with absolute ethanol.** Mix by inversion, and stir for at least 5 minutes to form a suspension of the residue. Centrifuge at 1900g (2610 r.p.m.) for 10 minutes and remove as much as possible of the clear supernatent by aspiration without disturbing the residue.

Add approximately **10ml acetone**, vortex mix and then **fill the tubes to approximately 50ml with acetone**. Stir for at least 5 minutes to form a suspension of the residue. Centrifuge at 1900g (2610 r.p.m.) for 10 minutes and remove as much as possible of the clear supernatent by aspiration without disturbing the residue. Place the tubes in a water bath at 80°C and use the magnetic stirrer until the residue and tube are completely dry. If aggregation has occurred in the sample then it should be broken up before the residue is completely dry.

6. ACID HYDROLYSIS:

Add 5ml **of 12M sulphuric acid** to the dry residue and immediately vortex mix, ensuring all the material is wetted. Take care that none of the sample adheres to the tube walls. Place the tubes in a 35°C oven (±1°C) for 1 hour with occasional mixing to disperse the cellulose.

Remove the tubes and then rapidly add **25ml water** and vortex mix. Place the capped tubes into a boiling water bath, cover and leave for 1 hour, stirring continuously. Cool in water at room temperature. Transfer the hydrolysates to 8-dram vials. If there is suspended solid present, then filter the hydrolysates using GF/C filter paper.

These hydrolysates may be stored at 5°C for 24 hours before alditol acetate derivation, or for several weeks before measurement of uronic acids.

7. PREPARATION OF THE ALDITOL ACETATE DERIVATIVES:

7.1 STAGE 1 - REDUCTION OF THE SUGARS:

Transfer 3ml hydrolysate to a 4-dram vial, add **1ml allose internal standard** and shake to mix. Place in ice water and add **2.4ml 12M ammonium hydroxide solution.** Ensure that the solution is alkaline. Prepare and add **0.4ml of sodium borohydride/ ammonium hydroxide solution,** and **5µl of octan-2-ol.** Shake to mix. Leave the vials lightly capped (to allow release of pressure) in a 40°C oven for 1 hour. Remove and add **0.8ml glacial acetic acid.**

These acidified solutions may be kept at room temperature for 2 to 3 days.

7.2 STAGE 2 - ACETYLATION:

Transfer 0.5ml of the acidified solution to an 8-dram vial. Add **0.5ml**

1-methylimidazole and **5ml acetic anhydride,** then shake to mix and leave capped for 10 minutes. Add **1.25ml** absolute ethanol, then shake to mix and leave capped for 5 minutes. Add **10ml water,** shake to mix and leave capped for 5 minutes.

Add **0.5ml bromophenol blue,** mix and place the vials in an ice water bath. Add **5ml 7.5M potassium hydroxide,** wait a few minutes and then add a further 5ml **7.5 M potassium hydroxide.** Cap the vials and mix by repeated inversion. Leave

for at least 15 minutes, until separation into the two phases is complete.

Draw the clear phase into the tip of an automatic pipette or pastette and transfer into a small autosampler vial with a 250µl micro-sert. Ensure that none of the blue phase is included in the auto-sampler vials. If any of the blue phase is drawn up allow the phases to separate and discard the blue phase before transferring the clear phase to the autosampler vial. The clear phase may be stored at 5°C in the auto-sampler vials for up to 2 weeks before analysis by GC.

8. URONIC ACID DETERMINATION:

Transfer 0.3ml of the hydrolysate obtained in section 7.3 to a scrupulously clean glass test tube. Dilute, if necessary, with 2M sulphuric acid to ensure that the uronic acid concentration does not exceed 100ppm (e.g. flour, no dilution; bran, dilute 1:2; most fruit and vegetables dilute 1:5). Add **0.3ml sodium chloride/boric acid solution**, mix, and then add **5.0ml concentrated sulphuric acid** and vortex mix immediately. Heat in a 70°C oven for 40 minutes.

Remove the tubes and cool in water at room temperature (the tubes may be left at room temperature for up to an hour).

Add **0.2ml 3-5 dimethylphenol solution** and vortex mix immediately. Wait 15 minutes and then measure the absorbance at 400nm and 450nm against a de-ionised water reference.

APPENDIX 2

CHAPTER 3 : CHARACTERISATION OF THE POLYSACCHARIDES

Calculation:

1. RESPONSE FACTORS:

$$RF = \frac{\text{Concentration of sugar in ext. standard (mg/ml)} * 3 * \text{Allose peak area}}{\text{Concentration of allose in int. standard (mg/ml)} * \text{Sugar peak area.}}$$

This formula can be re-arranged and used to check the concentration of a new external standard using old response factors or vice versa.

Current response factors are (29/5/1997)

Rhamnose	1.0756
Arabinose	0.9041
Xylose	0.8414
Mannose	0.9555
Galactose	0.8724
Glucose	0.8019

2. NEUTRAL SUGARS:

Conc. (%w/w) of sugar

$$= \frac{RF * 890 * \text{Conc. of Allose soln. (mg/ml)} * \text{Sugar peak area in sample}}{\text{Allose peak area} * \text{Sample weight (mg)}}$$

An extra factor of 1.5 should be applied for rhamnose calculation due to incomplete hydrolysis and acetylation.

3. URONIC ACID LEVELS:

Conc. (%w/w) of uronic acid in sample

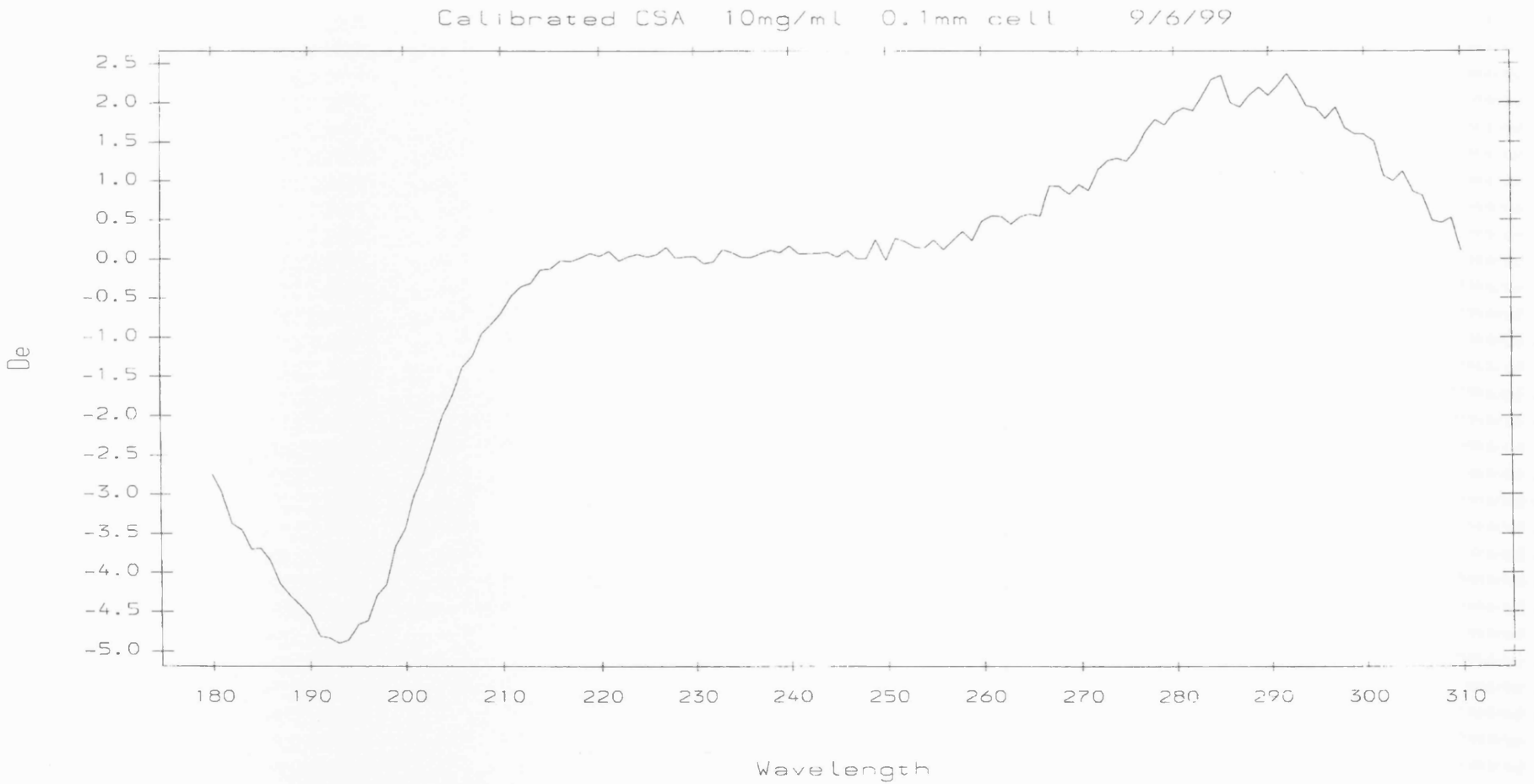
$$= \frac{(A_{450} - A_{400} - c) * 2.9 * 0.89}{m * \text{sample weight (mg)}}$$

where: A_{450} = absorbance at 450nm
A_{400} = absorbance at 400nm
c = y-intercept of calibration graph
m = gradient of calibration graph.

If dilution was necessary apply dilution factor to calculation.

Current values are (calibrated 29/5/1997)
c: -0.033101
m: 0.004582

Fibre content [% w/w] = sum of the concentrations of the six neutral sugars + uronic acid concentration



APPENDIX 3

CHAPTER 6 : CHARACTERISATION OF CONFORMATIONAL AND TRANSISTIONAL BEHAVIOUR USING HSDSC AND SCD