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Doctor of Philosophy

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DECLARATION

I hereby declare that the work is of the author alone except where due acknowledgment has been made. The content of the thesis is result of the work, which has been carried out at Department of Food Science, School of Applied Sciences, RMIT University, since the official commencement date of approved research program.

Omar Almrhag
17 June 2012
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Last but certainly not the least; I would love to take this opportunity to express my grateful to my parents and siblings for their continual love and support.
PUBLICATION AND PRESENTATIONS

Publications


Poster presentations

Almrhag, O., George, P., & Kasapis, S. Fundamental studies of dietary fibres and co-solute interaction in high-solid materials. Poster presented at the RMIT University 2010 HDR conference that was held on 20th of October in Melbourne, Victoria, Australia.

Almrhag, O., George, P., & Kasapis, S. Fundamental studies of protein/polysaccharide and sugar analogue interaction in high-solid materials. Poster presented at Australian Food Science Summer School that was held from 9th-11th February 2011 in University of Queensland, Brisbane, Queensland, Australia.

Almrhag, O., George, P., & Kasapis, S. Fundamental studies of protein/polysaccharide and sugar analogue interaction in high-solid materials. Poster presented at school research day that was held on 17th June 2011 in RMIT University, Victoria, Australia.

Almrhag, O., George, P., & Kasapis, S. Fundamental studies of dietary fibres and co-solute interaction in high-solid systems. Poster presented at the RMIT University 2011 HDR conference that was held on 21st of October 2011 in Melbourne, Victoria, Australia.

Almrhag, O., George, P., & Kasapis, S. (2012). Fundamental understanding of the glass transition behaviour in the hydrocolloid/polydextrose system for replacing sugar in high-solid formulation. Poster presented at the AIFST that was held from 1st-3rd February in Melbourne University, Melbourne, Victoria, Australia.
SUMMARY

High-solid biomaterials increasingly include a number of non-starchy polysaccharides, i.e. dietary fibre to deliver a range of properties such as structure, storage stability, processability, etc. This study aims to: i) develop methodologies and characterise the structure-function relationships in high-solid biopolymer systems and ii) organise a database of physicochemical measurements and viscoelastic properties of high-solid materials by utilising polydextrose as replacement of sugar based co-solute in formulations. Systems of interest are agarose and high methoxy pectin, and in comparison with gelatin that is widely used in high-solid products, in the presence of co-solute (polydextrose or glucose syrup) covering a wide range of solids in preparations.

In these systems, glass transition property is a major determinant of textural consistency, hence thermomechanical analysis was performed using small deformation dynamic oscillation in shear and modulated differential scanning calorimetry (MDSC) in order to characterise the vitrification behaviour of materials. The mathematical model of Williams, Landel and Ferry (WLF), which is built in tandem with the concept of free volume, and the modified Arrhenius equation (Andrade equation) were used to further model the vitrification behaviour of the systems thus ascribing physical significance to the prediction of glass transition temperature ($T_g$). To investigate the nature of physicochemical interactions between biopolymer molecules and co-solute, environmental scanning electron microscopy (ESEM), Fourier transform infrared spectroscopy (FTIR) and wide angle X-ray diffraction (WAXD) techniques were employed.

The first type of system in this Thesis consists of agarose and co-solute (polydextrose), with the investigation dealing with changes in network morphology of agarose when mixed with polydextrose from low to high-solid preparations. There was a central observation of decline in the mechanical strength of aqueous agarose preparation upon addition of high levels of polydextrose, which was accompanied by a reduced enthalpic content of the coil-to-helix transition in the polysaccharide network. Glass transition phenomena were observed at subzero temperatures in condensed preparations, hence further arguing for the formation of a lightly cross-linked agarose network with changing solvent quality.
The second system consists of high methoxy pectin and co-solute in the form of polydextrose or glucose syrup, with a view to examining the potential of replacing sugar with polydextrose in commercial formulations. Structural properties of pectin preparations were recorded in relation to the molecular weight and concentration of added co-solute in an acidic environment (pH ~ 3.0). High levels of co-solute induce formation of weak pectin gels at elevated temperatures (even at 95°C), which upon subsequent cooling exhibit increasing strength and convert to a clear glass at subzero temperatures. Glucose syrup is an efficient plasticiser leading to a reduction in the glass transition temperature of the pectin network, whereas polydextrose assists in the formation of stronger pectin gels in the rubbery state and accelerated vitrification properties.

The investigation on the third system, gelatin and polydextrose as the co-solute, focused on the understanding of structural behaviour in the presence of the non-sugar co-solute and in comparison with polysaccharides. A progression in the mechanical strength and thermal stability of the gelatin network was observed with the addition of polydextrose to the system, which was distinct from that of polysaccharide/co-systems. Combined thermomechanical and microscopy evidence argues for the development of phase separation phenomenon between protein and co-solute in high-solid preparations, where gelatin maintains helical conformation to provide network integrity as well as glassy consistency at subzero temperature. Again, that was distinct from the state of a single-phase system and “molecular dissolution” of polysaccharides in a high-content co-solute environment. At the high solids regime, glassy consistency was treated with theoretical frameworks from the synthetic polymer research to pinpoint the glass transition temperature of the gelatin/co-solute preparation.

The fourth system consisting of agarose, gelatin, and co-solute (polydextrose) was investigated from low to high levels of total solids, with a view to examining the phase behaviour of binary biopolymer mixtures that may lead to compositional adjustments in preparations. Agarose and gelatin form non-interactive bicontinuous phases in the aqueous environment. Systematic increase in the concentration of polydextrose prevents the formation of a stable agarose network, with the polysaccharide chains dispersing in the high-solid environment. Gelatin, on the contrary, retains its conformational stability even at a saturating co-solute environment through enhanced protein structuring. Vitrification studies on the high solids system at subzero temperatures provides information on the structural and molecular relaxation of these composites that can serve as a model system for further examination of binary polymeric systems in high co-solute environments.
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LIST OF ABBREVIATIONS

RMIT Royal Melbourne Institute of Technology

µm micrometer

ARG-2 Advanced Rheometer Generation-2

DSC Differential Scanning Calorimeter

MDSC Modulated Differential Scanning Calorimeter

Ca$^{2+}$ Calcium ions

cP centipoise

LVR Linear Viscoelastic Region

CRTs Cathode Ray Tubes

SEM Scanning Electron Microscopy

SE Secondary Electrons

ESEM Environmental Scanning Electron Microscope

VPSEM Variable-Pressure Scanning Electron Microscope
<table>
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<td>keV</td>
<td>Kilo electron volt</td>
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<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
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<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
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<tr>
<td>mM</td>
<td>milli Molar</td>
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pI  Isoelectric point

w/w  Weight by weight

TTS  Time-Temperature Superposition

WLF  William, Landel, and Ferry
# LIST OF SYMBOLS

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<th>Symbol</th>
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<tr>
<td>( \eta' )</td>
<td>Dynamic viscosity</td>
<td>Pa.s</td>
</tr>
<tr>
<td>( \sigma_0 )</td>
<td>Yield stress</td>
<td>Pa</td>
</tr>
<tr>
<td>( \eta^* )</td>
<td>Complex viscosity</td>
<td>Pa.s</td>
</tr>
<tr>
<td>( \phi_2 )</td>
<td>Volume fraction in dispersed phase</td>
<td>m³</td>
</tr>
<tr>
<td>( \phi_m )</td>
<td>Maximum packing fraction</td>
<td>m³</td>
</tr>
<tr>
<td>( \phi_X )</td>
<td>Phase volume of component X</td>
<td>m³</td>
</tr>
<tr>
<td>( \phi_Y )</td>
<td>Phase volume of component Y</td>
<td>m³</td>
</tr>
<tr>
<td>( c_X )</td>
<td>Effective concentrations (% w/w) of polymers X in the two phases</td>
<td>%</td>
</tr>
</tbody>
</table>
\( c_y \)  Effective concentrations (% w/w) of polymers Y in the two phases  \( \% \)

\( D \)  Relative density -

\( D_x \)  Relative density of phase X -

\( D_y \)  Relative density of phase Y -

\( G'' \)  Loss modulus  Pa

\( G' \)  Storage modulus  Pa

\( \tan \delta \)  Phase angle -

\( G_{ag} \)  Storage modulus of agarose  Pa

\( G_c \)  Modulus of composite gels  Pa

\( G_{exp} \)  Experimental storage modulus  Pa

\( G_L \)  Lower bound moduli  Pa

\( G_X \)  Modulus of phase X  Pa
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_y$</td>
<td>Modulus of phase Y</td>
<td>Pa</td>
</tr>
<tr>
<td>$k$</td>
<td>Einstein coefficient</td>
<td>-</td>
</tr>
<tr>
<td>$M_x$</td>
<td>Moduli of polymer X</td>
<td>Pa</td>
</tr>
<tr>
<td>$M_y$</td>
<td>Moduli of polymer Y</td>
<td>Pa</td>
</tr>
<tr>
<td>$n$</td>
<td>Square root of R</td>
<td>-</td>
</tr>
<tr>
<td>$p$</td>
<td>Solvent partition</td>
<td>-</td>
</tr>
<tr>
<td>$R$</td>
<td>Ratio of the two moduli, $M_y/M_x$</td>
<td>-</td>
</tr>
<tr>
<td>$S_x$</td>
<td>Fraction of solvent in the polymer X phase</td>
<td>m³</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>Maximum heat flow temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$t_{w,x}$</td>
<td>Total weights of phase X</td>
<td>kg</td>
</tr>
<tr>
<td>$t_{w,y}$</td>
<td>Total weights of phase Y</td>
<td>kg</td>
</tr>
<tr>
<td>$V_x$</td>
<td>Relative volume of region X</td>
<td>-</td>
</tr>
<tr>
<td>$V_y$</td>
<td>Relative volume of region Y</td>
<td>-</td>
</tr>
<tr>
<td>$w$</td>
<td>Total weight of water in system</td>
<td>kg</td>
</tr>
</tbody>
</table>
\( w_x \)  Weight of water in phase X  \( \text{kg} \)

\( w_y \)  Weight of water in phase Y  \( \text{kg} \)

\( T \)  Time over which the deformation is observed  \( \text{s} \)

Temperature  \( \text{K} \)

\( T_g \)  Glass transition temperature  \( ^\circ \text{C} \)

\( \tau \)  Characteristic time of the material  \( \text{s} \)
CHAPTER 1

INTRODUCTION

1.1 Hydrocolloids

The term hydrocolloids refer to a wide range of biopolymers of biological origin, such as protein, and polysaccharide. They are made up of large number of repetitive molecules for example, amino acids in proteins, and glucose units in starch. Hydrocolloids have been used extensively in various industrial applications to perform a number of functions such as, emulsion, thickening, stabilising and gelling aqueous solutions in food, biomedical and pharmaceutical industries (Phillips & Williams, 2000).

The biopolymers particles are spread throughout water and they behave as liquid or gel depending on the water availability. Due to the ability of biopolymers to form gel even in very low concentrations, they are used as emulsifiers, gelling agents and whipping agents. According to Lersh (2010), the simple definition of hydrocolloids is those substances which can form gel when contacted with water. Biopolymers contain many types of materials from different sources such as animals, plants and seaweeds. Seed-plant polysaccharide and mucilage are also good sources of hydrocolloids (Karazhiyan et al., 2011). The applications of hydrocolloids in food systems is wildly spread as gelling, thickening, texture modifiers, stabilisers and emulsifiers. Hydrophilic colloids or hydrocolloids are referred to high molecular weight polymers which are in long chain that disperse or dissolve in water to give gelling effect (Glicksman, 1978).

Biopolymers are very important food additives to provide thickening and stabilising properties, and they are come mostly from living organisms (Lmeson, 2010). Table 1.1 illustrates the most common hydrocolloids and their sources.
Table 1.1 Hydrocolloids and their sources (Lmeson, 2010).

<table>
<thead>
<tr>
<th>Source</th>
<th>Examples of Hydrocolloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botanical</td>
<td></td>
</tr>
<tr>
<td>Trees</td>
<td>Cellulose, and exudates tree gum such as, gum Arabic, gum karaya, gum ghattu and gum tragacanth</td>
</tr>
<tr>
<td>Plants</td>
<td>Starch, pectin, and cellulose</td>
</tr>
<tr>
<td>Seeds</td>
<td>Guar gum, locust bean gum, tara gum and tamarind gum</td>
</tr>
<tr>
<td>Tubers</td>
<td>Konjac mannan</td>
</tr>
<tr>
<td>Algae</td>
<td></td>
</tr>
<tr>
<td>Red seaweeds</td>
<td>Agar and  carrageenan</td>
</tr>
<tr>
<td>Brown seaweeds</td>
<td>Alginate</td>
</tr>
<tr>
<td>Microbial</td>
<td>Xanthan gum, curdlan, dextran, gellan gum and  cellulose</td>
</tr>
<tr>
<td>Animal</td>
<td>Gelatin, caseinate, whey protein and chitosan</td>
</tr>
</tbody>
</table>

Biopolymers have significant effect on food products; they are used as texture modifying agents, stabilisers, emulsifiers, gelling agents, and thickeners, are considered as a major food additives that significantly affect and control textural properties of food (Fonkwe, et al, 2003; Gabriele et al., 2009). The commonly used hydrocolloids in food industry are gelatin, agar, pectin, starch, guar gum, locust bean gum, carrageenan, alginate, cellulose and xanthan. Proteins and polysaccharides are widely used to create and modify texture in many processed food products. Gelatin was amongst preferred hydrocolloids, which has been used for many decades, due to its chemical and physical properties (Lmeson, 2010). Biopolymers have many applications in food manufacturing, and the usage of such biopolymers has been increased in the last few years. Even though they are added to food products in concentrations usually below 1%, they have significant effect on the textural and organoleptic properties of food products.

Food products can be produced in either complete or partial amorphous state by using different processing methods, for instance, spray drying, freeze drying, extrusion, and baking. The function and application of hydrocolloids in food products are shown in table 1.2.
Table 1.2 Application of hydrocolloids in food products (Glicksman, 1978).

<table>
<thead>
<tr>
<th>Function</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive</td>
<td>Bakery glaze</td>
</tr>
<tr>
<td>Binding agent</td>
<td>Sausages</td>
</tr>
<tr>
<td>Bulking agent</td>
<td>Dietetic foods</td>
</tr>
<tr>
<td>Crystallisation inhibitor</td>
<td>Ice cream and sugar syrup</td>
</tr>
<tr>
<td>Clarifying agent</td>
<td>Beer, wine</td>
</tr>
<tr>
<td>Clod agent</td>
<td>Fruit</td>
</tr>
<tr>
<td>Coating agent</td>
<td>Confectionary</td>
</tr>
<tr>
<td>Emulsifier</td>
<td>Salad dressing</td>
</tr>
<tr>
<td>Encapsulation agent</td>
<td>Powdered fixed flavours</td>
</tr>
<tr>
<td>Film former</td>
<td>Sausage casing and protective coatings</td>
</tr>
<tr>
<td>Flocculating agent</td>
<td>Wine</td>
</tr>
<tr>
<td>Foam stabiliser</td>
<td>Whipped topping and beer</td>
</tr>
<tr>
<td>Gelling agent</td>
<td>Puddings, desserts, aspics and mousses</td>
</tr>
<tr>
<td>Mould rebase agent</td>
<td>Gum drops and jelly candies</td>
</tr>
<tr>
<td>Protective colloid</td>
<td>Flavour emulsions</td>
</tr>
<tr>
<td>Stabiliser</td>
<td>Beer and mayonnaise</td>
</tr>
<tr>
<td>Suspending agent</td>
<td>Chocolate milk</td>
</tr>
<tr>
<td>Swelling agent</td>
<td>Processed meats</td>
</tr>
<tr>
<td>Syneresis inhibitor</td>
<td>Cheese and frozen foods</td>
</tr>
<tr>
<td>Thickening agent</td>
<td>Jams, pie fillings, sauces and gravies</td>
</tr>
<tr>
<td>Whipping agent</td>
<td>Topping and icings</td>
</tr>
</tbody>
</table>
Most hydrocolloids are linear, which are composed of one long chain with small side branches, however some of them are branched that is joined together in a bushy shape. Linear hydrocolloids exhibit higher viscosity than branched ones. Hydrocolloids have active groups on their main backbone chain for example amide and carboxylic acid groups in gelatin, and these groups will affect solubility, and gelling ability of polymers (Poppe, 1992).

1.2 Gels

According to Clark and Ross-Murphy (2009), gels are known as solvent swollen colloids. Ferry (1980) suggested that gel is a swollen polymeric system which shows no steady flows pattern. The hydrocolloid particles are aggregates together to form long chains which overlap on each other to form a network, whereas the aqueous phase dispersion gets trapped in the network. The covalent and non-covalent interaction between polymer molecules controls the polymer network formation (Clark & Ross-Murphy, 2009). According to Flory (1974) there are four different types of gel based on their structure and gelling behavior:

- Type 1: Well-ordered lamellar structure
- Type 2: Covalent polymer network (disordered)
- Type 3: Polymer network formed though physical aggregation (disordered but with regions of local order)
- Type 4: Particular, disordered junctions

Figure 1.1 shows three different types of polymer network such as, chemically cross-linked, junction zones and particulate or fibrillar.
1.3 Agarose

Agar was the first biopolymer used as food additive and it has been used for more than three centuries in the Far East (Armise´n et al., 2000). Agar is defined as a strong gelling hydrocolloid extracted from marine algae, class Rhodophyceae. It is a complex mixture of two or three polysaccharides with similar backbone structure but different degree of charged groups as shown in Figure 1.2. Chemically agarose is a linear co-polymer of alternating (1,3) $\beta$ – D- galactopyranose and -(1,4) 3,6- anhydro- $\alpha$ L- galactopyranose repeat units (Desezcynski et al., 2003). This chemical structure allows agarose to form rigid gels even at low concentrations (~0.1% w/w) (Nussinovitch, 1997).
Figure 1.2 Structural unit of agarose (Praiboon et al., 2006).

Agarose can form a gel in very low concentrations (≈0.1%) through hydrogen bonds and that makes the gel thermo-reversible. This gel is normally turbid, brittle and shows a marked degree of thermal hysteresis (Morris, 1986). The gelation of agarose solutions normally occurs around 38°C, and the gel strength increases by further cooling. The gel melts at temperature above 85°C (Phillips & Williams, 2000). In solution agarose molecules behaves as random coils, however when the solution is cooled the coils become ordered and with further cooling the agarose gel is formed as shown in Figure 1.3 (Arnott et al., 1974).

Figure 1.3 Agar gel formation (Lmeson, 2010).

Agarose is an important food ingredient to control food texture in developing sugar/biopolymers formulations (Deszczynski et al., 2003). Some chemical compounds within food ingredients have positive impact to set agar sols for example, inorganic salts to set agarose
sol, potassium sulfate accelerate the gelation of agarose solutions. Addition of sugars to agarose solutions up to 60% can enhance agarose gel strength through hydrogen bonding between hydroxyl groups in sugars and the polymer, and/or the changing of solvent “water” structure. Agar has been used in many food applications, such as baked goods, confections, meat and fish products (Nussinovitch, 1997). Table 1.3 shows the application of agars in food industry.

Table 1.3 Application of agars in Food (Padua, 1993).

<table>
<thead>
<tr>
<th>Products</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakery</td>
<td>Baked goods, icing and glazes</td>
</tr>
<tr>
<td>Confectionary and desserts</td>
<td>Jelly confections, particularly in Japan</td>
</tr>
<tr>
<td>Meat, fish and poultry</td>
<td>Canned products</td>
</tr>
<tr>
<td>Dairy</td>
<td>Stabiliser for sherbets and ice cream</td>
</tr>
<tr>
<td></td>
<td>Improves texture in cheese</td>
</tr>
<tr>
<td>Beverage</td>
<td>Finning and flocculating agent in wine and fruits</td>
</tr>
<tr>
<td></td>
<td>juice preparation</td>
</tr>
</tbody>
</table>

1.4 Pectin

Pectin refers to a group of diverse and complex polysaccharides found widely in the cell wall and intercellular space (middle lamella) of plant cells. Pectin is a natural compound of all terrestrial plants and it plays together with cellulose as a key role in the cell structure (Lmeson, 2010; Sharma et al., 2006; Lutz et al., 2009). Pectin has a linear chain $\alpha$- (1-4) -D-galacturonic acid residues (refer to Figure 1.4). Most pectin contains a number of sugars such as L- rhamnose, D-galactose and L- arabinose (Glicksman, 1978).
Pectin is a charged biopolymer, and as a result of that, pectin is very sensitive to the degree of pH, and also the presence of cations Ca$^{2+}$ in the system. Gelation of pectin can be classified as equilibrium state between solubility and sedimentation of a biopolymer. In this regard pectin can be categorised into two forms, high methoxyl pectin and low methoxyl pectin (May, 2000). Pectin has been widely used as gelling agent in fruit-based products, such as jams, jellies, yoghurts, marmalades, desserts and fruit filling for bakery products. Furthermore, pectin is used as stabiliser in acidic protein beverages, and juice drinks and also used in confectionery to improve gel structure.

**Low methoxy pectin**

Low methyl pectins with <50% esterified carboxyl groups can form a gel in a presence of divalent cations mainly calcium (Ca$^{2+}$) at pH around 3.0-3.5 (Sharma et al., 2006; Löfgren, & Hermansson, 2007). Low methoxyl pectin forms intermolecular junction zones homogalacturonic regions of different chains. The LM pectin’s ability of forming gels increases with decreasing degree of methylation. Gelation of low methyl pectin is mainly classified as interaction between pectin molecules and calcium ions. Calcium ions are important to form a gel, and their reactivity are controlled by percentage and the arrangement of carboxyl groups within the pectin chains (May, 2000).
High methoxyl pectin

High methoxyl pectin form gels by hydrophobic interactions and hydrogen bonds in a range of pH from 3.0-3.5 and in presence of high concentrations of co-solutes or sugars. Degree of esterification, concentration of co-solute, and pH are the major factors that govern the gel strength and setting temperature (May, 2000). Figure 1.5 illustrates the possible arrangements of pectin molecules in presence of sugar, where A and B pectin chains are less-ordered and high ordered pectin respectively (Lutz et al., 2009).

Figure 1.5 Possible arrangements of pectin gel formation, (A) less ordered pectin, (B) highly ordered pectin, the black sugar unit represents esterified galacturonic acid and the white sugar represents de-esterified galacturonic (Lutz et al., 2009).
1.5 Gelatin

Gelatin is a non-polysaccharide hydrocolloid obtained from hydrolysing collagen in skin, hides, bones and other animal tissues (Nemati et al., 2004; Barbooti et al., 2008). Gelatin contains high molecular weight aggregates (around 10 million) and polypeptides with a molecular weight of less than 80000 Da (Rose, 1987). Gilsenan and Ross Murphy (2000) stated that the sequence of amino acids in gelatin are varies from one source to another, however in general gelatin consists of large amount of glycine, proline and hydroxyproline as shown in Figure 1.6. Chemical properties of gelatin are governed by the animal species, tissues, amino acid composition (Figure 1.7) and molecular weight (Raja Mohd Hafidz et al., 2011; Zhou & Regenstein, 2006).

![Figure 1.6 Chemical configuration of gelatin (Poppe, 1992).](image-url)
This protein polymer has been used in various industries since decades ago and it has a wide range of applications, such as food (confectionary, dairy and meat products), pharmaceutical (capsules), photographic industry and other technical applications (Koepff, 1985; Poppe, 1992; Schacht et al., 1993). In food industry, gelatin has been used as a gelling agent, thickener, stabiliser, clarification agent, and coating hydrocolloid as shown in Table 1.4 (Djagny et al., 2001). The application of gelatin in food industry can be found in products such as candies, ice-cream, bakery goods, desserts and dairy product. However, there are some concerns regarding gelatin utilisation in food products which are mainly:

- Health issues like bovine spongiform encephalitis (BSE) and similar animal diseases can be transmitted to humans through the consumption of animal based food products; as a result, some people have turned to plant sources to avoid such risks.
- Religious aspects – some type of gelatin is derived from pig, therefore cannot be used by some religious groups such as Muslim and Jewish.
Table 1.4 Application of gelatin in Food (adapted from Ledward, 2000).

<table>
<thead>
<tr>
<th>Food product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen cream</td>
<td>To inhibit crystallisation of ice and sugar</td>
</tr>
<tr>
<td>Ice cream</td>
<td>To prevent crystallisation</td>
</tr>
<tr>
<td>Marshmallows, foam wafers</td>
<td>To increase viscosity of the system and stabilise the foam and also to prevent crystallisation</td>
</tr>
<tr>
<td>Lozenges, wafers, and sweet coating</td>
<td>To limit dissolution</td>
</tr>
<tr>
<td>Meat products</td>
<td>Holding water (retain the juices)</td>
</tr>
<tr>
<td>Novel dairy</td>
<td>To produce creamier stirred yoghurts, and gelled products with good organoleptic properties</td>
</tr>
<tr>
<td>Drinks industry</td>
<td>A flocculating agent</td>
</tr>
</tbody>
</table>

Based on extraction treatment, gelatin can be classified into two types type A and type B. Type A is produced by using acid treatment during extraction process in which the raw materials of collagen are washed by cold and diluted mineral acid (pH between 1.5 and 3.0). At the end of the process, the product is washed by water until the product become nearly neutral. On the other hand, type B gelatin is produced from alkaline treatment by washing raw materials with Ca(OH)$_2$ and pH around 12. Example of type A gelatin is gelatin produced from porcine skin, whereas gelatin manufactured from bovine is considered as type B gelatin (Ledward, 2000; Raja Mohd Hafidz et al., 2011). Figure 1.8 illustrates gelatin converted from collagen by acid processing. Payne et al. (1999) reported that, the properties of gelatin gel depends upon factors such as, concentration, molecular weight, temperature, pH and ionic interactions between gelatin chains.
Gelation of gelatin occurs by physical cross linking which then leads to the formation of junction zones and three dimensional branched networks (Clark and Murphy, 1987; Gilsenan & Ross-Murphy, 2000). Panouille & Larreta-Garde (2009) stated that gelatin forms solution at temperature higher than 50°C as a result of the coil conformation of its chains, whereas when the solution cooled to a temperature below 30°C the gelatin chains are in a coil-helix transition, hence, the liquid turns into a soft solid gel. A conformation of disorder-order transition occurs upon cooling and forms a thermo-reversible networks which created by triple helix formation of gelatin chains with unique cross-linked junction zones that are stabilised by hydrogen bonding. Formation of ordered semi-crystalline triple helical junction zones separated along the polymer through flexible regions characterises the initial start of gelatin gel formation. Helix content increases as temperature decreases due to reduction of helix stability (Kasapis et al., 2003). Figure (1.9) shows thermo-reversible gelation in coil-helix transition of polymers (Zandi., et al, 2007). At a given temperature and concentration, initial partial helices are formed and after cooling treatment (b), the helices aggregate into multiple junction zones (c) (Tanaka, 2004).
1.6 Polydextrose

Dietary fiber has been attracting much attention since 1970s because of its beneficial effects on human physiology and profound prebiotic effects (Jie et al., 2000). The dietary fiber consumption has been linked with decreasing of cardiovascular diseases, such as obesity, diabetes, and hypertension (Aderson et al., 2009). Polydextrose is widely used as bulking agent to replace sugar or fat in food manufacturing, thus in combination with other sweeteners it can help balance and/or modify the sweetness of foods and beverages. It provides only 1 kcal/g whereas sucrose gives 4 kcal/g (Carroll, 1990; Burdock & Flamm, 1999). This polysaccharide is constituted of 90% non-digestible and non-absorbable soluble fibers, a large amount of which is excreted in the feces (Witaicenis et al., 2010).
Polydextrose (PDX) is a white yellowish highly branched water soluble polymer with low caloric content. PDX has an appearance of non-crystalline powder and is used to provide texture to foods (Ribeiro et al., 2003). The chemical structure of polydextrose unit shown in Figure 1.10 exhibits randomly bonded condensation polymer of D-glucose, sorbitol and citric acid. PDX has a wide range of molecular weight (162 to 20000 Da) with an average of about 2000 Da and the degree of polymerisation is ~12. (Paul, 2009; AACC, 2000).

### 1.7 Glucose syrup

Glucose syrup can be derived from starch, maize, wheat and potatoes, however starch is generally considered as the main raw material for production of glucose syrup (Pontoh & Low, 1995). Glucose syrup has many applications in food industry, such as, confectionary, jams, soft drinks and ice cream. Despite its contribution in the sweetness of food, glucose syrup also helps stabilise other ingredients, such as, sucrose and gelatin, and provides mouthfeel and microbial stability (Jackson, 1999; Beltiz & Grosh, 1999). Glucose syrup also known as blood sugar and this co-solute can provide the essential energy for body activities (Whitney et al., 2005). The structure of glucose syrup consists of 1,4 \( \alpha \)-D glucosidic linkages with small percentage of 1,6 \( \alpha \)-D glucosidic linkages (Brich & Etheridge, 1973). Figure 1.11 illustrates the chemical structure of glucose molecule.
1.8 Water – polymer interaction

Water is a major component of food and it is an essential medium for chemical and microbiological reactions in food (Slade et al., 1991). Food properties are significantly influenced by moisture content because many chemical and enzymatic reactions (i.e. oxidation and non-enzymatic browning) are moisture dependent (Bell, 2007). Water content affects the shelf life of foods, therefore storage time can be extended by lowering water activity (a_w) to a point that inhibits or delays chemical and/or microbiological reactions. Therefore, addition of sugar or salt can aid in extending shelf life of food products by binding the water molecules (Belitz & Grosch, 1999).

The physical interaction of water with proteins, polysaccharides, lipids, and salts can contribute significantly to the texture of food, for example food tend to become plastic when their hydrophilic components are hydrated. In confectionary gels, water often acts as plasticiser to aid in gel formation (Burey et al., 2009). Variations in moisture content can lead to variations in quality for instance premature crystallization, stickiness, accelerated rancidity, and difference in hardness (Lees, 1980).

Figure 1.11 Chemical structure of glucose unit (Whitney et al., 2005).
1.9 Biopolymer mixtures

Biopolymers mixtures are mainly composed of two or more different types of biopolymers, for example protein and polysaccharide mixture. The resultant mixture is influenced by the nature of the biopolymers. According to Kasapis and Al-Marhoobi (2005), there are various types of biopolymer mixtures, for example, a fluid-fluid system, a solid dispersed in a fluid or a mixed gel system in which both biopolymers form gels. In addition, biopolymers mixtures can be categorised based on the network which is illustrated in Figure 1.12 as following:

a) *Interpenetrating networks*: each biopolymer gels individually, forming independent networks that interpenetrate into each other.

b) *Coupled separated network*: polymer form a single network which could be characterised by covalent bonds, and/or ionic interactions, or co-operative junction zones.

c) *Phase separated networks*: when the concentration of the two different types of polymers solutions are higher than the critical concentration, usually they phase separate as a consequence of thermodynamic incompatibility, which leads to less favourable interactions among different types of polymers segments. In this case each polymer excludes the other from its polymeric domain. In protein-polysaccharide-water system, phase separation usually happens when the concentration of total polymers is higher than 4%.
The thermodynamic incompatibility of protein/polysaccharide mixture is illustrated in Figure 1.13. System C is obtained by mixing solutions A and B in the volume proportion BC/AC (Tolstoguzov, 1995). The diagram bimodal curve which appears as solid line shows the co-solubility of biopolymers in the system, and the area under the curve (AB₁) is associated with one phase system, and the area above the curve (AB) correlates to two-phase system. F point represents the critical point which is the point at which the two coexisting phases are of the same composition and volume. However, (G) is the minimum biopolymers concentration required for phase separation to happen. D and E are the composition of the coexisting phases, and ED tie-line connects the binodal points (D and E) (Tolstoguzov, 1995).
Phase separation in protein polysaccharides mixtures remains one of important tools to achieve the required structural properties in a range of industrial products (Whorlow, 1992; Shrinivas et al., 2009). Binary phase separation is motivated by a diffusive process, thus, it is possible that the start of gelation would slow down the kinetics of the phase separation procedure (Kasapis et al., 1993). In biopolymers solution the phase separation can be easily noticed by observing the immediate turbidity that develops during mixing as a result of the formation of water-water emulsion, in which one phase behaves as continuous matrix whereas the other dispersed through it as liquid drops (Grinberg & Tolstoguzov, 1997; Polyakov et al., 1997). After the initial phase separation and as result of the density difference between the phases, mixtures of non-gelling polymers usually exhibit gradual resolution into two clear layers (Bamsil, 1993). However, in the case of gelling polymers the water-water emulsion structure can be trapped by network formation which then resulting in biphasic co-gel with continuous and dispersed phases. Furthermore, phase separation can also occur in the case where only one polymer forms a gel to develop a continuous network and the dispersed polymer is in solution state (Morris, 2009).
1.11 Glass transition

According to Roos (1995), based on the observation of discontinuities at the transition temperatures, phase transition can be classified into two major groups, first order and second order phase transition. First-order phase transition involves either absorption or release of latent heat as endothermic or exothermic reaction, while the second-order transition exhibits discontinuity in the primary variables, for example capacity and thermal expansion coefficient (Kasapis, 2006). However, glass transition sometimes is considered as a second-order transition, as it shows the changes in the heat capacity as well as thermal expansion coefficient. According to Liu et al. (2006), the occurrence of glass transition depends on the conditions of experiment and it should be a kinetic transition rather than thermodynamic changes “second-order transition” (Fennema, 1996). Even though glass transition occurs over a range of temperature, the phenomenon of transition is characterised by a specific temperature known as glass transition temperature \( T_g \). Depending on \( T_g \), a material behaves as a super cooled melt when it is above \( T_g \), however, bellow \( T_g \) it is known as a amorphous solid or a glass. Glass is characterised by the arrangement of liquid like molecules within the material with high viscosity about \( 10^{12} \) Pa-s (Walstra, 2003). Fennema (1996) states that, in the glassy state the viscosity of the material significantly increases due to the rotational and transitional motion of the material molecules are dramatically decreased. Thus, glass transition temperature is considered as one of major indicators during food processing and is used to monitor the changes in quality during shelf life. For further understanding of glass transition some principles should be taken in the consideration, for example:

a) Theory on kinetically determined glass transition using temperature and frequency postulates the relaxation timescales but not on molecular interaction.

b) Free volume theory which explains that when glass transition occurs in a polymer system with 30% free volume, only 3% of the total free volume remains at glass transition temperature

c) At the glass transition temperature the thermal expansion coefficient of free volume undergoes a discontinuity and this very temperature is taken as the glass transition temperature (Ferry, 1980).

Figure 1.14 shows the master curve of storage modulus and loss modulus. First region (I) of the curve shows the values of loss modulus \( G'' \) are higher than storage modulus \( G' \).
values, which is the result of the predomination of molecular flow in biopolymer solution and concentrated preparation of the co-solute. The second region (II) exhibits greater \( G' \) value compare to \( G'' \) value, due to the formation of rubbery or elastic network within the system. The third region illustrates the glass transition region in which the \( G'' \) value become higher than \( G'' \) again. Glass state region is shown in the last region of the chart (IV).

Figure 1.14 Variation of \( G' \) (■), \( G'' \) (□) and tan \( \delta \) (○) as function of temperature, frequency, molecular weight and concentration of polymers (Ong, Whitehouse, Abeysekera, Al-Ruqaie, & Kasapis, 1998).

1.12 The laws of Polymer blending

Based on the theoretical equation developed by Davis (1971) to explain the phase separated continuous networks, the modulus of composite gel (\( G_C \)) can be related to modulus of the individual phases (\( G_X \) and \( G_Y \)) as shown in equation (1.1).

Bicontinuous:

\[
(G_C)^{1/5} = \phi_X (G_X)^{1/5} + \phi_Y (G_Y)^{1/5} \]  

[1.1]
where, $\phi_x$ and $\phi_y$ refers to the volume fractions (phase volumes) of the constituent phases, X and Y (with $\phi_x + \phi_y = 1$).

The isostrain and isostress blending laws (equations [1.2] and [1.3]) are usually used to analyse the modulus of composites with dispersed phase in continuous matrix. In the parallel arrangement, the rigidity of the stronger material limits the deformation of the weaker polymer, therefore both components are deformed to the same extent (isostrain conditions), overall modulus is the weighted average of individual moduli (Morris, 2009).

(Isostrain): \[ G_c = G_x \phi_x + G_y \phi_y \] \[ 1.2 \]

When both components are exposed to the same stress (isostress) in the series arrangement, weaker component strength limits the force transmitted to the stronger polymer. Overall, the composite deformation ($J_c = 1/G_c$) is approximately weighted average of the individual compliance.

(Isostress): \[ 1/G_c = \phi_x / G_x + \phi_y / G_y \] \[ 1.3 \]

The three blending laws equations mentioned above (Bicontinuous, isostrain, and isostress) have the same general form:

\[ (G_c)^n = \phi_x (G_x)^n + \phi_y (G_y)^n \] \[ 1.4 \]

$n = 0.2$ for bicontinuous, $n = 1$ for isostrain, and $n = -1$ for isostress. Equation [1.4] can be applied to young’s modulus ($E$), $G^\|$, from oscillatory measurements, and to shear modulus ($G$) (Morris, 2009).

According to Morris (2009), rheological properties of biphasic co-gel can be described in rheological terms as follows:

- The strength of composite is determined by the continuous phase.
- There is a little direct effect of dispersed phase on the moduli composite, but it can contribute indirectly by occupying a part of the total volume, thus increases the concentration of the polymer in the continuous phase.
- Calculations of overall moduli can be made by using Takayanagi blending laws.
- Any large change would consider as an indication for the structure bi-continuous network.

- The effect of melting of strong dispersed gel on the reduction of overall moduli is unlikely to be more than a factor of four.

In 1987 Clark has introduced a factor \( p \) which is defined as the ratio of water/polymer in one phase divided by corresponding ratio for the other phase to describe the solvent partition.

\[
p = \frac{\text{water}_x / \text{polymer}_x}{\text{water}_y / \text{polymer}_y} \tag{1.5}
\]

In binary mixtures where weights of both biopolymers (X and Y) and water (\( \text{water}_x + \text{water}_y \)) are known, the \( p \) factor (equation [1.5]) defines phase volumes, hence, the effective concentrations and real moduli of each hydrocolloid are within its own phase (Kasapis, 2009).

**Solvent partition**

According to Morris (1992) the influence of solvent partition for a biphasic gel composed of polymers X and Y would have shear modulus of \( M_X \) and \( M_Y \) where, \( M_Y = n^2 M_X \), and \( n \) = the square root of R, as \( R = M_Y / M_X \). Thus the author suggested that, the upper \( (G_U) \) and lower \( (G_L) \) bound moduli can be calculated by using the application of isostrain and isostress blending laws as shown in equations [1.6] and [1.7]. Figure 1.15 illustrates the changes in calculated modulus as a function of \( S_X \). Solid line shows the upper and lower bounds for the polymer X, and the broken lines show the corresponding bounds for polymer Y.

Upper bound \( G_U = G_X \phi_X + G_Y \phi_Y \) \tag{1.6}

Lower bound \( (1 / G_L) = (\phi_X / G_X) + (\phi_Y / G_Y) \) \tag{1.7}
Kasapis et al. (1993) suggested that the calculation of total water in the mixture \((w)\) can be made by subtracting the combined weights of two components from the total weight, where, \(S_X\) refers to the solvent fraction in the polymer \(X\) phase. The following algorithm (equation [1.8] – [1.12]) was proposed for each possible distribution of solvent between the component phases of mixed-gel combinations. The weight of water in the phases \(X\) and \(Y\) is:

\[
\begin{align*}
 w_x &= S_x w \\
 w_y &= (1-S_x) w
\end{align*}
\]  

[1.8]

The total weights of phases are obtained by adding to the weight of the appropriate polymer.

\[
\begin{align*}
 t w_x &= x + w_x \\
 t w_y &= y + w_y
\end{align*}
\]  

[1.9]

Therefore the effective concentration \((%w/w)\) of polymer \(X\) and \(Y\) in the phases are:

\[
\begin{align*}
 c_x &= 100x/t w_x \\
 c_y &= 100y/t w_y
\end{align*}
\]  

[1.10]
The true phase volume can be obtained by adjusting the relative weights for the difference of the density between phases. The following equation [1.11] describes the relationship between the concentration of the polymer ($c$) and relative density ($D$).

$$D_X = 1.0 + Rc_X$$

$$D_Y = 1.0 + Rc_Y$$ [1.11]

As $R$ (equation 1.11) is the concentration coefficient particular to the experimental system, the calculation of relative volumes of $X$ and $Y$ can be made by using their total weights and their individual densities as described in equation [1.12].

$$V_X = \frac{w_X}{D_X}$$

$$V_Y = \frac{w_Y}{D_Y}$$ [1.12]

In terms of relative volumes, the phase volumes in the composite can be written as follows:

$$\phi_X = \frac{V_X}{V_X + V_Y}$$

$$\phi_Y = \frac{V_Y}{V_X + V_Y}$$ [1.13]

**Phase inversion**

Two-phase system usually contains continuous and dispersed phases, however, the both phases can be continuous or the phase can become inverted, the dispersed phase becomes the continuous phase. To include the phase inversion and to cover the wide range biphasic morphologies, Lewis and Nielson developed *Halpin-Tsai* equations (Nielsen, 1974). The following is *Halpin-Tsai* equations [1.14] and [1.15], and table 1.5 shows Einstein coefficients for composites:

$$\frac{G'}{G''} = \frac{1 + AB\phi_2}{1 - B\phi_2}$$ [1.14]
\[ B = \frac{G'_{2}/G' - 1}{G'_{2}/G' + A} \]  

[1.15]

Equations [1.14] and [1.15] subscript 1 and 2 refer to the continuous and dispersed phases respectively, \( \phi_2 \) is the volume fraction in the dispersed phase, \( k \) is generalised Einstein coefficient and it is very sensitive to the system, and \( A \) is a constant that determined by the morphology of the system, for dispersed spheres in an elastic matrix \( A=1.5 \), for example. Lewis and Nielsen (Nielsen, 1974).

\[ \frac{G'}{G_1'} = \frac{1 + AB\phi_2}{1 - B\psi\phi_2} \]  

[1.16]

\[ \Lambda = k - 1 \]  

[1.17]

\[ \psi = 1 + \frac{(1 - \phi_m)}{\phi_2} \phi_2 \]  

[1.18]

Furthermore, these following equations [1.19] to [1.21] can be used for systems with more rigid continuous phase (inverted systems) which are produced by rewriting the equation [1.15] to [1.17].

\[ \frac{G'_{1}}{G'} = \frac{1 + AB\phi_2}{1 - B\psi\phi_2} \]  

[1.19]

\[ B_i = \frac{G'_{1}/G'_{2} - 1}{G'_{1}/G'_{2} + A_i} \]  

[1.20]

\[ A_i = \frac{1}{A} \]  

[1.21]

\( G', G'_{1} \) and \( G'_{2} \) are shear moduli of the composite, continuous phase, and dispersed phase (filler) respectively, and \( \phi_2 \) is the phase volume of the filler. The Einstein coefficient in this case is shown as \( A_i \), equation [1.21].
Table 1.5 Einstein coefficients for composites (adapted from Nielsen, 1974).

<table>
<thead>
<tr>
<th>Filler phase</th>
<th>Modulus</th>
<th>Einstein coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spheres</td>
<td>$G$</td>
<td>$1 + \frac{(7 - 5v_i)}{(8 - 10v_i)}$</td>
</tr>
<tr>
<td>Large aggregates of spheres</td>
<td>$G$</td>
<td>$2.50/\varphi_a$</td>
</tr>
<tr>
<td>aggregates of two spheres</td>
<td>$G$</td>
<td>2.58</td>
</tr>
<tr>
<td>Rods-axial ratio 4</td>
<td>$G$</td>
<td>3.08</td>
</tr>
</tbody>
</table>

Note: $v_i$ Poisson’s ratio of matrix (for elastomer phase $v_i=0.5$ and for the rigid phase $v_i=0.35$)  
$\varphi_a$ Packing fraction of spheres in aggregate.

**Time-Temperature Superposition TTS**

The effect of temperature and frequency on the viscoelastic properties of a system are interchangeable, for example, the effect of increasing frequency can be obtained by decreasing temperature (Ferry, 1980). This property of viscoelasticity has been utilised in principles of TTS, in which frequency range that is not available for experimental measurements is obtained indirectly from accessible frequency range at another temperature. In *Time-Temperature Superposition*, frequency, which is a sequence of viscoelastic functions, covers a small range of time which were recorded at range of temperature range. $T_0$ is selected to be used as reference temperature. Master curve which is a composite of horizontally shifted curves taken at different temperatures, covers wider range of frequency. Master curve is also equivalent to the viscoelastic property of the system as a function of time (frequency) taken at $T_0$. The logarithm of frequency shifted for the individual curves are relative to the reference curve at the reference temperature is $\log a_T$, where $\log a_T$ is shift factor representing the ratio of any specific relaxation time at temperature $T$, to its value at $T_0$. 
Williams, Landel, and Ferry Equation and Ferry Free volume Theory (WLF)

Williams, Landel and Ferry (WLF) is an empirical equation that was introduced to describe the temperature dependence of \(a_T\) during glass transition (Williams, Landel & Ferry, 1955).

\[
\log a_T = -\frac{C_1^o(T - T_o)}{C_2^o + (T - T_o)} \tag{1.22}
\]

where, \(C_1^o\) and \(C_2^o\) are the WLF constants and \(T_o\) is the reference temperature.

Free volume theory states that the total \(v\) is the sum of the free volume \(v_f\) and the occupied volume \(v_o\), which includes van der waals radii and the volume associated with vibrational motions. The free volume offers an extra space which is required for the rotational and transitional motions amongst the system, and that relates to the processes of the mechanical relaxation. From the definition of shift factor, \(a_T\) which represent the ratio of any specific relaxation time at temperature \(T\) to its value at \(T_0\), with \(T\) refers to the relaxation time. Ferry (1980) stated that the relaxation time is proportional to \(\frac{\eta}{T_p}\), where \(\eta\) refers to the viscosity, \(T\) is representing the temperature and \(p\) is the destiny.

\[
a_T = \frac{\eta T_o \rho_o}{\eta_o T \rho} \tag{1.23}
\]

In equation [1.23], \(\eta_o\) is the viscosity and \(\rho_o\) is the density at the reference temperature.

Doolittle (1957) developed an empirical equation known as free space equation for viscosity, which is based on the concept that the free volume is the key factor for determining the molecular relaxation:

Free space equation for viscosity: \(\ln \eta = \ln A + B \frac{v_o}{v_f}\) \tag{1.24}

where, \(A\) and \(B\) are empirical constants that depend upon the nature of the material.
By rewriting, the equation [1.23] that expressed the shift factor $a_T$ and equation [1.24] together with the definition, $\nu = \nu_0 + \nu f$ and $f = \nu f / \nu$ equation [1.25] can be reproduced as follows:

$$\log a_T = \frac{B}{2.303} \left( \frac{1}{f} - \frac{1}{f_0} \right) + \log \left( \frac{T_0 \rho_0}{T \rho} \right)$$ \hspace{1cm} [1.25]

where, $f$ and $f_0$ are the fractional free volume at $T$ and $T_0$ respectively.

In practice the second part of the equation does not make any significant difference compared to the first one, therefore, the equation is almost used without it (Kasapis, 2008) and the equation is reduce to the following:

$$\log a_T = \frac{B}{2.303} \left( \frac{1}{f} - \frac{1}{f_0} \right)$$ \hspace{1cm} [1.26]

According to Ferry (1980), the fractional free volume increases linearly with temperature, consequently the equation [1.27] is produced.

$$f = f_0 + \alpha_f (T - T_0)$$ \hspace{1cm} [1.27]

where, $\alpha_f$ is the thermal expansion coefficient. Replacing equation [1.27] into equation [1.26] result in the equation [1.28];

$$\log a_T = -\frac{\left( \frac{B}{2.303 f_0} \right) (T - T_0)}{f_0 / \alpha_f + T - T_0}$$ \hspace{1cm} [1.28]

When equation [1.28] compared to equation [1.22], the WLF constants in equation [1.22] can be expressed in terms of fractional volume and thermal expansion coefficient the following two equations can be written:

$$C_1^\circ = \frac{B}{2.303 f_0}$$ \hspace{1cm} [1.29]

$$C_2^\circ = \frac{f_0}{\alpha_f}$$ \hspace{1cm} [1.30]
Based on equation [1.29] and [1.30], the WLF equation relates the temperature dependence of relaxation to the effect of the free volume. WLF equation makes the modeling process much easier by using computer.

Kasapis (2001) reported that, for temperature below glass transition region where the system attains a glassy state WLF equation cannot describe the stage therefore, to the progress in mechanical properties in at this region it is better to apply the modified Arrhenius equation. The following equation [1.31] is the general Arrhenius equation, whereas the modified Arrhenius equation is expressed in equation [1.32].

\[ K(E_a, T) = \ln(A) - \frac{E_a}{R.T} \]  \hspace{1cm} [1.31]

where, \( K \) refers to \( \ln(K) \), \( R \) is the gas constant \( (8.31447 \times 10^{-3} \text{kJ K}^{-1} \text{mol}^{-1}) \), \( T \) is temperature \( (K) \), \( A \) is the pre-exponential factor, and \( E_a \) is the activation energy \( (\text{kJ mol}^{-1}) \).

\[ \log a_T = \frac{E_a}{2.303R} \left( \frac{1}{T} - \frac{1}{T_0} \right) \]  \hspace{1cm} [1.32]
1.13 Reference


CHAPTER 2

METHODOLOGY

2.1 Rheology

The term rheology is defined as the study of flow behaviour of matters and gelation properties of materials. According to Barbosa-Canovas., et al. (1996), rheology focuses on the flow and deformation behaviour of materials in the transient between solids and fluids region. Furthermore, it also defines the relationship between the applied force/stress and the deformation of materials. Rheological measurement provides a better understanding in regards to the physics of amorphous solid, including cross-linked rubbers and polymeric glasses (Mcakenna, 2012). Rheological data is important for many aspects in the field of food manufacturing. It offers useful information about the raw materials, during processing and the final product (Steff, 1996; Dorbraszczyk & Morgenstern, 2003). Therefore the main aims of rheological measurements are:

i) To determine the functionality of food ingredients
ii) To evaluate the food texture and correlate with the sensory analysis
iii) To measure the quality of intermediate and/or final product
iv) To evaluate and test the shelf life of the products

Rheometer is an instrument that is capable to perform the rheological measurements, such as viscosity and viscoelasticity of fluids, semi solids as well as solids. In a large deformation rheological test or flow test, the geometry turns continuously in one direction, thus, forming a uni–directional shear to the sample. The speed of shear is the controlled variable known as shear rate ($\gamma$). Shear stress ($\sigma$) is measured as a function of shear rate, therefore as a consequence of the uni–directional shear, flow test is considered as destructive measurement and thus it is commonly used to analyse fluids (Rao, 1999).

According to Dorbraszczyk & Morgenstern (2003), the small deformation dynamic oscillation is one of rheological measurements that can be used to characterise the viscoelastic properties of materials as a function of time, temperature, strain or frequency. In these measurements, the geometry moves in two directions (bi–directional). The total resistance of materials to oscillatory shear is known as complex modulus ($G^*$) in Pascal unit. The complex
modulus consists of two components, $G'$ and $G''$, where $G^* = (G'^2 + G''^2)^{1/2}$. $G'$ or elastic modulus represents the strength of network and is also known as storage modulus. On the other hand, the viscous modulus or $G''$ is a measure of the flow properties for the sample in the structured state and is also known as the loss modulus. Furthermore, phase angle or $\tan \delta$ is a parameter associated with the degree of viscoelasticity of a sample, $\tan \delta = (G''/G')$. A high value of $\tan \delta$ indicates that the sample is more viscous or liquid-like, while low value of $\tan \delta$ means that the sample is more elastic or solid-like. The following table (2.1) shows the standard rheological parameters.

Table 2.1 Standard rheological parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Symbol</th>
<th>Units (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shear stress</td>
<td>Force per unit area</td>
<td>$\sigma$</td>
<td>Pa</td>
</tr>
<tr>
<td>Shear strain</td>
<td>Relative deformation in shear</td>
<td>$\gamma$</td>
<td>-</td>
</tr>
<tr>
<td>Shear rate</td>
<td>Change of shear strain per unit time</td>
<td>$\dot\gamma$</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Resistance to flow</td>
<td>$\eta$</td>
<td>Pa$s$</td>
</tr>
<tr>
<td>Shear storage modulus</td>
<td>Measure of elasticity of material</td>
<td>$G'$</td>
<td>Pa</td>
</tr>
<tr>
<td>Shear loss modulus</td>
<td>the ability of the material to dissipate energy</td>
<td>$G''$</td>
<td>Pa</td>
</tr>
<tr>
<td>Complex viscosity</td>
<td>Resistance to flow of the sample in the structured state, originating as viscous or elastic flow resistance to the oscillating movement</td>
<td>$\eta^*$</td>
<td>Pa$s$</td>
</tr>
<tr>
<td>Dynamic viscosity</td>
<td>Internal friction of liquid</td>
<td>$\eta$</td>
<td>Pa$s$</td>
</tr>
<tr>
<td>Phase angle</td>
<td>Degree of viscoelasticity</td>
<td>$\tan \delta$</td>
<td>-</td>
</tr>
</tbody>
</table>

The measurements of linear viscoelastic region (LVR) can be done by using rheological measurements which can be conducted in the regions where the viscoelastic properties observed are independent of imposed stress and strain levels (TA Instruments, n.d-a). The materials’ LVR should be established before starting the dynamic tests. The determination of LVR of a material can be done by increasing the amplitude of oscillation and observation of the magnitude of phase lag. In dynamic oscillation test measurements, in order to verify that the results are real and not merely artifacts, it is extremely vital that all the test are carried out on amplitude within the linear viscoelastic region of the sample. The principle behind this is that if the deformation is small or applied slowly, the molecular arrangements
are never far from equilibrium. The mechanical response is then just a reflection of dynamic processes at the molecular level which go on constantly, even for a system at equilibrium. Within this domain of linear viscoelasticity, the magnitudes of stress and strain are related linearly, and the behaviour for any liquid is completely described by single function of time. According to Rao (2007), viscoelastic properties of materials can be analysed using dynamic rheological tests such as:

- **Temperature sweep**
  Storage modulus \( (G') \) and loss modulus \( (G'') \) are measured as a function of temperature at fixed frequency and strain. This test provides useful information about the gel formation (e.g. protein) and gelatinisation of starch dispersion.

- **Time sweep**
  Time sweep test is used to analyse viscoelastic properties of materials as a function of time in which the strain, frequency and temperature are kept constant. The importance of a time sweep is to determine if the properties of a system changes over fixed time. This test is also known as a gel cure experiment. A curing time is usually necessary for gels in order to reach equilibrium state and it varies from gel to gel. For example, like most other biopolymer systems, the coil to helix transformation in agarose gels occur very fast which resembles a true first order phase transition. A short curing time is therefore sufficient in the case of agarose gels. However, in the case of gelatin gels, the initial phase lasts several hours thus requires a much longer curing time before a pseudo equilibrium state is achieved.

- **Frequency sweep**
  Frequency is the time required to complete one oscillation. A frequency sweep usually follows a time sweep and this test can provide information regarding the viscoelastic properties of materials as a function of frequency at a constant strain and temperature. The data obtained from frequency sweeps helps in determining under which category a sample can be classified, for example, a dilute solution, an entangled solution, a weak gel or a strong gel. Derived parameters such as complex viscosity \( (\eta^*) \) and \( \tan \delta \) provide useful information about the nature of the system that being tested. In addition, data from frequency sweeps is used in time-temperature superposition in order to gauge long term properties or extremely high/low frequencies beyond the scope of the instrument or reasonable experimental time. This concept uses a direct equivalency between time (frequency of measurement) and temperature.
Strain sweep

A strain sweep helps determine the extent to which a sample undergoes deformation and is mostly used to determine the LVR of the system. In this test, the material response to increasing amplitude at a constant frequency and temperature is measured. Sample is assumed to be stable before performing a strain sweep. An unstable sample is subjected to time sweep prior to strain sweep to determine the stability.

2.2 Differential Scanning Calorimetry (DSC)

The term Differential Scanning Calorimetry (DSC) refers to the thermal analysis of a sample by measuring the difference in the heat that is either absorbed or expelled from a sample and the reference during the experiment. Nowadays there are two types of DSC instruments, heat flux DSC and power compensation DSC. In heat flux DSC, the sample and reference pans are placed in the same furnace and heated by the same heating source. The temperature difference between sample and reference is measured and converted back to heat flow. In power compensation DSC, the sample and reference pans are placed in two isolated furnace and heated by different sources. Temperature difference of the sample and reference pans are maintained at zero by adjusting the heat supply to the sample pan, and the difference and heat supply is recorded as a function of temperature (Coleman & Craig, 1996).

Modulated Differential Scanning Calorimetry (MDSC) is an improved DSC where a small amplitude sine wave temperature modulation is applied to the standard linear temperature program. When subjected to DSC programmed temperature, samples undergo either reversible process or irreversible process. Reversible processes are those which can be reversed by small modification of a variable, and the heat flow associated with these processes is dependent upon the rate of the temperature change. On the other hand, irreversible kinetically controlled processes are not in equilibrium with the temperature program and the heat flow signal is dependent on the absolute temperature (Reading, Luget, & Wilson, 1994). Separating the reversing heat flow and non-reversing heat flow can be done by using MDSC because it has the capability of analysing the overlapping of some disentangle thermal events and can enhance sensitivity. In addition, MDSC provides accurate determination of \( T_g \) by using divertive in time reversing component signal (Ronkart et al., 2006).
2.3 Fourier Transform Infrared (FT-IR) spectroscopy

FTIR is a technique used to achieve an infrared spectrum from absorption, emission, photoconductivity of gas, liquid, or solid materials. This technique helps to identify the chemical bonds in a molecule by generating an infrared absorption spectrum (Yang & Zhang, 2011). According to Rees (2010), FTIR can be used for inorganic and organic compounds and has many advantages such as rapid, non-destructive, sensitive and simple sample preparation. The general components of FTIR (refer to 2.1) are described as follow (Thermo Nicolet, 2001):

- **The energy source:**
  
  Infrared energy is produced from a glowing black-body. The produced beam goes through a small hole which controls the amount of the energy exposed to the sample, and eventually to the detector.

- **The interferometer:**
  
  After the beam passed of the surface of the sample, it absorbs specific frequencies of the energy which specifies the characteristic of the sample. Afterwards, the beam goes through the interferometer where the spectral encoding is placed, after which the signal exit the interferometer.

- **The detector:**
  
  Finally the beam passes to the detectors which are designed to detect and measure the signal of interferogram.

- **The computer:**
  
  The Fourier transformation takes place in a computer, which designed to receive the signal from the detector, and then it produces the final infrared spectrum.
2.4 Wide Angle X-ray Diffraction (WAXD)

In general, X-ray diffraction is used to provide information of materials’ structure at atomic resolution (I’Ason et al., 1987). WAXD scattering can be used to measure the degree of crystallinity and to access the structure at the unit cell level whereas the small angle scattering provides information at the lamellar level (Mano, 2007). According to Liebhafsky et al. (1966), the basic principle of all X-ray diffraction phenomena is scattering by X-ray electrons. In a wide-angle X-ray diffraction pattern of a tendon collagen, a reflection corresponding to distance between neighbouring amino acids along the helix is observed (Sasaki & Odajima, 1996).

When a tungsten target bombarded by constant rate of electrons, it will show readings of the intensity on the detector as an angular position of the crystal monochromator. These positions will correspond to known wavelengths and specified in terms of \(2\theta\), the angle between incident and reflected beam and twice angle. X-ray diffraction is usually taken within certain angles \(2\theta\) at constant rate per time unit (min) and the relative crystallinity is calculated by dividing the area of the peak by the total area (Rindav-Westling et al., 1998).

Based on the theory behind X-ray diffraction, the solid matter can be described as amorphous or crystalline. In amorphous, the atoms are randomly aggregate, but in crystalline the atoms are aggregate systematically. When an X-ray beam bombards an atom the electrons in the orbits around the atom start to oscillate with the same frequency as the incoming beam, mostly in all directions. This result in destructive interference, that is the combining waves are out of phase and in this case there is no resultant energy leaving the solid sample. On the other hand, the atoms in crystal aggregate in a regular pattern and in a limited directions leading to
constructive interference. The waves will be in phase and there will be well defined X-ray beams radiating from the sample in different directions, thus the angle $\theta$ is determined according to the distance between planes (Jenkins & Snyder, 1996). Bonds within the materials can also influence the signal, for example, if the energy of the particles is greater than the binding energy of the atomic electron, it is possible that the atomic electron will be ejected from its atomic position (Arnaud et al., 2011). Jenkins (2000) reported that if a particle with high energy, such as electron, bombards a bound atomic electron and the energy of that particle is higher than the binding energy of the atomic electron, it is very likely that the atomic electron will be ejected from the atomic position and it will depart from the atom with a kinetic energy ($E - \phi$) equal to the difference between the energy ($E$) of the original particle and the binding energy ($\phi$) of the atomic electron.

### 2.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is a unique technique to study and analyse the microstructure of materials. SEM is able to obtain two-dimensional images of the surface of a variety of materials and high-resolution micrographs. The magnification range of SEM is 10-10,000X. SEM generally consists of lens system, electron gun, electron detector, visual and photorecording cathode ray tubes (CRTs) as shown in figure 2.2. The beam from electron gun is passed through electron lenses in order to decrease spot size, thus producing a clear image. Subsequently, the signals from the beam-specimen from different locations are collected by the electron detector. Afterwards, the electron detector converts the collected signals to point–by–point intensity changes on the observing monitor, and produces an image. The secondary electron (SE) and backscattered electron (BSE) are the two signals most often used to produce SEM image (Kimseng & Meissel, 2001).

The recent developments in SEM are variable pressure scanning electron microscope (VPSEM) and environmental scanning electron microscope (ESEM). VPSEM can study the surfaces of wet or dry specimen because it can function while its specimen chamber contains a gas or a vapor at a pressure range from approximately 10 to 2500 Pa (0.1-20 torr). ESEM is quite similar to VPSEM except that ESEM offers a propriety secondary electron detector for use in gas. VPSEM relies only on BSE detection for imaging when there is gas in the chamber (Goldstein et al., 2003).
2.6 Materials

2.6.1 Agarose

Agarose was supplied by Sigma Aldrich (A0576) (Sydney Australia). The determination of moisture, ash and sulfate contents according to the supplier were less than 5%, 0.25%, 0.10% (w/w) respectively.
2.6.2 **Pectin**

High Methoxy pectin (HM pectin) was provided by Sigma Aldrich (Sydney, Australia). According to the supplier the polysaccharide content was 92.0% on dry weight basis of which 85.1% is galacturonate with a degree of methyl esterification (DE) of 70.3%, and chromatographic analysis carried out by the supplier, using an ultrahydrogel linear column and eight pullulan standards, produced a number average molecular weight (Mn) for the pectinic material of this investigation of 154 kDa.

2.6.3 **Gelatin**

Gelatin with gel strength of 300 bloom was purchased from Sigma Aldrich (G2500) (Sydney, Australia). It was the first extract from the single batch of pigskin produced by acid hydrolysis of collagen (Type A). Tender collagen from young animals were used which allowed mild and rapid hydrolysis. The isoelectric point of gelatin is close to the collagen isoelectric point (pH 9.4).

2.6.4 **Polydextrose**

Polydextrose used in this study was supplied by TATE & LYLE (Illinois, USA). According to the supplier the product was of 90% purity, with moisture content of 4%. Polydextrose used in this study was obtained by using thermal polymerisation of D-glucose in the presence of sorbitol and phosphoric or citric acid.

2.6.5 **Glucose syrup**

Glucose syrup used in the study was a Cerestar product (Manchester, UK). The dextrose equivalent of the sample is equal to 42, and the total level of solids is 82%. The polydisperse nature of glucose syrup was established using gel permeation chromatography data provided by the supplier, and the product converts from a thick solution at ambient temperature to a transparent glass at subzero temperatures.
2.7 Instruments

2.7.1 Advanced Rheometer Generation 2 (ARG-2)

The Advanced Rheometer Generation 2 is an instrument with patented thrust bearing technology for ultra-low, nano-torque control. The instrument shown in the figure 2.3 is an advanced rheometer that is capable to measure several of mechanical properties of the materials sophisticatedly, such as direct controlled strain. ARG-2 is also equipped with a peltier plate that can hold temperature between -20 °C and 200 °C.

Figure 2.3 Advanced Rheometer Generation 2 (ARG-2) at RMIT.

2.7.2 Modulated Differential Scanning Calorimeter (MDSC) Q2000

MDSC Q2000 is a sophisticated Differential Scanning Calorimeter (DSC), with high sensitivity, resolution and stability. The features of Q2000 are Advanced Tzero™ and Modulated DSC™ technologies. Moreover, it is also equipped with an autosampler with 50 positions, and Platinum™ software that allows users to schedule tests automatically at off-
work periods (TA Instruments, n.d-b). Figure 2.4 illustrates MDSC Q2000 instrument at RMIT.

![Figure 2.4 Modulated Differential Scanning Calorimeter (MDSC) Q2000 at RMIT.](image)

### 2.7.3 Fourier Transform Infrared (FT-IR) spectroscopy Spectrum 100

FT-IR spectroscopy from Perkin Elmer Spectrum 100 spectrometer shown in figure 2.5, is equipped with MIRacle™ ZnSe single reflection ATR plate (Perkin-Elmer, Norwalk, CT). Spectra were obtained in absorbance mode for the wavelength range of 600 – 4000 cm⁻¹ with a resolution of 4 cm⁻¹. This was corrected against the background spectrum of the solvent at ambient temperature.

![Figure 2.5 FT-IR Spectroscopy- spectrum100 at RMIT.](image)
2.7.4 Wide angle X-ray diffraction: WAXD

BRUKER AXS D8 Advance Diffractometer shown in figure 2.6 is from Karlsruhe, Germany and equipped with Cu-Kα (1.54 Å) radiation. The accelerating voltage and current of 40 kV and 40 mA respectively were employed. Freeze dried samples were located on a tray holder and scanned continuously to obtain diffractograms within a 2θ range of 5° and 90° at a measuring interval of 1°. The Bruker Advanced X-Ray Solutions software, DIFFRAC+ Evaluation (Eva), version 10.0 revision 1 enables the user to schedule tests automatically at off work time.

Figure 2.6 BRUKER AXS D8 Advance Diffractometer at RMIT.

2.7.5 Quanta™ 200 Scanning Electron Microscope

Quanta™ 200 as shown in figure 2.7 was used in this study to provide tangible evidence of changes in network morphology and phase topology of samples. Quanta™ 200 from FEI is a sophisticated performance instrument with three imaging modes, low vacuum, high vacuum and ESEM.
2.8 Sample Preparation

2.8.1 Agarose and polydextrose mixtures

Agarose samples were formulated with concentration of 1.5% (w/w) with varying concentration of polydextrose from 0.0 until 78.5% (w/w). The agarose solutions were prepared by dissolving the agarose powder in distilled water at 85°C using rapid stirring for 20 minutes. Polydextrose samples were dissolved in distilled water at room temperature. After both systems are completely dissolved and the clear solutions were obtained, the agarose samples were held at 70°C and then polydextrose samples were added into agarose at 70°C and stirred for 20 minutes using magnetic stirrer.

2.8.2 Pectin and polydextrose mixtures

Pectin solutions were prepared by dissolving the powder at 2% (w/w) solids in distilled water at 95°C with gentle stirring until clear solution of pectin were obtained. The temperature of the solution was reduced for the addition of the required amount of polydextrose (40%, 50%, 60%, 70 and 78%). The samples were kept at 75°C until the cosolute was completely dissolved and the desired level of total solids was obtained by evaporating the excess water from the system. The pH of the system was then adjusted to 3.0.
using 0.2 M HCl solution to create the required gelling conditions for subsequent experimentation.

2.8.3  *Pectin and glucose syrup mixtures*

The polysaccharide solutions were prepared by dissolving the powder at 2% (w/w) solids in distilled water at 95°C with gentle stirring. Pectin solutions were held at 75°C for the addition of glucose syrup (40%, 50%, 60%, 70 and 78%). Samples were kept at 75°C until the system was homogenised and at the desired level of total solid. The pH of the mixture was then adjusted to 3.0 using 0.2 M HCl solution to provide the required gelling conditions for subsequent experimentation.

2.8.4  *Gelatin and polydextrose mixtures*

Gelatin solution was obtained by dissolving the powder at 15% (w/w) in distilled water at 50°C with gentle stirring on a hot plate. The required amount of polydextrose for each formulation (2.5%, 5%, 7.5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% and 65%) was added to the protein solution at the same temperature and stirred until a clear solution was achieved. The excess water was evaporated with temperature of sample never exceeding above 50°C.

2.8.5  *Agarose, gelatin and polydextrose mixtures*

The mixture of agarose and gelatin samples was prepared by dissolving 1.5% (w/w) agarose powder at 85°C on a hot plate and they were stirred gently until the clear solution of agarose was obtained. The temperature of the solution was lowered to 60°C and then gelatin 7.5% (w/w) was added to formulate a binary mixture. In this experiment both polymers were dissolved within half-an-hour forming an aqueous system. The mixture remained at 60°C for the addition of polydextrose (2.5%, 5%, 7.5%, 10%, 15%, 20%, 25% 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% and 71%), followed by stirring till the samples were completely mixed and homogenised, and excess water was gently evaporated to form preparations from low to high levels of solids.
2.9 Methods

2.9.1 Rheology

A controlled strain rheometer was used for small deformation dynamic oscillation measurements, with 40 mm diameter parallel-plate geometry and 1 mm gap and a thin layer of silicon fluid (50 cS) was added to expose edge of the sample to prevent moisture loss. The analysis was aimed to determine the storage modulus $G'$ and loss modulus $G''$, viscosity of the network, complex viscosity $(\eta^*)$, and $\tan \delta$ where, $\tan \delta = G''/G'$. 

Experimental for agarose with polydextrose mixtures consists of cooling ramp runs from 85°C to 0°C with a ramp rate of 1°C /min, followed by an isothermal segment for 30 min at 0°C at a frequency of 1 rad/s, followed by time sweep at 0°C for 60 minutes at a frequency of 1 rad/s, then followed by frequency sweep from 0.1 to 100 rad/s at a strain of 0.1%, and finally the mixtures were heated up to 85 °C at a ramp rate of 1°C/min and a frequency of 1 rad/s. For the samples with high level of total solids of 80% solids, a measuring geometry of 10 mm in diameter was used within an extended experimental temperature range to analyse the vitrification behaviour of the agarose network. Molten samples were loaded on the preheated peltier plate at 85°C and cooled to -60°C at 1°C/min thus covering the melting, rubbery, glass transition and glassy state of the mixtures using a strain of 0.01%. At an interval of four degrees centigrade, frequency sweeps were performed within a range of 0.1–100 rad/s to examine the time dependent mechanical properties of the matrix.

Experimental for pectin with polydextrose mixtures, the polysaccharide with co-solute either (polydextrose or glucose syrup) samples were loaded on the preheated peltier plate at 95°C and then cooled to 0°C at a ramp rate of 1°C/min followed by an isothermal segment for 30 min at 0°C and a heating run to 95°C at 1°C/min. An oscillatory frequency of 1 rad/s with a strain of 1% was executed throughout the experiments. For concentrated mixtures with total solids of 80%, a measuring geometry of 10 mm in diameter was used within an extended experimental temperature range to analyse the vitrification behaviour of the pectin network. Molten samples were loaded on the preheated peltier plate at 95°C and cooled to -60°C at 1°C/min thus covering the melting, rubbery, glass transition and glassy state of the mixtures using a strain of 0.01%. At an interval of four degrees centigrade, frequency sweeps were performed within a range of 0.1–100 rad/s to investigate the time dependent mechanical properties of the condensed matrix.
Gelatin with polydextrose samples were loaded on the peltier plate at 50°C and cooled to 0°C at a ramp rate of 1°C/min followed by a 1 hour isothermal run, a frequency sweep from 0.1 to 100 rad/s at 0°C and a heating run at 1°C/min to 60°C, and the plate diameter for the parallel-plate measuring geometry was 40 mm. In the high-solid system, the experimental temperature was extended from 60°C to -60°C in order to observe the transformation from the melt through the rubbery plateau to the glassy state. The parallel-plate measuring geometry used is 20 mm in diameter. Cooling and heating scan rate was 1°C/min and a frequency range of 0.1 to 100 rad/s covered at regular temperature intervals of four degrees centigrade utilising a fixed amplitude strain of 0.01%.

Low solids samples of agarose, gelatin, and polydextrose mixtures were loaded on the preheated peltier plate at 70°C and analysed using parallel plate geometry of 40 mm in diameter and 1 mm gap. The edges of the samples were covered by a thin layer of silicon fluid (50 cS) to prevent moisture loss, samples were cooled to 0°C at a ramp rate of 1°C/min, and then followed by an isothermal run for 60 minutes at 0°C, frequency sweep between 0.1–100 rad/s at 0°C, and frequency sweep between 0.1–100 rad/s at the same temperature and heating to 90°C at 1°C/min, afterward heating run to 90°C at 1°C/min. For samples with 20% moisture the temperature was extended from -60 to 90°C and 10 mm diameter parallel-plate geometry was used. The ramp rate of temperature was obtained at 1°C/min for both cooling and heating runs. Frequency sweeps were performed at constant temperature intervals of 4°C between 0.1–100 rad/s.

2.9.2 Modulated differential scanning calorimetry (MDSC)

Modulated Differential Scanning Calorimeter (MDSC) Q2000 includes a refrigerated cooling system (RCS 90) to obtain temperatures down to -90°C and a nitrogen DSC cell purge at a flow rate of 50 ml/min. Calibration of the heat flow signals using a traceable indium standard ($\Delta H_f = 28.3$ J g$^{-1}$) and the heat capacity response using a sapphire standard enabled accurate measurements. Hermetic aluminium pans were used in the study and an empty pan was the reference cell.

For agarose-polydextrose, pectin-polydextrose, and pectin-glucose syrup mixtures, samples with total solids of 80% (w/w) were weighed (about 10 mg) and sealed in hermetic aluminum pans which were cooled from 90°C to -70°C at 1°C/min. A modulation rate of 0.53°C for every 40s was applied throughout the experiments. Mixtures with low solids below 70% were cooled from 90°C to 0°C.
For gelatin and polydextrose mixtures, a small amount of the sample (10 mg) was heated to 60°C and cooled to 0°C for low solid samples, whereas the high solid samples were cooled to -90°C, and then followed by heating to 60°C at a scan rate of 1°C/min throughout the experimental routine. A modulation rate of 0.53°C for every 40 s was applied.

For agarose, gelatin, and polydextrose mixtures, a modulation rate of 0.53°C for every 40 s was applied throughout the calorimetric study of the samples. A small amount of sample (about 10 mg) was heated to 90°C (to exclude the thermal history during sample preparation and loading) followed by fast cooling to 60°C at 10°C/min to minimise protein denaturation, and then the samples were cooled at scan rate of 1°C/min to 0°C or -90°C for low solids high solids respectively.

All samples were prepared as summarised in Table 2.2.

2.9.3 Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy was used to identify potential molecular interactions between the individual constituents. Samples of five systems (agarose with polydextrose, pectin with polydextrose, pectin with glucose syrup, gelatin with polydextrose, and mixture of agarose and gelatin with polydextrose) were examined to identify the nature of molecular interactions between the two constituents in the mixture. Absorbance mode was used to obtain FTIR spectra, within the wavelength range of 600 – 4000 cm$^{-1}$ and a resolution of 4 cm$^{-1}$. This was corrected against the background spectrum of the solvent at ambient temperature.

2.9.4 Wide angle X-ray diffraction: WAXD measurements

An accelerating voltage and current of 40 kV and 40 mA, respectively, were used. Samples of five different mixtures that mentioned earlier in section 2.8 were placed on tray holders and continuously scanned to achieve the raw data for the required diffractograms. These were recorded in a 2θ range between 5° and 90° in measuring intervals of 0.1°, and subsequently analysed using the Bruker Advanced X-Ray Solutions software, DIFFRACplus Evaluation (Eva), version 10.0 revision assisting in the elucidation of network characteristics from low to high solids of the samples of different mixtures that were mentioned earlier.
2.9.5 Environmental scanning electron microscopy (ESEM)

ESEM was used to provide tangible evidence of changing in the morphology and topology of the systems’ network. Samples were freeze dried, gold plated, and then examined by using Quanta™ 200 under a high vacuum mode and accelerating voltage of 30 kV. This experiment protocol allows imaging of the binary biopolymer mixtures, and it shows the morphology of system and the influence of increasing co-solute levels.

Table 2.2 Formulation of the polymers used in this study

<table>
<thead>
<tr>
<th>Biopolymer %</th>
<th>Co-solute</th>
<th>Concentration of co-solute (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose (1.5%)</td>
<td>Polydextrose</td>
<td>(0 - 78.5%)</td>
</tr>
<tr>
<td>Pectin (2.0%)</td>
<td>Polydextrose</td>
<td>(40 - 78%)</td>
</tr>
<tr>
<td>Pectin (2.0%)</td>
<td>Glucose syrup</td>
<td>(40 - 78%)</td>
</tr>
<tr>
<td>Gelatin (15.0%)</td>
<td>Polydextrose</td>
<td>(0 - 65%)</td>
</tr>
<tr>
<td>Gelatin (7.5%) + Agarose (1.5%)</td>
<td>Polydextrose</td>
<td>(0 - 71%)</td>
</tr>
</tbody>
</table>
2.10 References


CHAPTER 3

CHANGING NETWORK MORPHOLOGY IN HIGH-SOLID AGAROSE/POLYDEXTROSE MIXTURES

3.1 Abstract

The present investigation deals with the changing network morphology of agarose when mixed with polydextrose from low to high solid preparations. Thermomechanical analysis and micro-imaging was performed using small deformation dynamic oscillation in shear, modulated differential scanning calorimetry and environment scanning electron microscopy. To investigate the nature of physicochemical interactions between agarose and polydextrose molecules, fourier transform infrared spectroscopy (FTIR) and wide angle x-ray diffraction (WAXD) techniques were employed. We observed a decline in the mechanical strength of aqueous agarose preparation upon addition of high levels of polydextrose, which was accompanied by a reduced enthalpic content of the coil-to-helix transition of the polysaccharide network. Glass transition phenomena were observed at subzero temperatures in condensed preparations, hence further arguing for the formation of a lightly cross-linked agarose network with changing solvent quality.
3.2 Introduction

The functional property of gelling polysaccharides is an area of interest in science and technology since they are utilised as texturisers to impart stability and viscoelastic flow in a variety of industrial applications. Scientific understanding on gelation mechanism, phase behaviour and emulsification properties of non-starch polysaccharides like agarose, alginates, carrageenans, pectins, etc. in aqueous preparations has been widely explored in the past (Stewart-Knox & Mitchell, 2003). More recently, the altered physicochemical interaction and network morphology of these polysaccharides in a changing solvent environment by the addition of sugar as the co-solute has been investigated (Tsoga, Kasapis, & Richardson, 1999).

The present treatise deals with the changing morphology of agarose networks by the addition of polydextrose, as the co-solute at various concentrations to prepare formulations from low to high solids. Agarose is one of the polysaccharides considered by major ingredient and product manufacturers for the development of, for example, high sugar/low polysaccharide formulations, which are central to many confectionery products. It is a linear polymer of alternating (1→3)-β-D galactopyranose and (1→4)-3,6-anhydro-α-L galactopyranose subunits (Arnott, Fulmer, & Scott, 1974). Agarose gels are formed by the aggregation of helical molecules into junction zones, which are further stabilised by hydrogen bonds from the surrounding aqueous environment (Deszczynski, Kasapis, MacNaughton, & Mitchell, 2003).

Changes in the physicochemical environment of the agarose networks were investigated in the presence of sugar (glucose syrup or glucose syrup/sucrose mixtures), which was found to dramatically alter the morphology of three dimensional structures leading to the formation of rubbery gels (Kasapis, Al-Marhoobi, Deszczynski, Mitchell, & Abeysekera, 2003; Sworn & Kasapis, 1998). As an extension to the above observation, we now formulate the system with polydextrose that can serve as a bulking agent and sugar replacer in low-calorie foods. Polydextrose is a branched glucose polymer with an average degree of polymerisation of 10 or 12 glucose residues and a mixed combination of α and β linkages that prevent intermolecular association and gelation of the material. Its physiological benefits of dietary fibre, low glycemic index, and prebiotic effects make it a useful ingredient in novel food and nutraceutical applications (Craig SAS, 1998; Julian, 2009).

Conceptually, the present work is presented in two parts. Thus we undertake a systematic study of the effect of polydextrose on the thermomechanical properties of agarose
gels covering a range of solids up to 80% in formulations. A series of ideas is developed to discuss changes in viscoelasticity with increasing levels of co-solute from the dilute to the condensed state. This is further aided by FTIR and X-ray studies that identify the nature of agarose/polydextrose interactions at the molecular level. In the second part, we implement a time-resolved study of the relative stability of the enthalpic/low-solid and entropic/high-solid networks of the polysaccharide/co-solute mixtures. The phenomenon of glass transition is of importance to industry, as the glassy consistency of materials is a fundamental indicator of storage quality, and our results are discussed in this context as well.

3.3 Materials and method

3.3.1 Materials

Agarose: The sample of this investigation was supplied by Sigma-Aldrich Co. (Sydney, Australia). According to the supplier, the content of water, ash and sulphate were 7, 0.25 and 0.12% respectively. It is a material of high gel strength, which achieves elastic modulus ($G'$) values of $3.75 \times 10^4$ Pa at 0°C (aqueous preparation of 1.5% in Figure 3.1a.

Polydextrose: The material used was a product from TATE & LYLE (Illinois, USA). The powder was of 90% purity with 4% moisture and has passed the microbiological testing under the food grade standards. It is a readily soluble, amorphous polymer with an average degree of polymerisation of twelve glucose residues.

3.3.2 Methods

Sample preparation: The polysaccharide solution was prepared by dissolving agarose in distilled water at 80°C by gentle stirring on a hot plate. After obtaining a clear solution of agarose, the temperature was lowered to 65°C for the addition of the required amount of polydextrose. The temperature of the system was maintained at 65°C on the hotplate until the polydextrose was fully dissolved. Thus, mixtures were prepared with 1.5% (w/w) agarose and increasing concentrations of polydextrose up to a total level of solids of 80% (w/w).

Rheology: Low amplitude oscillatory measurements were performed in shear to obtain the storage modulus ($G'$) and loss modulus ($G''$) which are parts of the complex shear modulus ($G^* = G' + iG''$), and tan δ ($G''/G'$). The analysis was performed on AR-G2, which is a controlled strain rheometer with a magnetic thrust bearing technology (TA Instruments, New...
Samples with low and intermediate levels of solids (< 60%; w/w) were loaded on the preheated Peltier at 60°C using a 40 mm parallel plate, with edges covered in silicone oil from BDH (50 cS) to prevent moisture loss. According to the experimental protocol, samples were cooled to 0°C at a scan rate of 1°C/min followed by a 30 min isothermal run and a frequency sweep (0.1 – 100 rad/s) at the same temperature. These were then heated to 90°C at 1°C/min. Materials with high levels of solids intended for vitrification studies were loaded at 60°C using a 10 mm parallel plate and subjected to extended cooling until -50°C. At an interval of every 4°C, frequency sweeps were performed within the range of 0.1 – 100 rad/s. A constant strain of 0.1% was maintained for samples of low solids and 0.01% for the condensed counterparts, which were modeled for estimation of the glass transition temperature at subzero temperatures.

Modulated differential scanning calorimetry: Thermal measurements were performed on MDSC Q2000 (TA instruments, New Castle, DE). The instrument used a refrigerated cooling system to achieve temperatures down to -90°C and a nitrogen DSC cell at 50 mL/min to purge condensation. Samples were loaded in hermetic aluminium pans. Calibration of the heat flow signals using a traceable indium standard (rHf = 28.3 J g⁻¹) and the heat capacity response using a sapphire standard enabled accurate measurements. Samples of 10 to 12 mg were analysed at modulation amplitude of 0.53°C at every period of 40 s. These were equilibrated at 80°C and cooled to 0°C for low-solid or to -90°C for high-solid analysis at a scan rate of 1°C/min.

Fourier transform infrared spectroscopy: Perkin Elmer Spectrum 100 spectrometer was used to obtain the FT-IR spectrum, attached with ZnSe single reflection ATR plate (Perkin Elmer, Norwalk, CT). Agarose, polydextrose and their mixtures at concentrations described earlier were analysed to identify potential physicochemical interactions at low and high solid environments. Absorbance spectra were recorded within a range of 600 to 4000 cm⁻¹ with a scan number of 8 and a resolution of 4 cm⁻¹.

Wide angle X-ray diffraction: These measurements were carried out using a D8 Advanced Bruker AXS (Karlsruhe, Germany) attached with Cu-Kα radiation (0.1542 nm). The instrument operates at an accelerating voltage and current of 40 kV and 40 mA, respectively. Freeze dried samples of agarose, polydextrose and their mixtures were placed on a tray holder, which was then continuously scanned to obtain the diffractograms within a 2θ range of 5° and 90°.
Environmental scanning electron microscopy: Imaging of the changing polysaccharide network in aqueous and high-solid preparations was performed using FEI Quanta 200 ESEM (Hillsboro, Oregon, USA). Freeze dried samples were attached on sample holder followed by gold plating for the analysis. Under the operating conditions of a high vacuum mode with an accelerating voltage of 30 kV, images of several magnifications were recorded.

3.4 Results and discussion

3.4.1 Qualitative observations of changes in the mechanical properties of agarose networks with increasing additions of polydextrose

In the present series of experiments, agarose was used at a fixed concentration of 1.5% and the polydextrose content was varied from 10 to 78.5% in the mixture to prepare samples of low, intermediate and high levels of solids. In an effort to understand the mechanical properties of the agarose network in the aqueous and the polydextrose environments, the molten state of the binary mixture was cooled at a fixed scan rate of 1°C/min from 50°C to 0°C at a constant oscillatory frequency of 1 rad/s and strain amplitude of 0.1%.

As shown in Figure 3.1a, there is up to 4.5 log increase in values of the elastic component of the network upon cooling as the temperature falls below 35°C representing the coil-to-helix transition of the agarose molecules. At low concentrations of polydextrose addition (10 - 20%), agarose networks become reinforced, as observed by the increased values of storage modulus traces that conclude at 0°C. Entering the intermediate level of solids, i.e. > 30% polydextrose in formulations, a gradual decrease in network strength becomes visible. This leads to a considerable reduction in network strength of preparations, which at 70% co-solute have lost two orders of magnitude compared with the aqueous counterparts.

Earlier work on gelling polysaccharides in mixture with sugars as the co-solute observed a similar variation in shear modulus, and the suggestion there was that polysaccharide helices cannot attain thermodynamic stability in a highly “unhydrated” environment (Gekko & Kasuya, 1985; Gekko, Mugishima, & Koga, 1985; Kasapis et al., 2003). This pattern of modulus variation as a function of polydextrose concentration is also shown in Figure 3.1b that illustrates the dissociation of network chains on temperature ramping from 0 to 90°C. Structures collapse entirely at temperatures approaching 90°C, with the helical aggregates dispersing into coils leading to the formation of a viscoelastic liquid. The thermal hysteresis between cooling and heating profiles in Figures 3.1a and 3.1b is
attributed to the formation of extensive aggregation in the agarose network (Aymard et al., 2001).

3.4.2 Thermal explanation for the changing morphology of the agarose network in the presence of polydextrose

To broaden our understanding on the changing property of the agarose network, thermal events were recorded using the heat flow signals of differential scanning calorimetry. Change in enthalpy ($\Delta H$) during the first order transition of the agarose molecules in aqueous preparations provides information on the thermodynamic state of the system (Rahman, 2006; Nishinari, 1990). Furthermore, calorimetric explanations have been sought earlier for the changing morphology of polysaccharide gels in a co-solute environment from low to high solids (Papageorgiou, 1995).

Figure 3.2a illustrates the trace of heat flow signals for the agarose/polydextrose system during cooling at 1°C/min from 50°C to 5°C. Thermograms sketch an upward peak corresponding to the exothermic process of coil-to-helix transition during agarose gelation. These sol-gel transitions ($T_{max}$), calculated as the midpoint transition temperature during the exothermic process, were found to be about 30°C for the aqueous agarose preparation. They shift to higher temperature along with broadening of the peak as we introduce polydextrose at increasing concentrations (0 to 78.5%) in the mixture. Using the area enclosed under the peak of the thermal event during gelation and by drawing a baseline, it is possible to calculate the change in enthalpy ($\Delta H$) associated with helix formation.

Figure 3.2b depicts the trend for values of $\Delta H$ as it increases for concentrations of polydextrose until 20% followed by a gradual decline with further additions of the co-solute in the mixture. The above observation compliments the mechanical behaviour of the agarose/polydextrose network discussed in Figure 3.1a, and is congruent with the hypothesis of reduced cooperativity for structure development in polysaccharide networks in a high sugar environment (Kasapis, Giannouli, Hember, Evageliou, Poulard, Tortbourgeois, & Sworn, 1999).
3.4.3 Understanding the glassy phenomena in agarose/polydextrose systems using a viscoelastic approach

This section deals with the properties of condensed agarose/polydextrose systems at a total level of solids of 80%. Upon cooling to subzero temperatures, these materials undergo vitrification phenomena, since the low experimental temperatures seize molecular rotation/vibration of the chain segments within the amorphous matrix. The glassy consistency requires investigation to assist in the development of novel materials with superior functionality. Figure 3.3 represents the trace of heat flow at subzero temperatures for systems being thermally treated at a rate of 1°C/min. The gradient of the heat-flow traces illustrates the reduction in specific heat, as the material undergoes cooling and *vice versa* on heating. The onset of calorimetric glass transition in the binary composite during cooling and heating has been recorded at -32°C and -56°C, respectively, with the mid-point glass transition temperature (*T_g*) being pinpointed in both cases at -43°C.

Calorimetric work on the vitrification patterns of our system lays the ground for changing the mode of investigation, which allows delivering mechanical insights into the behaviour of these supersaturated systems. In doing so, we adapt the techniques of “advanced polymer science” that elaborates on the changes of viscoelastic properties of the materials during the transition from the rubbery to the glassy state (Mitchell, 2000; Kasapis, & Sablani, 2005). In Figure 3.4, small amplitude oscillatory measurements on the system of 1.5% agarose with 78.5% polydextrose, upon controlled cooling to subzero temperatures, produce a spectacular development in the values of elastic and viscous modulus. Thus the variation in *G’* and *G”* traces as a function of temperature represents the master curve of viscoelasticity for this preparation in the passage from the solution state at temperatures > 20°C to the gel state. On further cooling, modulus values increase rapidly, with the sample entering the glass transition region at subzero temperatures where the viscous component of the network (*G”*) becomes dominant. At the very end of the cooling routine, there is yet another transformation and the sample is found in the glassy state, for example at 40°C, where the values of storage modulus dominate exceeding $10^9$ Pa. Reduced mobility in the glassy state reflects ‘β transitions’ and the stretching or bending of chemical bonds (Ward, & Hadley, 1993).

Experimental measurements in Figure 3.4 recognise a preliminary connection between the temperature and frequency dependence of dynamic mechanical properties of our material. Given current technology, we cannot obtain mechanical measurements at times shorter of a tenth of a second and experiments that are longer than $10^5$ s are prohibitive in terms of
equipment availability. Nevertheless, investigators are interested in combining rheological data at different temperatures and frequencies in an attempt to expand the window of observations of physicochemical phenomena (Ronan, Alshuth, Jerrams, & Murphy, 2007). In an effort to synchronise the factors of temperature and time in this work, we implement the so-called time temperature superposition principle (TTS) over the experimentally accessible range of temperature (Farhat, Mousia, & Mitchell, 2003).

As shown in Figures 3.5a and Figure 3.5b, frequency sweeps were performed at fixed temperature intervals of four degree centigrade within the range of -12°C to -40°C. The values of storage and loss modulus at the lower and upper range of temperature represent the pathway of transition from the glassy to the rubbery state (Tobolsky, 1956). Horizontal superposition of the mechanical spectra around an arbitrary reference temperature ($T_o = -24°C$) reproduces a master curve of viscoelasticity as a function of an extended frequency range of $10^{-4}$ to $10^{7}$ rad/s (refer to Figure 3.6). Superposing the mechanical spectra horizontally along the x axis results in a set of shift factors, $a_T$. Figure 3.7 represents those shift factors plotted as a function of temperature thus unveiling a fundamental relationship between patterns of viscoelastic relaxation and temperature (Maltini, & Anese, 1995).

In synthetic polymer research, several systems follow the principles of thermorheological simplicity, as demonstrated in Figures 3.5 and Figure 3.6 presently. In these systems, the free volume theory describes the concept of vacant spaces within the network arising from the irregularities in polymeric arrangement. As postulated in the literature, during the vitrification process, the vacant space facilitating the chain and molecular motion collapses, thus inducing an arrest on polymer chain dynamics (Ferry, 1991). Williams, Landel and Ferry postulated a mathematical model on the concept of free volume along with the thermal expansion coefficient of amorphous synthetic polymers (van der Put, 2010). Modification of the above described mathematical model of WLF, to integrate the dynamic mechanical data in shear, delivers the following mathematical design (Ferry, 1980):

$$\log a_T = \log \left[ \frac{G'(T)}{G'(T_o)} \right] = - \frac{(B/2.303f_o)(T - T_o)}{(f_o/\alpha_f) + T - T_o}$$  \hspace{1cm} (3.1)$$

where, $f_o$ represents the fractional free volume (free to total volume of the molecule), $\alpha_f$ is the thermal expansion coefficient and $B$ is set to 1. $B/2.303f_o$ and $f_o/\alpha_f$ are considered to be the WLF parameters and termed as $C_1$ and $C_2$ respectively.
During the glass transition region, shift factors follow equation (3.1) based on macromolecular free volume, hence for the system of 1.5% agarose and 78.5% polydextrose the WLF parameters were calculated to be 13.73 and 53 deg, respectively. However, modelling based on the WLF concept becomes unrealistic for the shift factors in the glassy state and, instead, it follows the mathematical expression of Andrade (modified Arrhenius) through which the activation energy needed for an elementary flow can be obtained (Gunning, Parker, & Ring, 2000):

\[ \log a_T = \frac{E_a}{2.303 R} \left( \frac{1}{T} - \frac{1}{T_o} \right) \]  

(3.2)

where, \( E_a \) represents the activation energy and \( R \) is the gas constant. There is a notable point of discontinuity in the arrangement of the shift factors in Figure 3.7. This corresponds to a threshold from WLF to Arrhenius type kinetics and can be considered as the absolute boundary between the glass transition region and the glassy state, which reflects the mechanical glass transition temperature (\( T_g = -27°C \)).

It is expected that the calorimetric glass transition temperature (-30°C in Figure 3.3) is below that of the mechanical glass transition temperature (-27°C in Figure 3.7), since the former describes a micromolecular dimension whereas the latter is governed by the relaxation of the network forming polymer, agarose in this case. Accelerated mechanical vitrification has been reported earlier for several systems of polysaccharide or gelatin in the presence of sugar as the co-solute (Kasapis, 2006).

3.4.4 Microscopic evidence of changing morphology in the agarose/polydextrose network

In an attempt to visualise the morphology of agarose networks in various concentrations of polydextrose, we performed environmental scanning electron microscopy (ESEM) on freeze dried samples of the polysaccharide, co-solute and their mixture. Figure 3.8a reproduces the image of the aqueous preparation of 1.5% agarose, which can be described as an irregular arrangement of folded ‘sheets’ due to aggregated helices. Figures 3.8b and 3.8c argue that polysaccharide associations become even denser upon addition of low amounts of polydextrose (10 and 20%, respectively) in accordance to our rheology and calorimetric observations.
In contrast, formulations including polydextrose at concentration above 30% produce relatively amorphous matrices, which are dominated by the physicochemical environment of the co-solute (refer to Figures 3.8c to 3.8h). These images can find parentage in the featureless background of the single polydextrose preparation in Figure 8i. Overall, observations offer tangible evidence of the diminishing mechanical strength of the agarose network recorded rheologically in parallel with the reduction in the DSC enthalpic content of its coil-to-helix associations at high levels of polydextrose. Micrographs of gelling polysaccharides when mixed with sugar at high concentrations depicted a congruent image with reduced aggregation of the cross-linker (Kasapis, Al-Marhoobi, Deszczynski, Mitchell, & Abeysekera, 2003).

3.4.5 Micromolecular assessment of interactions in the agarose/polydextrose mixture

To explore the molecular basis of potential interactions between agarose and polydextrose in mixture with residual water molecules, we performed Fourier transform infrared spectroscopy. Figure 3.9 illustrates these FTIR spectra, where among the pronounced vibrations within the chemical groups of agarose, C-O-C vibration of the 3,6 anhydrogalactose bridge (1038 cm$^{-1}$) and O-H coupled with C-H stretching vibrations (3600-2986 cm$^{-1}$) have been identified (Sekkal, Huvenna, Legrand, Sombre, Mollet, Givernaud, & Verdus, 1993). Polydextrose, a glucose based polymer, produces characteristic peaks representing stretching vibration of C-O-C glycosidic linkages (1180-927 cm$^{-1}$), C=O stretching vibration of aldehyde (1627 cm$^{-1}$), C-H stretching (2900 cm$^{-1}$) and O-H stretching vibrations (3640 – 2978 cm$^{-1}$) (Mickova, Copikova, & Synytsya, 2007). Mixtures of agarose and polydextrose reproduce the spectra of the individual components within the combination spectra of the binary mixtures, thus arguing for the absence of covalent linkages between the individual constituents in low and high solids preparations.

Finally, diffractograms from wide angle X-ray scattering were obtained for each of the individual components and their binary mixtures shown in Figure 3.10. The absence of crystalline domains in single and mixed preparations was confirmed by the presence of broad peaks extending between 10 and 55°. These peaks are correlated to the high density of materials as a result of freeze drying during sample preparation. In general, the amorphous character of diffractograms supports the conclusions of the viscoelastic analysis for these materials that was based on the concept of a rubber-to-glass transition.
Figure 3.1 Cooling (a) and heating (b) profiles of storage modulus for 1.5% agarose with 0 (■), 10 (□), 20 (♦), 30 (◊), 40 (▲), 50 (△), 60 (●) and 70% (○) polydextrose (scan rate: 1°C/min; frequency: 1 rad/s; strain: 0.1%).
Figure 3.2 DSC exotherms (a) for 1.5% agarose with 0, 10, 20, 30, 40, 50, 60, 70, 75 and 78.5% polydextrose, and (b) trend for change in enthalpy ($\Delta H$) during gelation of 1.5% agarose with varying concentrations of polydextrose.
Figure 3.3 DSC cooling and heating profile for 1.5% agarose with 78.5% polydextrose at subzero temperatures (scan rate: 1°C/min).
Figure 3.4 Cooling profiles of storage (■) and loss (□) modulus for 1.5% agarose with 78.5% polydextrose scanned at 1°C/min (frequency: 1 rad/s; strain: 0.01%).
Figure 3.5 Frequency variation of $G'$ (a) and $G''$ (b) for 1.5% agarose with 78.5% polydextrose; bottom curve is taken at -12°C (■), other curves successively upwards -16°C (□), -20°C (♦), -24°C (◊), -28°C (▲), -32°C (Δ), -36°C (●) and -40°C (○).
Figure 3.6 Master curve of reduced shear moduli ($G'_p$ and $G''_p$) as a function of reduced frequency of oscillation ($\omega_a T$) based on the frequency sweeps of the preparation in Figure 5 (reference temperature = -24°C).

Figure 3.7 Temperature variation of the factor $a_T$ within the glass transition region ($\Delta$) and glassy state ($\square$) for 1.5% agarose with 78.5% polydextrose, with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors throughout the vitrification regime (dashed line pinpoints the mechanical Tg prediction).
Figure 3.8 Micrographs for 1.5% agarose with 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g) and 70% (h) polydextrose in mixture, and in 80% (i) single polydextrose preparations.
Figure 3.9 FTIR absorbance spectra for 1.5% agarose with 0, 20, 40, 60 and 80% polydextrose in mixture, and an 80% single polydextrose preparation arranged successively upwards.
Figure 3.10 X-ray diffractograms for 1.5% agarose with 0, 20, 40, 60 and 80% polydextrose in mixture, and an 80% single polydextrose preparation arranged successively upwards.
3.5 Conclusion

This work dealt with the changing network morphology of agarose in an environment of varying solvent quality induced by the addition of polydextrose as co-solute from low to high-solid contents. Thermomechanical spectra along with micrographs strongly argue that agarose networks possess less order in a high polydextrose environment. The change in solvent quality with introduction of polydextrose prevents full helix development and systems appear to be increasingly amorphous. Changes in structural dynamics allows the lightly cross-linked networks to undergo vitrification at subzero temperatures that can be treated with theoretical models according to the synthetic polymer research. Formation of single-phase systems where the polysaccharide is “dissolved” within the co-solute environment is in direct contrast to the phase morphology of the gelatin/polydextrose preparation where phase separated domains were observed (refer to Chapter 5 in this Thesis).
3.6 References


CHAPTER 4

ANALYSIS ON THE EFFECTIVENESS OF CO-SOLUTE ON THE NETWORK INTEGRITY OF HIGH METHOXY PECTIN

4.1 Abstract

Co-solute requirements for high methoxy pectin gelation were observed by the addition of glucose syrup and polydextrose at concentrations varying from 50 to 78% (w/w). Pectin content was fixed at 2% (w/w) in formulations. Studies from small deformation dynamic oscillation in shear, modulated differential scanning calorimetry and environmental scanning electron microscopy are reported presently. Structural properties of pectin preparations were recorded in relation to the molecular weight and concentration of added co-solute in the acidic environment (pH ~ 3.0). High levels of co-solute induce formation of weak pectin gels at elevated temperatures (even at 95°C), which upon subsequent cooling exhibit increasing strength and convert to a clear glass at subzero temperatures. Fourier transform infrared spectroscopy and wide angle x-ray diffraction were practised to examine the nature of interactions between polymer and co-solute, and the extent of amorphicity of preparations. Glucose syrup is an efficient plasticiser leading to a reduction in the glass transition temperature ($T_g$) of the pectin network, whereas polydextrose assists in the formation of stronger pectin gels in the rubbery state.
4.2 Introduction

Among the series of gelling polysaccharides, high methoxy pectin shares a unique feature of requiring high concentrations of co-solute (mainly sugars) to induce network formation. This functional feature has been abundantly utilised in the food industry to formulate high-solid embodiments of a small aqueous phase (Evageliou, Richardson, & Morris, 2000). Sucrose has been a widely preferred co-solute to assist in pectin gelation, whereas the influence of other glucose based co-solutes on the gelation mechanism of pectin has been less investigated or understood (Christensen, 1986; May, 1990). Recently, there has been a resurgent interest to study the pattern of network formation of high methoxy pectin in the presence of glucose syrup and contrast this with the function of polydextrose, which can be used as sugar replacer.

Pectin is a linear polymer of (1-4) linked α-D-galacturonate interspersed periodically with 1,2-linked rhamnosyl residues (de Vries, Rombouts, Voragen, & Pilnik, 1982). Gelation of pectin in the presence of co-solute provides viscoelastic structure, which is a combination of thermally reversible and hydrophilic interactions of the pectinic acid network at low temperatures along with the hydrophobic association of methyl ester substituents developed at high temperatures (Oakenfull, 1991; Oakenfull, & Scott, 1984; Walkinshaw, & Arnott, 1981). The above mentioned network integrity has been widely explored for pectins of different chemical classes but, as mentioned above, research is still needed to ascertain structure-function relationships as a function of the multifaceted chemistry of various co-solutes.

Polydextrose has been increasingly regarded as a potential replacer of sugar with multiple health benefits, which, of course, creates an interest in examining the structural, textural and sensory characteristics of its systems. It is a randomly bonded, bulk polymer of glucose residues with an average degree of polymerisation of ten or twelve, which is obtained by the thermal polymerisation of D-glucose in the presence of sorbitol and phosphoric acid. The randomly bonding nature of polydextrose creates a high number of possible combinations of α and β linkages, making this polydispersity a retardant to intermolecular associations leading to the formation of a “permanent” network (Ribeiro, Zimeri, Yildiz, & Kokini, 2003). These chemical and conformational characteristics combined with the physiological benefits of dietary fibre, low glycemic index and prebiotic nature make polydextrose a potentially interesting replacer of sugar in food formulations (Craig SAS, 1998; Julian, 2009).
The development of structural characteristics and the thermodynamic state of high methoxy pectin is usually examined in the presence of at least 50% (w/w) co-solute solids so that gel formation is induced. Techniques of investigation range from macromolecular rheology and thermal analysis that pinpoints first-order phase transitions to micromolecular infrared spectroscopy and x-ray diffraction. This affords investigation of a range of textural consistencies as a function of polymer concentration, amount of added co-solute, pH range and experimentally accessible temperature in relation to industrial production and commercial storage of products.

Indeed, condensed systems of high methoxy pectin with co-solute upon cooling to subzero temperatures undergo vitrification whose molecular relaxations, monitored via the free volume theory, are of scientific interest since they relate directly to the design of novel product functionality (Kasapis, Al-Alawi, Guizani, Khan, & Mitchell, 2000). Headway in this type of research is achieved by adapting theoretical frameworks from the “sophisticated” synthetic polymer research, which allows determination of viscoelastic functions in relation to time or temperature of observation (Dannhauser, Child, Ferry, & 1958). The current investigation utilises such theoretical concepts to build molecular understanding by contrasting the structural properties of high solid pectin/glucose syrup and pectin/polydextrose mixtures in terms of co-solute concentration and molecular weight.

4.3 Materials and methods

4.3.1 Materials

High Methoxy Pectin: The citrus peel pectin was purchased from Sigma Aldrich Co. (Sydney, Australia). Polysaccharide content was 92.0% on dry weight basis of which 85.1% is galacturonate with a degree of methyl esterification (DE) of 70.3%. Chromatographic analysis carried out by the supplier, using an ultrahydrogel linear column and eight pullulan standards, produced a number average molecular weight ($M_n$) for the pectinic material of this investigation of 154 kDa.

Polydextrose: The product used in this investigation was purchased from TATE & LYLE (Illinois, USA). According to the supplier, the sample was 90% pure containing 4% moisture. It facilitated glass related research in mixture with the pectin polysaccharide, since
it is a readily water soluble, glucose based bulk polymer, which has passed the standard microbiological testing under the food grade standards.

**Glucose syrup:** Glucose syrup used was a product of Cerestar (Manchester, UK) with 42% of glucose residues present as reducing end groups (dextrose equivalent, de = 42). The total level of solids was 82%, and percentages in formulations of this investigation refer to dry solids. The polydisperse nature of glucose syrup was established using gel permeation chromatography data provided by the supplier, and the material converts from a thick solution at ambient temperature to a transparent glass at subzero temperatures.

### 4.3.2 Methods

**Sample Preparation:** Pectin solutions were prepared by dissolving the polysaccharide at 2% (w/w) solids in distilled water at 95°C using hot magnetic plate and gentle stirring. On obtaining the clear solution of pectin, the temperature was lowered to 75°C for the addition of the required amount of glucose syrup or polydextrose. The solution was maintained at 75°C until the co-solute was fully dissolved and the desired level of total solids was obtained by evaporating slowly excess water. The pH of the system was then adjusted to 3.0 using 0.2 M HCl solution to create the required gelling conditions for subsequent experimentation.

**Rheological Analysis:** Measurements were executed on ARG-2 (TA Instruments, New Castle, DE), a controlled strain rheometer with magnetic trust bearing technology. Under the small amplitude oscillatory experimental mode of the instrument, the linear viscoelastic properties of samples were assessed by varying conditions of temperature, frequency and strain amplitude. Samples with total solids up to 70% (w/w) were loaded on the preheated Peltier at 95°C using a 40 mm parallel plate with edges covered with silicone oil from BDH (50 cS), thus preventing moisture loss. Samples were then cooled to 0°C at a controlled rate of 1°C/min followed by an isothermal segment for 30 min at 0°C and a heating run to 95°C at 1°C/min. An oscillatory frequency of 1 rad/s with a strain of 1% was executed throughout the experiments. For condensed samples with 20% moisture, a measuring geometry of diameter 10 mm was used within an extended experimental temperature range to analyse the vitrification behaviour of the pectin network. Molten samples were loaded on the preheated Peltier at 95°C and cooled to -60°C at 1°C/min thus covering the melt, rubbery, glass transition and glassy state of the sample using a strain of 0.1%. At an interval of four degrees
centigrade, frequency sweeps were performed within a range of 0.1 – 100 rad/s to study the
time dependent mechanical properties of the condensed matrix.

**Modulated Differential Scanning Calorimetry:** Thermal measurements were performed
using Q 2000 (TA instruments, New Castle, DE). Instrumentation includes a refrigerated
cooling system (RCS 90) to achieve temperatures down to -90°C and a nitrogen DSC cell
purge at a flow rate of 50 ml/min. Calibration of the heat flow signals using a traceable
indium standard ($\triangle H_f = 28.3 \text{ J g}^{-1}$) and the heat capacity response using a sapphire standard
enabled accurate measurements. Samples with total solids of 80% (w/w) were weighed (about
10 mg) and sealed in an hermetic aluminium pan which was cooled from 90°C to -70°C at
1°C/min. A modulation rate of 0.53°C for every 40 s was applied throughout the experiments.

**Fourier Transform Infrared Spectroscopy:** Readings were obtained from Perkin Elmer
Spectrum 100 using ZnSe single reflection ATR plate (Perkin Elmer, Norwalk, CT). Samples
of high methoxy pectin with polydextrose or glucose syrup as the co-solute were analysed to
identify the nature of molecular interaction between the two constituents, i.e. polymer and co-
solute, from the absorbance spectra. These were taken within a range of 600 – 4000 cm$^{-1}$ from
an average scan number of 8 and a resolution of 4 cm$^{-1}$.

**Wide Angle X-ray Diffraction:** Diffractograms were obtained using D8 Advanced
Bruker AXS (Karlsruhe, Germany) from a Cu-Kα (1.54 Å) radiation source. Freeze dried
samples of pectin with polydextrose or glucose syrup were scanned continuously using an
accelerating voltage and current of 40 kV and 40 mA, respectively. Raw data within a 20
range of 5 and 90° were analysed using DIFFRAC$^plus$ Evaluation (Eva), version 10.0 revision
1, which is a Bruker Advanced X-Ray Solutions software.

**Environmental Scanning Electron Microscopy:** Micrographs of the pectin network
with various levels of added co-solute were obtained using FEI Quanta 200 ESEM (Hillsboro,
Oregon, USA). Gold plated samples under the conditions of high vacuum and an accelerating
voltage of 30 kV produced magnified microscopic images of the binary mixtures thus
assisting in the elucidation of network characteristics from low to high solids.
4.4 Results and discussion

4.4.1 Analysis of linear viscoelastic properties of the pectin/co-solute mixture during network formation

Viscoelastic understanding of the materials of this investigation was carried out at a fixed concentration of high methoxy pectin (2%, w/w) with varying additions of co-solute, i.e. polydextrose and glucose syrup from 50 to 78% (w/w). Using the technique of small amplitude dynamic oscillation in shear, the development of structural properties of the high solid mixture as a function of temperature was studied first, as samples in the molten state were cooled from 95°C to 0°C at 1°C/min under a fixed frequency of 1 rad/s and 1% strain.

Figure 4.1a represents the gelling behaviour of the polysaccharide under varying concentrations of glucose syrup as noted by the development of elastic modulus (G'), as the material is cooled steadily from high temperatures. Observations on the gelling behaviour of pectin with polydextrose, a glucose based co-solute of higher molecular weight than glucose syrup, can be visualised in Figure 4.1b. The requirement of co-solute for the gelation of pectin even under the ideal acidic pH of 3.0 is evident from the observations in both figures, as pectin by itself in an aqueous environment fails to develop a convincing three-dimensional structure throughout the examined temperature range. High concentrations of co-solute (> 50%, w/w) induce gelation of pectin at temperatures as high as 70°C due to the hydrophobic association between methyl ester substituents, with networks being developed further on subsequent cooling (Chronakis, Kasapis, & Abeysekera, 1997).

Indeed, controlled cooling to temperatures below ambient is met with a relatively sharp increase in the values of storage modulus, which is at a temperature close to 20°C for preparations with 50% co-solute. This phenomenon of hydrophilic association, leading to formation of structures that include the polymer in a helical configuration, shifts to higher temperature with increasing the concentration of co-solute (e.g. at about 40°C in the case of 70% glucose syrup). Observations on the effectiveness of sugar type or concentration on pectin gelation have been previously reported (Evageliou, Richardson, & Morris, 2000), and it is widely believed that the onset temperature of conformational ordering during cooling is strongly dependent on the specific interaction between polymer and co-solute (Nilsson, Piculell, & Malmsten, 1990).
4.4.2 Experimental studies on the molecular and structural relaxation of pectin/co-solute mixtures

Figure 4.2 depicts the heat flow (W/g) trace for the high solid system of polysaccharide and co-solute (78% glucose syrup and 78% polydextrose). The continuous and sigmoidal drop in the signal of heat flow with controlled cooling results in a relatively broad transition that is considered as the calorimetric manifestation of a glass transition. The onset and mid-point glass transition temperature of the pectin/glucose syrup preparation in Figure 4.2 was calculated to be at -35°C and -48°C, respectively. High solid systems of pectin/polydextrose produced earlier transformations recorded at -31°C for onset and -42°C for mid-point transition temperatures. Perfect reversal of the above observed transition can be obtained by heating the system from the glassy to the rubbery state, as the disorganisation of molecular structure follows a linear relationship on the thermal effect.

Besides calorimetric studies, mechanical work provides complementary understanding on the structural relaxation of biomaterials throughout the glass transition region. To achieve this, we take our lead from the so-called “sophisticated” synthetic polymer science approach that gives a well-established pattern of viscoelasticity through the master curve of melt-to-glassy state (Mitchell, 2000; Kasapis, & Salblani, 2005).

Figure 4.3 reproduces a rather spectacular development in the viscoelasticity of pectin/glucose syrup as the rubbery state of the material at temperatures above 0°C, where $G'$ is above $G''$, transforms into the state of a high-viscosity liquid ($G'' > G'$). On further cooling, there is yet another development with the solid component overtaking again the viscous element of the network and the material entering the glassy state at about -34°C. Figure 4.3 also illustrates the corresponding master curve of viscoelasticity for the pectin/polydextrose preparation at 80% (w/w) total level of solids. As observed in Figure 4.3, the glass transition region of pectin/polydextrose sample maintains a dominant elastic component, apparently, due to the relatively high molecular weight of polydextrose, as compared with glucose syrup. This effect of molecular weight is also seen in an early vitrification of pectin/polydextrose, with the onset of the glassy state being at about -20°C. In both cases, the storage modulus approaches the value of 1010 Pa well within the glassy state indicating that molecular dynamics have reached a state of pseudo-equilibrium.

Besides the temperature dependence of viscoelasticity, we are interested in the complimentary time dependence of fundamental functions, since, for example, experimentation of that nature could overcome drawbacks associated with prohibited lengthy
requirements for the use of laboratory facilities (Tobolsky, 1956). To obtain the time function, the time-temperature superposition (TTS) principle was implemented by synchronising time scale observations within the experimentally accessible temperature (Farhat, Mousia, & Mitchell, 2003). Logistics dictate that frequency sweeps within a range of 0.1 to 100 rad/s are obtained at an interval of every four degrees centigrade between -43°C and -15°C for the high solid system of pectin with glucose syrup and between -32°C and -4°C for the pectin/polydextrose preparations shown in Figures 4.4a and 4.4b, respectively. These mechanical spectra of loss modulus as a function of frequency of oscillation represent the glass transition region at the upper temperature range and the glassy state at the low temperature range (data of storage modulus was also been obtained but not shown here).

In an attempt to integrate the concepts of time and temperature within a single pictorial representation and subsequent mathematical expression, frequency sweeps were shifted horizontally along the x-axis in accordance with an arbitrary chosen reference temperature (To = -31°C for pectin/glucose syrup and -20°C for pectin/polydextrose high solid systems). Such superposition demonstrates that the values of storage and loss modulus fit into a master curve of viscoelasticity, as shown for the pectin/glucose syrup sample and pectin/polydextrose system in Figure 4.5a and 4.5b, respectively. This clearly reproduces the glass transition region (G'' > G') and the glassy state (G' > G'') as a function of frequency (or timescale) of observation.

Systematic shifting of mechanical spectra in Figures 4a and 4b generates a set of shift factors, $aT$, which can be plotted as a function of temperature to provide tangible evidence of the variation in mechanical relaxation of a given material during vitrification. This is shown in Figure 4.6 for the pectin/glucose syrup and pectin/polydextrose systems covering a temperature range of -5°C to -45°C. The above procedure of horizontal superposition was performed for the pectin/polydextrose mixture using corresponding frequency sweeps obtained every four degrees centigrade.

Shift factors are fundamental descriptors of the nature of vitrification patterns in biomaterials, a concept that invites treatment of data with a suitable theoretical framework of thought. The concept of free volume, as derived from the materials science, gives a mechanistic understanding of the transformation of a system from the rubbery to the glassy state. It is based on the role of vacant spaces within a physical network arising from irregularities within the polymeric matrix. Progression in the vitrification process dictates that the free volume within the system collapses resulting in freezing-in effects of the chain
segment (Ferry, 1991). The mathematical model proposed by Williams, Landel and Ferry that includes the concepts of free volume and thermal expansion co-efficient of amorphous synthetic polymers can be adapted to integrate dynamic mechanical data in shear, as follows (Ferry, 1980; van der Put, 2010):

\[
\log a_T = \log \left[ \frac{G'(T)}{G'(T_0)} \right] = -\frac{(B/2.303f_0)(T - T_0)}{(f_0/\alpha_f) + T - T_0} \quad (4.1)
\]

where, the fractional free volume (i.e. the ratio of free to total volume of the polymeric segment) is represented by \(f_0\). \(\alpha_f\) is the thermal expansion coefficient and \(B\) is usually set to the value of 1. \(B/2.303f_0\) and \(f_0/\alpha_f\) are known as the WLF parameters, which are denoted as and for a given reference temperature, \(T_0\).

We have found that in our systems, the mechanics of the glass transition region as seen in the factor \(a_T\) are described by equation 4.1. This generates a set of WLF parameters (i.e. \(C_1\) and \(C_2\)) for high methoxy pectin/glucose syrup and high methoxy pectin/polydextrose of 11.70 and 53 deg, and 12.27 and 53 deg, respectively. As systems enters the glassy state, modelling based on the concept of free volume becomes inappropriate (i.e. empirically derived shift factors do not fit) but, instead, data follow the mathematical design of modified Arrhenius equation (Andrade equation according to Gunning, Parker, & Ring, 2000):

\[
\log a_T = \frac{E_a}{2.303 R} \left( \frac{1}{T} - \frac{1}{T_0} \right) \quad (4.2)
\]

where, \(E_a\) denotes the activation energy and \(R\) is the gas constant. It becomes apparent that in both samples, the free volume theory of equation (1) describes the glass transition region, whereas molecular processes in the glassy state are followed by the predictions of the reaction rate theory of equation (4.2).

Consequently, the experimental arrangement of shift factors in Figure 4.6 and ensuing theoretical treatises argue for a critical discontinuity from one molecular pathway to another. This threshold in mechanical relaxation can be considered as the mechanical or network glass transition temperature (\(T_g\)), which for 80% solids is recorded at -34°C for pectin/glucose syrup and -22°C for pectin/polydextrose. Systems containing polydextrose exhibit an early vitrification as the co-solute contains on average of ten to twelve glucose molecules per species. Thus the higher molecular weight of polydextrose facilitates an early glass transition
temperature also observed in thermograms of Figure 4.2. Glucose syrup, as the low molecular weight co-solute, acts as an effective plasticiser delaying the process of glass transition in high methoxy/pectin networks.

4.4.3 Aspects of network topology and micromolecular characterisation of pectin/co-solute mixtures

It is always desirable to provide a visual outlook or tangible evidence of the molecular characteristics of biomaterial matrices so that it complements theoretical modeling of the preceding section, and in an effort to do so, we resorted to electron microscopy and infrared/x-ray experimentation. In the case of microscopy, single pectin preparations (2%, w/w) and mixtures of the polysaccharide with 60% (w/w) glucose syrup or polydextrose were freeze dried ready for image taking.

Figures 4.7a – 4.7c reproduce images obtained with environmental scanning electron microscopy. Freeze dried strands of pectin molecules are clearly shown in the absence of added co-solute in Figure 4.7a, i.e. in a thermodynamic regime that the polysaccharide is unable to form a coherent hydrated network, as discussed earlier using rheology evidence in Figures 4.1a and 4.1b. In contrast to the polysaccharide morphology of this micrograph, Figures 4.7b and 4.7c illustrate sheet-like fields of what is essentially an amorphous pectin network in the presence of co-solute at sixty percent solids. Thus, microscopy images provide additional confirmation in support of the thermomechanical analysis presented earlier for the polysaccharide forming a firm network that progressively vitrifies with increasing co-solute content.

Presentation of data thus far in terms of gelation or glass transition behaviour was made on the assumption that there is no creation of chemical interactions between polymer and co-solute that could change the underlying physics of the two constituents in the binary mixture. To identify potential intermolecular associations of a covalent nature, mixtures of pectin and co-solute at varying concentrations of residual water were prepared for inspection under fourier transform infrared spectroscopy (refer to Figure 4.8). The inherent chemical linkages of pectin in both figures were identified as O-H stretching vibrations coupled with C-H stretching vibrations at 3681 - 2900 cm\(^{-1}\), and the esterified (COO-R) along with non-esterified (COO\(^{-}\)) carboxyl groups at 1756 – 1500 cm\(^{-1}\) (Monsoor, Kalapathy, & Proctor, 2001).
Besides pectin, Figure 4.8 depicts peaks of the starch derived glucose syrup with reference to C-H stretching along with O-H stretching vibrations at 3645 - 2845 cm\(^{-1}\), C=O stretching vibration of aldehyde at 1624 cm\(^{-1}\), C-O stretching and bending at 1493 - 1173 cm\(^{-1}\), and C-O-C glycosidic linkages at 1172 – 875 cm\(^{-1}\) (Demiate, Dupuy, Huvene, Cereda, & Wosiaki, 2000). Polydextrose in Figure 8 produces characteristic peaks of C-O-C glycosidic linkages at 1196 - 912 cm\(^{-1}\), C-O stretching and bending at 1493 – 1173 cm\(^{-1}\), C=O stretching vibration of aldehyde at 1621 cm\(^{-1}\), and C-H stretching along with O-H stretching vibrations at 3652 – 2843 cm\(^{-1}\) (Mickova, Copikova, & Synytsya, 2007).

Finally, the extent of amorphicity in systems of individual components and binary mixtures was analysed using X-ray diffraction studies. Figure 4.9 illustrates the diffractograms of pectin and/or glucose syrup, with the corresponding pectin/polydextrose preparations. In both cases, single fingerprints of broad peaks between 14 and 30° are recorded, which indicate high density non-crystalline assemblies, whose free volume between adjacent polymeric segments depends on the processing protocol of freeze drying used presently for material preparation. Taking into account the infrared and x-ray spectra of our materials, it appears that the binary mixture of pectin and co-solute (polydextrose or glucose syrup) reproduces in mixture patterns of individual components. This highlights the absence of covalent interactions between chemical groups of polymer and co-solute and, in addition, argues strongly for the absence of sizeable crystalline entities in matrices, which can be considered largely as amorphous systems.
Figure 4.1 Cooling profiles of storage modulus for 2% pectin with 0 (■), 50 (□), 60 (▲), 70% (△) glucose syrup (Figure 1a), and 0 (■), 50 (□), 60 (▲), 70% (△) polydextrose (Figure 1b) at pH 3.0 (scan rate: 1°C/min; frequency: 1 rad/s; strain: 1.0%).
Figure 4.2 DSC cooling and heating profile for 2% pectin with 78% glucose syrup and 78% polydextrose at subzero temperatures (scan rate: 1°C/min).
Figure 4.3 Cooling profiles of storage ($G'$, closed symbols) and loss ($G''$, open symbols) modulus for 2% pectin with 78% glucose syrup (■, □), and 2% pectin with 78% polydextrose (▲, △) scanned at 1°C/min at a constant frequency of 1 rad/s and strain 0.01%.
Figure 4.4 Frequency variation of $G''$ for 2% pectin with 78% glucose syrup (Figure 4a), bottom curve is taken at -15°C (■), other curves successively upwards: -19°C (□), -23°C (♦), -27°C (◊), -31°C (▲), -35°C (Δ), -39°C (●) and -43°C (○); and for 2% pectin with 78% polydextrose (Figure 4b), bottom curve is taken at -4°C (■), other curves successively upwards: -8°C (□), -12°C (♦), -16°C (◊), -20°C (▲), -24°C (Δ), -28°C (●) and -32°C (○).
Figure 4.5 Master curve of reduced shear moduli $G'_p$ (closed symbols) and $G''_p$ (open symbols) as a function of reduced frequency of oscillation ($\log \omega a_T$) for 2% pectin with 78% glucose syrup (Figure 4.5a) with reference temperature - 31°C and 2% pectin with 78% polydextrose (Figure 4.5b) with reference temperature - 20°C, based on the frequency sweeps of the preparation in Figure 4.
Figure 4.6 Temperature variation of the factor $a_T$ within the glass transition region (closed symbols) and glassy state (open symbols) for 2% pectin with 78% glucose syrup (triangles), and 2% pectin with 78% polydextrose (squares) with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors throughout the vitrification regime (dashed line pinpoints the mechanical $T_g$ prediction).
Figure 4.7 Micrographs for 2% pectin (Figure 4.7a), and binary mixture of 2% pectin with 60% glucose syrup (Figure 7b) and 60% polydextrose (Figure 4.7c).
Figure 4.8 FTIR absorbance spectra for 2% pectin with 0, 50, 60 and 70% glucose syrup in mixture, an 80% single glucose syrup preparation, 50, 60 and 70% polydextrose in mixture, and an 80% single polydextrose preparation (spectra arranged successively upwards).
Figure 4.9 X-ray diffractograms for 2% pectin with 0, 50, 60 and 70% glucose syrup in mixture, an 80% single glucose syrup preparation, 50, 60 and 70% polydextrose in mixture, and an 80% single polydextrose preparation (diffractograms arranged successively upwards).
4.5 Conclusions

Pectin is quite unique among other polysaccharides, since in a state of high degree of esterification is capable of exhibiting a double mode of gelation including a hydrophobic structure at high temperatures and another one due to hydrophilic interactions at lower temperatures. This work demonstrated first the double structuring functionality of high methoxy pectin, which then was utilised to assess vitrification phenomena in the presence of two distinct types of co-solute. Concentration and molecular weight of glucose syrup or polydextrose as co-solutes in mixture with the polysaccharide influenced molecular relaxations upon cooling of condensed materials to subzero temperatures. Classical patterns of a thermoreversible glass transition were recorded using thermomechanical analysis in materials that remained amorphous throughout the experimentally accessible temperature range and maintained in mixture the physicochemical parentage of the individual components. The high molecular weight of polydextrose induced firm gel formation at ambient temperature and early vitrification on further cooling, whereas glucose syrup with a relatively low molecular weight increased the plasticity of the system retarding molecular relaxation to a lower temperature.
4.6 References


CHAPTER 5

PHASE BEHAVIOUR OF GELATIN/POLYDEXTROSE MIXTURES AT HIGH LEVELS OF SOLIDS

5.1 Abstract

This investigation focuses on understanding the phase behaviour of gelatin when mixed with polydextrose (co-solute) primarily at high solid concentrations. The experimental work was carried out using small deformation dynamic oscillation in shear, modulated differential scanning calorimetry, Fourier transform infrared spectroscopy, wide angle x-ray diffraction and environmental scanning electron microscopy. A progression in the mechanical strength and thermal stability of the gelatin network was observed with the addition of polydextrose to the system. Combined thermomechanical and microscopy evidence argues for the development of phase separation phenomenon between protein and co-solute in high-solid preparations, where gelatin maintains helical conformation to provide network integrity as well as glassy consistency at subzero temperature. At the high solids regime, glassy consistency was treated with theoretical frameworks from the synthetic polymer research to pinpoint the glass transition temperature of the system.
5.2 Introduction

Gelatin has been used in the food and pharmaceutical industry for decades, with its distinct functional properties over other gelling polysaccharide systems (Hawkins, Lawrence, Williams, & Williams, 2008). However, scientific understanding on the phase behavior of gelatin with co-solute in systems of high solids has been least understood (Kasapis, Al-Marhoobi, Deszczynski, Mitchell, & Abeysekera, 2003). Using the sophisticated material and synthetic polymer science approach, exploratory work into gelling biopolymer/co-solute systems at high solids has provided an introduction to this field based on physical interaction, phase behavior and vitrification pattern. Thermodynamic and physical state of gelatin, at varying levels of sugar (e.g. glucose syrup) as co-solute, has been investigated for it’s thermodynamic state and network integrity thus covering the transformation from the melt to the rubbery plateau and the glass transition region (Kasapis et al., 2003).

In terms of industrial applications, current trend of designing healthy foods, by partially/totally replacing sugar in foods thus creating formulations with low GI, finds great market value and consumer interest. However, fundamental understanding on the structure-function relationship of gelatin with potential sugar analogues or mimetics in confectionary products is largely unknown (Bayarri, Durán, & Costell, 2004). The present investigation focuses on understanding the structural properties and phase behaviour of gelatin at 15% (w/w) solids in the presence of various concentrations of polydextrose (0 – 65%, w/w), which in the high solids regime can serve as a model system for confectionery applications.

Polydextrose is an oligomeric compound with the physiological functions of dietary fiber, prebiotic, low GI and various other health benefits (Julian, 2009). Earlier, researchers describe polydextrose as a highly branched indigestable glucose polymer with an average degree of polymerization of ten or twelve glucose molecules which is soluble in water (Craig SAS, 1998). The diverse and essentially amorphous physical property (as opposed to the crystalline state) of polydextrose has been proposed as a functional ingredient in confectionary products, baked goods and frozen dairy desserts (Julian, 2009).

Further, vitrification studies on systems of high solids with gelatin and polydextrose (e.g. at eighty percent solids) could provide information on molecular and chain relaxation aspects at subzero temperatures in protein based biological glasses. Previous studies conducted on formulations involving sugar (glucose syrup and sucrose/glucose syrup mixtures) as the co-solute unveil unexpected phase behavior for gelatin at high levels of solids
(> 60% w/w), as opposed to that of gelling polysaccharides in mixture with sugar (Kasapis & Sablani, 2005). The present investigation extends this framework of thought in gelatin/polydextrose mixtures to contrast thermomechanical and glass transition profiles with those reported for sugar based formulations.

5.3 Materials and methods

5.3.1 Materials

**Gelatin:** The protein sample was supplied by Sigma-Aldrich Co. (Missouri – USA). It was a high quality first extract from the acidic extraction of porcine skin (Type A) with a mass average molecular weight of 162,000, a bloom value of 295-315 and an isoelectric point of about 8.0.

**Polydextrose:** The material used was a TATE & LYLE (Illinois - USA) product. According to the supplier, the product was of 90% purity with 4% moisture and has passed the food grade standard of microbiological testing. It was obtained from the thermal polymerisation of D-glucose in the presence of sorbitol and phosphoric or citric acid, with the random degree of polymerisation making it a mixed combination of α and β linkages. It is essentially non sweet but can be used to provide bulk and mouthfeel in confectionery products with a caloric content of 1 kcal per gram.

5.3.2 Methods

**Sample preparation:** The protein solution was made by dissolving gelatin in distilled water at 50°C with gentle stirring on a hot plate. Required amount of polydextrose as per each formulation (ranging from 0 – 65% w/w) was then added piecemeal to the gelatin solution keeping the same temperature and stirred until a clear solution was obtained. Small amounts of excess water were removed, with the hydration temperature of gelatin or sample preparation temperature never exceeding 50°C.

**Rheology:** Small deformation oscillatory measurements were performed in shear using a controlled strain rheometer with magnetic-trust bearing technology (ARG-2; TA instruments, New Castle, DE). Low-solid samples were loaded on the preheated Peltier plate (50°C), with their edges being covered in silicone fluid from BDH (50 cS) to minimise
moisture loss. Samples were then cooled to 0°C at 1°C/min. This was followed by a 60 minute isothermal run, frequency sweep from 0.1 - 100 rad/s at 0°C and a heating scan at 1°C/min to 60°C. In high-solid samples, the experimental temperature was extended from 60 to -60°C, in order to observe the transformation from the melt through the rubbery plateau to the glassy state. Scan rate for cooling or heating was 1°C/min and a frequency range of 0.1 to 100 rad/s was covered at regular temperature intervals of four degrees centigrade utilising a fixed amplitude strain of 0.01%. The plate diameter for the parallel-plate measuring geometry was either 40 mm for low solid or 20 mm for high solid systems.

**Modulated differential scanning calorimetry:** Thermal measurements were performed on Q2000 MDSC (TA instruments, New Castle, DE). The instrument used a refrigerated cooling system (RCS 90) to achieve temperatures down to -90°C and a nitrogen DSC cell purge at 50 ml/min. Hermetic aluminium pans were used in the present work and an empty pan was the reference cell. Small amounts of the material (10 mg) were heated to 60°C and cooled to 0°C (low solids), or to -90°C for high solids, followed by heating to 60°C at a rate of 1°C/min throughout the experimental routine. A modulation rate of 0.53°C for every 40 s was applied.

**Fourier transform infrared spectroscopy:** FT-IR spectroscopy was performed on Perkin Elmer Spectrum 100 spectrometer equipped with MIRacle™ ZnSe single reflection ATR plate (Perkin-Elmer, Norwalk, CT). Preparations of gelatin with various concentrations of polydextrose were examined to identify the nature of molecular interactions between the two constituents. Spectra were obtained in absorbance mode for the wavelength range of 600 – 4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\). This was corrected against the background spectrum of the solvent at ambient temperature.

**Wide angle X-ray diffraction:** WAXD measurements were performed using BRUKER AXS D8 Advance Diffractometer (Karlsruhe, Germany) equipped with Cu-K\(\alpha\) (1.54 Å) radiation. An accelerating voltage and current of 40 kV and 40 mA, respectively, were employed. Samples were placed on tray holders and continuously scanned to obtain the raw data for the required diffractograms. These were recorded in a 20 range between 5° and 90° in measuring intervals of 0.1°, and subsequently analysed using the Bruker Advanced X-Ray Solutions software, DIFFRAC\(^{\text{plus}}\) Evaluation (Eva), version 10.0 revision 1.

**Environmental scanning electron microscopy:** ESEM images were used to provide tangible evidence of changes in network morphology and phase topology of gelatin/polydextrose mixtures as a function of thermal treatment and polymer/co-solute
composition (FEI Quanta 200 ESEM, Hillsboro, Oregon, USA). In general, mixtures for imaging were from freeze dried and gold plated preparations under a high-vacuum mode. Observing the microstructure of the high moisture-content materials, i.e. in the presence of relatively low concentrations of polydextrose, requires exposure to a gaseous secondary electron detector (GSED) at an accelerating voltage of 30 kV.

5.4 Results and discussion

5.4.1 Observed variation on the structural morphology of gelatin by the addition of co-solute.

Detailed investigation on the effect of sugar addition to gelling polysaccharides at concentration levels of industrial interest demonstrated that there is a transformation from highly enthalpic and aggregated networks to lightly crosslinked and entropic structures with increasing levels of co-solute in mixtures. In contrast, structural properties in gelatin/sugar gels is governed by phase separation phenomena resulting in clearly discernable protein or co-solute rich domains (Kasapis, et al., 2003; Kasapis, Mitchell, Abeysekera, & MacNaughtan, 2004).

In investigating physicochemical changes in the gelatin system with polydextrose as the co-solute, we made a start by considering cooling profiles of the mixtures at a standard frequency of oscillation (1 rad/s) and strain amplitude (1%). As shown in Figure 5.1a, controlled cooling at 1°C/min from 50 to 0°C of 15% gelatin with increasing levels of polydextrose (up to 50%) exhibits a trend of enhanced network strength and thermal stability. The sigmoidal profile that takes off at about 30°C in the case of the aqueous gelatin preparation is attributed to a cooperative coil-to-helix transition discussed extensively in the literature (Tolstoguzov, 2003). There is a fifteen-degree displacement of this transition to higher temperatures as the level of co-solute increases to 50% in the mixture. Towards the end of the cooling run, there is a gradual leveling off in the values of storage modulus ($G'$, elastic component of the network), which shapes up a four-decade increase in rigidity. Gelatin networks develop dynamically with time due to ongoing reconfiguration of the three-stranded super helices of its networks (Fonkwe, Narsimhan, & Cha, 2003).

Mechanical studies on temperature-dependent ordering of gelatin with or without co-solute were extended, as shown in Figure 5.1b, where the continuous gelatin network is
subjected to linear ramping at 1°C/min from 0 to 50°C. Gradual reduction in $G'$ accelerates to a sharp drop in mechanical strength of gels at temperature above 10°C, which convert to viscoelastic liquids at temperatures above 50°C. Complete disordering from helical to coil configuration is expected with heating, with network melting being displaced to elevated temperatures with increasing amounts of polydextrose in the mixture. There is a certain thermal hysteresis between the cooling and heating spectra of each sample depicted in Figures 5.1a and 5.1b (five to six degrees centigrade), which due to the lack of aggregation in the protein network, is well below what has been observed for aqueous polysaccharide or polysaccharide co-solute systems (Nickerson, & Paulson, 2005).

5.4.2 Further evidence from the thermal behaviour of the gelatin/polydextrose

Rheological studies were complemented by thermal work on the gelatin/polydextrose formulations using modulated differential scanning calorimetry. The emphasis here was to pinpoint the temperature range of first-order thermodynamic transitions and calculate the change in enthalpy ($\Delta H$) in exothermic or endothermic processes from the area under the peak of the thermal event (Rahman, 2006).

Figure 5.2a depicts results from cooling runs of gelatin samples at a rate of 1°C/min, to imitate rheological routines, from 50 to 5°C. The upward peak is considered to be the exothermic process of the coil-to-helix transition for the gelatin molecules. The DSC gelling point calculated as the mid-point transition temperature of the thermal event, $T_{\text{max}}$, is about 21°C for the aqueous protein preparation. As for the rheology results, this shifts to higher temperatures with addition of co-solute reaching a temperature of about 35°C at 65% polydextrose. Critically, thermograms become broader at high levels of co-solute in the mixture indicating a reduction in the cooperativity of the gelatin-molecule association in a low-moisture environment.

Figure 5.2b reproduces the heating profiles of materials from 5 to 50°C at the same scan rate, where pronounced endothermic events reflecting the helix-to-coil transition of gelatin gels are recorded. Similar to cooling runs, spectra broaden up with increasing additions of polydextrose and maintain a thermal hysteresis, as for the corresponding profiles of the rheological analysis discussed in the preceding section. Exothermic events were utilised to estimate the change in enthalpy, which is depicted as a function of polydextrose
concentration in Figure 5.3a. There is a clear increase in \( \Delta H \) that exceeds 3.3 J per gram of the protein in condensed preparations.

It is evident from this graphical representation that inclusion of co-solute in the gelatin network raises its structural functionality leading to enhanced molecular association. It is suggested that this increase in gelatin ordering is an attempt by the protein to reduce the interfacial contact with polydextrose. This drive for self-association leads to a phase separated system with a reduced chemical potential that reflects a less unfavorable state thermodynamically. Results in gelatin/sugar mixtures are also suggestive of this type of phase morphology (Al-Marhoobi & Kasapis, 2005); (Firoozmand, Murray, & Dickinson, 2009).

5.4.3 Structural and molecular relaxation in high-solid gelatin/polydextrose systems

In this section of the work, we focus on concentrated systems at 80% solids, which are close to industrial application, and feel that for the present ingredients there should be glassy phenomena manifest at subzero temperatures. Such creation of an energy barrier, by cooling to subzero temperatures, which inhibits molecular rotation and leads to considerable changes in heat capacity has been observed first in the DSC heat-flow trace in Figure 3b. 80% polydextrose and 15% gelatin with 65% polydextrose produces identical mid-point glass transition temperature of -45°C during cooling and heating scans.

Since the molecular packing of materials in the glassy state is of great theoretical and practical importance to the food and pharmaceutical industries, we extend our work by using well established theoretical concepts from the synthetic polymer research (Mitchell, 2000). Besides research on synthetics, work on gelatin/sugar mixture has been reported in the literature at different concentrations of the protein and co-solute utilising this framework of thought, which then was applied to industrial processing (Kasapis & Sablani, 2005).

A high-solid system for the mechanical manifestation of vitrification phenomena has been formulated with 15% gelatin and 65% polydextrose including a residue of 20% water. Figure 4 reproduces small-deformation oscillatory profiles for this mixture and a single system of polydextrose at a similar total level of solids, i.e. 80% (w/w). The mechanical relaxation of both systems can be assessed by monitoring the traces of \( G' \) and \( G'' \) (viscous component of the network) as a function of temperature (Marshall & Petrie, 1980). Upon cooling at a controlled rate of 1°C/min, frequency of 1 rad/s and a small amplitude of
oscillatory strain (0.01%), the values of shear modulus represent different consistencies within
the viscoelastic master curve.

Polydextrose being a bulky amorphous material shares a similar pattern to that of
amorphous sugar preparations (e.g. 80% glucose syrup) with a progressive transformation
from a melt to a glass reproduced in Figure 5.4. In the glassy state, the $G'$ trace crosses over
that of $G''$ seen at temperature below -30°C. In the case of the protein/co-solute system, there
is a clear progression from the rubbery state at temperatures above 30°C to the glass transition
region culminating in the glassy state at the low end of the experimental temperature range (<
-20°C). Clearly, partial replacing of polydextrose with a high molecular-weight material, i.e.,
gelatin, has accelerated the vitrification of the mixture at the same total level of solids. For
both systems, values of $G'$ dominate in the glassy state exceeding $10^9$ Pa at -40°C, and this
reinforcement of the solid-like element of the network is attributed to the stretching or
bending of chemical bonds and pendant group ‘β transitions’ (Ward, 1993).

To delve deeper into theoretical aspects of this field, the concept of a true glass
transition is a derivative of a combined time and temperature effect, and this synchronisation
between temperature and time scale of observation must be resolved to identify the real
contribution of each molecular process to a given system (Ronan, Alshuth, Jerrams, &
Murphy, 2007). In doing so, the so-called time-temperature superposition principle (TTS) has
been practiced successfully to extend the time scale of observation via the experimentally
accessible temperature range (Farhat, Mousia, & Mitchell, 2003). Such methodology was
adopted on the gelatin/polydextrose system of 80% solids to obtain mechanical spectra, as
shown in Figures 5.5a and 5.5b covering the temperature range from -8 to -36°C.

Frequency sweeps were obtained within the range of 0.1 to 100 rad/s at constant
temperature intervals of four degrees centigrade and shear modulus data at the bottom and top
of the figures reflect the glass transition region and glassy state for this mixture.
Corresponding data were also recorded for the 80% single polydextrose preparation but these
are not shown here. Implementation of TTS requires that experimental frequency sweeps are
shifted horizontally only, i.e. along the x-axis of frequency of oscillation, in relation to the
data of an arbitrarily chosen reference temperature, $T_o$, until they superpose onto a single
master curve. Good superposition of both elastic and viscous modulus traces is a requirement
for valid application of this approach to a given system. In the case of our mixture,
mechanical spectra were superposed using a reference temperature of -20°C. This results in a
viscoelastic master curve over an extended frequency range of ten decades (from $10^{-4}$ to $10^6$
rad/s) highlighting the passage from the glass transition region to the glassy state of the material (refer to Figure 5.6).

Shifting of mechanical spectra left or right to produce the master curve of viscoelasticity generates a set of shift factors, $a_T$, which possess a fundamental value by describing the patterns of molecular relaxation of the system within the glass transition region (Maltini & Anese, 1995). Plotting the shift factors as a function of temperature, therefore, unveils the progress in viscoelasticity for the gelatin/polydextrose system shown in Figure 5.7. TTS was reproduced for single polydextrose preparations at 80% solids and corresponding results of factor $a_T$ are also shown in this figure.

The approach used extensively by material scientists to develop a mechanistic understanding of the glassy transformation is based on the concept of macromolecular free volume. According to Ferry (1991), holes between the packing irregularities of long chain segments or the space required for their string-like movements accounts for free volume. In polymer melts the proportion of free volume is usually 30% of the total volume and the theory predicts that free volume collapses to about 3% of the total volume at the glass transition temperature thus making free volume the governing process of molecular dynamics in the glass transition region. Williams, Landel and Ferry have produced a mathematical expression that includes the concept of free volume thus being able to test the theory against the mechanical profile of a plethora of amorphous synthetic polymers (van der Put, 2010).

This way of thinking generates the WLF equation, which for the storage modulus in shear recasts in the following mathematical form (Ferry, 1980):

$$\log a_T = \log \left[ \frac{G'(T)}{G'(T_o)} \right] = \frac{(B/2.303f_o)(T - T_o)}{(f_o/\alpha_f) + (T - T_o)}$$

(5.1)

Where, the fractional free volume, $f_o$, is the ratio of free to total volume of the molecule, $\alpha_f$ is the thermal expansion coefficient, and $B$ is usually set to one. The assumption of a rapid and linear development of the fractional free volume upon heating at temperatures above the glass transition can be considered in terms of the thermal expansion coefficient of the material (Peleg, 1992). The terms $B/2.303f_o$ and $f_o/\alpha_f$ are known as the WLF parameters of $C_1$ and $C_2$, respectively.
Application of the WLF equation to the gelatin/polydextrose mixture at 80% total solids generates WLF parameters of $C_1^0$ and $C_2^0$, which were found to be 13.91 and 52 deg, respectively, i.e. according to experience from synthetic and biopolymer research (Ferry, 1980; Kasapis, & Sablani, 2005). We found that the WLF equation provides a good fit of the empirically derived shift factors in the glass transition region of the protein/co-solute mixture, which extends to temperatures as low as -20°C. As shown in Figure 5.7, however, the shift factors of mechanical spectra in the glassy state unveil a pattern of behaviour that cannot be followed by the WLF equation. Instead, progress in mechanical properties at the region of the lowest temperatures ($<-20°C$) is better described by the mathematical expression of Andrade (Gunning, Parker, & Ring, 2000):

$$\log a_T = \frac{E_a}{2.303R} \left( \frac{1}{T} - \frac{1}{T_0} \right)$$

(5.2)

This yields the concept of activation energy (Ea) for an elementary flow process in the glassy state, which is independent of temperature. Within the glassy state, the factor $aT$ is an exponential function of the reciprocal absolute temperature, so the logarithmic form with a constant energy of activation for an elementary flow process can be used for calculating numerical values (Matveev, Grinberg, & Tolstoguzov, 2000).

The point of discontinuity in Figure 5.7 reflects a threshold of transformation from the free volume theory (WLF equation) to the predictions of the reaction rate theory (Andrade equation) thus defining a glass transition temperature with physical significance. This was found to be ~22°C for the mixture of 15% gelatin with 65% polydextrose. Similar analysis was performed for the polydextrose preparation at 80% solids to yield a $T_g$ value of -30°C in Figure 5.7. It appears, therefore, that unlike the DSC thermograms where glass transition estimates are determined by the total level of solids in formulations (Kasapis, Al-Marhoobi, & Mitchell, 2003), the rheological $T_g$ is affected by the nature of the biopolymer and cannot be predicted by the basic theoretical frameworks for mixed systems such as the Couchman-Karasz equation (Couchman, & Karasz, 1978). The apparent acceleration of vitrification observed for the gelatin/polydextrose mixture in Figure 7, as compared to the single polydextrose preparation, is related to the ability of the biopolymer to form a network. This makes the rheological $T_g$ synonymous to a network $T_g$, as opposed to the DSC $T_g$ that describes a micromolecular index of vitrification.
5.4.4 Tangible evidence on the phase morphology of gelatin/polydextrose preparations

Environmental scanning electron microscopy (ESEM) was used presently to provide images of the phase morphology in gelatin gels with a variable content of polydextrose. As illustrated in Figure 5.8a, 15% gelatin networks in an aqueous medium form uniformly spread assemblies of a super-helical configuration that can be readily visualised. Addition of the co-solute from low to intermediate levels of solids reflects a “serial dilution” in the density of the helical strands of the protein with the mixtures adopting a distinctly amorphous three-dimensional structure (refer to Figures 5.8b to 5.8f). Further enrichment of the mixture with polydextrose reaching a high-solid regime reveals condensed gelatin assemblies that should be attributed to phase separation phenomena at 60 and 65% co-solute in these gels (refer to Figure 5.8g and 5.8h). This drive to self-association and reduction in the gelatin/polydextrose interface has been proposed as the governing mechanism of reinforcement of the mechanical strength and energy content in gelatin networks recorded rheologically and calorimetrically.

5.4.5 Further probing into the physicochemical characteristics of gelatin/polydextrose gels

This section deals with micromolecular aspects of the two constituents and the nature of their molecular interactions using Fourier transform infrared spectroscopy (FTIR) and wide angle x-ray diffraction. Figure 9 reproduces the FTIR spectra of single gelatin or polydextrose preparations and their mixtures. A variety of molecular events are unveiled, which correspond to specific chemical linkages within the polydextrose molecule: O-H stretching (3500 cm\(^{-1}\)), C-H stretching (2900 cm\(^{-1}\)), C=O stretching of aldehyde (1627 cm\(^{-1}\)) and stretching vibration of COC glycosidic linkage (1180 – 930 cm\(^{-1}\)) (Mickova, Copikova, & Synytsya, 2007). The gelatin spectrum depicts the characteristic peptide linkages of C=O stretching vibration from amide I (1642 cm\(^{-1}\)), C-N stretching combined with N-H bending from amide II (1542 cm\(^{-1}\)) and amide A due to N-H stretching vibration (3290 cm\(^{-1}\)) (Bandekar, 1992; Ahmad, & Benjakul, 2011). The profile of these absorptions in the gelatin/polydextrose mixture matches the expected spectra for the individual components, hence arguing against the presence of chemical (covalent) interactions between the two constituents that would invalidate the working protocol of a phase separated model proposed presently.

Finally, Figure 5.10 shows the diffractogram from wide angle x-ray scattering for single or mixed systems of gelatin and polydextrose that were analysed for amorphous
character and the presence of traces of crystallinity. Gelatin exhibits a broad peak at 22°
characteristic of a non-crystalline network. A broad peak recorded at 22° with shouldering
until 50° for the co-solute and protein/co-solute samples corresponds to the signature of
amorphous materials, with their characteristic dense morphology being shaped up from the
processing conditions of freeze drying used in preparing these samples (Payne, McCormick,
& Francis, 1999). Overall, the absence of sharp peaks in the diffractogram argues against the
presence of considerable crystalline entities in the constituents and mixtures of this work. This
supports the experimental observations of rheology and calorimetry and the working
framework of glass transition theory employed in this work.
Figure 5.1 Cooling (a) and heating (b) profiles of storage modulus for 15% gelatin with 0 (■), 10 (□), 20 (▲), 30 (△), 40 (♦) and 50% (◊) polydextrose (scan rate: 1°C/min; frequency: 1 rad/s; strain: 1%).
Figure 5.2 DSC exotherms (a) and endotherms (b) for 15% gelatin with 0, 10, 20, 30, 40, 50, 60 and 65% polydextrose successively upwards. (scan rate: 1°C/min).
Figure 5.3 (a) Trend of the change in enthalpy for 15% gelatin with varying concentrations of polydextrose, and (b) DSC cooling and heating profiles for 80% polydextrose and 15% gelatin with 65% polydextrose at subzero temperatures (scan rate: 1°C/min).
Figure 5.4 Cooling profiles of storage and loss modulus for 15% gelatin with 65% polydextrose (right spectrum; G' [▲], G'' [■]), and 80% polydextrose (left spectrum; G' [△], G'' [□]) scanned at 1°C/min (frequency: 1 rad/s; strain: 0.01%).
Figure 5.5 Frequency variation of $G'$ (a) and $G''$ (b) for 15% gelatin with 65% polydextrose preparations. Bottom curve is taken at -8°C (■), other curves successively upwards -12°C (□), -16°C (♦), -20°C (◊), -24°C (▲), -28°C (△), -32°C (●), -36°C (○).
Figure 5.6 Master curve of reduced shear moduli ($G'_p$ and $G''_p$) as a function of reduced frequency of oscillation ($\omega_a T$) based on the frequency sweeps of the preparation in Figure 5 (reference temperature = -20°C).
Figure 5.7 Temperature variation of the factor $a_T$ within the glass transition region ($\square$), and the glassy state ($\triangle$) for 15% gelatin with 65% polydextrose, and the glass transition region ($\blacksquare$) and the glassy state ($\triangle$) for 80% polydextrose, with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors throughout the vitrification process (dashed lines pinpoint the $T_g$ predictions).
Figure 5.8 Micrographs for 15% gelatin with (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50, (g) 60 and (h) 65% polydextrose.
Figure 5.9 FTIR absorbance spectra for 15% gelatin with 0, 20, 40 and 60% polydextrose in mixture, and an 80% polydextrose preparation arranged successively upwards.
Figure 5.10 X-ray diffractograms for 15% gelatin with 0, 20, 40 and 60% polydextrose in mixture, and an 80% polydextrose preparation arranged successively upwards.
5.5 Conclusions

The present work deals with the molecular interactions, phase behaviour and glass transition properties of gelatin as we change the solvent quality of its physicochemical environment. This is achieved with the addition of polydextrose as the co-solute at various contents. It appears that there are no direct, chemical interactions between the two constituents in the mixture, which is also free of crystalline domains in high-solid preparations cooled to subzero temperatures. This allows treatment of the protein/co-solute mixture on the basis of phase separated domains where gelatin networks retain or enhance their structural cohesion and thermal stability. Further, condensed preparations of gelatin and/or polydextrose are amenable to treatment by classic viscoelastic theory in the rubber-to-glass transformation thus allowing pinpointing of the mechanical and DSC glass transition temperatures for these systems.
5.6 References


CHAPTER 6

PHASE BEHAVIOR OF GELATIN/AGAROSE MIXTURE IN AN ENVIRONMENT OF REDUCED SOLVENT QUALITY

6.1 Abstract

Investigation on the phase behaviour of a biopolymer mixture has been performed using gelatin and agarose in the presence of variable amounts of polydextrose as the co-solute from low to high levels of total solids. Mechanical observation of the system was performed using small deformation dynamic oscillation in shear along with thermal studies using modulated differential scanning calorimetry. Micrographs provided images of the changing morphology of the network with the addition of co-solute. Fourier transform infrared spectroscopy was used to analyse potential direct interaction between polymers and co-solute. The extent of amorphicity in the system was confirmed using wide angle x-ray diffraction. Agarose and gelatin form non-interactive bicontinuous phases in the aqueous environment. Systematic increase in the concentration of polydextrose prevents the formation of a stable agarose network, with the polysaccharide chains dispersing in the high solids environment. Gelatin, on the other hand, retains its conformational stability even at a saturating co-solute environment through enhanced protein structuring. Vitrification studies on the high solids system at subzero temperatures provides information on the structural and molecular relaxation identified as a glass transition phenomenon.
6.2 Introduction

Biopolymers have been widely applied as functional ingredients in food formulations to impart better texture, specific flow behaviour and enhance the nutritional aspects of food. Effective usage of these functional and mostly natural additives needs better understanding of their physicochemical properties and molecular interactions in binary/tertiary mixtures when present in processed foods (van de Velde et al., 2003). Biopolymer mixtures depending on processing conditions (e.g., temperature, time and shear) create various thermodynamic states that range from coupled to interpenetrating and phase separated topologies (de Jong & van de Velde, 2007). Today, the industrialist understands that knowledge of the thermodynamic state of polymeric phases during mixing and subsequent processing greatly assists in tailoring functional systems that find application in a wide variety of desirable textures (Kasapis, 2000; Norton & Frith, 2001).

Among the three distinct phase topologies of biopolymer mixtures mentioned in the preceding paragraph, it appears that the molecular architecture of micro phase separation is the most promising for the engineering of structures with a view to replacing lipids or sugars in formulations. Consequently, research has been extensively performed and principles of phase separation in biopolymer mixtures have been widely understood in systems of relatively low solids (< 10% w/w). Conformational chemistry of biopolymer chains leading to gelation with thermal treatment has been identified as the governing factor determining the type and complexity of phase separation (Antoniou et al., 2010). Starting from single-phase solutions at the high temperatures of mixing, materials commence phase separation upon subsequent cooling due to molecular ordering via the coil-to-helix transition. This leads to thermodynamical incompatibility and the creation of a segregative gel state where usually the faster gelling constituent forms the continuous phase that supports discontinuous inclusions of the second and slower gelling polymeric component. Gelled composites are kept in a state of “dynamic equilibrium”, since the high viscosity of the two coexisting networks prevents molecular rearrangements or polymeric diffusion within normal experimental constraints (Harrington et al., 2009).

In composite gels made of two biopolymers, the overall mechanical strength is dictated by the corresponding strength of the individual polymeric phases. Aspects of phase topology of the binary mixture, such as the phase volume of the continuous phase and the size or shape of discontinuous inclusions, should be taken into account in predictions of the composite’s strength according to the blending law theory (Tolstoguzov, 2003). It has been reported that
for systems of protein-polysaccharide-water, phase separation can be induced when polymeric constituents are mixed at concentrations in excess of 4% (Fitzsimons, Mulvihill & Morris, 2008). Further, increasing concentrations of the second component in a binary mixture (e.g. maltodextrin), in the presence of a continuous gelatin network at a fixed concentration, leads to phase inversion where the carbohydrate forms the continuous phase in the gel (Kasapis, Morris, Norton & Clark, 1993). Phase inversion is a common phenomenon in mixed biopolymer gels leading to variable amounts of solvent (mainly water) distribution between the two phases, which affects the overall strength and plasticity of the end product.

In contrast, there has been limited experimental work in the literature for mixed systems with a total level of solids that is above 60% (w/w) in formulations. These find application in the confectionery, ice cream and chocolate industries but, despite the obvious commercial interest, fundamental aspects of polymer and/or co-solute interactions in such high-solid materials remain underresearched. An interesting development in this concentration regime is the departure of molecular dynamics from classical gelation-theory considerations to the postulates of the modified Rouse theory (Kasapis, 2004). This is commonly manifested as the rubber-to-glass transformation where the process of decreasing free volume within the polymer interstices holds sway in determining molecular interactions (Rogers, Roos & Goff, 2006). Completion of the process of vitrification is highlighted by an index of convenience known as the glass transition temperature ($T_g$). In view of the above, the present investigation aims to identify the network morphology, composite topology and glass transition phenomena in gelatin/agarose mixtures in the presence of polydextrose for potential use of this mixture in high-solid sugar-free formulations of soft confections.

6.3 Materials and methods

6.3.1 Materials

**Agarose:** The sample of this investigation was supplied by Sigma Aldrich Co (Sydney, Australia). Purity of the material was 92.5% on a dry weight basis, with water, ash and sulphate content being 7.0, 0.25 and 0.12%, respectively, as notified by the supplier.

**Gelatin:** The material used was a Sigma Aldrich Co (Missouri, USA) product, which is a TYPE-A high quality first extract from porcine skin. It possesses a mass average molecular weight of 162,000 and an isoelectric point ($pI$) of about 8.0 with a bloom value of 295-395.
Polydextrose: The sample was purchased from TATE & LYLE (Illinois, USA). On a dry weight basis the product was of 90% purity with 4% moisture. The random degree of polymerisation with $\alpha$ and $\beta$ linkages, through the thermal treatment of D-glucose in the presence of sorbitol and phosphoric or citric acid, makes polydextrose a totally amorphous compound, which is suited for the high-solid investigations of the present work. According to the supplier, it has passed the standard food grade microbial testing.

6.3.2 Methods

Sample preparation: These were made by dissolving agarose in distilled water at 85°C with gentle stirring on a hotplate. Once a clear solution was obtained, the temperature was lowered to 60°C for the addition of gelatin to create the binary mixture. Using this experimental protocol, both polymers were dissolved within half-an-hour forming an aqueous system. Required amounts of polydextrose were added to the mixture at 60°C followed by stirring and excess water was gently evaporated to form preparations from low to high levels of solids.

Rheology: A controlled stain rheometer incorporating a magnetic bearing technology (TA instruments, New Castle, DE) under the operational mode of small-deformation dynamic oscillation in shear was used for the mechanical analysis of the samples. Low-solid systems were loaded on the preheated Peltier plate at 70°C using a parallel plate geometry of 40 mm diameter and 1 mm gap. Edges of the measuring geometry were sheltered with silicone fluid from BDH (50 cS) to minimise the moisture loss during experimentation, which covered an extensive temperature regime. Samples were then cooled at a rate of 1°C/min to 0°C, followed by an isothermal run for 60 min at 0°C, frequency sweep between 0.1 - 100 rad/s at the same temperature and heating to 90°C at 1°C/min. In order to examine the structural properties of high-solid samples that undergo vitrification at subzero temperatures, the temperature range of this part of investigation was extended from -60 to 90°C using a relatively small parallel-plate geometry of 10 mm. Ramping of temperature was achieved at 1°C/min for cooling or heating. Frequency sweeps were performed at constant temperature intervals of 4°C between 0.1 – 100 rad/s.

Modulated differential scanning calorimetry: Sample measurements were performed using Q2000 MDSC (TA instruments, New Castle, DE) attached with a refrigerated cooling system (RCS 90). Hermetic aluminium pans were used for the experimental study and an
empty cell served as the reference pan. Nitrogen was used to purge the DSC cells at a flow rate of 50 ml/min. A small amount of sample (about 10 mg) was heated to 90°C (to eliminate the thermal history during sample preparation and loading) followed by rapid cooling to 60°C at 10°C/min to minimise protein depolymerisation. From 60°C, samples were cooled to 0°C/min at 1°C/min for low solids or -90°C for high solids (same scan rate). In both cases, the experimental routine was wrapped up by heating to 90°C at 1°C/min to match the rheological counterpart. A modulation rate of 0.53°C for every 40 s was applied throughout the calorimetric work.

Fourier transform infrared spectroscopy: Data were obtained using Perkin Elmer Spectrum 100 spectrometer attached with MIRacle™ ZnSe single reflection ATR plate (Perkin-Elmer, Norway, CT). Single systems or mixtures of agarose and gelatin with polydextrose as co-solute were analysed to identify potential molecular interactions between the individual constituents. Spectra were obtained within the wavelength range of 600 – 4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) in absorbance mode.

Wide angle X-ray diffraction: These measurements were recorded using BRUKER AXS D8 Advance Diffractometer (Karlsruhe, Germany) equipped with Cu-K\(\alpha\) (0.1542 nm) radiation. Freeze dried samples were placed on a tray holder and continuously scanned to obtain diffractograms within a 2\(\theta\) range of 5° and 90° at a measuring interval of 1°. Current and accelerating voltage of 40 mA and 40 kV, respectively, were applied.

Environmental scanning electron microscopy: Micrographs of our preparations were captured using FEI Quanta 200 ESEM (Hillsboro, Oregon, USA). Freeze dried and gold plated samples under a high-vacuum mode allowed imaging of the binary biopolymer mixtures that depict distinct network morphologies and composite topologies as a function of increasing additions of co-solute. An accelerating voltage of 30 kV was applied for the experimental work regardless of the level of solids in preparations.
6.4 Results and discussion

6.4.1 Changes in the phase morphology of the agarose/gelatin mixture by the addition of co-solute as observed thermomechanically

There has been extensive work on single polymeric preparations with various sugars as the co-solute in the past, but scant information is found in the literature on the binary mixtures of polymers in the presence of co-solute ranging from low to high solids. The investigation focuses on the mixture of biopolymers as opposed to single biopolymer systems in the presence of co-solute in a high solid environment. In doing so, we focus on the phase morphology of agarose/gelatin mixtures gelled by cooling and the addition of co-solute (i.e. polydextrose) from 0 to 71% (w/w).

Agarose is a linear polymer of \((1\rightarrow3)\beta-D\text{-galactopyranose and } (1\rightarrow4)\text{-3,6 anhydro-}\alpha-L\text{-galactopyranose units, which upon cooling in an aqueous environment undergoes structuring through a coil-to-helix transition to form a rigid network. Gelatin structuring, on the other hand, involves the formation of a triple helix upon cooling at temperatures below 30°C (Barrangou et al., 2006; Hawkins et al., 2008). In particular, the transformation of a dispersed coil to an ordered structure with junction zones as the structural knots of the network for single systems of 1.5% (w/w) agarose and 7.5% (w/w) gelatin has been reported to be at 35 and 25°C, respectively, upon controlled cooling (Sharma, George, Button, May & Kasapis, 2011).}

Figure 6.1a depicts the temperature variation of storage modulus of the network, \(G'\), for 1.5% agarose and 7.5% gelatin as a function of increasing concentrations of polydextrose. For preparations until 40% of the co-solute in the mixture, the trace of the solid-like component of the network produces a wave of structure development around the temperatures of 35 and 25°C, as recorded for single preparations of the two polymers mentioned in the preceding paragraph. This constitutes strong evidence of independent coil-to-helix transformation for each network in the mixture as it would be in the case of single agarose or gelatin gels. At higher levels of co-solute, i.e. above 40% (w/w), a gradual development of storage modulus can be observed as approaching lower temperatures, which is unlike the relatively sharp sol-gel transition discussed for the low solids regime. This change in the progress of viscoelasticity at 50% polydextrose in the mixture removes the obvious fingerprints of a bimodal structure formation discussed earlier thus signifying a change in the molecular dynamics of structure formation at this concentration range. Finally, at even higher

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levels of co-solute (i.e. 60%, w/w), a drop in the values of storage modulus is observed about half an order of magnitude ($G' \approx 10^{4.1}$ Pa at 0°C) compared to their counterparts at 50% polydextrose in the mixture.

The cooling routine was followed by a heating scan to ascertain changes in structural properties with temperature, and Figure 6.1b illustrates mechanical profiles of 1.5% agarose with 7.5% gelatin at 1°C/min. A bimodal melting profile is observed, which strongly argues for mixtures where each component forms independently its own network. Similar results have been previously documented for the binary mixture of agarose and gelatin in the presence of glucose syrup, where the initial drop in the value of storage modulus between 35 and 45°C has been regarded as the melting of gelatin followed by disintegration of the agarose network at temperatures higher than 85°C (Sharma, George, Button, May & Kasapis, 2011). Intermediate levels of co-solute (20 - 50%, w/w) show a trend wherein the values of storage modulus increase after the initial drop following gelatin melting. This could be attributed to the progressive migration of non-gelling concentrations of agarose chains from the liquefied gelatin phase that join the helical strands of the polysaccharide network, thus inducing further reinforcement of its phase prior to eventual melting at the end of the heating run.

Cooling exotherms of the binary mixture of agarose and gelatin, as recorded by DSC, assist in categorising the nature of physical interactions in the mixture, and the outcome of such experimentation is reproduced at the right hand side of Figure 6.2. Traces of heat flow of the mixture show two distinct peaks representing the gelation of agarose and gelatin with maximum (midpoint) heat-flow temperatures ($T_{\text{max}}$) at 35°C and 19°C, respectively. This supports the mechanical observations in Figures 6.1a and 6.1b that the two components undergo a co-operative conformational transformation without direct interactions with each other in the blend. Addition of 20% polydextrose shifts the values of $T_{\text{max}}$ to higher temperatures for both systems representing increased thermal stability of networks. Further addition of polydextrose (40%, w/w) shows only an exothermic peak due to the coil-to-helix transition of gelatin, which becomes very broad at 60% co-solute, with both samples failing to record a noticeable thermal event for agarose.

Heat flow signals between temperatures of 50 and 0°C in Figure 6.2 argue that increasing concentrations of co-solute prevent extensive aggregation of the agarose chains, which results in a weaker thermal event in comparison to the aqueous counterparts. There is evidence from previous work on polysaccharide/sugar mixtures that at intermediate levels of
solids (40 to 60%), polymeric segments dissolve within the sugar saturated medium forming lightly crosslinked junction zones (Kasapis et al., 2003). On the other hand, gelatin retains its ability to form a three-dimensional structure in the presence of polydextrose, an outcome that argues for the protein being able to maintain helix formation in this environment of “reduced solvent quality”. Gelatin interactions with co-solute (i.e. sugars and it appears from this work polydextrose as well) are of a non-specific nature that raise the chemical potential of the interface. This requires additional structuring in the protein chains in order to maintain a thermodynamically favourable topology in the binary mixture (Gekko & Kasuya, 1985; Gekko, Mugishima, & Koga, 1985).

Overall by considering results of the first two figures, it is proposed that agarose and gelatin form single networks in the presence of polydextrose without direct interactions between the two chemical moieties. We further propose that agarose being the fastest gelling component of the two does so by forming a continuous network, which is an anticipated outcome for the constituent that gels first in binary biopolymer mixtures (Morris, 1992). This is also supported by the long melting profile of the composite gel upon heating to temperatures close to the boiling point of water. It appears that gelatin forms a continuous network as well due to its structural stability in the saturating environment of the co-solute. There is also a considerable drop in the values of storage modulus of the composite gel at temperature below 40°C (from about $10^4$ to $10^2$ Pa in Figure 6.1b), which would be physically unrealistic if gelatin was a discontinuous phase according to the theoretical analysis of blending laws (Picout, Richardson & Morris, 2000). Therefore, there is a strong argument from this work that cooling of the agarose/gelatin solution creates a bicontinuous arrangement where both constituents contribute considerably to the overall modulus of the composite gel.

6.4.2 Structural and molecular relaxation in the binary mixture of agarose/gelatin in the presence of polydextrose

Once the low and intermediate levels of co-solute addition to the agarose/gelatin mixture have been examined in terms of structural property and ingredient interaction, work has been extended to the condensed regime by preparing a system with 80% (w/w) total level of solids. Thermograms at sub-zero temperature in Figure 6.2 represent the trace of heat flow for the mixed system undergoing vitrification during cooling and its reversal on heating at a controlled rate of 1°C/min examined with differential scanning calorimetry. During the process of vitrification, the energy barrier created by the experimentally accessible low
temperatures seizes the molecular rotation/vibration and lowers the specific heat of the material, which results in a sigmoidal downfall of the heat flow signal and *vice versa* upon subsequent heating. The onset of the calorimetric glass transition temperature in the composite mixture for cooling and heating has been recorded at about -25 and -55°C, respectively, with the mid-glass transition temperature ($T_g$) coinciding at -45°C. The latter observation will be considered as an empirical index of quality control for subsequent discussions in relation to the rheological work in this material.

DSC results were complimented with a rheological study, in an effort to understand the complementary mechanical properties of the sample upon vitrification. In doing so, we have adapted the protocol of “advanced polymer science”, which portrays the viscoelastic property of the material undergoing structural relaxation with a glassy consistency (Mitchell, 2000; Kasapis & Salblani, 2005). In terms of pictorial rheology, Figure 6.3 is a typical example of the transformation in the cooling profile of 1.5% agarose and 7.5% gelatin along with 71% polydextrose using small amplitude oscillatory measurements in shear between temperatures of 60 and -32°C. A rubbery plateau is recorded at the upper range of temperatures, for example at 40°C, with modulus values varying between $10^4$ and $10^5$ Pa. On further cooling at temperatures below 30°C, a dramatic increase in the progress of viscoelasticity is recorded with the $G''$ trace dominating over $G'$. This is the so-called glass transition region where the modulus values increase four decades from $10^5$ to $10^9$ Pa.

Towards the end of the cooling run, there is another development. As shown in Figure 6.3, at temperature below -20°C, values of $G'$ become higher than for $G''$ and approach $10^{10}$ Pa at the end of the experimental routine (-30°C), which is the mechanical manifestation of the glassy state. Structural relaxation in this last part of the viscoelastic master curve in relation to temperature reflects $\beta$ transitions and the stretching or bending of chemical bonds (Ward, & Hadley, 1993). Overall, we were able to monitor experimentally the thermomechanical transformation from rubber to glass in the agarose/gelatin/polydextrose mixture allowing us to treat results using appropriate theoretical frameworks.

### 6.4.3 Theoretical treatment of the experimental observations in condensed systems using a classical viscoelastic approach

Insights into the contribution of molecular processes from the rubbery to the glassy state can be obtained by an approach that synchronises the effects of temperature and time.
scale of observation into a single and physically significant working protocol (Ronan, Alshuth, Jerrams & Murphy, 2007). This approach overcomes drawbacks of current technology or the requirement for lengthy experimentation in relation to normal laboratory operations. In a nutshell, we employed the mathematical concept of time-temperature superposition (TTS) wherein an extended frequency range can be obtained through experimentally accessible temperature (Farhat, Mousia & Mitchell, 2003).

In terms of logistics, successful TTS application requires that we obtain frequency sweeps at constant temperature intervals spanning the entirety of the viscoelastic master curve. In doing so, we recorded mechanical spectra within the range of 0.1 to 100 rad/s every four degrees centigrade for temperatures varying from -4 to -32°C (data not shown). Values of storage and loss modulus at the high range of temperatures represent the glass transition region thus showing pronounced frequency dependence, whereas at the low temperature end these become flat reflecting the glassy state of our material, and as reported for amorphous synthetic polymers (Tobolsky, 1956).

The theory demands further that a master curve of viscoelasticity as a function of extended time scale is put together to allow elucidation of molecular dynamics in vitrified materials. This is achieved presently by shifting horizontally the mechanical data from the frequency sweep of each temperature in relation to an arbitrarily chosen reference temperature within the glass transition region ($T_o = -20$). The outcome of such operation is well superposed data of storage and loss modulus creating in Figure 6.4 a viscoelastic master curve over eight decades of frequency from $10^{-4}$ to $10^4$ rad/s. Superposing the mechanical spectra along the frequency axis delivers a set of shift factors ($a_T$), which when plotted as a function of temperature represent the fundamental relationship between structural relaxation and thermal treatment in the system (Maltini & Anese, 1995). Figure 6.3 reproduces at subzero temperatures the set of factors, $a_T$, for our system, which “converts” experimental data in Figure 6.4 into theoretical functions of the molecular processes governing vitrification.

The concept of molecular free volume has been an effective tool in synthetic polymer research to examine the relaxation phenomenon that deals with void spaces arising from irregularities in polymeric arrangement. During the vitrification process, reduction in free volume leads to collapsing molecular motion and structural arrest of overall chain dynamics (Ferry, 1991). William, Landel and Ferry harnessed this approach via the well-known WLF equation thus obtaining fractional free volume and thermal expansion coefficient estimates for amorphous synthetic polymers (van der Put, 2010). We employ a modification of the WLF
model to integrate the mechanical data in shear, as suited to our experimental conditions, which is achieved with the following mathematical expression:

$$\log a_T = \log \left[ \frac{G'(T)}{G'(T_o)} \right] = -\left( \frac{B/2.303f_o(T - T_o)}{(f_o/\alpha_f) + T - T_o} \right)$$  \hspace{1cm} (6.1)

where, $\alpha_f$ represents the thermal expansion coefficient, $f_o$ is the fractional free volume and $B$ is treated having the value of one. The WLF parameters $C_1^o$ and $C_2^o$ are calculated from the functions $B/2.303f_o$ and $f_o/\alpha_f$, respectively (Ferry, 1980).

During the process of glass transition, it appears that molecular dynamics are governed by the concept of molecular free volume, as outlined in the patterns of shift factor in Figure 6.3 that follow equation (6.1). This allows calculation of the WLF parameters for the mixture of 1.5% agarose and 7.5% gelatin with 71% polydextrose producing values of 12.8 and 53 deg for $C_1^o$ and $C_2^o$, respectively. However, the concept of WLF was found to become inappropriate at conditions where the system enters the glassy state. In this case, the arrangement of shift factors is better described by the mathematical expression of Andrade in terms of a set of two temperatures (Gunning, Parker, & Ring, 2000):

$$\log a_T = \frac{E_a}{2.303 R} \left( \frac{1}{T} - \frac{1}{T_o} \right)$$  \hspace{1cm} (6.2)

where, $Ea$ represents the activation energy needed for an elementary flow within the glassy state and $R$ is the gas constant.

Application of the aforementioned body of theory generates a point of discontinuity at the junction of the predictions of the free volume theory and the reaction rate (Arrhenius) theory. This bridging temperature between the two physicochemical approaches, in regards to the shift factors in Figure 6.3, can be considered as the mechanical glass transition temperature ($T_g = -23^\circ C$). It signifies the point where free volume considerations become negligible, i.e. below three percent of the total molecular volume. Finally, the mechanical observation delivered an early glass transition temperature in comparison to the calorimetric data representing a late mid-point transition of -45°C in Figure 6.2. This is now established as a valid outcome of the analysis, since dynamic mechanical data in shear correspond to
network relaxation whereas DSC describes mainly the micromolecular dimensions of saturating levels of co-solute during vitrification.

6.4.4 Analysis on the microstructural domains of the agarose/gelatin mixture in the presence of co-solute

Environmental scanning electron microscopy was used to obtain tangible evidence on the changing morphology of agarose/gelatin mixtures with the addition of polydextrose. As shown in Figure 5a, the mixture of 1.5% agarose and 7.5% gelatin produces a structured network with a dense and uniformly spread configuration of helical strands. This compliments the observation of mechanical and calorimetric studies on low levels of solids that the mixture of agarose and gelatin undergoes a co-operative and classical gelling process via the coil-to-helix transition. Upon addition of 20% polydextrose, the polymeric mixture retains the ability of network formation as represented in the broad but distinct structural knots of a three dimensional structure in Figure 6.5b. Micrographs in Figures 6.5c and 6.5d are for systems with 40 and 60% polydextrose, and in contrast with earlier images, an ongoing reduction in the number and size of polymeric aggregates is visualised. This follow earlier arguments from thermomechanical analysis that agarose fails to produce an extensive agglomerate configuration in a high solids environment of reduced solvent quality, which contributes to the development of a largely amorphous matrix undergoing vitrification at subzero temperatures.

6.4.5 Physicochemical characteristics of the polymeric mixture with polydextrose

This section describes the molecular nature of polymeric constituents and their chemical fingerprints within the composite gel using Fourier transform infrared spectroscopy (FTIR) and wide angle x-ray diffraction (WAXD) in an effort to identify potentially unusual molecular events. Figure 6 represents the infrared spectra for the sample of agarose/gelatin, its blend with polydextrose and a single polydextrose system. A series of chemical moieties can be observed for the polymeric mixture, where gelatin is represented by the characteristic peptide linkages of C=O stretching vibration from amide I (1655 cm\(^{-1}\)), C-N stretching combined with N-H bending from amide II (1594 cm\(^{-1}\)) and amide A due to N-H stretching vibration (3290 cm\(^{-1}\)) (Bandekar, 1992; Ahmad & Benjakul, 2011).
Agarose molecules on the other hand exhibit O-H signals coupled with stretching vibrations at 3600-2986 cm\(^{-1}\) (Sekkal, Huvenna, Legrand, Sombret, Mollet, Givernaud & Verdus, 1993). Single polydextrose preparations have a pronounced vibration of C-O-C glycosidic linkage (1202-927 cm\(^{-1}\)), C=O stretching vibration of aldehyde (1659 cm\(^{-1}\)), O-H stretching vibrations (3640-2978 cm\(^{-1}\)) and C-H stretching vibration at 2946 cm\(^{-1}\) (Mickova, Copikova, & Synytsya, 2007). Results for the polymeric components and polydextrose in the mixture are identical with those reported in earlier chapters of this Thesis for individual constituents. Overall, mixtures of agarose, gelatin and polydextrose reproduce only the characteristic peaks of the individual preparations, an outcome that rules out the possibility of covalent linkages between the components of the gel following mixing and processing (cooling or heating) of the materials.

Finally, the extent of amorphicity in the composite gel was examined using diffractograms that were obtained as before for the binary mixture of agarose and gelatin, tertiary systems with co-solute and single polydextrose materials. As depicted in Figure 6.7, the absence of sharp crystalline peaks within the range of 10 to 90° confirms the largely amorphous nature of all our preparations. Broad peaks observed between 10 and 55° are attributed to the high density of the material resulting from freeze drying of the samples prior to experimentation. Thereby, we can attest to the previously described concepts of amorphous matrices undergoing glass transition in the high solid preparations of this work.
Figure 6.1 Cooling (a) and heating (b) profiles of storage modulus for 1.5% agarose and 7.5% gelatin with 0 (■), 10 (□), 20 (●), 30 (◊), 40 (▲), 50 (△) and 60% (○) polydextrose (scan rate: 1°C/min; frequency: 1 rad/s; strain: 0.1%).
Figure 6.2  DSC exotherms for 1.5% agarose and 7.5% gelatin with 0, 20, 40 and 60% polydextrose arranged successively upwards between 0 and 50°C, and thermograms for 1.5% agarose and 7.5% gelatin with 71.5% polydextrose at subzero temperatures (scan rate: 1°C/min).
Figure 6.3 Cooling profiles of storage (■) and loss (□) modulus for 1.5% agarose and 7.5% gelatin with 71% polydextrose scanned at 1°C/min (frequency: 1 rad/s; strain: 0.01%), and temperature variation of the factor $a_1$ within the glass transition region (△) and glassy state (▲) for the same sample with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors throughout the vitrification regime (dashed line pinpoints the mechanical $T_g$ prediction).
Figure 6.4 Master curve of reduced shear moduli ($G'_p$ and $G''_p$) as a function of reduced frequency of oscillation ($\omega a_T$). (reference temperature = -20°C).
Figure 6.5 Micrographs for 1.5% agarose and 7.5% gelatin with 0 (a), 20 (b), 40 (c) and 60% (d) polydextrose.
Figure 6.6 FTIR absorbance spectra for 1.5% agarose and 7.5% gelatin with 0, 20, 40 and 60% polydextrose in mixture, and an 80% single polydextrose preparation arranged successively upwards.
Figure 6.7 X-ray diffractograms for 1.5% agarose and 7.5% gelatin with 0, 20, 40 and 60% polydextrose in mixture, and an 80% single polydextrose preparation arranged successively upwards.
6.5 Conclusions

It has been proposed that mixtures of agarose and gelatin form a bicontinuous phase separated system in aqueous environment based on experimental observations from thermomechanical analysis, electron microscopy and the postulates of blending-law theory. Addition of polydextrose as co-solute at varying concentrations shows a characteristic influence on the overall phase morphology of the blend through two distinct molecular processes. Polysaccharide chains appear to dissolve in a condensed polydextrose environment that hinders extensive aggregation, whereas the conformational chemistry of gelatin is better suited to achieve thermodynamic stability in a system with reduced solvent quality. Condensed preparations of agarose/gelatin with polydextrose exhibit classical rubber-like and glass transition phenomena as the temperature is dropped to ambient or subzero conditions. All in all, the fundamentals of the agarose/gelatin/polydextrose mixture are understood following this work and appear to be similar to the sugar containing counterpart. Therefore, the performance of the material as a sugar replacer in confections merits further investigation in relation to textural properties, sensory attributes and colour/flavour retention.
6.6 References


(CSLM) and covalent labelling techniques. *Colloids and Surfaces B: BioInterfaces, 31*, 159-168.

CHAPTER 7
CONCLUSIONS AND FUTURE WORK

The overall aim of this Thesis is to continue the debate on the formulation of a scientific basis for an optimal choice of constituents and composition in high-solid materials comprising mixtures of biopolymers and small-molecule co-solute with industrial relevance. Biopolymers have been widely applied as functional ingredients in food formulations to impart better texture, specific flow behaviour and enhance the nutritional aspects of food. Effective usage of these functional and mostly natural additives needs better understanding of their physicochemical properties and molecular interactions in binary/tertiary mixtures when present in processed foods. Biopolymer mixtures depending on processing conditions (e.g., temperature, time and shear) create various thermodynamic states that range from coupled to interpenetrating and phase separated topologies. Therefore, knowledge of the thermodynamic state of polymeric phases during mixing and subsequent processing can greatly assist in tailoring functional systems that find application in a wide variety of desirable textures with increased likelihood of acceptance by the demanding consumer of the present era.

The ingredients in this investigation, i.e. agarose, gelatin and pectin in the presence of co-solute (polydextrose or glucose syrup) are found or are expected to have a wide range of high-solid industrial applications. In terms of industrial applications, current trend of designing healthy foods, by partially/totally replacing sugar in foods thus creating formulations with a low glycemic-index (GI), finds great market value and consumer interest. However, fundamental understanding on the structure-function relationship of the ingredients mentioned previously with potential sugar analogues or mimetics in confectionary products is largely unknown. Polydextrose has been increasingly regarded as a potential replacer of sugar with multiple health benefits, which, of course, creates an interest in examining the structural, textural and sensory characteristics of its system. The physiological benefits of dietary fibre, low GI and prebiotic nature make polydextrose a potentially interesting replacer of sugar in novel food formulations.

Work of this Thesis has tried to address a common observation among artisans in the field that by and large, manufacturing of low and high-solid materials containing two or more ingredients as structuring agents is regarded as being craft based. For example, we
documented that in the case of agarose and polydextrose as co-solute, agarose networks possess less order in a high polydextrose environment due to the addition of polydextrose that prevents full helix development and hence the systems appear to be increasingly amorphous. On the other hand, the protein/co-solute mixture can be treated on the basis of phase separated domains where gelatin networks retain or enhance their structural cohesion and thermal stability. This may pose some issues in commercial replacing of the protein with polysaccharide in terms of creating true analogues to existing confectionary formulations.

This issue prompted us to delve deeply into the nature of the governing molecular process in these systems and, in doing so, work was carried out using an approach that synchronises the effects of temperature and time scale of observation into a single and physically significant working protocol. This approach overcomes drawbacks of current technology or the requirement for lengthy experimentation in relation to normal laboratory operations. The so-called time-temperature superposition principle (TTS) has been practiced successfully to extend the time scale of observation via the experimentally accessible temperature range. As described in this Thesis, a number of theoretical treatments from the realm of synthetic polymer research were developed for use in biomaterials including a qualitative blending law, the WLF/free volume equation and the modified Arrhenius/energy of activation equation. The outcome of such theoretical modelling was a strong endorsement of the position that gelatin or polysaccharides in the presence of polydextrose or glucose syrup form comparable rubbery gels that convert to a true glass at subzero temperatures thus unveiling opportunities for novel product development.

The present investigation, therefore, provides valuable information on the phase behaviour of protein and polysaccharide in the presence of co-solute such as glucose syrup and its mimetic, polydextrose. It documents the manner by which the presence of co-solute reduces the solvent quality of the system, hence altering the structural behaviour of gelling polysaccharides in a condensed environment. This type of fundamental research can be used as a point of reference to improve manufacturing processes and create competitive advantage for the food industry in contributing to the development of a variety of added value materials in the market. Based on this work, polydextrose, in particular, appears to have great potential as sugar replacer in high-solid formulations and thus it merits further research in relation to textural properties, sensory attributes and colour/flavour retention in prototypes that reproduce commercial concepts and end products.
Networks of polysaccharides with hydrophilic and hydrophobic characteristics in the presence of co-solute

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ABSTRACT

The present investigation deals with the changing network morphology of agarose and high methoxy pectin when mixed with polystyrene as co-solute at concentrations varying up to high level of solids. Thermomechanical analysis and micro-imaging were performed using small deformation dynamic oscillation in their, modulated differential scanning calorimetry and environment scanning electron microscopy. Fourier transform infrared spectroscopy and wide angle X-ray diffraction were practiced to examine the nature of interactions between polymer and co-solute, and the extent of amorphization of preparations. We observed a decline in the mechanical strength of agarose pectin preparations upon addition of high levels of polystyrene, which should be attributed to reduced entanglement content of the coil-to-helix transition of the polysaccharide network. Glass transition phenomena observed at subzero temperatures in controlled preparations, hence further arguing for the formation of a tightly cross-linked agarose network with changing solvent quality. High levels of co-solute induce formation of weak pectin gels at elevated temperatures (even at 0°C), which with lowering temperature exhibit increasing strength. This results in the formation of rubbery pectin gels at ambient temperature, which upon controlled cooling to subzero temperatures convert to a clear glass earlier than the agarose counterparts.

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1. Introduction

The functional property of gaining polysaccharides is an area of interest in science and technology, since they are utilized as texturizers to impart stability and viscoelastic flow in a variety of industrial applications. Scientific understanding on gelation mechanisms, phases behavior and emulsion properties of non-starch polysaccharides like agarose, alginate, carrageenans, pectins, etc. in aqueous preparations has been widely explored in the past [1-11].

Agarose and pectin are polysaccharides widely considered by major ingredients and product manufacturers for the development of, for example, high sugar low polysaccharide formulations, which are central to many confectionery products. Agarose is a linear polymer of alternating (1 → 3)-β-D-galactopyranose and (1 → 4)-3,6-anhydro-α-L-galactopyranose subunits forming junction zones via the aggregation of helical chains that are further stabilised with hydrogen bonds from the surrounding aqueous environment [1,8]. Pectin is a linear polymer of (1 → 4)-linked α-D-galacturonic interosed periodically with 1,2-linked rhamnosyl residues [7]. Gelation of pectin in the presence of co-solute provides viscoselastic structure, which is a combination of thermally reversible and hydrophilic interactions of the pectic acid network at low temperatures along with the hydrophilic association of methyl ester substituents developed at high temperatures [26,27].

Changes in the physicochemical environment of the agarose and pectin networks were investigated in the presence of sugar (glucose syrup, fructose syrup, sucrose mixtures), which was found to dramatically alter the morphology of the dimensional structures leading to the formation of gels with rubbery viscoelasticity [32]. Further, high levels of sugar (≥ 80%, w/w) are unable to maintain a thermodynamically stable polysaccharide network with extensive aggregation which converts into a lightly cross-linked system undergoing vitrification at subzero temperatures [30]. As an extension to the above observations, we now formulate the system with polystyrene that can serve as a bulking agent and sugar replacer in low-calorie foods. Polystyrene is a branched glucose polymer with an average degree of polymerization of 10 or 12 glucose residues and a mixed combination of α and β linkages that prevent intermolecular association and gelation of the material. Its physiological benefits of dietary fibre, low glycemic index, and prebiotic effects...
methyl esterification (DE) of 70.3%. Chromatographic analysis carried out by the supplier, using an ultrahydrogel linear column and eight pulsed standards, produced a number average molecular weight (Mn) for the pectic material of this investigation of about 154000.

2.1.3. Pectinex

The material used was a product from TATE & LYLE (IL, USA). The powder was of 90% purity with 30% moisture and passed the microbiological testing under the food grade standards. It is a readily soluble, amorphous polymer with an average degree of polymerisation of twelve glucose residues.

2.2. Methods

2.2.1. Sample preparation

The polysaccharide solutions were prepared by dissolving agaropectin at 1.5% (w/w) and pectin at 2% (w/w) solid in distilled water at 80 and 95°C respectively, by gentle stirring on a hot plate. After obtaining a clear solution, the temperature was lowered to 65°C in the agaropectin solution and to 75°C in the pectin solution for the addition of the required amount of pectinex. The temperature of the system was maintained at 65°C for the agaropectin solution and at 75°C for the pectin solution on the hot plate until polydextrose was fully dissolved and the desired level of total solids was obtained by evaporating slowly excess water. The pH of the pectin system was then adjusted to 3.0 using 0.1 M HCl solution to create the required gelling conditions for subsequent experimentation.

2.2.2. Rheology

Low amplitude oscillatory measurements were performed in shear to obtain the storage modulus (G') and loss modulus (G'') which are parts of the complex shear modulus (G = G' + iG''). The analysis was performed on AR-G2, which is a controlled strain rheometer with a magnetic thrust bearing technology (TA Instruments, New Castle, DE). Samples with total solids up to 70% (w/w) were loaded on the preheated Peltier at 60°C using a 45mm parallel plate, with edges covered in silicone oil from DHK (30-60) to prevent moisture loss. According to the experimental protocol, samples were cooled to 0°C at a rate of 1°C/min (followed by a 30 min isothermal run and a frequency sweep (0.1-100 rad/s)) at the same temperature. These were then heated to 95°C at 1°C/min. An oscillatory frequency of 1 rad/s with a strain of 0.1% for agarose and 1% for pectin samples was executed throughout the experiments. Strain sweeps identified the linear viscoelastic region, which indicated that the agarose network at least an order of magnitude more strain sensitive than the pectin counterpart.

For concentrated samples with 20% moisture, a measuring geometry of diameter 10mm was used within an extended experimental temperature range to analyse the viscoelastic behaviour of the network. Modified agarose samples were loaded on the preheated Peltier at 60°C and subjected to extended cooling until -50°C, whereas high-solids preparations of pectin were loaded at 95°C and cooled to -60°C at 1°C/min. At an interval of 4°C frequency sweeps were performed within the range of 0.1-100 rad/s. A constant strain of 0.01% for agarose and 0.1% for the pectin counterpart was maintained, and results were modelled for estimation of the glass transition temperature at subzero temperatures.

2.2.3. Modulated differential scanning calorimetry

Thermal measurements were performed on MDSC Q2000 (TA Instruments, New Castle, DE). The instrument used a refrigerated cooling system to achieve temperatures down to -90°C and an amygdalin DSC cell at 50mL/min to purge condensation. Samples were loaded in hermetic aluminium pans. Calibration of the heat flow signals using a traceable indium standard (ΔH = 28.3J·g⁻¹) and the
heat capacity response using a sapphire standard enabled accurate measurements. Both types of samples were weighed (about 10 mg) and analysed at modulation amplitude of 0.53°C at every period of 40 s. Agarose preparations were equilibrated at 80°C and cooled to −90°C for high-solid analysis, whereas the pectin counterpart was cooled from 90°C to −70°C. The cooling ramp rate for both samples was 1°C/min.

2.2.4. Fourier transform infrared spectroscopy

Perkin Elmer Spectrum 100 spectrometer was used to obtain the FT-IR spectrum, attached with ZnSe single reflection ATR plate (Perkin Elmer, Norwalk, CT). Agarose, pectin, polydextrose and their mixtures at concentrations described earlier were analyzed to identify potential physicochemical interactions at low and high solid contents. Absorbance spectra were recorded within a range of 800−1000 cm\(^{-1}\) with a scan number of 8 and a resolution of 4 cm\(^{-1}\).

2.2.5. Wide angle X-ray diffraction

These measurements were carried out using a Brüker AXS D8 Advanced (Karlsruhe, Germany) equipped with Cu-Kα radiation (0.1542 nm). The instrument operates at an accelerating voltage and current of 40 kV and 40 mA, respectively. Freeze dried samples of agarose, pectin, polydextrose and their mixtures were placed on a glass holder, which was then continuously scanned to obtain the diffraction patterns within a 2θ range of 5° and 90°.

2.2.6. Environmental scanning electron microscopy

Imaging of the changing polysaccharide network in aqueous and high-solid preparations was performed using FEI Quanta 200 ESEM (Hillsboro, OR, USA). Freeze dried samples were attached on sample holder followed by gold plating for the analysis. Under the operating conditions of a high vacuum mode with an accelerating voltage of 30 kV, images of several magnifications were recorded.

3. Results and discussion

3.1. Qualitative observations of changes in the mechanical properties of agarose and pectin networks with increasing additions of polydextrose

Heat capacity was at a fixed concentration of 1.5% (w/w), high methoxy pectin at 2% (w/w) and the polydextrose content was varied from 10 to 75% in the mixture to prepare samples of low, intermediate and high levels of solids. In an effort to understand the mechanical properties of the agarose and pectin networks in the agarose and the polydextrose environments, the molten state of the binary mixture was cooled at a fixed scan rate of 1°C/min and a constant oscillatory frequency of 1 Hz. Applied strains of 0.1% for agarose/polydextrose samples were held from 50 to 0°C, and from 95 to 0°C with applied strain of 1% for the pectin/polydextrose counterparts.

As shown in Fig. 1, there is up to 4.5 log increase in values of the elastic component of the network upon cooling at the temperature falls below 25°C representing the coil-to-helix transition of the agarose molecules. At low concentrations of polydextrose addition of 10–20% (w/w), agarose networks become reinforced by the increased values of storage modulus traces that conclude at 0°C (data not shown). Extending the intermediate level of solids, i.e. >30% polydextrose in formulations, a gradual decrease in network strength becomes visible. This leads to a considerable reduction in network strength of preparations, which at 70% co-solute have lost two orders of magnitude compared with the aqueous counterparts. Earlier work on gelation polysaccharides in mixtures with sugars as the co-solute observed a similar variation in shear modulus, and the suggestion there was that polysaccharide helices cannot attain thermodynamic stability in a highly "unhydrated" environment [11,14,20].

The pattern of modulus variation as a function of polydextrose concentration has also been detected as a function of temperature ramping from 0 to 50°C (data not shown). Structures collapse entirely at temperatures approaching 50°C, with the helical aggregates dispersing into coils leading to the formation of a viscous plastic liquid. The thermal hysteresis between cooling and heating profiles is attributed to the formation of extensive aggregation in the agarose network [2].

Results in Fig. 1 are congruent to earlier calorimetric studies on agarose in mixture with glucose syrup as the co-solute, which reported a gradual decline in enthalpy values with increasing additions of the co-solute in the mixture [8]. Observations on glucose syrup as the co-solute also complement the mechanical behaviour of the agarose/polydextrose networks described presently, and support the hypothesis of reduced cooperativity for structure development in polysaccharides in high sugar environments [19].

In comparison with agarose/polydextrose systems, the requirement of co-solute for the gelation of pectin, even under the ideal acidic pH of 3.0, is evident from the observations in Fig. 1, as pectin by itself is an aqueous environment fails to develop a convincing three-dimensional structure throughout the examined temperature range. High concentrations of co-solute (>50%, w/w) induce gelation of pectin at temperatures as high as 70°C due to the hydrophobic association between methyl ester substituents with networks being developed further on subsequent cooling [4].

Indeed, controlled cooling to temperatures below ambient is met with a relatively sharp increase in the values of storage modulus, which is at a temperature close to 20°C for preparations with 50% co-solute. This phenomenon of hydrophobic association, leading to formation of structures that include the polymer in a helical configuration, shifts to higher temperature with increasing the concentrations of co-solute. Observations on the effectiveness of sugar type or concentration on pectin gelation have been frequently reported [9], and it is widely believed that the onset temperature of conformational ordering during cooling is strongly dependent on the specific interaction between polymer and co-solute [25].

3.2. Experimental studies on the molecular and structural relaxation of gelation polysaccharides/co-solute mixtures

This section of the work deals with the structural and molecular relaxation patterns of condensed pectin and agarose/co-solute mixtures at total solids of 80% (w/w), with the concentration of high methoxy pectin being fixed at below 2% (w/w) and the concentration of agarose at 1.5% (w/w). Controlled cooling of the mixture to subzero temperatures results in a brittle and clear matrix that exhibits glassy consistency. Theory argues that such material vitrification is due to an energy barrier setting molecular motion and leading to a structural arrest [29]. An expedient way to monitor conformational changes affecting the molecular arrangement of the condensed material is the heat flow signal observed by differential scanning calorimetry [2].

Fig. 2 depicts the heat flow (W/g) trace for the high solid systems of polysaccharide and co-solute. The continuous and sigmoidal drop in the signal of heat flow with controlled cooling results in a relatively broad transition that is considered as the calorimetric manifestation of a glass transition. The onset and mid-point glass transition temperature of the pectin/polydextrose preparation in Fig. 2 was calculated to be at −21°C and −42°C, respectively. High solid systems of agarose/polydextrose produced comparable transformations recorded at −32°C for onset and −43°C for mid-point transition temperatures. Similar readings, i.e. −34°C and −45°C for the onset and mid-point transition temperatures,
were observed for single polydextrose systems arguing that the presence of polyacrylamide in these preparations in a mere cross-contamination with the technique recording primarily the glass transition temperature of the small molecules in the system. Perfect reversal of the above observed transition can be obtained by heating the system from the glassy to the rubbery state, as the dis-organisation of molecular structure follows a linear relationship on the thermal effect.

Calorimetric work on the vitrification patterns of our system lays the ground for changing the mode of investigation, which allows delivering mechanical insights into the behaviour of these supersaturated systems. In doing so, we adapt the techniques of “advanced polymer science” that elaborate on the changes of viscoelastic properties of the material during the transition from the rubbery to the glassy state [23, 18].

In Fig. 3, small amplitude oscillatory measurements on the system of 1.5% agarose with 78.5% polydextrose and 20% high mol. wt. pectin with 78% polydextrose, upon controlled cooling to sub-zero temperatures, produce a spectacular development in the values of the elastic and viscous modulus. Thus the variation in G’ and G” traces as a function of temperature represents the master curve of viscoelasticity for these preparations. In the case of agarose, for example, there is a passage from the solution state at temperatures > 30 ºC to the gel state. On further cooling, modulus values increase rapidly, with the sample entering the glass transition region at sub-zero temperatures where the viscous component of the network (G”) becomes dominant. At the very end of the cooling routine, there is yet another transformation and the sample is found in the glassy state where the values of storage modulus dominate exceeding 10^9 Pa (at about –40 ºC for agarose). Reduced mobility in the glassy state reflects β transversals and the stretching of bending chemical bonds [22]. Similar profiles are recorded for pectin/polydextrose but displaced to higher temperature arguing for accelerated vitrification in this system. In both cases, the spectral development in viscoelasticity is thermally reversible and is governed by configurational vibrations of segments of the polymer molecule, which are shorter than the distance between cross-links or points of entanglement.

Experimental measurements in Fig. 3 recognize a preliminary connection between the temperature and frequency dependence of dynamic mechanical properties of our materials. Given current technology, we cannot obtain mechanical measurements at times shorter than a tenth of a second, and experiments that are longer than 10^5 s are prohibitive in terms of equipment availability. Nevertheless, investigators are interested in combining rheological data at different temperatures and frequencies in an attempt to expand the window of observations of physicochemical phenomena [28]. In an effort to synchronize the factors of temperature and time in this work, we implement the so-called time temperature superposition principle (TTP) over the experimentally accessible range of temperature [10].

As shown in Figs. 4a and b, frequency sweeps were performed at fixed temperature intervals of four degree centigrade within the range of −4 to −32 ºC for the high solid system of pectin and polydextrose, and between −12 and −40 ºC for the agarose with polydextrose preparations respectively. The values of storage and loss modulus at the lower and upper range of temperature represent the pathway of transition from the glassy to the rubbery state [23]. Horizontal superposition of the mechanical spectra around an arbitrary reference temperature (T_r = −24 ºC for agarose/polydextrose and T_r = −21 ºC for pectin/polydextrose high solid preparations) reproduces a master curve of viscoelasticity as a function of an extended frequency range of 10^−2 to 10^4 rad/s (Fig. 5). Superposing the mechanical spectra horizontally along the x axis results in a set of shift factors, or Fig. 6 represents those shift factors plotted as a function of temperature thus unveiling a fundamental relationship between patterns of viscoelastic relaxation and temperature [21].

In synthetic polymer research, several systems follow the principles of thermorheological simplicity, as demonstrated in Figs. 4 and 5. Presently, in these systems, the free volume theory describes the concept of vacant spaces within the network arising from the irregularities in polymeric arrangement. As postulated in the literature, during the vitrification process, the vacant space facilitating the chain and micromolecular motions collapses, thus inducing an arrest on polymer chain dynamics [12]. Williams, Landel and Ferry postulated a mathematical model on the concept of
Fig. 4. Frequency variation of storage modulus ($G'$) for (a) 2% pectin with 78.5% poly-
dextrose, bottom curve is taken at -4°C (●); other curves successively upwards: -8°C (□), -12°C (■), -16°C (△), -20°C (▲), -24°C (▲), -28°C (▲), and -32°C (▲); and (b) 1.5% agarose with 78.5% polydextrose, bottom curve is taken at -12°C (●); other curves successively upwards: -16°C (□), -20°C (▲), -24°C (▲), -28°C (▲), -32°C (▲), -36°C (▲), and -40°C (▲).

free volume along with the thermal expansion coefficient of amorphous synthetic polymers [7]. Modification of the above described mathematical model of WLF, to integrate the dynamic mechanical data in shear, delivers the following mathematical design [11]:

$$\log \alpha = \log \left( \frac{G(T)}{G(T_0)} \right) = -\left( \frac{B(2.303T)}{T_0} \right) + \left( \log \alpha_0 \right)$$

(1)

where $f_s$ represents the fractional free volume (free to total volume of the molecule), $\alpha$ is the thermal expansion coefficient and $B$ is set to 1. If $2.303T$ and $f_s/\alpha_0$ are considered to be the WLF parameters and termed as $C_1$ and $C_2$, respectively.

Fig. 5. Master curve of reduced shear moduli $G'\prime$ (closed symbols) and $G''\prime$ (open symbols) as a function of reduced frequency of oscillation (ω*) for 2% pectin with 78.5% polydextrose (squares) and 1.5% agarose with 78.5% polydextrose (triangles) based on the frequency sweeps acquired in Fig. 4.

Fig. 6. Temperature variation of the factor $\alpha$ within the glass transition region (closed symbols) and glassy state (open symbols) for 2% pectin with 78.5% polydextrose (squares) and 1.5% agarose with 78.5% polydextrose (triangles) with the solid lines reflecting the WLF and modified Arrhenius fit of the shift factors throughout the identification region (dashed line plots the mechanical $T_g$ predictions).

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Fig. 7. ESEM micrographs for (a) 5.5% agarose, (b) 2% pectin, (c) 5.5% agarose with 5% polydextrose and (d) 2% pectin with 10% polydextrose.

of modelling i.e. based on the WLF concept, becomes unrealistic for the shift factors in the glassy state and, instead, progress in viscoelasticity follows the mathematical expression of Arrhenide (modified Arrhenius) through which the activation energy needed for an elementary flow can be obtained [15]:

$$\log a_t = \frac{E_a}{2.303 R} \left( \frac{1}{T_0} - \frac{1}{T} \right)$$  \hspace{1cm} (2)

where, $E_a$ represents the activation energy and $R$ is the gas constant. As a result of this, there is a notable point of discontinuity in the arrangement of the shift factors in Fig. 6. This corresponds to a threshold from WLF to Arrhenide type kinetics and can be considered as the absolute boundary between the glass to rubber transition region and the glassy state, which reflects the mechanical glass transition temperature ($T_g$). Application of the combined WLF/free volume theoretical framework yields $T_g$ estimates of $-27^\circ C$ for the agarose/polydextrose sample and $-22^\circ C$ for the pectin/polydextrose preparation.

The calorimetric glass transition temperature for both mixtures (around $-42^\circ C$ in Fig. 2) is below that of the mechanical glass transition temperatures, since the former describes a microstructural dimension whereas the latter are governed by the relaxation of the network forming polymer, agarose and pectin in this case. Accelerated mechanical vitrification has been reported earlier for several systems of polysaccharide or gelatin in the presence of sugars as the co-solute in comparison to calorimetric predictions [17].

Regarding comparisons between the mechanical spectra in Fig. 6, high solid systems with pectin/polydextrose exhibit an early vitrification in relation to agarose co-solute materials due to the presence of hydrophilic interactions that are further supported by hydrophobic associations in the pectin molecule. Agarose, as a polysaccharide with only hydrophilic association of units, demonstrates a relative delay in the process of glass transition at high-solid polydextrose matrices. The polysaccharide has also been observed to be a slow vitrifier in the presence of sugar [17].

3.2. Aspects of network topology and microstructural characterisation of geling polysaccharide co-solute mixtures

It is desirable to provide a visual outlook or tangible evidence of the molecular characteristics of biomaterial matrices so that it complements theoretical modeling of the preceding section, and in an effort to do so, we resorted to electron microscopy and infra red/X-ray experimentation. In the case of microscopy, single pectin (2%, w/w) or agarose (1.5%, w/w) preparations, and mixtures of the polysaccharide with 50% (w/w) polydextrose were freeze dried ready for image taking.

Fig. 7a-d reproduces images obtained with environmental scanning electron microscopy. Freeze dried strands of agarose and pectin molecules are clearly shown in the absence of added co-solute in Fig. 7a and 7b, respectively, i.e. in a thermodynamic regime where agarose is able to form a three dimensional structure but pectin forms mainly helices in solution, as discussed earlier using rheological evidence from Fig. 1. In contrast to the

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polysaccharide morphology in aqueous environments. Fig. 7c and d illustrates sheet-like fields of what is essentially an amorphous agarose and pectin network in the presence of co-solute at sixty percent solids. Thus, microscopy images provide additional confirmation in support of the thermomechanical analysis presented earlier for the polysaccharide forming a firm network that progressively diminishes with increasing co-solute content.

Presentation of data thus far in terms of gelation or glass transition behavior was made on the assumption that there is no development of chemical interactions between polymer and co-solute that could change the underlying physics of the two constituents in the binary mixture. To explore the molecular basis of potential interactions between gelating polysaccharide and polydextrose in mixture with residual water molecules, we performed Fourier transform infrared spectroscopy. Fig. 8 illustrates these FTIR spectra, among which are the pronounced vibrations within the chemical groups of agarose: C=O–C vibration of the 3,6-anhydrogalactose bridge (1028 cm⁻¹) and O–H coupled with C–H stretching vibrations (3600–2850 cm⁻¹) have been identified [30]. Besides agarose, Fig. 8 depicts peaks of pectin with reference to O–H stretching vibrations coupled with C–H stretching vibrations at 3810–2000 cm⁻¹ and esterified (COO⁻), carboxyl groups at 1754–1500 cm⁻¹ [24].

Polydextrose, a glucose based polymer, produces characteristic peaks representing stretching vibration of C=O–C glycosidic linkages (1160–827 cm⁻¹), C=O stretching vibration of aldehyde (1627 cm⁻¹), C=O stretching (2930 cm⁻¹) and O–H stretching vibrations (3640–2978 cm⁻¹) [22]. Mixtures of both polysaccharides and polydextrose reproduce the spectra of the individual components within the combination spectra of the binary mixtures, thus arguing for the absence of covalent linkages between the individual constituents in low and high solids preparations.

Finally, the extent of amorphogen in systems of individual components and binary mixtures was analyzed using X-ray diffraction studies. Fig. 9 illustrates the diffractograms of gelating polysaccharide and/or polydextrose preparations. In both cases, single fingerprints of broad peaks between 14° and 25° are recorded, which indicate high density non-crystalline assemblies, whose free volume between adjacent polymeric segments depends on the processing protocol of freeze drying used presently for material preparation. Taking into account the infrared and X-ray spectra of our materials, it appears that the binary mixture of gelating polysaccharide (agarose or pectin) and co-solute reproduces in mixture patterns of individual components. This highlights the absence of covalent interactions between chemical groups of polymer and co-solute and, in addition, argues strongly for the absence of sizeable crystalline entities in matrices, which can be considered largely as amorphous systems.

4. Conclusions

This work dealt with the changing network morphology of agarose and high methoxyl pectin in an environment of varying solvent quality induced by the addition of polydextrose as cosolute up to high-solids content. Thermomechanical spectra along with microscopic strongly argue that agarose network persists less order in a high polydextrose environment. The change in solvent quality with introduction of polydextrose prevents full helix development and systems appear to be increasingly amorphous. On other hand, pectin demonstrates the double structuring functionality, which is due to its capability of exhibiting a double mode of gelation including a hydrophilic structure at high temperatures and hydrophobic interactions at lower temperatures. Type of geling polysaccharide in mixture with polydextrose as co-solute influenced molecular relaxations upon cooling of condensed materials to subzero temperatures. Classical patterns of a thermoreversible glass transition were recorded using thermomechanical analysis in materials that remained amorphous throughout the experimentally accessible temperature range and maintained in mixture the physicochemical parentage of the individual components. High methoxyl pectin with binary structuring functionality induced firm gel formation at ambient temperature and early vitrification on further cooling, whereas the hydrophilic interactions of agarose increased the plasticity of the system retaining molecular relaxation at a lower temperature.

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Phase behaviour of gelatin/polydextrose mixtures at high levels of solids

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ABSTRACT

This investigation focuses on understanding the phase behaviour of gelatin when mixed with polydextrose (co-solute) primarily at high solid concentrations. The experimental work was carried out using small deformation dynamic oscillation shear, modulated differential scanning calorimetry, rheometer, transmission infrared spectroscopy, wide angle X-ray diffraction and environmental scanning electron microscopy. A synergistic effect in the mechanical strength and thermal stability of the gelatin network was observed with the addition of polydextrose to the system. Combined thermomechanical and microscopic evidence argues for the development of phase separation phenomena between protein and co-solute in high-solid preparations, where gelatin maintains helical conformation to provide network integrity as well as glassy consistency at similar temperature. At the high solids regime, glassy consistency was treated with theoretical frameworks from the synthetic polymer research to propose the glass transition temperature of the system.

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1. Introduction

Gelatin has been used in the food and pharmaceutical industry for decades, with its distinct functional properties over other polysaccharide systems (Hankins, Lawrence, Williams, & Williams, 2008). However, scientific understanding on the phase behaviour of gelatin with co-solute in systems of high solids has been least understood (Kasapis, AlMrhhothi, Doszynski, Mitchell, & Abyeyeke, 2003). Using the sophisticated material analysis techniques such as light microscopy, QCM, and rheology has given new insight into the structure–function relationship of gelatin with potential sugar analogues or mimetics in confectionery products is largely unknown (Bayart, Duru, & Costello, 2004). The present investigation focuses on understanding the structural properties and phase behaviour of gelatin at 15% (w/w) solids in the presence of various concentrations of polydextrose (4-65%, w/w), which in the high solids regime can serve as a model system for confectionery applications.

Polydextrose is an oligomeric compound with the physiological functions of dietary fibre, probiotic, low GI and various other health benefits (Julian, 2000). Earlier researchers describe polydextrose as a highly branched indigestible glucose polymer with an average degree of polymerisation of ten or twelve glucose molecules which is soluble in water (Craig, Holdem, Trup, Aurbach, & Fried, 1984). The diverse and essentially amorphous physical property (as opposed to the crystalline state of polydextrose) has been proposed as a functional ingredient in confectionary products, baked goods and frozen dairy desserts (Julian, 2000).

Further, verification studies on systems of high solids with gelatin and polydextrose (e.g. at eighty percent solids) could provide information on molecular and chain relaxation aspects at extreme temperatures in protein based biological gels. Previous studies conducted on formulations involving sugar (glucose syrup and sucrose/glucose syrup mixtures) as the co-solute unveil unexpected phase behaviour for gelatin at high levels of solids (>60% w/w), as opposed to that of gelatin/polyacrylate in a mixture with sugar (Kasapis & Sasiadi, 2005). The present investigation extends this framework of thought in gelatin/polydextrose mixtures to contrast thermomechanical and glass transition profiles with those reported for sugar based formulations.
2. Materials and methods

2.1. Materials

2.1.1. Gelatine

The protein sample was supplied by Sigma-Aldrich Co. (MO - USA). It was a high quality first extract from the acetone extraction of porcine skin (Type A) with a mass average molecular weight of 102,000, a bloom value of 255-315 and an isoelectric point of about 5.6.

2.1.2. Polydextrose

The material used was a Tate & Lyle (IL - USA) product. According to the supplier, the product was of 99% purity with 4% moisture and has passed the food grade standard of microbiological testing. It was obtained from the thermal polymerisation of d-glucose in the presence of tartaric and phosphoric or citric acid, with the random degree of polymerisation making it a mix of x and y linkages. It is essentially non sweet but can be used to provide bulk and mouthfeel in confectionery products with a caloric content of 1 kcal per gramme.

2.2. Methods

2.2.1. Sample preparation

The protein solution was made by dissolving gelatin in distilled water at 50 °C with gentle stirring on a hot plate. Required amount of polydextrose was then added to each formulation (ranging from 0% to 5% w/w) in a glass beaker. Samples were then left until the gelatin solution had the same temperature and stirred until a clear solution was obtained. Small amounts of excess water were removed, with the hydration temperature of gelatin or sample preparation temperature never exceeding 50 °C.

2.2.2. Rheology

Small deformation oscillatory measurements were performed in shear using a controlled strain rheometer with magnetic-rotating heating technology (AR-G2, TA Instruments, New Castle, DE). Low-shear samples were based on the preheated Petter plate (50 °C), with the liquid being covered in silicone fluid from B&H (50 cS) to minimize moisture loss. Samples were then cooled to 0 °C at 1 °C/min. This was followed by a 60 min isothermal ramp, frequency sweep from 0.1 to 100 rad/s at 50 °C and a heating scan at 1 °C/min to 60 °C. In high-shear samples, the experimental temperature was extended from 50 to 70 °C in order to observe the transformation from the melt through the rubbery plateau to the glassy state. Scan rate for cooling or heating was 1 °C/min and a frequency range of 0.1 to 100 rad/s was covered at regular temperature intervals of four degrees centigrade utilising a fixed amplitude strain of 0.01%. The plate diameter for the parallel-plate measuring geometry was either 40 mm for low solid or 30 mm for high solid systems.

2.2.2.1. Modulated differential scanning calorimetry

Thermal measurements were performed on Q2000 DSC (TA Instruments, New Castle, DE). The instrument used a refrigerated cooling system (RCS 00) to achieve temperature down to −80 °C and a nitrogen gas cell purge at 50 ml/min. Hermetic aluminium pans were used in the present work and an empty pan was the reference cell. Small amounts of the material (10 mg) were heated to 60 °C and cooled to 0 °C (low solids), or to −80 °C for high solids, followed by heating to 60 °C at a rate of 1 °C/min throughout the experimental routine. A modulation rate of 0.63 °C for every 40 s was applied.

2.2.4. Fourier transform infrared spectroscopy

FT-IR spectroscopy was performed on Perkin-Elmer Spectrum 100 spectrometer equipped with Micronelle™ ZnSe single reflection ATR plate (Perkin-Elmer, Norwalk, CT). Preparations of gelatin with various concentrations of polydextrose were examined to identify the nature of molecular interaction between the two components. Spectra were obtained in absorbance mode for the wavelength range of 4000-400 cm⁻¹ with a resolution of 4 cm⁻¹. This was corrected against the background spectrum of the solvent at ambient temperature.

2.2.5. Wide angle X-ray diffraction

WAXD measurements were performed using BRUKER AXS D8 Advance Diffractometer (Karlsruhe, Germany) equipped with Cu Kα (1.54 Å) radiation. An accelerating voltage and current of 40 kV and 40 mA, respectively, were employed. Samples were placed in tray holders and continuously scanned to obtain the raw data for the required diffractionograms. These were recorded in a 2θ range between 5° and 40° in 0.01° increment intervals of 0.3°’ and subsequently analysed using the Bruker Advanced X-ray Solutions software, EVA software, version 10.0 revision 1.

2.2.6. Environmental scanning electron microscopy

ESEM images were used to provide tangible evidence of changes in network morphology and phase topology of gelatin/polydextrose mixtures as a function of thermal treatment and polymer/co-solute composition. FEI Quanta 200 ESEM FEG, Hillsboro, OR, USA. In general, mixtures for imaging were from freeze-dried and gold plated preparations under a high vacuum mode. Observing the microstructure of the high moisture-content materials, presence of relatively low concentrations of polydextrose, requires exposure to a gauge secondary electron detector (GSED) at an accelerating voltage of 30 kV.

3. Results and discussion

3.1. Observed variation on the structural morphology of gelatin by the addition of co-solute

Detailed investigation on the effect of sugar addition to gelling polysaccharides at concentration levels of industrial interest demonstrated that there is a transformation from highly entangled and aggregated networks to lightly conformed and entropic structures with increasing levels of co-solute in mixtures. In contrast, structural properties in gelatin/sugar gels is governed by phase separation phenomena resulting in clearly discernible protein or co-solute rich domains (Kasapi, Mitchell, Atkins, & McNaughton, 2004; Kasapi et al., 2003).

In investigating physicochemical changes in the gelatin system with polydextrose as the co-solute, initially the cooling profiles of the mixtures at a standard frequency of oscillation (1 rad/s) and strain amplitude (1%) were considered. As shown in Fig. 1a, controlled cooling at 1 °C/min from 50 to 0 °C of 15% gelatin with increasing levels of polydextrose (up to 50%) exhibits a trend of enhanced network strength and thermal stability. The significant profile that takes off at about 30 °C in the case of the aqueous gelatin preparation is attributed to a cooperative coacervation transition discussed extensively in the literature (Tijocused, 2003). There is a fifteen-degree increase in the transition temperature of the gel at co-solute increases to 50% in the mixture.

Towards the end of the cooling run, there is a gradual levelling off in the values of storage modulus (G', elastic component of the network), which shapes up a four-decade increase in rigidity. Gelatin networks develop dynamically with time due to ongoing
reconfiguration of the three-stranded super helices of its networks (Pandey, Narisingh, & Cha, 2003).

Mechanical studies on temperature-dependent ordering of gelatin with or without co-solute were extended, as shown in Fig. 1b where the continuous gelatin network is subjected to linear ramping at 1 °C/min from 0 to 50 °C. Gradual reduction in G’accelerates to a sharp drop in mechanical strength of gels at temperature above 10 °C, which convert to viscous elastic liquids at temperature above 50 °C. Complete disordering from helical to coil configuration is expected with heating, with network melting being displaced to elevated temperatures with increasing amounts of polydextrose in the mixture. There is a certain thermal hysteresis between the cooling and heating spectra of each sample depicted in Fig. 1a and 1b (5-6 °C), which is due to the lack of aggregation in the protein network, as well as the time required for observations to have been observed for aqueous polysaccharide or polysaccharide co-solute systems (Nickerson & Paulson, 2005).

3.2. Further evidence from the thermal behaviour of the gelatin/polydextrose system

Theological studies were complemented by thermal work on the gelatin/polydextrose formulations using modulated differential scanning calorimetry. The emphasis here was to pinpoint the temperature range of first-order thermodynamic transitions and calculate the changes in enthalpy (AH) in exothermic or endothermic processes from the area under the peak of the thermal event (Raima & others, 2006).

Fig. 2 depicts results from cooling rate of gelatin samples at a rate of 5 °C/min to imitate rheological routines, from 50 to 5 °C. The upward peak is considered to be the exothermic process of the coil-to-helix transition for the gelatin molecule. The DSC gelation peak calculated at the mid-point transition temperature of the thermal event, Tgmax, is about 21 °C for the aqueous protein preparation. As for the rheology results, this shifts to higher temperatures with addition of co-solute reaching a temperature of about 35 °C at 65% polydextrose. Critically, endotherms become broader at higher levels of co-solute in the mixture indicating a reduction in the cooperativity of the gelatin molecule association in a low-moisture environment.

Fig. 2b reproduces the heating profiles of materials from 5 to 50 °C at the same scan rate where endothermic events reflecting the helix-to-coil transition of gelatin gels are reported. Similar to cooling runs, spectra broaden with increasing additions of polydextrose and maintain a thermal hysteresis, as for the corresponding profiles of the rheological analysis discussed in the preceding section. Isothermal events were utilized to estimate the change in enthalpy, which is depicted as a function of polydextrose concentration in Fig. 3a. There is a clear increase in ΔH that exceeds 3.2 J per gram of the protein in condensed preparations.

It is evident from this graphical representation that inclusion of co-solute in the gelatin network raises its structural functionality leading to enhanced molecular association. It is suggested that this increase in gelatin ordering is in attempt by the protein to reduce the interfacial contact with polydextrose. This drive for self-association leads to a phase separated system with a reduced chemical potential that reflects a less unfavourable state thermodynamically. Results in gelatin/sugar mixtures are also suggestive of this type of phase morphology (Al-Refaie & others, 2005; Finoeczky, Murney, & Dinson, 2009).

3.3. Structural and molecular interaction in high-solid gelatins/polydextrose systems

In this section of the work, the concentrated systems at 80% solids were focused on, which are close to industrial application. Also for the present ingredients there should be gummy phenomena manifest at subzero temperatures. Such creation of an energy barrier, by cooling to subzero temperatures, which inhibits molecular rotation and leads to considerable changes in heat capacity, has been observed first in the DSC heat flow trace in Fig. 3b. 80% polydextrose and 15% gelatin with 65% polydextrose produces identical mid-point glass transition temperature of ~ 45 °C during cooling and heating scans.

Since the molecular packing of materials in the gummy state is of great theoretical and practical importance to the food and
pharmaceutical industries, the work was extended by using well established theoretical concepts from the synthetic polymer research (Mitchell, 2000). Besides research on synthetic work on gelatin/sugar mixtures has been reported in the literature at different concentrations of the protein and co-solutes utilizing this framework of thought, which then was applied to industrial processing (Kosapin & Sathan, 2006).

A high-solid system for the mechanical manifestation of vitrification phenomena has been formulated with 15% gelatin and 65% polydextrose including a residue of 20% water. Fig. 4 reproduces small-deformation oscillatory profiles for this mixture and a single system of polydextrose at a similar total level of solids, i.e. 80% (w/w). The mechanical relaxation of both systems can be assessed by monitoring the traces of G and G' (viscous component of the network) as a function of temperature (Marshall & Pérez, 1980). Upon cooling at a controlled rate of 1°C/min, frequency of 1 rad/s and a small amplitude of oscillatory strain (0.01%), the values of shear modulus represent different consistencies within the viscoelastic master curve.

Polydextrose being a bulky amorphous material shares a similar pattern to that of amorphous sugar preparations (e.g. 80% glucose syrup) with a progressive transformation from a solid to a glass reproduced in Fig. 4. In the glassy state, the G' trace crosses over that of G' even at temperature below -40°C. In the case of the protein/co-solute system, there is a clear progression from the rubbery state at temperatures above 30°C to the glass transition region culminating in the glassy state at the low end of the experimental temperature range (-20°C). Clearly, partial replacing of polydextrose with a high molecular-weight material, i.e., gelatin, has accelerated the vitrification of the mixture at the same total level of solids. For both systems, values of G' dominate in the glassy state exceeding 10^6 Pa at -40°C, and this reinforcement of the solid-like
element of the network is attributed to the stretching or bending of chemical bonds and pendant group β transitions (Ward & Hadley, 1993).

To delve deeper into the theoretical aspects of this field, the concept of a true glass transition is a derivative of a combined time and temperature effect, and this synchronisation between temperature and time scale of observation must be resolved to identify the real contribution of each molecular process to a given system (Ronsin, Aldush, Jermain, & Murphy, 2007). In doing so, the so-called time-temperature superposition principle (TTS) has been practised successfully to extend the time scale of observation via the experimentally accessible temperature range (Farrall, Mountz, & Mitchell, 2003). Such methodology was adopted on the gelatin/polydextrose system of 80% solids to obtain mechanical spectra, as shown in Fig. 5a and b covering the temperature range from -8 to -36°C.

Frequency sweeps were obtained within the range of 0.1-100 rad/s at constant temperature intervals of four degrees centigrade and shear modulus data at the bottom and top of the figures reflect the glass transition region and glassy state for this mixture. Corresponding data were also recorded for the 80% single polydextrose preparation but not shown here. Implementation of TTS requires that experimental frequency sweeps are shifted horizontally only, i.e. along the x-axis of frequency of oscillation, in relation to the data of an arbitrarily chosen reference temperature, Tref, until they superpose onto a single master curve. Good superposition of both elastic and viscous modulus traces is a requirement for valid application of this approach to a given system. In the case of our mixture, mechanical spectra were superposed using a reference temperature of -20°C. This results in a viscoelastic master curve over an extended frequency range of ten decades (from 10^-4 to 10^6 rad/s) highlighting the passage from the glass transition region to the glassy state of the material (Fig. 6).

Shifting of mechanical spectra left or right to produce the master curve of viscoelasticity generates a set of shift factors, α, which possess a fundamental role by describing the patterns of molecular relaxation of the system within the glass transition region (Malini & Anese, 1985). Plotting the shift factors as a function of temperature therefore reveals the progress in viscoelasticity for the gelatin/polydextrose system shown in Fig. 7. TTS was reproduced for single polydextrose preparations at 80% solids and corresponding results of factor α are also shown in this figure.

The approach used extensively by material scientists to develop a mechanistic understanding of the glassy transformation is based on the concept of macromolecular free volume. According to Ferry (1994), holes between the packing irregularities of long chain segments or the space required for their string-like movements accounts for free volume. In polymer melts the proportion of free volume is usually 80% of the total volume and the theory predicts that free volume collapses to about 3% of the total volume at the glass transition temperature thus making free volume the governing process of molecular dynamics in the glass transition region. Williams, Landel and Ferry have produced a mathematical
expression that includes the concept of free volume thus being able to test the theory against the mechanical profile of a plethora of amorphous synthetic polymers (van der Put, 2010).

This way of thinking generates the WLF equation, which for the storage modulus in shear at a constant temperature appears in the following mathematical form (Ferry, 1980):

$$\log{\alpha_T} = \log{\left(\frac{C(T)/C(T_0)}{C(T_0)}\right)} = \frac{(B/2.303)(T - T_0)}{\left[(T_0 - T) + T - T_0\right]}$$

(1)

where, the fractional free volume, $f_{\text{v}}$, is the ratio of free to total volume of the molecule, $\alpha_T$ is the thermal expansion coefficient, and $B$ is usually set to one. The assumption of a rapid and linear development of the fractional free volume upon heating at temperatures above the glass transition can be considered in terms of the thermal expansion coefficient of the material (Peleg, 1992). The terms $B/2.303$ and $f_{\text{v}}$ are known as the WLF parameters of $C_T$ and $C_T$, respectively.

Application of the WLF equation to the gelatin/polydextran mixture at 80% total solids generates WLF parameters of $C_T$ and $C_T$, which were found to be 13.91 and 52 deg, respectively, i.e., according to experience from synthetic and biopolymer research (Ferry, 1980; Kassopis & Sahlin, 2006). The WLF equation provides a good fit of the empirically derived shift factors in the glass transition region of the protein/co-solute mixture, which extends to temperatures as low as $-20\,^\circ$C. As shown in Fig. 7, however, the shift factors of mechanical spectra in the glassy state reveal a pattern of behavior that cannot be followed by the WLF equation. Instead, progress in mechanical properties at the region of the lowest temperatures ($<-30\,^\circ$C) is better described by the mathematical expression of Andrade (Gunnery, Parker, & Ring, 2000):

$$\log{\alpha_T} = \frac{E_g}{2.303} \left[\frac{1}{T} - \frac{1}{T_0}\right]$$

(2)

This yields the concept of activation energy ($E_g$), for an elementary flow process in the glassy state, which is independent of temperature. Within the glassy state, the factor $a_T$ is an exponential function of the reciprocal absolute temperature, so the logarithmic form with a constant energy of activation for an elementary flow process can be used for calculating numerical values (Matveev, Ginberg, & Tolstoguzov, 2000).

The point of discontinuity in Fig. 7 reflects a threshold of transformation from the low volume theory (WLF equation) to the predictions of the reaction rate theory (Andrade equation) thus defining a glass transition temperature with physical significance. This was found to be $-22\,^\circ$C for the mixture of 15% gelatin with 85% polydextran. Similar analysis was performed for the polydextran preparation at 80% solids to yield a $T_g$ value of $-30\,^\circ$C in Fig. 7. It appears, therefore, that unlike the DSC thermograms where glass transition estimates are determined by the total level of solids in formulations (Kassopis, Al-Mashoudi, & Mitchell, 2002), the rheological $T_g$ is affected by the nature of the biopolymer and cannot be predicted by the basic theoretical framework for mixed systems such as the Courteen-Karasz equation (Courteen & Karasz, 1978). The apparent acceleration of vitrification observed for the gelatin/polydextran mixture in Fig. 7, as compared to the single polydextran preparation, is related to the ability of the biopolymer to form a network. This makes the rheological $T_g$ synonymous to a network $T_g$, as opposed to the DSC $T_g$ that describes a micromolecular index of vitrification.

3.4. Tangible evidence on the phase morphology of gelatin/ polydextran preparations

Environmental scanning electron microscopy (ESEM) was used presently to provide images of the phase morphology in gelatin gels with a variable content of polydextran. As illustrated in Fig. 8a, 15% gelatin networks in an aqueous medium form uniformly spread assemblies of a super-uniform configuration that can be readily visualized. Addition of the co-solute from low to intermediate levels of which reflects a "serial dilution" in the density of the helical strands of the protein with the mixtures adopting a distinctly amorphous three-dimensional structure (Fig. 8b-d). Further enhancement of the mixture with polydextran reaching a high solid regime reveals condensed gelatin assemblies that should be attributed to phase separation phenomena at 60% and 65% co-solute in these gels (Fig. 8g and h). This drive to
Fig. 8: Micrographs for 2% gelatin with (a) 0%, (b) 1%, (c) 2%, (d) 3%, (e) 4%, (f) 5%, (g) 6%, and (h) 7% polyelectrolyte.

Self-association and reduction in the gelatin/polyelectrolyte interface has been proposed as the governing mechanism of reinforcement of the mechanical strength and energy content in gelatin networks recorded rheologically and calorigraphically.
3.5. Further probing into the physicochemical characteristics of gelatin/polydextrose gel

This section deals with nanomolecular aspects of the two constituents and the nature of their molecular interactions using Fourier transform infrared spectroscopy (FTIR) and wide angle X-ray diffraction. Fig. 9 reproduces the FTIR spectra of single gelatin or polydextrose preparations and their mixtures. A variety of molecular events are unravelled, which correspond to specific chemical linkages within the polydextrose molecule: D–H stretching (3500 cm⁻¹), C=O stretching (1700 cm⁻¹), C–O stretching of aldehyde (1677 cm⁻¹) and stretching vibration of COC glycosidic linkage (1180–1200 cm⁻¹). (Miczewska, Gópska, & Symonenko, 2003). The gelatin spectrum depicts the characteristic peptide linkages of C=O stretching vibration from amide I (1652 cm⁻¹), C=O stretching combined with N–H bending from amide II (1542 cm⁻¹), and amide A due to N–H stretching vibration (3250 cm⁻¹) (Ahmed & Benjakul, 2011; Bandelker, 1992). The profiles of these absorptions in the gelatin/polydextrose mixture matches the expected spectra for the individual components, hence arguing against the presence of chemical (covalent) interactions between the two components that would invalidate the working protocol of a phase separated model proposed previously.

Finally, Fig. 10 shows the diffractogram from wide angle X-ray scattering for single-armed systems of gelatin and polydextrose that were analysed for amorphous character and the presence of traces of crystallinity. Gelatin exhibits a broad peak at 22° characteristic of a non-crystalline network. A broad peak recorded at 22° with shouldering until 30° for the co-solute and protein-co-solute samples corresponds to the signature of amorphous material with their characteristic dense morphology being shaped up from the processing conditions of freeze drying used in preparing these samples (Payne, McCormick, & Franks, 1958). Overall, the absence of sharp peaks in the diffractogram argues against the presence of considerable crystalline entities in the constituents and mixtures of this work. This supports the experimental observations of morphology and calorimetry, and the working framework of glass transition theory employed in this work.

4. Conclusions

The present work deals with the molecular interactions, phase behaviour and glass transition properties of gelatin as we change the solvent quality of its physicochemical environment. This is achieved with the addition of polydextrose as the co-solute at various contents. It appears that there are no direct, chemical interactions between the two constituents in the mixture, which is also free of crystalline domains in high-solid preparations cooled to subzero temperatures. This allows treatment of the protein/co-solute mixture on the basis of phase-separated domains where gelatin networks retain or enhance their structural cohesion and thermal stability. Further, concentrated preparations of gelatin and/or polydextrose are amenable to treatment by classic viscoelastic theory in the rubber-to-glass transformation thus allowing pinpointing of the mechanical and DSC glass transition temperatures for these systems.

References


