

RHEOLOGY OF PECTIN SOLUTIONS CONTAINING MONTMORILLONITE NANOPARTICLES

by

JOSHUA LIANG

(Under the Direction of Jake Mulligan)

ABSTRACT

Commercial high and low-methoxy pectins were blended with seven different modified montmorillonite clays and the resulting solutions were characterized by dynamic and transient rheology utilizing Couette geometry. Pectin solutions without clay nanoparticles served as control. In all cases, montmorillonite clay-pectin dispersions possessed altered rheological properties, with more highly hydrophilic nanoparticles imparting higher moduli and stress values, along with more highly shear thinning characteristics, while clays with a more hydrophobic modification imparted higher moduli and stress values to a lesser degree, and tended to cause a more Newtonian-like flow. These results are coupled with NMR relaxometry and ζ -potential, providing a more complete picture of the properties of the pectin/montmorillonite dispersions. Particle size varied in the pectin/clay dispersions; however, the zeta potential was nearly invariant with and without clay, regardless of clay modification. T2 spin-spin relaxometry tests showed a trend in which the hydrophilic samples had shorter relaxation time than the hydrophobic samples.

INDEX WORDS: Rheology, Pectin, Montmorillonite, Zeta Potential, Nuclear Magnetic Resonance, T2 Relaxation

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CHAPTER 1

INTRODUCTION

The processing of certain materials can change certain properties of food or food related materials. In any case, it is the flow property changes that will affect material processing, which produces changes in the material's structure and stability. As a result, certain applications can become either desirable or undesirable, as in the shelf life of a certain product. When it comes to the evaluation of food products, one of the more effective ways is the instrumental method of sensory evaluation due to its reduction in time and cost for expert sensory panelists. One such replacement for human sensory is the use of texture evaluation that could imitate human actions (Henry, 1983). The flow properties involve the combination of different types of pectin with clay nanoparticles, which then affects the flow properties when in dispersions. This works by nanoparticles affecting the rheological properties of polymers, which then had an effect on flow properties. Nanoparticles are typically mixed in with another material to form nanocomposites that result the nanoparticle dimension of the composite to be less than 100nm (Rao, 2007).

The ideal behind the application is in liquid food rheology. The comprehension of the pectin/montmorillonite rheology is important in the food industry. Liquid food rheology in the past has been a heavily explored subject because of the importance in the liquid derivatives of fruits that goes into the making of juice (Gratão, Silveira & Telis-Romero, 2007). Some of rheological studies in the past involving juice are *Malus floribunda* (crab apple), cherry juice, sloe juice, and raspberry juice (Cepeda & Villarán, 1999; Giner, Ibarz, Garza & Xhian-Quan, 1996; Ibarz, Garvin & Costa, 1996; Ibarz & Pagán, 1987). These studies on the rheological characteristic of such previously mentioned foods facilitate the understanding in the flow

property of liquid food products. Therefore, it is applicable to real industry applications such as the processing of viscosity and other rheological properties, ultimately affecting the juice fluidity through pipes. Another application involves the shipping of juice on trucks. The vibrations from the roads on the cargo influence the stability of the juice systems. Furthermore, shelf life has an importance on the components of the juice, with an example such as pectin. Moreover, even the act of drinking juice or eating fluid food can be considered of significant importance. In short, these rheological studies on real and industrial applications can characterize and control the flow property of such fluid foods.

One type of factors that influence into the stability of the juice is pectin, which are basically found in all vegetable and fruits (Rooker, 1928). In plants, they are used for strength and adhesion for the cell walls (Van Buren, 1991). They are also considered to be cheap because of the pomace and marc leftover from juicing processes (Rooker, 1928). Studies have shown that pectins are in fruit juices and even in concentrates for various purposes (Ibarz, Garvin & Costa, 1996; Jerome A. Klavons, 1994).

The indication of pectin in fruit juices provides the opportunity to add nanoparticles that can interact with pectin to control the flow properties of pectin solution and ultimately, the flow properties of liquid foods. Montmorillonite clay is considered a nanoparticle because of the 1 nm thick clay sheets (Hrachova, Komadel & Fajnor, 2007). The mineral size, less than 2 μm in particle ranging from 0.1 μm to 2 μm (Lagaly & Ziesmer, 2003) allows for reinforcements and diffusion into the polymer particles (Kornmann, Berglund, Sterte & Giannelis, 1998). This type of polymerization with montmorillonite is usually set for plastics and other non-food related polymers. However, montmorillonite itself can be considered a food product. There has been a market, in the pharmaceutical industry, for montmorillonite to be used as anti-diarrheal

treatment, bowel movement, and even nutrition. The montmorillonite clay, with its nanocomposite forming abilities, can affect the rheological properties liquid foods, primarily fruit juices because of the pectin content.

This study gives insight to the rheological characteristics of high-methoxy and low-methoxy pectins with montmorillonite clays, which are investigated to an extensive end. The emphasis is on the rheology validated with zeta potential, particle size, and NMR T2 relaxation. The pectin interacts with the clay by intercalating between clay sheets and exfoliation, separation of clays sheets. As a result, it becomes a more viscous and stable sample. Seven different clays differing in hydrophobic grades, including one hydrophilic, are used with each pectin type.

Rheological tests used in this study include dynamic strain sweep, dynamic frequency sweep, and transient shear rate ramp test. Experiments for rheology are performed at 25°C and 50°C. The different in temperature is to control flow properties with the imitation of food processing in which foods go through thermal treatment. The strain sweep is performed to give an indication the amount of interaction which results in the amount of exfoliation in the sample. The frequency sweep gives the indication a more solid-like or liquid-like characteristic of the sample. Also, the dependence on frequency against the modulus determines the stability of the samples. The idea of the frequency dependency gives insight from life to industry applications, such as mastication all the way to thermal processing of fruit juices. The shear rate ramp test gives the viscosity measurements between each pectin/clay sample, the type of flow (Newtonian or non-Newtonian), and shear thinning or shear thickening characteristics. All of these tests can then be correlated with the zeta potential, particle size, and T2 relaxation. The end results are observed with the hydrophilic clay having the highest viscosity and modulus. The lower hydrophobic clay samples give higher viscosities and modulus than the hydrophobic samples,

thus indicating that the less hydrophobic and more hydrophilic samples created have a higher amount of interaction with the clay and thus, creating a high degree of exfoliation. Furthermore, T2 relaxation tests and particle size analysis validate it. All the samples of the same pectin type have the same zeta potential, showing invariance of particle size to system stability.

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CHAPTER 2

LITERATURE REVIEW

Pectin

Pectin or pectic substances are complex polysaccharide mixtures that are in a third of the cell-wall make up when it comes to dicotyledonous and monocotyledonous plants, which contain one or two embryonic leaves in the seeds. The substances are part of both cell wall mechanical strength and the adhesion (Van Buren, 1991). Furthermore, the substances group together from higher plants in the primary cell wall and also in the intercellular regions (Voragen A.G.J., 1995). Pectins are basically found in all fruits and vegetables with main sources of interest in apples, lemons, and oranges (Rooker, 1928). It can be extracted from agro-byproducts like apple pomace and citrus peels (Schols, Huisman, Bakx & Voragen, 2003). Previously, these pectins were considered to be useless in the food industry, but items like fruit pomace and marc, leftover from the juicing process, are considered to be a very cheap source for pectins (Rooker, 1928). The pectin structure is complex, consisting of a homogalacturonan (smooth region) backbone with rhamnogalacturonan I and II (hairy region) that has neutral sugars attached as side chains (Voragen A.G.J., 1995; Yoo, Fishman, Hotchkiss & Lee, 2006).

Pectins are not homopolysaccharides, meaning they do not have the same repeating monosaccharide chains. *Figure 2.1* shows an example backbone structure of pectins. The structure consists of α -(1 $\rightarrow$ 4)-linked D-galacturonic acid with interrupted insertions of (1 $\rightarrow$ 2)-linked L-rhamnopyranosyl residues (Voragen A.G.J., 1995). These are placed either in alternate or adjacent positions of the polysaccharide chains. The side chains that are also attached to the pectin include both neutral (Yoo, Fishman, Hotchkiss & Lee, 2006) sugars and nonsugar

substituents (Voragen A.G.J., 1995). The most common sugars side chains are D-galactose, D-xylose, and L-arabinose while D-glucose, L-fucose, D-mannose, and glucuronic acid are not as common. The nonsugar substituents are mainly acetic acid, methanol, phenolic acids, and amide groups (Voragen A.G.J., 1995). The galacturonic acid residues may be replaced with the methoxyl esters, methanol (Voragen A.G.J., 1995), at the C6-carboxyl group, and sometimes acetyl group substitute the C3 or C2-hydroxyl groups (Yoo, Fishman, Hotchkiss & Lee, 2006). It occurs more frequently with methyl ester substituents rather than the acetyl groups (Yoo, Fishman, Hotchkiss & Lee, 2006). This type of substitution is called methylation. There are 2 types of methylated pectins: high-methoxy pectins (HM) and low-methoxy (LM) pectins. The HM pectins are defined as having more than 50% of the carboxyl groups in the polysaccharide backbone being methylated while the LM pectins contains less than 50% methyl esters in the backbone (Voragen A.G.J., 1995).

Due to the ability of the pectin to form jellies and gels, applications for pectin include jams, marmalades, jellies, and confectionary products. Over 50% of the pectin production goes towards the making of these products (Beach, Davis, Ikkala & Lundby, 1986). It can be used as stabilizers in acidified milk drinks as well. In the pharmaceutical and medical field, it can facilitate anti-diarrheal effects, as in regulation and protection of the gastrointestinal tract. In addition, pectin can also be used as a dietary fiber as well as lowering blood cholesterol levels (Voragen A.G.J., 1995).

Montmorillonite Clay

Clay minerals are fairly prevalent in marine and land environments. The clay minerals have very thinly separated structure with a great surface area (per unit mass). This fine division creates the clay's surface reaction to contain sturdy ecological and biochemical properties in

water and soil. Along with these properties, clay minerals have great industrial application such as thermal insulators, concrete additives, packing materials, and plaster (Abollino, Giacomino, Malandrino & Mentasti). Since clay minerals are fairly abundant, the cost of the material is cheaper. Moreover, it is also thermally and chemically stable (Rao, 2007). Clay can also be used in the medical field with clinical treatments and even help with pollution removal (Abollino, Giacomino, Malandrino & Mentasti). Montmorillonite clay itself has been used as landfill barriers to prevent leachate contamination from the subsoil and groundwater (Abollino, Giacomino, Malandrino & Mentasti).

Montmorillonite or MMT is a type of clay that can be used to form nanocomposites because of the nanoparticle size in the MMT clay (Kornmann, Berglund, Sterte & Giannelis, 1998). Montmorillonite minerals are the most abundant of the smectite clay group (Lagaly & Ziesmer, 2003). Structurally, MMT is composed of silicate layers that are 1 nm thick (Hrachova, Komadel & Fajnor, 2007). The MMT particles are not of regular shape. It has the look of sheets of paper that have been torn to pieces (Lagaly & Ziesmer, 2003). The sheets are layered with each other in the shape of a sandwich, in which two tetrahedral sheets are layered onto the top and bottom of an octahedral sheet (Hrachova, Komadel & Fajnor, 2007). These sandwich-type structures are called phil-silicate (Chang, An, Cho & Giannelis, 2003). For the montmorillonite, the sheet lengths are about 218 nm (Chang, An, Cho & Giannelis, 2003). The MMT clay mineral is less than 2 μm in particle size, from 0.1 μm to 2 μm (Lagaly & Ziesmer, 2003), that allows for reinforcements and diffusion into the polymer particles (Kornmann, Berglund, Sterte & Giannelis, 1998). This can then form what are known as nanocomposites. *Figure 2.2b* gives an idea of the foliated sheets that are sandwiched or layered on top of one another. *Figure 2.2a*

shows the exchangeable cation, which can be interact with polymers or be modified to have the cation replaced with modifiers like nitronium salts and hydrogenated tallows.

Nanocomposites

Polymer composites have applications that can include electrical insulation with mineral-filled epoxies or cultured marble. The reason for using these mineral fillers is that it often helps reduce cost (Kornmann, Berglund, Sterte & Giannelis, 1998) while improving mechanical properties. However, these composites are based on a microscale fortification (Kornmann, Berglund, Sterte & Giannelis, 1998). The nanocomposite often presents combined physical and mechanical properties at lower concentrations than conventional composites. A composite becomes a nanocomposite when at least one dimension of composite phase is less than 100 nm that is derived from ultrafine inorganic particles and dispersed homogeneously in the polymer composite matrix (Chang, An, Cho & Giannelis, 2003; Rao, 2007). The sheer small size of the particle can have a significant effect on the macroscopic properties of the composite due to the atomic and molecular interactions. One of the subcategories of nanocompsites is clay nanocomposites. The clay particles are the ones at the nanosize and are used for the reinforcement of the polymer composite structure (Rao, 2007).

Interaction is essential to the rheological properties of the nanocomposite. *Figure 2.3* shows a schematic of the three different types of interactions that can occur. The first interaction depicts a poor reaction. The interaction should have occurred between the sheets of smectic clay. However, poor interactions do not allow for but rather an encasing of the pectin around the clay which then creates macromolecules or tactoids. The solid-like state and rheological properties decrease, as in the elasticity. On the other side of the spectrum, a very attractive interaction can only produce intercalation but only to a minimum where the clay sheets separate

slightly. Intercalation is where the pectin can enter and interact between the clay sheets. The strong interaction does not allow for full exfoliation because of the strong attraction within of the pectin to the sheets. The pectin is strongly attracted to both the modifier and the clay sheets so the exfoliation cannot occur. The rheological properties of this type of interaction suggest an increase in elastic modulus, average chain stiffness of the mixture, and a decrease in entanglement density. Idealistically, a mildly attractive interaction is what is desired. Mild attractions allow the pectins to be attracted to the clay modifier, but the clay sheet will display repulsion against the pectins. As a result, full exfoliation and separation of the clay sheets are allowed, which then provide a more effective surface area. This type of mixture gives an increase in the elastic modulus, in the strength of the chain stiffness, and produces significantly low entanglement density (Mulligan).

Some of the advantages of clay nanocomposite include better mechanical properties, increased heat distortion temperature, decreased gas permeability, application as absorbent, flame-retardant property improvement (used as an additive in rheological control), and as an additive use for antistatic material (Rao, 2007). This research study is to show the effect of clay nanoparticles and nanoparticle surface modification on the rheology of pectin solutions. Also, zeta potential and T2 spin-spin relaxometry is performed to show the effect of hydrophobic and hydrophilic differences.

Rheology

Rheology is utilized to measure the dynamic parameters of the pectin solution montmorillonite clay for dispersive purposes. The rheometer employed here is the concentric cylinder rheometer, invented by Maurice Couette in 1890, which measures Couette flow (Macosko, 1994). The reason for utilizing the concentric cylinder is due to the presumption that

the mixture will have low viscosity, which is advantageous for this type of rheometer (Mulligan, 2007). The Couette flow performs by have a rotating outer cup with the sample in the cup and by having an inner cylinder placed in a rotating cup. The torque is measured on the inner cylinder, and the angular velocity of the outer cup is measured as well (Macosko, 1994). The torque, using working equations, relates to the shear stress while the angular velocity relates to the shear rate (Macosko, 1994). These equations can be located in Christopher W. Macosko's *Rheology Principles, Measurements, and Applications*. The actual concentric cylinder used is slightly different from the conventional concentric cylinder rheometer. The actual rheometer is a dynamic stress rheometer with Couette stress attachments. The outer cup of the rheometer does not rotate while the inner cylinder rotates for the reason that the purpose of the built is to have the base, outer rotating cup, not spin at all. The workings are the same and help produce G' and G'' , which are values used to calculate the elastic response and the viscous response. The strain is the change in length, or deformation, while shear stress is the amount of force that is in a unit of area (Macosko, 1994). It is used to measure the dynamic viscosity and moduli of the mixture at different concentrations and temperatures. The measurements also depend on the flow of the mixture as well.

An aqueous solution also has either Newtonian or non-Newtonian flow. A Newtonian flow exists if the shear rate is in proportion to the shear stress. So therefore, it has a linear plot that starts at the origin (Rao, 1999). A non-Newtonian flow does not have the shear rate be directly proportional to the shear stress, resulting the flow to be non- linear on the plot, which may or may not start at the origin (Rao, 1999). Examples of Newtonian fluids may include water, oil, and other foods that feature a low molecular weight. Polymers like starches

(cornstarch), proteins (egg whites), and pectins have higher molecular weight and therefore, exhibit non-Newtonian flow (Rao, 1999).

The concentric cylinder rheometer is designed to give sinusoidal oscillations that translate to the sample deforming at a continuous rate with the same frequency. The oscillation produces a stress phase that is sinusoidal, and certain attributes correlate with the oscillating waves that are outputted. The strain is correlated with the peak of the wave; therefore, the strain adding on to the frequency causes the stress (Macosko, 1994). Since this is a viscoelastic sample, the stress consists of two parts or two responses, elastic and viscous response. The elastic response is in correlation with sine of the oscillation and so, in phase. The viscous response is considered out of phase and correlates with the cosine of the frequency (Macosko, 1994). After several derivation and calculations to get the values of each response, the dynamic moduli is calculated (Macosko, 1994). G' , elastic modulus, is the elastic stress response per strain rate of the sample, and G'' , viscous modulus, is the viscous stress response per strain rate. Essentially, G' and G'' are used to figure out the phase shift as well as the viscoelastic response (Macosko, 1994). The G' and G'' indicate elastic and viscous ability of materials along with the viscosities as well. The rheometer produces the phase shift, and the shear stress produces the response (Mulligan, 2007).

An important concept to understand is the linear viscoelastic regime. This is the region of strain in which the structure of the dispersed system is still structurally intact. At a critical strain, the G' and G'' decrease drastically, and the structural breakdown of the system starts to occur and continues beyond the critical strain point. The results then factor into the frequency sweep in which the strain in the linear viscoelastic regime is inputted into the parameters for the frequency sweep. The G' , G'' , and viscosity may help assess the degree of intercalation, pectin

interaction between clay sheets, and exfoliation (separation) of clay sheets. In other words, the higher the value of G' , G'' , and viscosity, the stability of dispersion becomes greater, which mean high intercalation and exfoliation (Mulligan, 2007).

Rheology of Pectin

An example of rheology on pectin was the low-methoxyl pectin testing, starting from the solution to gel stage. Low methoxyl pectins were tested with treatments of calcium chloride (divalent cation) using Fast Field Cycling Relaxometry and dynamical rheology (Dobies, Kozak & Jurga, 2004). The LM pectin gel formation with calcium was known as “egg-box” binding, which is the formation of intermolecular junction zone that occurred between the homogalacturonic (smooth) regions of the pectin (Voragen A.G.J., 1995).

This type of gel network was characterized by Dobies, Kozak, and Jurga using nuclear magnetic relaxation dispersions (NMRD) to separate the state and dynamic behavior of the pectin solution. The Fast Field Cycling Relaxometer was used to measure the dispersion of the spin-lattice relaxation time. All were tested at different stages of the solution to gel transition (Dobies, Kozak & Jurga, 2004). The rheological testing proved the existence of the gel network, especially when rheology demonstrated the solid-like response and gel-like character to be more apparent with higher amounts of calcium cations (Dobies, Kozak & Jurga, 2004).

Additional studies performed on low methoxyl pectin gels with the effect of sugars (Fu & Rao, 2001; Grosso & Rao, 1998). Calcium was known for its influence; however, no studies yet have shown the effect of sugar that increases the G' . Moreover, studies had shown that different types of sugar and sweeteners could affect G' differently in the gels produced containing sugar. The strongest gel containing sugars started with sucrose samples followed by glucose, fructose,

plain, and the lowest being sorbital (Grosso & Rao, 1998). The sugars worked together with the calcium to increase the G' of the gel (Fu & Rao, 2001; Grosso & Rao, 1998).

For the nanocomposite studies, there are numerous rheological studies that dealt with polymers or mixtures of suspensions involving montmorillonite clay or pectin solutions/gels. Some studies involved rheology showed the gelation of protein mixed with low-methoxy pectin in the presence of sodium and/or calcium ions (Donato, Garnier, Novales & Doublier, 2005). The proteins alone, β -lactoglobulin (β -Lg) and bovine serum albumin (BSA), formed into a gel when treated with heat. The gel strength of the protein increased with increasing sodium or calcium concentration. Gel strength of β -Lg decreased when both salts were present, but for the BSA gel strength, it was slightly increased. When the LM pectin was added into the mixture, different results occurred. The presence of sodium and calcium together enhanced the strength better than the either salt individually, which meant that other variations could occur as well (Donato, Garnier, Novales & Doublier, 2005). In short, the rheological features of a pectin mixture could heavily be influenced by factors other than the original mixed particles mixed with pectin.

Furthermore, there had been experimental studies performed on high-methoxy pectins. High-methoxy pectins did not gel the same way as the low-methoxy pectins. While low-methoxy pectins became gel in the presence of divalent cations, calcium, high-methoxy pectins became gel with sugar and acid (Voragen A.G.J., 1995). Such reactions could be applied to real applications in the industry. In food colloids, high-methoxy pectins were usually used in acidic liquid milk products as stabilizers, which occurred through the molecular interactions between the pectin and casein in acidic conditions (Dickinson, Semenova, Antipova & Pelan, 1998). The rheological study proved that the pectin could give protection to sodium caseinate, α -casein, and

β -casein from forming mass aggregates. In addition, it proved that the pectin (high-methoxy) could fasten to the emulsion droplets at pH over the isoelectric point of the caseinate (Dickinson, Semenova, Antipova & Pelan, 1998). The end results demonstrated that at a pH of 5.5, pectin influence was able to integrate polysaccharides around the emulsion droplet and into the layer with interfacial interactions of the α -casein, and β -casein, and sodium caseinate with the pectin, which was not possible at a pH of 7. The rheological properties, in which the surface shear viscometry was measured, proved that this was the case. Along with this confirmation, the study also showed with low ionic strength, all three protein samples exhibited emulsions with pectin at a secure level. However, at higher ionic strength, the α -casein presented salt-induced flocculation that could not have been reduced even with the presence of pectin incorporated (Dickinson, Semenova, Antipova & Pelan, 1998). The idea behind these studies was to realize the influence pectin had unto other types of particles or items, such as protein.

Another case study was the effect of sucrose and calcium mixed with pectin on the rheological properties. Dispersions were created by mixing sucrose, calcium and pectin at varying concentrations and setting up for rheological tests. The experiments performed were controlled stress rheometry. Dynamic frequency swept along with oscillatory measurements with cooling temperatures using cone-and-plate geometry. As the result showed, increased in pectin, calcium, and sucrose increased the rheological values, G' and G'' values. The greatest factor though was the increasing pectin concentration. Individually, each factor was investigated in its interaction of the samples. Increasing calcium amount at low pectin concentration increased the G' for all of the temperatures but only at lower temperatures for high pectin concentrations. The sucrose addition, at higher pectin concentrations had much higher G' whereas at low concentration, there was no significant effect. These outcomes were just some of

the observations made. In general, the greater the amount of pectin, calcium, and sugar, the higher the G' and G'' values (Norziah, Kong, Abd Karim & Seow, 2001).

Moreover, a low-methoxy pectin study by Kjønksen, Hiorth, and Nyström involved oscillatory shear measurements of pectin solutions with water and with urea. The pectin/water solution demonstrated shear thickening at low shear rates, but with urea, no shear thickening was observed. It was also indicated that the pectin/water had much higher viscosity than the pectin/urea mixture as the polymer concentration increased. One test revealed the effect of salt additions on the samples of pectin/water solutions. The results showed an increase in the viscosity and a greater degree of clarity on the shear thickening affect. This was to conclude that partial screening of repulsive forces or electrostatic interactions influenced the growth of energetic cross-links or structures that were hydrogen bonded. The idea was to demonstrate how viscosity could change based on different factors like pectin concentration, urea concentration that could break hydrogen bond, or addition of salt that promoted hydrogen bonding (Kjønksen, Hiorth & Nyström, 2005).

Rheology of Montmorillonite Clay

Montmorillonite studies had been conducted and researched to similar extent as rheological studies. Some studies included viscosity studies performed on sodium montmorillonite in alkaline states (Miyahara, Adachi & Nakaishi, 1998). Other studies took the same type of clay and applied the viscosity study with electrostatic conditions (Adachi, Nakaishi & Tamaki, 1998). One rheological study performed on natural montmorillonite clay to investigate the characteristic of the clay during activation with magnesium hydroxy carbonate and sodium carbonate. The bentonite and montmorillonite clay were treated with each of these salts. The montmorillonite clay sample had high viscosity readings. The conclusion was that the

activation compounds enhanced the rheological properties for the bentonite for viscosity. The montmorillonite increased in viscosity because the interaction of the exchangeable cations caused the interaction by the activation of the salt compounds, which then produced structural changes. The concept was to have approach to study colloidal systems involving montmorillonite clay and the dispersive system (Radojevic & Mitrovic, 2007).

These studies had viscosity and elasticity properties performed onto montmorillonite suspensions with other compound. One example was hydrotalcite-like compound (HTlc) with sodium montmorillonite (MT) (Li & Liu, 2007). The R value was taken into account of the rheological, and viscoelastic research performed on the suspension. The R value was the weight ratio for HTlc:MT. The resulting data were proportional strength, and network increased with the increase in R value. Meaning that as the R value increased, the strength of the suspension increased and the network of the suspension structure became more firm (Li & Liu, 2007). This study consisted of montmorillonite concentrations being subjected to different changes by varied HTlc weights (Li & Liu, 2007). Some montmorillonite research had studied the testing degree of flocculation in the montmorillonite suspensions. This was due to the fact that since the suspensions were disperse systems, the amount of flocculation was strongly associated with the particles of the montmorillonite clay. The particles itself had heavy influence on the structures of the flocculi (Ramos-Tejada, Arroyo, Perea & Duran, 2001). To test for these degrees of flocculation, many parameters were applied and tests were performed to establish a decent analysis of the flocculation properties. Some of these tests included oscillatory tests, creep recovery, and steady state viscometry, and these parameters included change in pH and ionic strength (Ramos-Tejada, Arroyo, Perea & Duran, 2001). After thorough studies and research, there was a revelation that scaling laws could be recognized between the particle concentration

of the montmorillonite suspensions and the result from the rheological experiments. The suspension used in this case was sodium montmorillonite suspensions. In summary, the suspension ionic strength of 10^{-2} M gave the greatest degree of flocculation, and for all other NaCl concentrations that were used in the experiment, it led to favorable conditions or systems, which meant that other changes in the ionic strength had a system where there was a lesser degree of flocculation (Ramos-Tejada, Arroyo, Perea & Duran, 2001).

Even more, studies have researched montmorillonite suspensions at low concentrations. The montmorillonite suspension was compared to the kaolin suspension. Both of these suspensions were made with diethylene triaminomethylated polyacrylamide (DTAA). The measurement used was the Rheotest rheometer (Kislenko & Verlinskaya, 2001). Using different equations such as $\eta' = \eta_0(1 + \phi c)$, c = volume fraction, η_0 = viscosity of dispersion medium, and ϕ = constant, one could describe the viscosity and the Newtonian properties of the suspension. Other factors and parameters were measured as well like the constant at low concentration of the suspension, which correlated with the hydrodynamic resistance changes (Kislenko & Verlinskaya, 2001). The resistance was usually started to decrease with low concentration of polymers and then increased drastically with higher concentration. The P_c value then was determined based on the particle properties of the kaolin and montmorillonite materials. The end result showed that when comparing the kaolin and montmorillonite suspensions, flocculation or aggregation of the suspension was very high with DTAA. On the other hand, the aggregation or flocculation of kaolin was at a much slower rate than that of montmorillonite. The kaolin suspension aggregation also had a small size (Kislenko & Verlinskaya, 2001).

Poly(ethylene terephthalate) (PET) was combined with different types of clay that included Somasif MEE and Closite 10A, and unmodified clay Somasif ME100. The clays were

dispersed in water with a dispersion of ionomers that had great compatibility of the poly(ethylene terephthalate). Water was removed, and the sample was finished in preparation by a melt compounding route. The PET with the ionomer gave a higher amount of exfoliation of clay sheets within the nanocomposite system. Tests performed included trans-electron microscopy, X-ray diffraction, TGA-MS, and melt rheology. The focus was that the melt rheology, addition of 10A and the unmodified clay with ionomers, had a much higher viscosity. It correlated with the TEM tests in which the ionomer gave more exfoliation which then gave higher viscosities (Ammala, Bell & Dean, 2008).

Moreover, a polymer study was intercalated nanocomposites between montmorillonite clay and poly(butyl methacrylate) (PBMA). The composite was done at both low and high clay contents. At low clay content the shear viscosity did not show significant shear-thinning but did show more Newtonian flow at lower temperatures. However, at higher temperatures a more significant shear-thinning behavior was observed; where with high clay contents, shear-thinning with curves were observed at all temperatures and shear rates. Viscoelastic tests concluded that the nanocomposites had more of a solid-like characteristic to the structure. The X-ray diffraction also was confirmed by indicating the exfoliation of the clay layers that induced the solid-like formation of the composite. As a result, the resulting solid-like formation was concluded to cause the unusual terminal viscoelastic and shear thinning behavior (Yang & Hu, 2006).

An important study to mention was the unpillared montmorillonite and five organoclays that included Southern Clay Products' 25A and 30B, which were used to have a melt-blend with poly(propylene carbonate) (PPC). XRD and TEM were utilized to inspect the dispersion and the interaction of the polymers formed. The important concept was the thought of rheological measurements. The samples had dynamic strain sweep and dynamic frequency sweeps

performed, using parallel plate geometry at 140°C. The strain sweep was set at a range of 0.1% to 100% strain at 1 Hz frequency. The frequency sweep was set at .05 Hz to 100 Hz with a 1% strain. The results revealed that the organoclay/PPC nanocomposite had a narrower linear viscoelastic region than just the PPC or the PPC combined with PGV, which included a higher complex viscosity, storage modulus, and loss modulus along with higher shear thinning properties as well. Moreover, the results demonstrated a more solid-like behavior with the nanocomposite samples (Zhang, Lee, Lee, Heo & Pittman Jr, 2008).

The combination of montmorillonite and pectins would be an interesting study to conduct. One of the important differences in various types of pectins, as mentioned above, was the degree of methylation in the pectin. The degree of methylation could affect how it would react with cations. One study that depicted this type of effect was the modification of high-methoxy pectins with pectin methylesterase (PME). The PMEs used specifically were U-PME and B-PME, which are from Valencia oranges. Each type of PME from Valencia had a different set of characteristics. The important difference between the U-PME and B-PME was the difference of affinity for cation exchange columns and peptide composition. The PME in the study modified pectins to have similar total charge but to have different distributions between the modified pectins. The modified pectins also became calcium sensitive that gave rise to different characteristics to gelling. The gelling properties of the modified and unmodified pectins were tested through zeta potential, texture profile analysis, and rheological properties (Kim & Wicker, 2007).

In the zeta potential tests, the influence of calcium chloride gave less of a magnitude of zeta potential than pectins in the absence of CaCl_2 . At higher pH values, the zeta potential became more negative. With zeta-potential ranged with O-PME, modified pectins were to be the

highest of the three pectins and the unmodified to be the lowest of the three pectins. Fractionated B-Pec and unmodified (U-Pec) had lower zeta-potential than the unfractionated (Kim & Wicker, 2007), giving the indication that modification of pectins beyond de-esterification could affect the zeta-potential. For the textural profile analysis, unmodified (O-Pec) and some of the fractionated pectins did not gel with the calcium chloride. Therefore, texture profile analysis could not be tested on O-Pec. For the U-Pec and B-Pec, all of the fractionated pectins had lower textural profile than unfractionated pectins.

The rheological tests were completed on a cone and plate using controlled dynamic stress rheometry. In the absence of calcium chloride, the unfractionated pectins showed shear-thinning behavior, which meant it became less viscous as increasing stress was applied to it. With the presence of calcium chloride, the overall G' and G'' was higher in the modified pectin than the unmodified pectin. Overall, these differences from the tests demonstrated the different functional properties, which were most likely due to the different distribution of the free carboxyl groups in pectins (Kim & Wicker, 2007). However, as previously mentioned, the modified and unmodified pectins had different sensitivity to the calcium chloride, which meant that the degree of esterification might relate to the interactions with cations. This became especially important when testing with the montmorillonite because of the cation modifiers that would be on the clay. Overall, there were different types of cation modifiers that could cause different rheological properties depending on both the type of pectin modification and the type of cation exchange modifier.

Zeta Potential

Matter consists of three existing states: gas, liquid, and solid. When one is dispersed into another, a colloidal system is formed (Anonymous, 2001-2005). These colloidal systems then

have many applications that include emulsions, suspensions, aerosols, and so forth (MRK654-01). When the two phases are put together, there is a potential difference between them. If there are ions in one or both phases, there will be tendencies for electric charges to distribute throughout in a non-uniformed way. The region between the two phases always has an electrically charged separation which causes an excess of charge in one of the phases, and the opposing balancing charge distributes throughout the surface of the other phase. Depending on the type of charge, the potential of one phase will go positively or negatively with respect to the other phase. This is what is known as electrostatic potential (Hunter, 1981). This zeta potential, as mentioned, occurs due to the electrically charged colloid particles caused by the dipolar attributes and ionic characteristics. The ion distribution of one phase will cause oppositely charged ion from particle of the other phase to concentrate (Anonymous, 1998-2006). Zeta potential then becomes colloidal particle charge, measured in magnitudes (Tholstrup Sejerssen, Salomonsen, Ipsen, Clark, Rolin & Balling Engelsen, 2007).

The significance of this potential is the ability to determine, directly or indirectly, different properties of the colloidal system (Hunter, 1981). One of the properties is the colloidal stability of the system. An example is the colloid's ability to aggregate based on the particle dispersion. This aggregation can increase in size which then can settle out due to gravity. There are two types of aggregation that can form: flocculation and coagulation (MRK654-01). Flocculation occurs when the colloid initially aggregates and can be a reversible process. Coagulation is when the aggregates become denser and is no longer reversible (Anonymous, 2001-2005; MRK 654-01). The potential distribution of the two phases results in the determination of the particle interaction energy, which is the correlation to the zeta potential and the stability of the particles to coagulation (Hunter, 1981). Colloidal stability theory is

developed by Derjaguin, Verwey, Landau, and Overbeek (DLVO theory) in the 1940s. The theory states that the stability is determined by the balance of the repulsive and attractive forces of the particles. It is recognized by the equation $V_T = V_A + V_R + V_S$, where V_T = the overall balance, V_S is potential energy caused by the solvent, V_A = attractive potential, and V_R = is the repulsion potential. The attractive force is caused by van der Waals reaction, and the repulsion potential is dependent upon the degree of double layer overlap of the phases (MRK654-01).

Zeta potential is measured upon electrokinetic effects or phenomenon. This includes electrophoresis, electro-osmosis, streaming potential, and sedimentation potential. All four effects are based on one phase moving tangentially past the other phase (Hunter, 1981). Electrophoresis is when the liquid or gas phase is stationary, and the liquid or solid particles moves when an electric field is introduced. Electro-osmosis is the opposite in which the solid charged particles remain stationary while the liquid phase has movement. The streaming potential is the excess charge or electric field that is produced when the liquid is forced to move by a pressure gradient. The excess of charges is near the wall that is carried by the moving liquid, which then builds up to an electric field that drives a current back against the liquid flow. The potential difference is measured. Sedimentation potential is the electric field that is produced due to the charged particles that settle, under gravity or in centrifugal field, to the bottom of the liquid. This sedimentation creates a potential difference that can then be measured (Anonymous, 2001-2005; MRK 654-01).

When measuring the zeta potential, the important focus is on the magnitude of the zeta potential and not whether it is positive or negative. The larger the magnitude of the zeta potential, the more repulsion the phases will have with each other, and the less coagulation will occur, which brings about dispersion stability. If the zeta potential is low, then there are forces

to repel the particles from one another, and the dispersion instability will give way to aggregation. A typical line that divides stability and instability is at +30 mV or -30 mV. The higher the magnitudes beyond those values, the more stable the colloidal system. However, if there is a change in density, the stable zeta potential can change (Anonymous, 2001-2005; MRK 654-01). In this study, the particle size analyzer and zeta potential analyzer are used to figure out stability of the dispersion system.

Zeta Potential Studies

On colloid particles, which the pectin/clay solution will be, differences in particles can result in different surface properties. One example was using zeta potential to investigate bitumen-clay interactions, which bitumen was a type of oil. Interaction was tested by finding bitumen recovery from the oil sands with different factors added to it. This included montmorillonite, kaolinite, illinite sands, and calcium. After different combinations of the factors, it was suggested and concluded that calcium ions combined with montmorillonite clay produced a significant decrease in the bitumen recovery. However, calcium and montmorillonite alone did not have a significant impact on decreasing bitumen recovery. It was then suggested that that might have been possible synergistic effect occurring between calcium ions and the montmorillonite clay. Although previous experiments like doping method, bitumen flotation, and impinging jet experiments had been used to suggest these conclusions, the zeta potential was utilized to further confirm it. The results demonstrated that with montmorillonite/bitumen mixture and calcium ions, the zeta potential distribution had only one peak, which indicated a clear strong interaction rather than separate peaks, which indicated weak interactions. The more separated peaks on the zeta potential distribution was shown when combining the kaolinite together with bitumen, even with calcium chloride. This technique, although used specifically

for bitumen/clay systems, could be performed for any binary hetero-coagulation colloidal system (Liu, Zhou, Xu & Masliyah, 2002). Therefore, zeta potential correlated with rheological results and conclusions of change in rheological properties in pectin/clay solutions. The clay modifiers, although not calcium ions, were where the primary interactions between the montmorillonite and pectin would occur. The zeta potential would test for stability and confirm the particle interactions in which the rheometry results would suggest. Also, with the study showing interactions with montmorillonite and a modifier, calcium, it could significantly correlate with what the interactions of pectins might have. Seeing that the ions, modifiers would have an influence on the clay/pectin interactions, the zeta potential would be an important tool for analysis.

Moreover, there had been experiments where zeta potential was tested due to the effect of aqueous phase properties on clay particles. In particular, the clays included bentonite, silty-clay soil and kaolinite. The aqueous phase properties included pH, ionic strength, and divalent cation concentrations. For kaolinite, the pH was found to be a strong function to zeta potential. With different treatments (DDI, HCl, KCl, and untreated) the zeta potential had similar behaviors when the pH increased. It all increased in negativity as the pH increased. However, bentonite did not react over the pH increase as much. The silt-clay had a result that was of intermediate between the kaolinite and bentonite. The kaolinite zeta potential was heavily dependent on the pH, divalent cations, but not as much on the ionic strength. Bentonite had strong function to zeta potential with the influence of divalent metal ion concentrations but had a weak function of pH and KCl concentration (Vane & Zang, 1997). Since kaolinite was similar to montmorillonite behavior, it could be assumed that a significant amount of zeta potential with different parameters would be similar as well.

In addition, there Tholstrup and others did studies that involved the use of high-methoxy pectin, which was used to stabilize acidified milk drinks. The zeta potential was measured with increasing pectin content at 75°C for 20 minutes. The observed result was a change from a positive to more negative zeta potential value. Also, the heat treatment gave a decrease in the magnitude of the zeta potential that indicated partial desorption of the pectin from the acidified milk protein aggregates (Tholstrup Sejerssen, Salomonsen, Ipsen, Clark, Rolin & Balling Engelsen, 2007). As well, it might be an indication that heat decreased the stability of the pectin/casein system.

Along with the rheological characterization of PME de-esterification of the high-methoxy pectin and modified pectins mentioned earlier, Kim, Teng, and Wicker did a study on action pattern experiments completed to different types of fractionated and unfractionated pectins. One main focus was the zeta potential that measured the zeta potential of 0.4% unfractionated pectin (at different de-esterifications) dispersions in deionized water. The pectins used were O-Pec, original pectin at 73% DE, B-Pec at 63% DE, and U-Pec at 61% DE. All of the values started in the negative and increased in magnitude. The U-Pec and B-Pec had a greater magnitude in zeta potential than the O-Pec, which meant that the negative zeta potential was enhanced by blockwise charge distribution. As well, tests with NMR and IEX had given the similar conclusions (Kim, Teng & Wicker, 2005).

Nuclear Magnetic Resonance

In every product there are elements that contain what are known as spins. These can be 1, $\frac{1}{2}$, $1\frac{1}{2}$, and so forth. Typically, most of the elements will have a $\frac{1}{2}$ spin. The spin can also be represented as I , which is also known as the angular momentum (Friebolin, 2005). The NMR will have a magnetic field applied to the sample, which then causes the spin to occur (Hornak,

1997-1999). The angular momentum correlates with magnetic moment in which an angular momentum of 0 has no magnetic moment at all. I is established by halves on the x, y, and z axis. The higher the angular momentum, the more magnetic or directional quantum numbers are presented, which translates to more vectors spinning and more energy values. The spin number correlates with the number of energy values in increments of 1. So a spin of $\frac{1}{2}$ has 2 energy values because $\frac{1}{2} - (-\frac{1}{2})$, and a spin of 1 has 3 energy values because $1 - 0$ and $0 - (-1)$ (Friebolin, 2005). *Figure 2.4a* depicts a spin of $\frac{1}{2}$. The equation $(2I + 1)$, I is the spin, is used to locate the number of directional quantum magnetic numbers, m . So for a spin of $\frac{1}{2}$, there are two values for m and for a spin of 1. Shown in *Figure 2.4b*, there are three values for m . For this study, the ^1H proton is measured in the NMR, which has a spin of $\frac{1}{2}$ (Friebolin, 2005).

Different spins set different results on the NMR spectroscopy that has to be accounted for. There are many different types of tests that can be performed on a NMR spectroscopy, one of which is the relaxation test. Relaxation is when pulse of applied magnetic field is turned off, and the vector that is spinning relaxes back to zero on the z-axis (Friebolin, 2005). In other words, it is the time it takes for the deformation of a sample to go back to equilibrium. There are two types of relaxation times, known as T_1 and T_2 . T_1 is known as spin-lattice or longitudinal relaxation time while T_2 is known as spin-spin or transverse relaxation time. T_1 or spin-lattice relaxation focuses on the spinning vector around the z-axis. This is shown in *Figure 2.5a* as the proton vector shown spinning about the z-axis on the x-y axis plane. The magnetic field pushes the magnetization vector, M_0 , from the z-axis either 90° towards the y axis or 180° towards the z-axis. At the same time, the vector will be spinning about the z axis. The T_1 relaxation occurs when the applied magnetic field or pulse is removed. The relaxation is the time it takes for the spinning vector to go back to equilibrium, back to the z axis, which is M_0 , same as M_z . T_2

relaxation, shown in *Figure 2.5b*, has vectors, dipoles, bunched into one, spreading apart around the z-axis upon an applied magnetic field. The population of dipoles spreading out decreases the transverse signals. T_2 relaxation occurs when the magnetic pulse is turned off, and the vectors go back to a single bunch of vectors in order to reach equilibrium. M_y then increases to have the strongest signal to indicate T_2 (Friebolin, 2005). In this study, T_2 relaxation tests are performed due to the rate of speed in obtaining data. Plus, these tests are based on the comparison between hydrophobic and hydrophilic samples.

NMR Relaxation Studies

Nuclear magnetic resonance relaxation tests have not only been done on pectins but other carbohydrates as well. One of which is water T_2 relaxation of sugar solutions. Many sugars, carbohydrates interactions were heavily influenced by hydroxyl group properties. NMR can help determine the structure and dynamics, but the relaxation tests gained information by inquiring the dynamics of the molecules of the water and the sugar solutions (Fabri, Williams & Halstead, 2005). For this study, the molecular dynamics being investigated are the pectin/montmorillonite solutions. The experiment performed with NMR are transverse relaxation times, which, by the Carver and Richards model, depend on different parameters: chemical shifts, number of exchangeable proton, exchange rate, intrinsic spin-spin relaxation of the hydroxyl protons, and inter-pulse (90° - 180°). Many sugars that included sugar alcohols, reducing sugars, non-reducing sugars, and polysaccharides, have the T_2 relaxation performed on it with all the mentioned parameters. Generally, T_2 relaxation depended on the proton-proton dipole strength of both inter-molecular and intra-molecular of the molecules along with time dependence of both. The strength of the dipolar interaction for the sugar solutions were about the same in the study (Fabri, Williams & Halstead, 2005). For the pectin study, it is assumed for now that this is

the case as well. However, even with the same strength, the fluctuation rate depends on the internal motion of the molecule and the motion of the tumble. Specific motion, the slower it is, drives the effectiveness in the spin-spin relaxation. Therefore, the molecular tumbling and the motions of the backbone molecular chains are the primary relaxation mechanism. This is more predominant than the O-H bond reorientation that is faster around the C-O axis. The tumbling rate also depended on the size and shape of the molecule while the backbone motions depended on the rigidity of the chain. The overall conclusion of the experiments was that the Carver and Richards (CR) exchange model was fitting to interpret ^1H protons for small sugar solutions in water (Fabri, Williams & Halstead, 2005).

Whereas a study that is quite similar to the one that is currently completed is the evaluation of powdered food ingredients' solubility. The study's objective was to evaluate the solubility of the powder and to monitor the hydration over time under different conditions of mixing. This was done by NMR relaxometry, which monitored the hydration and solubility of the powders. In this case, the ingredients included glucose, β -lactoglobulin, zein, and cellulose. The hydration of soy protein was also performed to investigate the effect of mixing rate and time, because many factors could affect the hydration of the ingredients, such as chemistry, solvent nature, wettability, dispersibility, and sinkability of the powders. Before NMR, several methods had been used in the analytical study of powder solubility: High Performance Liquid Chromatography (HPLC), spectrophotometry, and filtration and drying. The problem with these techniques was they did not apply well to in-line or in situ measurements. Other methods that had been proposed were the use of ultrasounds on sugar solutions and comparisons based on refractive index-based method. A rotation viscometer was proposed but the lumping and the

sticking of samples to the stirrer produced problems. The NMR was ultimately utilized to analyze the effects of concentration, stirring rate, and additives.

The transverse (T_2) relaxation time correlated with the water molecule mobility, which powder particles had free movement (free water) with slow relaxation time, and restricted particle movement (restricted water) had fast relaxation times. The samples were prepared with β -lactoglobulin (soluble) and zein (insoluble) as the protein controls while glucose and cellulose were the soluble and insoluble carbohydrate control samples. The effect of stirring on hydration was performed using soy protein concentrate. The result for the protein and carbohydrate solution testing revealed that solubility as well as interaction of the ingredients with water had an effect on the relaxation time. Glucose (high solubility) and zein (low solubility and little interaction) had similar fast relaxation times that were produced by the relative amount of free water. For the soy protein solutions, T_2 measurements were done at different mixing rates and times. At higher mixing rates, the initial drop in T_2 was smaller than at slower mixing rates. Time to reach certain solubility was also recorded as well using different methods. The results were fairly similar to each other. In short, the study shows that NMR transverse relaxometry did not necessarily correlate with solubility, at least in the protein and carbohydrate case, but rather with the interaction. Some of the powdered ingredients had significant differences in T_2 times but the same solubility due to the interaction based on the free and restricted water (Granizo, Reuhs, Stroshine & Mauer, 2007). Like the pectin/clay solution, the solubility was not the critical factor, but rather the interaction of the clay modifier with the pectin was the resulting factor in the solubility and viscoelastic properties. Also, the protein concentrate tests showed that the solubility with NMR relaxometry did correlate when using one ingredient having different mixing rates and times (Granizo, Reuhs, Stroshine & Mauer, 2007).

Nuclear magnetic resonance had also been used on high-methoxy pectins (HMP) and low-methoxy pectins (LMP) in solution. T2 (CPMG) or transverse relaxation test was completed at both LMP and HMP in different concentration solutions of 0.025%, 0.1%, and 1% pectin. Relaxation curves were resolved into a short and long time constant due to different physical environments for water. In both pectin solutions, the transverse relaxation rate was much faster at 1% pectin which could be explained by the increase viscosity and the much tighter binding of the pectin to water, restricting the water molecule motion (Kerr & Wicker, 2000).

To see how it could affect an applicable study in the industry, pectin was added to acidified milk drinks at increasing concentration of 0.0%, 0.1%, 0.3%, and 0.5% pectin along with a protein, skim milk powder. The pectin used was high-methoxy pectins with low-field nuclear magnetic resonance. The T2 relaxation test performed was the Carr-Purcell-Meiboom-Gill (CPMG). From the resulting observation, one revealed that 0.5% pectin had a much faster relaxation rate than that of 0.1%, which meant, like in the previously mentioned study, that tighter bound protons in the higher pectin concentration would prevent free movements to have a much faster relaxation rate. In short, the significance of this study was to prove how low-field nuclear magnetic resonance was a very useful tool in the food industry (Salomonsen, Sejersen, Viereck, Ipsen & Engelsen, 2007).

NMR tests were completed on montmorillonite clay nanoparticles. Kunigel-V1 bentonite, consisted of montmorillonite, was analyzed by using proton nuclear magnetic resonance where both T1 and T2 relaxation tests were performed by inversion recovery with Carr-Purcell-Meiboom-Gill (CPMG) method on bentonite gels. Other tests included surface relaxivity and water self-diffusion coefficient. The concentration here was that the T1 and T2 relaxation times were decreasing with increasing bentonite concentrations. The reason for this

was the pore size was decreasing which resulted in increase of bentonite weight, collision frequency between clay particles, and diffusing water. As a result, it correlated with the decrease in the relaxation time as the bentonite weight increased, which translated to a tighter bound of clay to water created in the gel (Nakashima, 2004).

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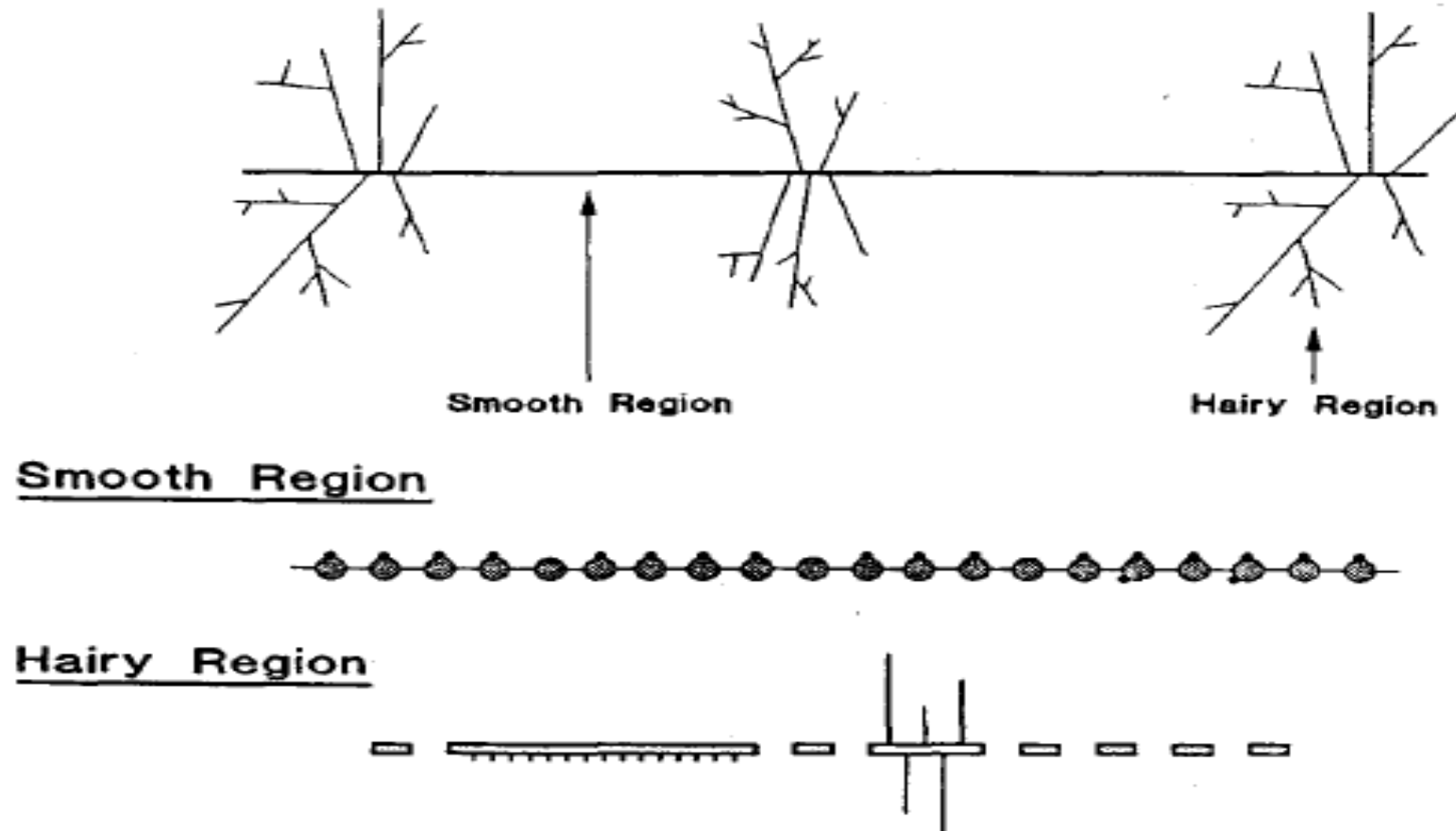
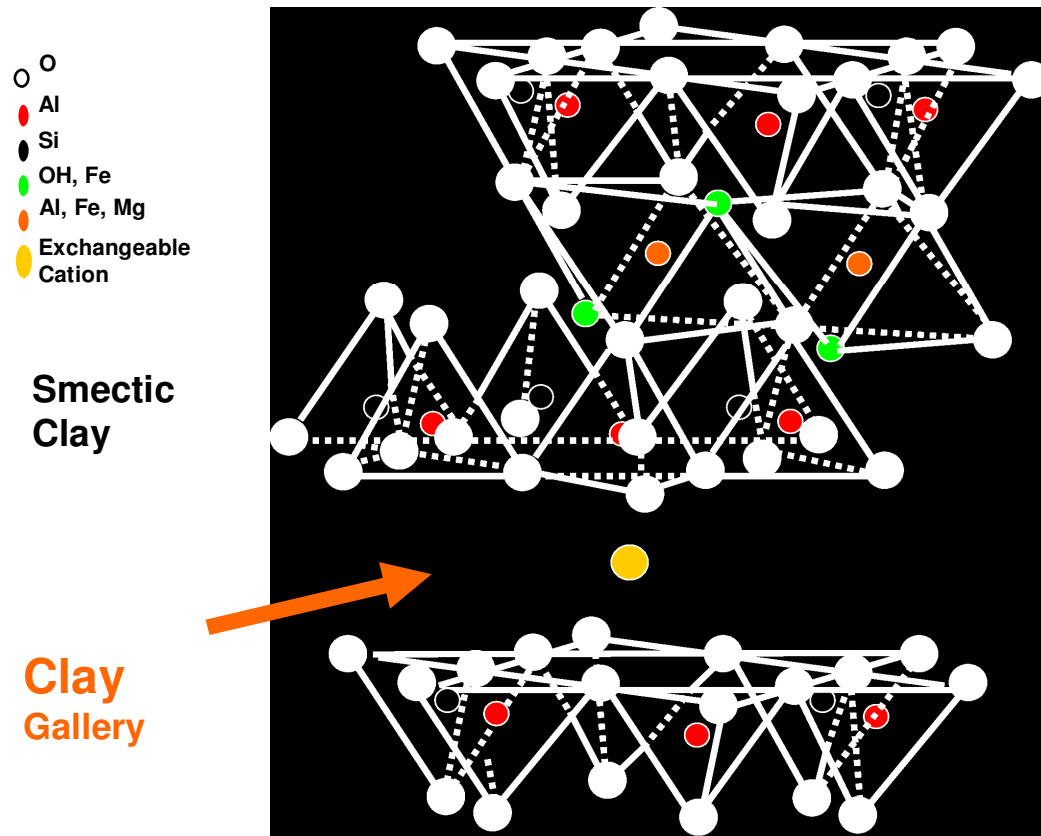


Figure 2.1 (Voragen A.G.J., 1995)

Backbone structure of pectin with smooth regions and hairy regions that branch out (Voragen A.G.J., 1995).

(a)



(b)

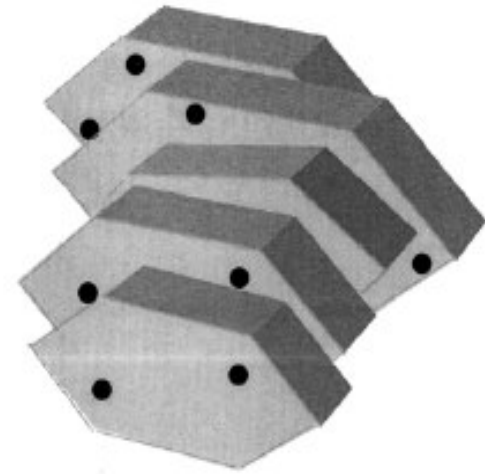


Fig. 2.2 (Lagaly & Ziesmer, 2003; Mulligan)
 Montmorillonite Structures (a & b) (Lagaly & Ziesmer, 2003; Mulligan)

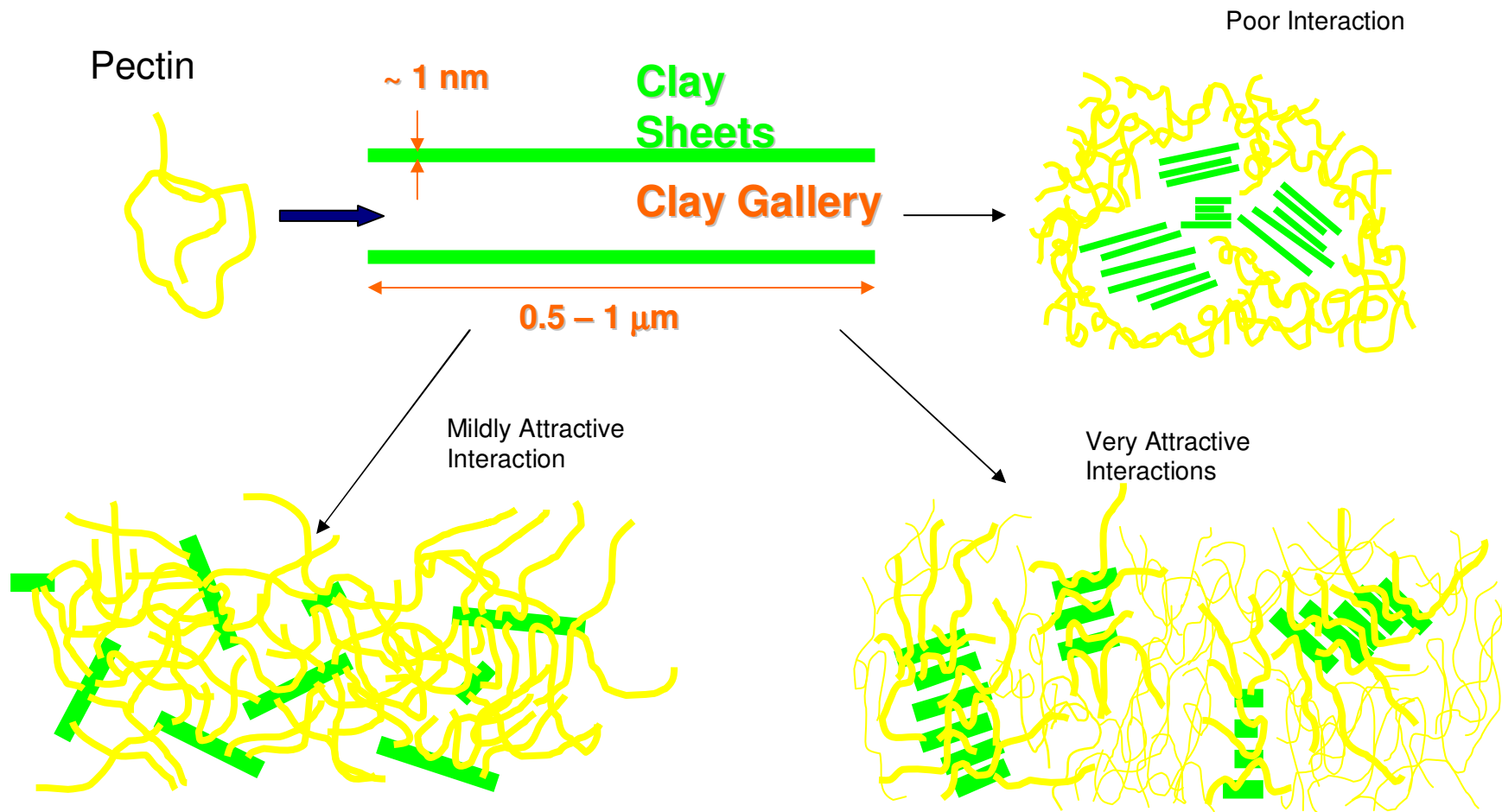


Figure 2.3 (Mulligan)

Different interactions that can occur when pectin and clay interacts. Best type of interaction is the mildly attractive interaction because it creates full exfoliation which gives it more effective surface area (Mulligan).

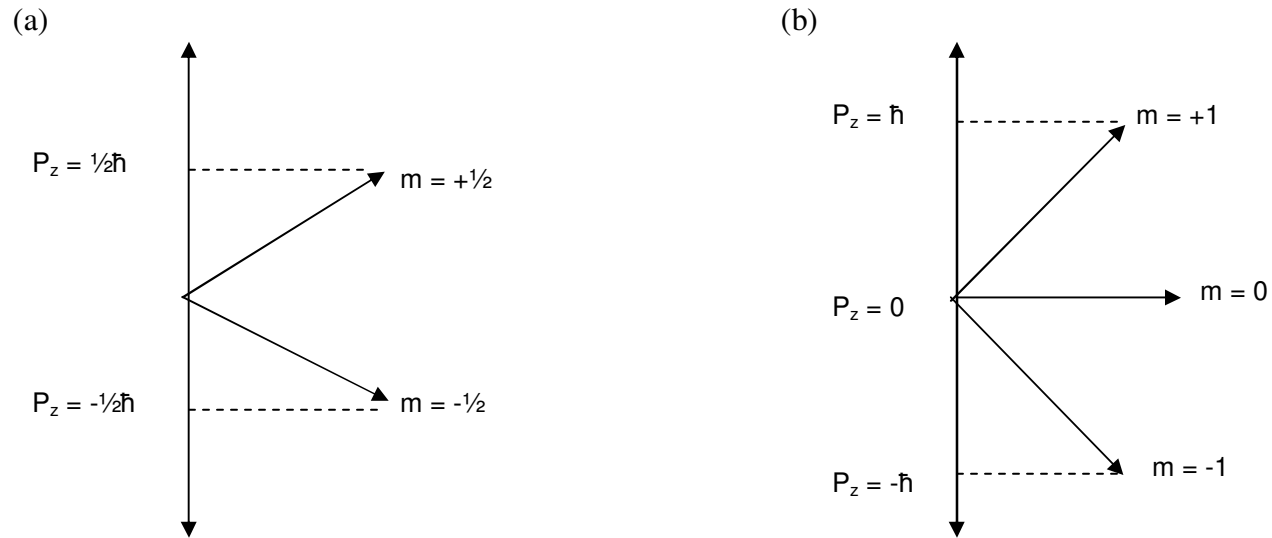
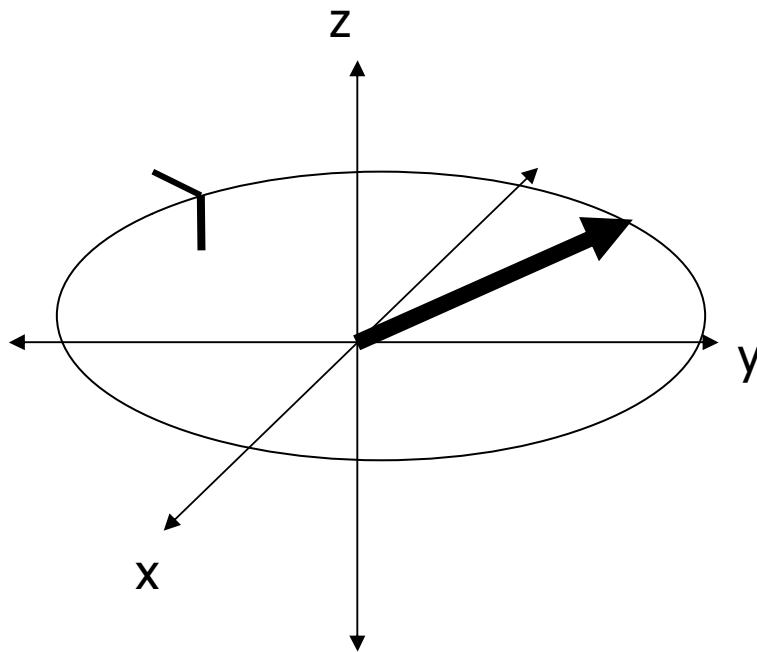


Fig 2.4 (Friebolin, 2005)

Showing the directional quantization at a spin of $I = \frac{1}{2}$ (a) and $I=1$ (b). P_z = angular momentum, $\hbar$ = integral or half-integral multiple, m =magnetic or directional quantum number (Friebolin, 2005).

(a)



(b)

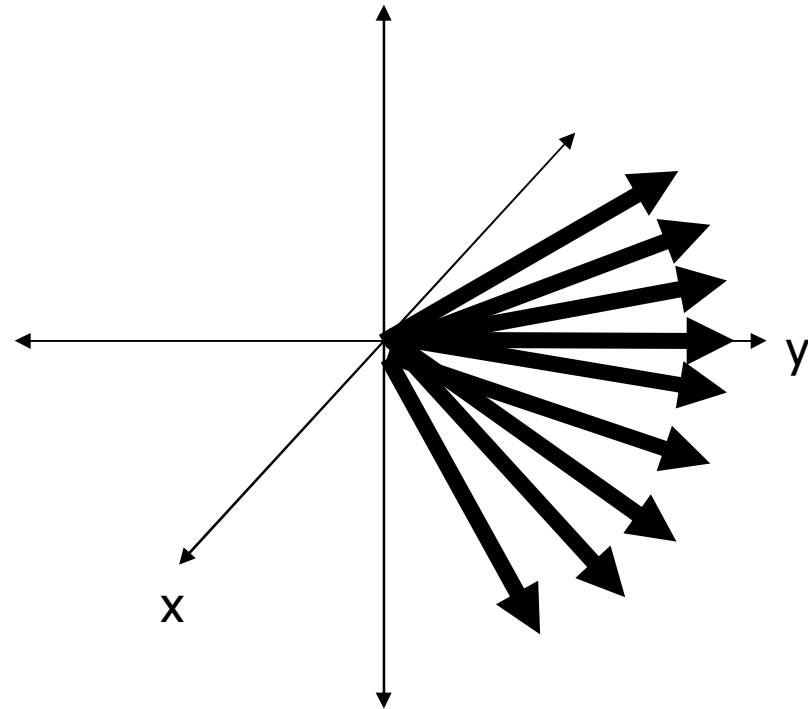


Fig. 2.5

(a) shows T_1 or longitudinal relaxation time with the vector spin around the z axis on the x - y axis plane until the relaxation brings it back to the z axis. (b) shows T_2 or transverse relaxation time in which the vectors first spread out on the x - y axis about the z axis upon magnetization and relaxes back to looking like one vector.

CHAPTER 3

RHEOLOGICAL TESTS TO HIGH-METHOXY PECTIN/CLAY NANOPARTICLES

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Abstract

Pectin/modified montmorillonite clay dispersions were characterized by rheology, nuclear magnetic resonance, and zeta potential. Modified montmorillonite clays were blended with high-methoxy pectins at 2% w/v pectin/1% w/v clay dispersions in 0.1M acetate buffer. Several clays were used with differing levels of hydrophobicities and the sodium (Na^+) montmorillonite was the only hydrophilic clay. Dynamic strain sweep, dynamic frequency sweep, and transient shear rate ramp tests were performed at 25°C and 50°C. Results indicated higher viscosities, G' , and G'' modulus for the least hydrophobic 10A/pectin and hydrophilic Na^+ /pectin samples. Frequency sweep G' values at lower frequencies were greater at 50°C than at 25°C. Particle size analysis and T2 relaxation validated rheological results with faster relaxation rates for Na^+ clay and smaller particle size in the suspensions. Zeta potential indicated stability of dispersion system is invariant with clay type.

Introduction

High-methoxy pectins are complex polysaccharides that are found in all fruits and vegetables (Rooker, 1928). They are heteropolysaccharides, meaning they consist of non-repetitive monosaccharide chains. Pectin structure consists of smooth regions (homogalacturonan) with hairy regions (rhamnogalacturonan I and II) containing neutral sugars as side chains (Voragen A.G.J., 1995). High-methoxy pectins have methylation of carboxyl groups to a degree greater than 50%. High-methoxy pectin is commonly used as a viscosifying agent in acidified milk drinks, and in pharmaceuticals (Voragen A.G.J., 1995). Montmorillonite clay particles consist of silicate layers that are 1 nm thick (Hrachova, Komadel & Fajnor, 2007). Between each sheet are exchangeable cations that can be modified so that they can interact with macromolecules to form homogenous nanocomposites. Nanocomposite dispersions are defined

where one phase of the dispersion is less than 100 nm (Chang, An, Cho & Giannelis, 2003; Rao, 2007).

The nanoparticles can potentially change the rheological properties of macromolecules, including: moduli, frequency dependence, and the overall viscosity. There have been previous studies using montmorillonite in other polymer systems. However, the blending of montmorillonite in food macromolecules such as has yet to be studied. The objective of this study is to characterize the effects of montmorillonite with varying surface modification on the rheology of high-methoxy pectin solutions. The characterization used in this study consisted of strain sweep, frequency sweep, and shear rate ramp tests for viscosity measurements. Zeta potential (colloidal stability), particle size, and NMR T2 relaxation (proton spin) tests were performed to agree with the rheological experimental results.

Methodology

Sample Preparation

The commercially available high-methoxy pectins used in this study were donated by CP Kelco Company (Copenhagen, Denmark). The montmorillonite donated by Southern Clay Products Inc. (Gonzales, TX, USA), were mixed in with the pectin. Hydrophobicity of the clay arranged from most hydrophobic to most hydrophilic grade was:

15A>20A>93A>25>10A>30B>Na⁺ with Na⁺ clay, or sodium montmorillonite is considered hydrophilic. Pectin control solutions were used as reference values compared to the other montmorillonite clay/pectin dispersions. The pectin concentration used was 2% (w/v) (Kar & Arslan, 1999). The clay concentration was 1% (w/v). Pectin control and pectin/clay were dispersed in 0.1 M acetate, pH 4.0. The clays were slowly added onto the surface of buffer at 50°C while stirring on a magnetic stir plate. The clay suspension was hydrated overnight at 4°C.

The pectin was then added to the hydrated clay dispersion under moderate agitation at 50°C which was acceptable temperature based previous preparation trials. Other studies have shown preparatration at higher temperatures (Fu & Rao, 2001). After mixing, the samples were stored at 4°C overnight. The pH was then measured to ensure hydration. The pH ranged from 3.8-4.0. Samples were made in triplicate.

Strain Sweep

Dynamic strain sweep was used to determine the linear viscoelastic region of the sample. This was determined by plots of the elastic modulus (G') versus strain. At a critical strain at which the structure started to break down, G' changed with strain; this is the non-linear viscoelastic regime (Herh, Tkachuck, Wu, Bernzen & Rudolph, 1998; Ramos-Tejada, Arroyo, Perea & Duran, 2001). The strain sweep also helped differentiate the G'' and G' within the intrinsic structure of the sample (MRK788-01, 2006). Strain sweeps were dynamic measured in Couette flow geometry under dynamic conditions in the Rheometric Scientific SR-5000 rheometer. The amplitude or strain sweeps were measure at 25°C, 1 Hz frequency (MRK788-01, 2006), initial strain at 0.1332%, final strain at 100.0%, and 10 points per decade of measurements. The initial strain was based on the lowest bound of this rheometer's range. The samples were also tested for strain sweep at 50°C to see the effect at high temperature as well. Strain sweeps at both 25°C and 50°C were performed in triplicate.

Frequency Sweep

Frequency sweeps were performed in the SR-5000 rheometer. Samples were tested at both 25°C and 50°C with initial frequency at 0.1 Hz, final frequency at 79 Hz, and strain of 0.1332% to keep within the linear viscoelastic region (MRK602-01). Frequency sweeps at both 25°C and 50°C were performed in triplicate.

Shear Rate

Shear rate tests were used to determine the shear dependency of viscosity. Transient shear rate ramp tests were performed in the SR-5000 rheometer. All samples were tested at 25°C and 50°C, at final shear rate of 30/s for 60 seconds. Shear rate ramp test at both 25°C and 50°C were performed in triplicates.

Zeta Potential

For zeta potential, the samples were diluted from 20 mg/mL (pectin control) or 30 mg/mL (pectin/clay) to 0.30 mg/mL (Tholstrup Sejersen, Salomonsen, Ipsen, Clark, Rolin & Balling Engelsen, 2007). Final samples were diluted from 0.1 M to 0.01 M acetate buffer samples. The pH, 4.0, was then measured. The particle size was measured through the Brookhaven particle size analyzer. Both of these obtained parameters were then used to determine the zeta potential. All samples, zeta potential were measured using the Brookhaven instrument.(Holtsville, NY), were performed in average of 5 runs and done triplicates for the 15A, 30B, Na+, and pectin control samples.

T2 Spin-Spin Relaxation

NMR relaxometry was performed by T2 spin-spin relaxation tests on undiluted dispersions. About 6 mL of samples were used for these measurements using the Carr–Purcell–Meiboom–Gill (CPMG) (Kerr & Wicker, 2000; Meiboom & Gill, 1958), using a Maran 20 MHz proton NMR with Resonance RI NMR software (Resonance Instruments, UK). Each sample ran over an average of 8 scans. The relaxation data was fit with a single exponential peak for each of the sample dispersions. The final relaxation time was determined along with each sample's respective intensity using the Winfit software. All samples were performed in triplicate for the 15A, 30B, Na+, and pectin control samples.

Results and Discussion

Dynamic Strain Sweep

Figures 3.1-3.4 show dynamic strain sweeps at 25°C and 50°C. G' and G'' at 25°C shows the Na⁺ and 10A clay/pectin samples have the highest modulus indicating higher G' and G'' values with the hydrophilic (Na⁺) and least hydrophobic of the nitronium ion modified clay (10A) samples. At 50°C, the Na⁺ dispersions had the highest value G' and G'' . This is an indication of greater degree of interactions between the high-methoxy pectins and the clay. The higher G' and G'' likely indicate a better dispersion of the clay into the pectin solution. This could be due to the intercalation and exfoliation of the clay sheets by the pectin, which increases surface area of the clay that interacts with the pectin, thereby stiffening the pectin molecules. The data also shows some of the samples going into the nonlinear viscoelasticity for both temperatures around a critical strain of about 10-100%. More hydrophobic samples showed nonlinear viscoelastic regime at lower critical strain. This result indicates a breakdown in structure at lower strains in the most hydrophobic samples. Similar previous research was done with poly (propylene carbonate) polymers using 25A and 30B clay nanocomposites under similar testing parameters except with parallel plates. The results showed the 25A clay/polymer to have higher G' and G'' than the 30B clay/pectin polymer (Zhang, Lee, Lee, Heo & Pittman Jr, 2008), which is similar to the results in this study in which the 25A sample had higher G' and G'' than the 30B clay samples at both 25°C and 50°C. The explanation was that the hydroxyl groups in the 30B clay bind to the aqueous phase, much more favorably than to the polymer phase. The better dispersed system usually cleaves the PPC polymer during the blending process with the clay. If there were enough cleavage in the polymer the lower G' and G'' and viscosity values would show on the rheology (Zhang, Lee, Lee, Heo & Pittman Jr, 2008). This same phenomenon could be

applied to the pectin/clay samples in which the blending caused some possible cleavages in the pectin/clay dispersions. Other possibilities may have been that since 30B was less hydrophobic clay than 25A, it may have indicated a result in which the modifier in the 25A clay was less susceptible in preventing pectin from interacting with the exchangeable cation between clay sheets of the Cloisite 25A clay.

Figure 3.5 shows examples of G' and G'' vs. strain for 15A (most hydrophobic) Na^+ (hydrophilic) clay. The samples at 25°C possess G'' higher than the G' . This indicates that at room temperature the rheology of pectin/clay dispersion is dominated by the liquid phase. At 50°C , G' is higher than G'' . This result indicates a more dominant solid phase contribution to rheology, which may possibly be due to hydrophobic interaction with water. Apolar elements cluster together with water to form cage-like structures. The increase temperature to 50°C may have broken the cage-like structure and cause dissociation of the nanoparticles back to dispersion (Steventon, Parkinson, Fryer & Bottomley, 1990), resulting in a phase differential for the Na^+ sample at 50°C . This means the storage modulus that has been higher than the loss modulus eventually transitions to a situation where G'' is greater than G' . This indicates a breakdown in network structure at a critical strain level. The behavior of samples containing 10A grade clay (not shown) is similar to that of samples containing Na^+ montmorillonite, while other clay grades yield behavior closer to 15A grade clay.

Frequency Sweep

Figures 3.6-3.9 show that at 25°C , the G' and G'' are about the same for all samples with the exception of the Na^+ clay sample, which shows a slightly higher G'' with increasing frequency. The elastic modulus was higher than the viscous modulus at both temperatures. Also, at 25°C , both of the G' and G'' are dependent on the frequency indicating possible

sedimentation due to lower colloidal stability. At 50°C, however, and especially in the cases of 30B, 10A, and 15A, pectin/montmorillonite dispersions have lower frequency dependence. At 50°C, there was clear change G' and G'' due to the temperature change for the least hydrophobic of the nitronium modified clay samples 30B and 10A. 15A also show an exceptional increase that correlates to more interactions between clay sheets and pectin to increase intercalation and/or exfoliation based on heat. Surprisingly, the Na^+ sample does not show much of a change in the G' and G'' which may show that thermal treatment may not have a significant effect on the interaction or possibly where interaction of the pectin and Na^+ clay is at an optimum based on the heat. Other studies have shown that 30B, even though more dispersed than the 25A nanocomposites, had a much smaller storage modulus and viscous modulus. Zhang et al. explained that this may occur because of the polymerization agent, poly(propylene carbonate), had resistance to hydrolytic cleavage but was much better with the less hydrophobic 30B clay causing a lower molecular weight for PPC (Zhang, Lee, Lee, Heo & Pittman Jr, 2008). However, with pectin, the lesser hydrophobic clay does not have the same effect at higher temperatures. This may be due to the lack of resistance that the high-methoxy pectins have and nothing to due with its molecular weight but more with the amount of interaction between the pectin and clay at 50°C than 25°C that likely resulted in the higher G' and G'' values. Also, M.H. Norziah and others showed increase in G' and G'' of pectin with addition of sucrose and calcium cations (Norziah, Kong, Abd Karim & Seow, 2001). This then may explain the higher values with the hydrophilic samples in which the exchangeable cations between the clay sheets are able to interact more easily with the pectin that created the intercalation and exfoliation of the clay sheets leading to a better dispersed system, which then results in greater overall G' and G'' values at 50°C.

Viscosity Measurements

The viscosity as a function of shear rate is shown in *Figures 3.10-3.11*. These results show that temperature has little effect on viscosity in the range of 25-50°C. At 50°C, the Na⁺, 10A, and pectin control all show initial relatively sharp shear thinning effect at 50°C. This may be typical as I-Kuan Yang and Chi-Cheng Hu has seen with their polymer study using montmorillonite clay showing a more Newtonian flow at low temperatures and a more shear-thinning characteristic with increasing temperatures (Yang & Hu, 2006). Other studies in nanocomposites using 10A clay polymer composite at high temperatures were consistent with the results shown with the pectin/10A clay samples done in this study showing higher viscosities because of the higher exfoliation (Ammala, Bell & Dean, 2008). This also correlates with hydrophilic and less hydrophobic clay increase that exhibit greater interactions between the pectin and clay which then increased exfoliation and higher viscosities. As mentioned on the strain sweeps, the highest G' and G'' were the 10A and Na⁺ dispersions. This correlates with the viscosity readings in which the 10A and Na⁺ had the highest viscosities. Therefore, the higher the viscosities are, the higher the G''. The interactive state of the pectin in which shows interactions with cations. This was proven by Kjøniksen et al. by adding certain amount of salts to pectin which catalyzed cross-links leading to a higher viscosity observation (Kjøniksen, Hiorth & Nyström, 2005). The hydrophilic clay used for this study has the exchangeable cations between the clay galleries, Na⁺. However, the results are still the same with an increase in the viscosity.

Zeta Potential

Particle size from *Table 3.1* shows higher size in more hydrophobic, 15A, samples than hydrophilic Na⁺ samples. This indicates a lot less interaction between the high-methoxy pectin

and 15A clay. The hydrogenated tallow in the 15A could be preventing the pectin to interact between the clay sheets, preventing the clay sheets to be exfoliated, separated, and stay in a more clumped form. This then give a much greater particle size. Whereas the Na⁺ hydrophilic clay, which has no hydrogenated tallow, has a much lesser particle size indicating a greater exfoliation of clay nanoparticles preventing the montmorillonite clay from staying in the tactoid form.

The ζ -potential for the samples at pH 4.0 is all about the same shown in *Table 1* giving the indication that the regardless of hydrophobic, hydrophilic interactions, or particle size, the surface charge is not affected by these parameters. Other studies have shown where the additions of high-methoxy pectins to acidified milk drinks at pH 4.0 have taken the ζ -potential from a positive to negative value because of electrosorption (Tholstrup Sejersen, Salomonsen, Ipsen, Clark, Rolin & Balling Engelsen, 2007), showing the same observation with ζ -potential of the clay samples and pectin control to be in the negative range. pH was also a factor in which at pH 4.0 all of the samples range from (-24)-(-26) mV. Other studies had shown pectin samples to be around the same range (Kim & Wicker). The similar values in ζ -potential may indicate the same surface charge of the pectin/clay system which then reveals a lack of change in the stability of the system.

T2 Spin-spin Relaxation

NMR T2 relaxometry was performed on the samples shown in *Table 3.2*. The pectin control had a significantly higher relaxation time, at almost 0.9 seconds, than samples mixed with different clays. Although the samples were at 2% pectin, it was similar but higher time than other relaxation tests performed before with 1% pectin and faster relaxation rate with increasing concentration of pectins (Kerr & Wicker, 2000; Salomonsen, Sejersen, Viereck, Ipsen & Engelsen, 2007). As the sample character becomes more hydrophilic the samples have a shorter

T2 relaxation time indicating a longer decay time. The added montmorillonite results in lower relaxation time compared to the pectin control as previous studies have shown with water mixed with increasing clay amounts showing a faster relaxation time meaning a more tightly bound system (Nakashima, 2004) with higher viscosity. This correlates with the greater interaction in the Na⁺ and 30B samples giving a much shorter relaxation time due to it. This also correlates with the previous rheological tests in which the Na⁺ samples shows higher G' and G'' values along with having the highest viscosity. The higher rheological values, compared with the T2 relaxation results, indicates longer decay time and the limited mobility of the free water because of the higher rheological values whereas the 15A hydrophobic samples shows a longer T2 relaxation time indicating a shorter decay time and more mobility of free water. The much lower rheological values from previous rheological experiments correlate with the relaxation time of the 15A clay dispersion sample.

Conclusion

There is a significant difference from the hydrophobic to hydrophilic samples where the hydrophilicity had higher G', G'', and viscosity values. This is an indication of greater interaction between the modified montmorillonite clay nanoparticles and high-methoxy pectin. The most hydrophobic sample, 15A, showed signs of increased interactions of pectin and clay at lower frequencies at 50°C. The dynamic strain sweep show a much higher reading in the hydrophilic Na⁺ and 10A samples due to greater degree of interactions caused by the more homogenous dispersions and exfoliation of clay sheets by the high-methoxy pectins. This is corroborated by the shear rate tests at which Na⁺ clay/pectin dispersions had the highest viscosity. The increase in temperatures gave a higher G' and G'' for the more hydrophilic samples because of the greater bound system. With the more tightly bound system, the higher

degree of intercalation of the clay sheets due to the hydrophilicity gives way to higher observed viscosity, 10A and Na⁺, and a more shear thinning sample at higher temperatures. The particle size and T2 relaxation tests correlated with rheological data in which the Na⁺ clay samples had the shortest time relaxation rate compared to the most hydrophobic 15A samples correlating to longer decay time and tighter binding of the pectin to the clay particles. Zeta potential results in moderate colloidal stability for all samples tested. It is not affected by the particle size of the nanocomposites. However, the higher 15A grade hydrophobic sample's particle size does indicate sedimentation due to gravity because of apolar particles in water that create dissociation in the colloidal dispersion.

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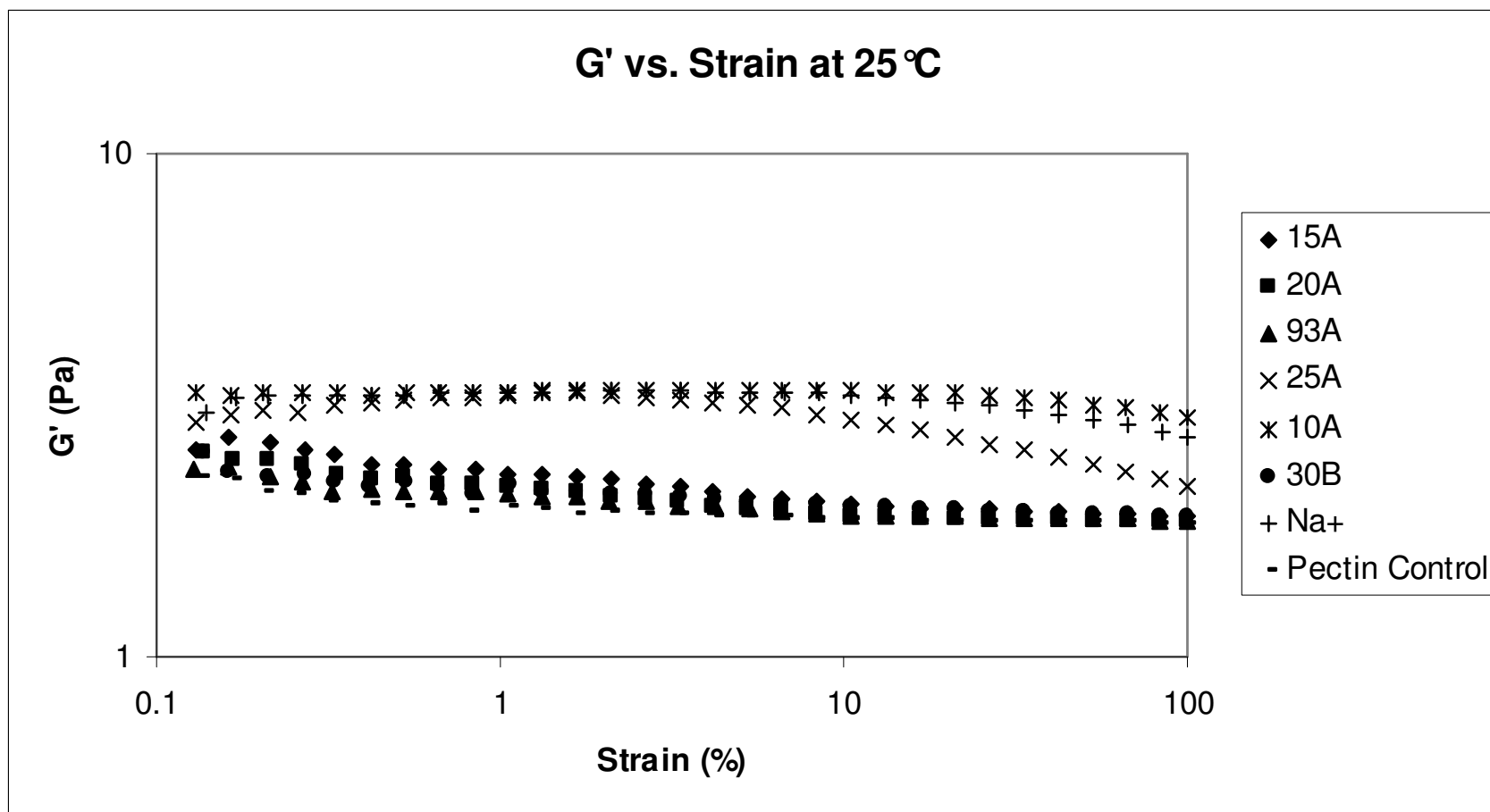


Figure 3.1

G' pectin/clay dispersions dynamic strain sweep at 25°C. G' vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

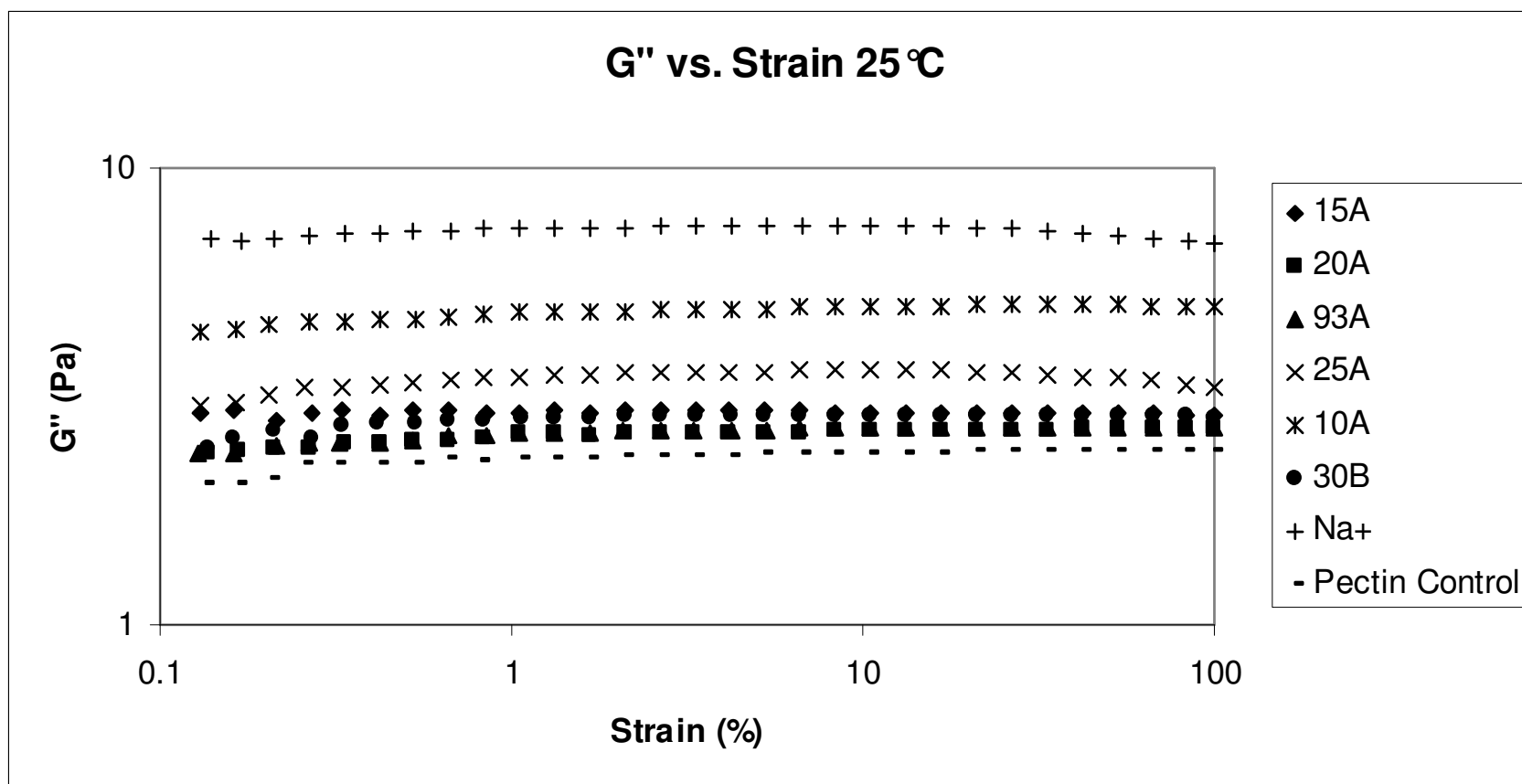


Figure 3.2

G'' pectin/clay dispersions dynamic strain sweep at 25°C. *G'* vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

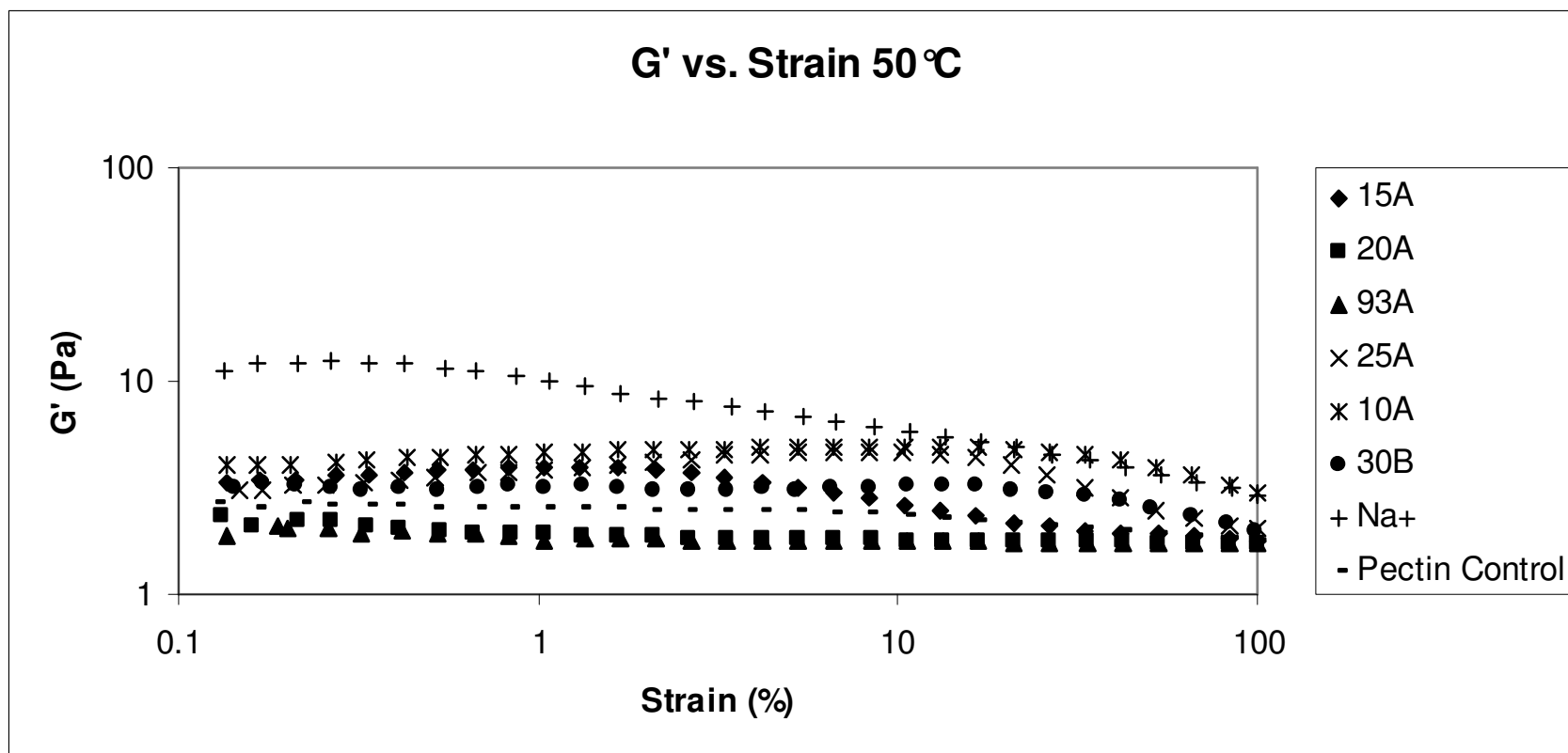


Figure 3.3

G' pectin/clay dispersions dynamic strain sweep at 50°C. *G'* vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

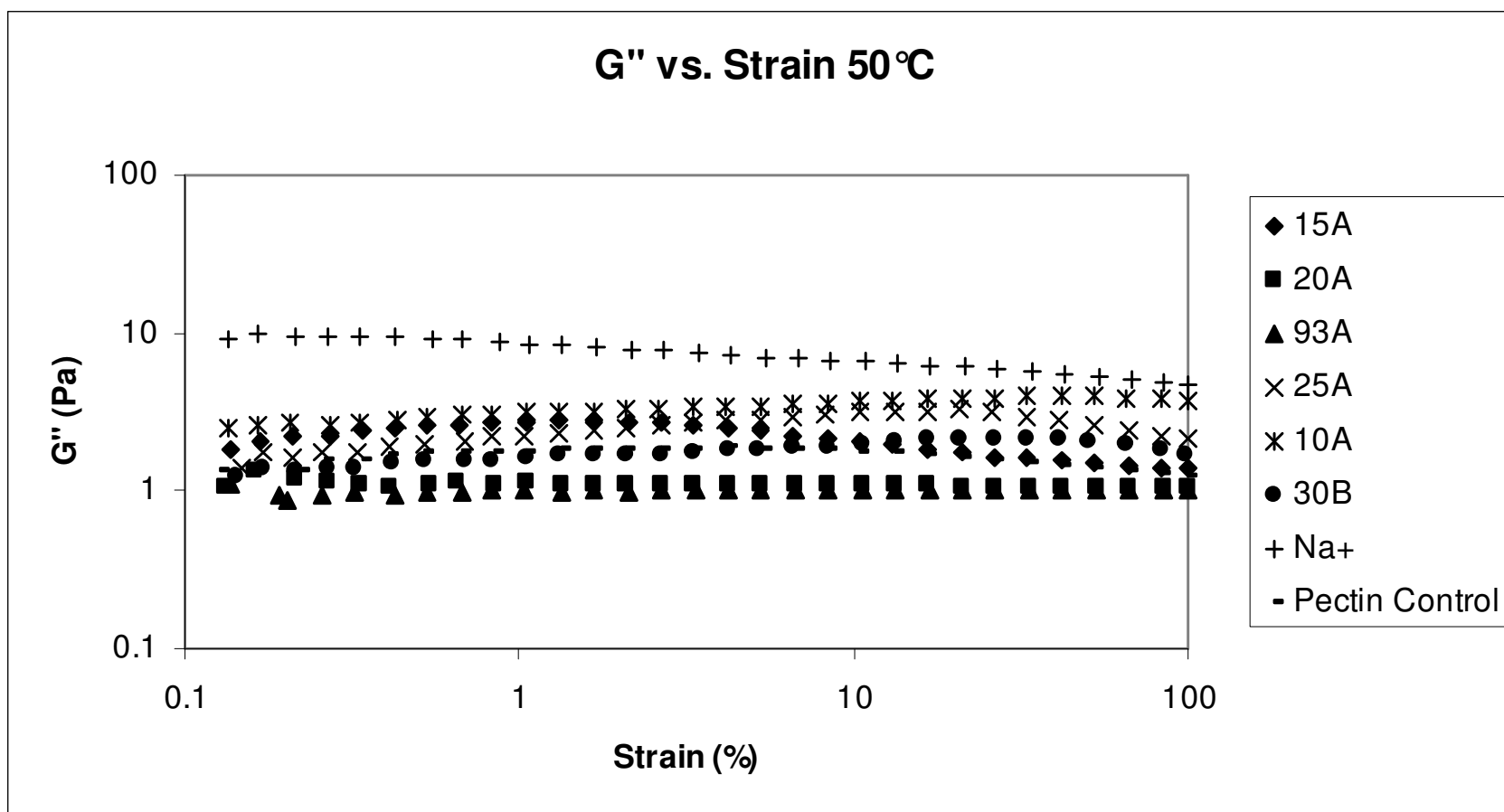


Figure 3.4

G'' pectin/clay dispersions dynamic strain sweep at 50°C. *G''* vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

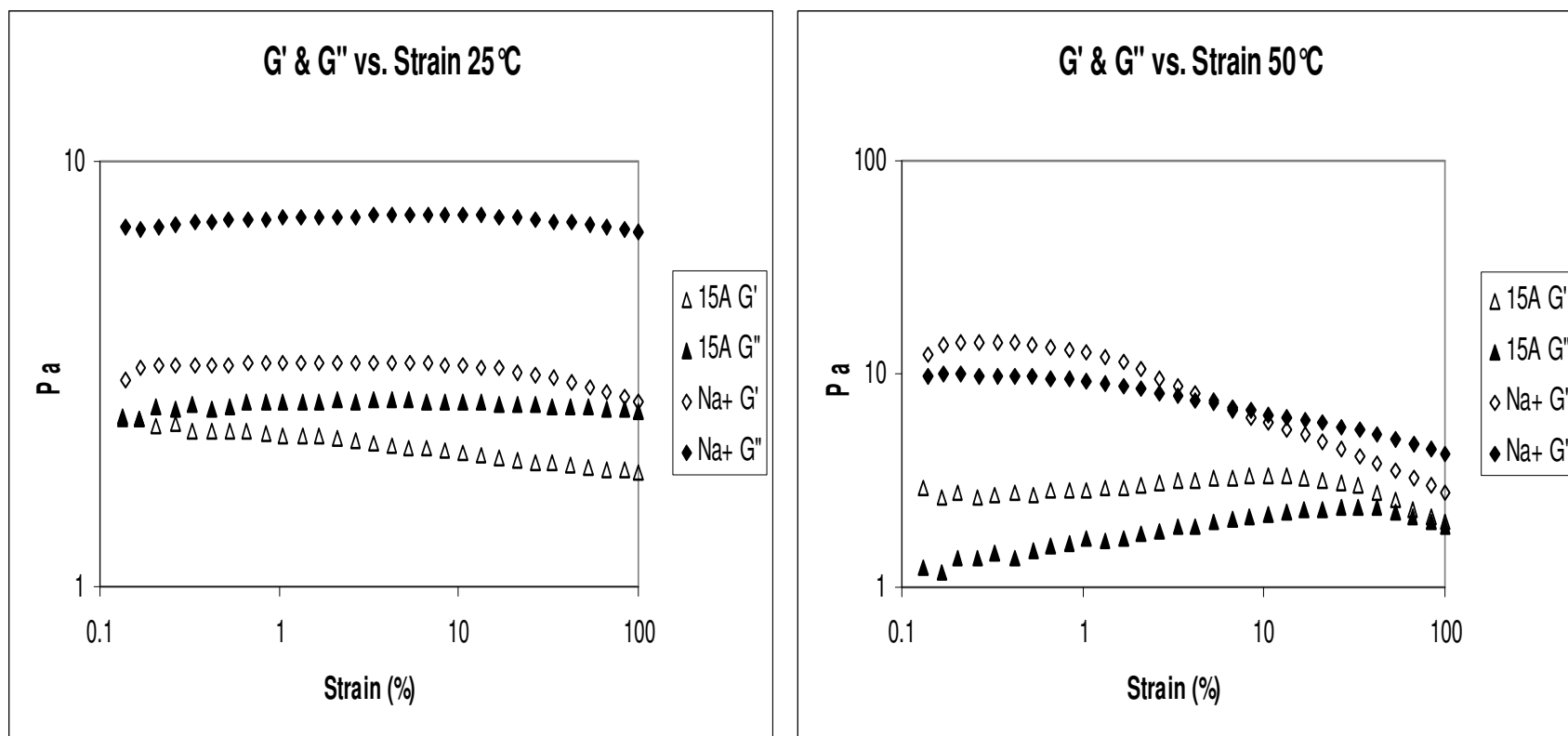


Figure 3.5

Example G' and G'' comparison based on 25°C and 50°C for 15A clay/pectin dispersion. Change is at 50°C, which the G' is higher than the G'' for the 15A and Na^+ whereas the G'' was higher than the G' at 25°C

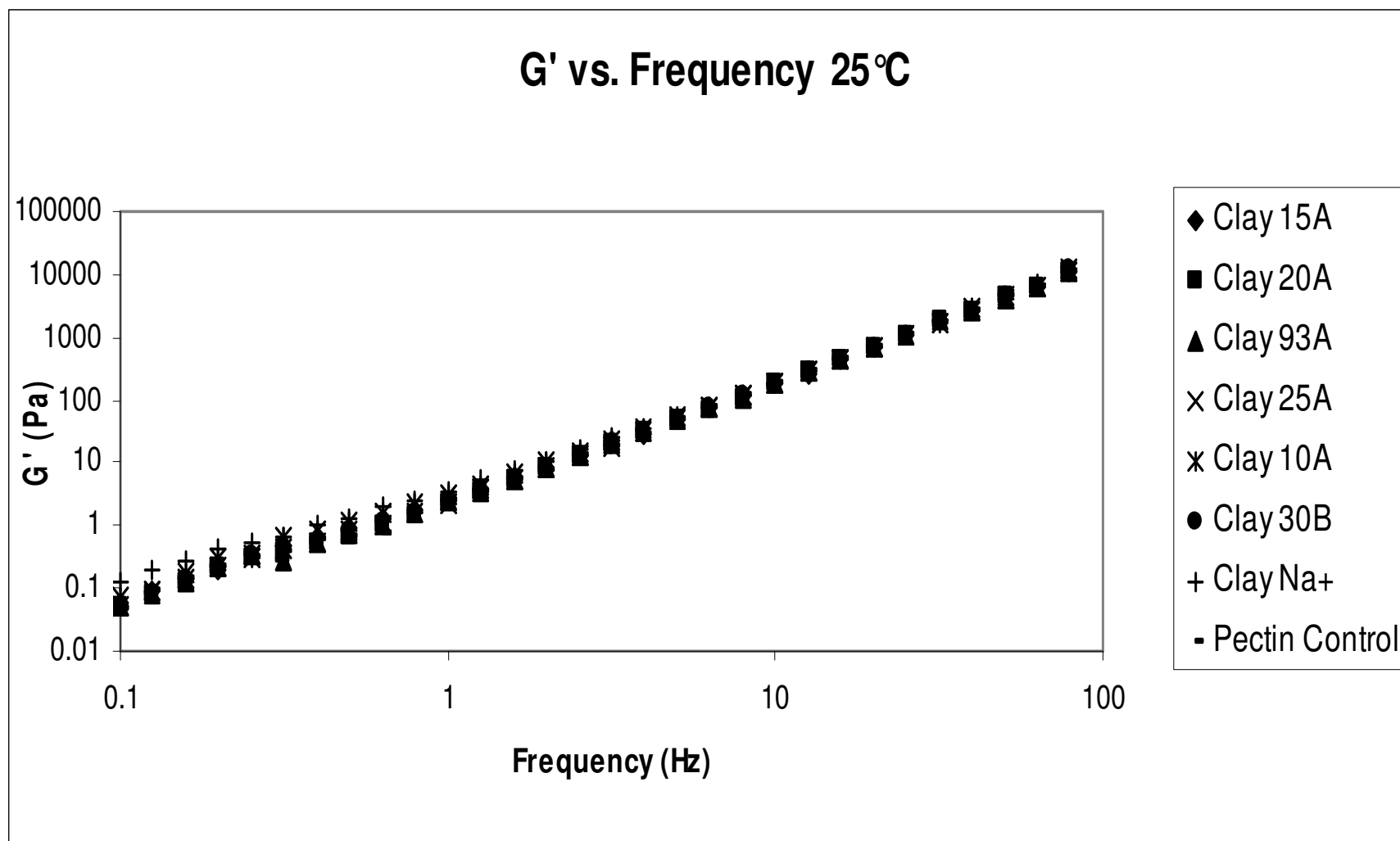


Figure 3.6

G' pectin/clay dispersions dynamic frequency sweep at 25°C. G' vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

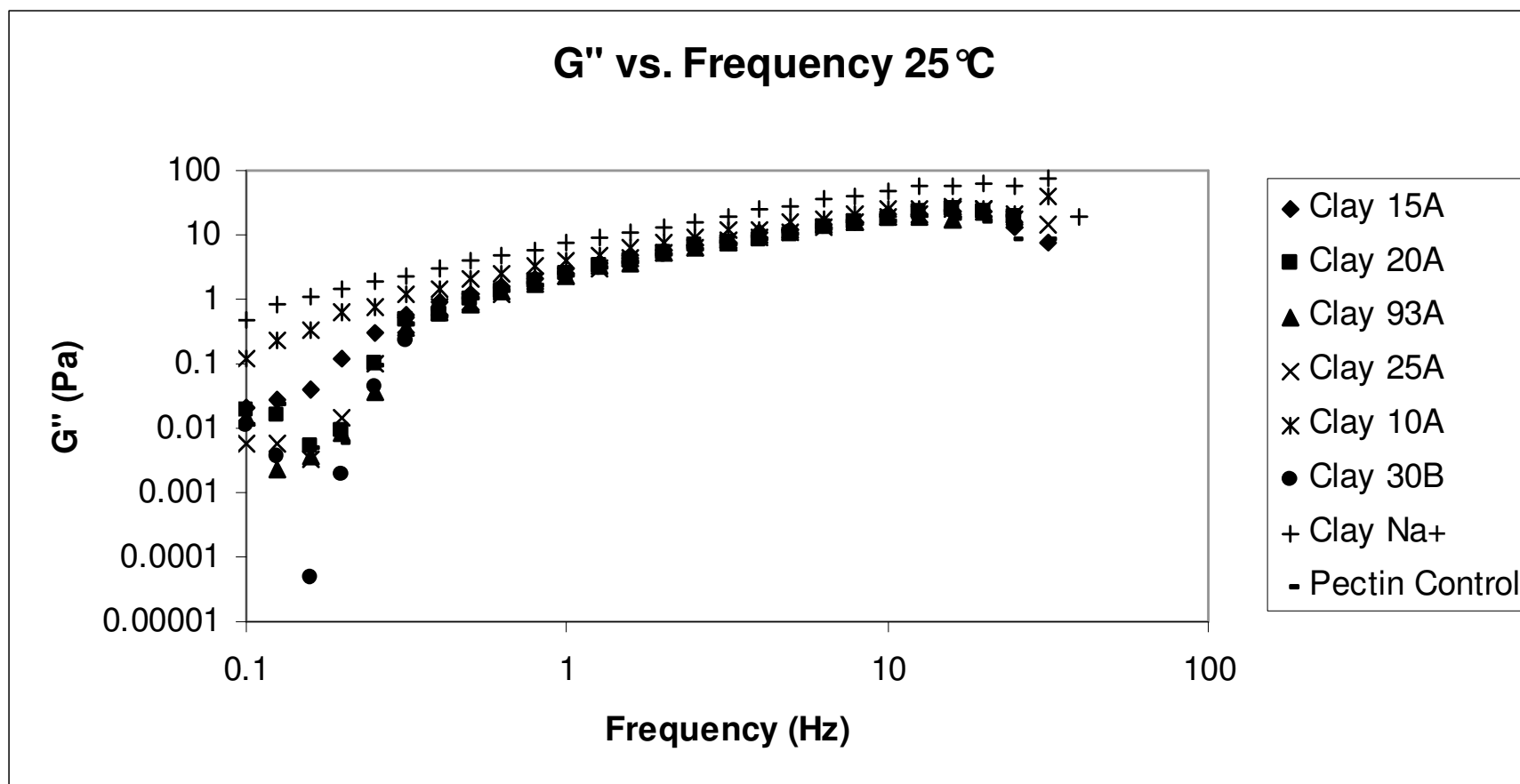


Figure 3.7

G'' pectin/clay dispersions dynamic frequency sweep at 25°C. G'' vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

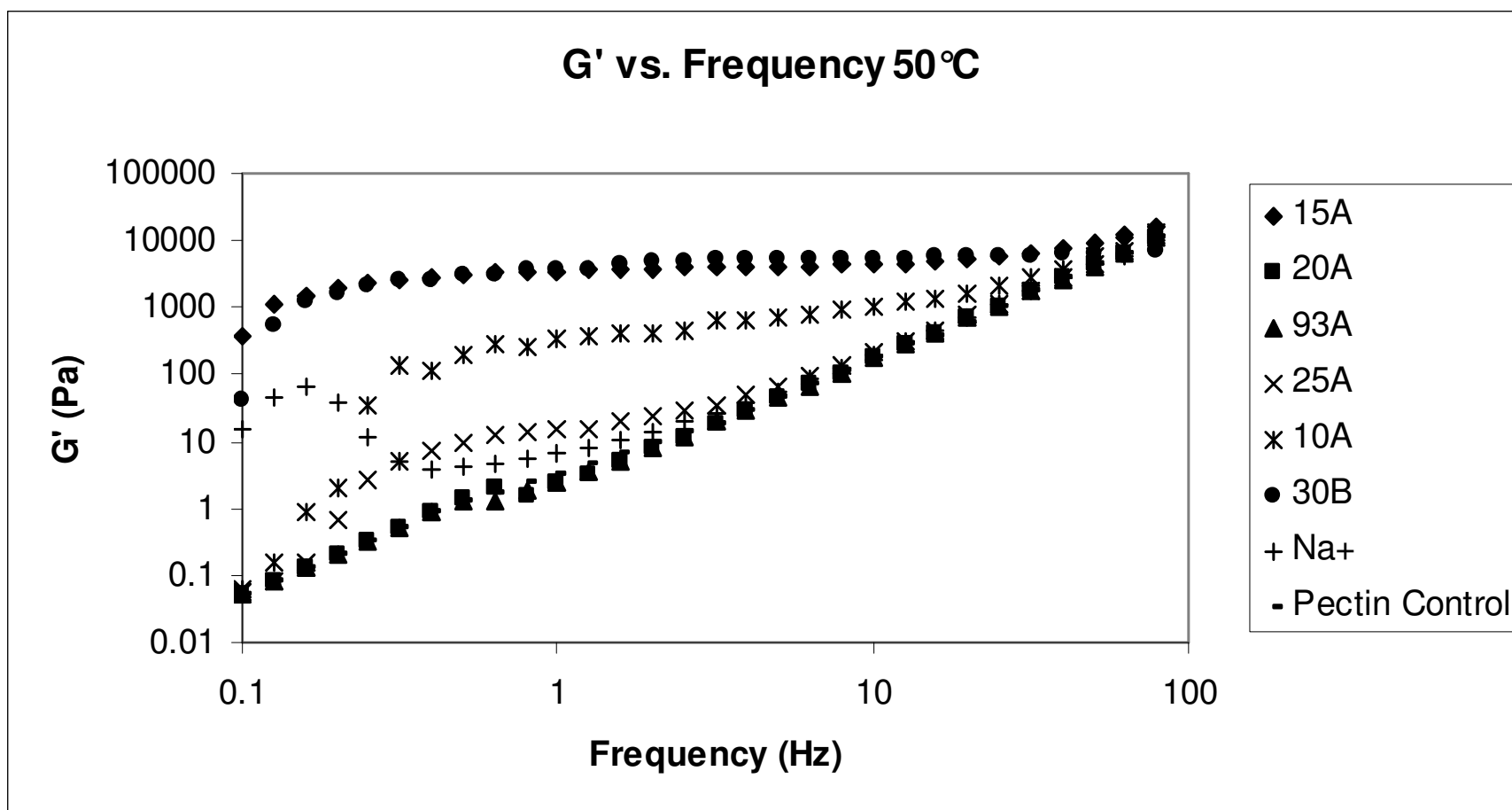


Figure 3.8

G' pectin/clay dispersions dynamic frequency sweep at 50°C. *G'* vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

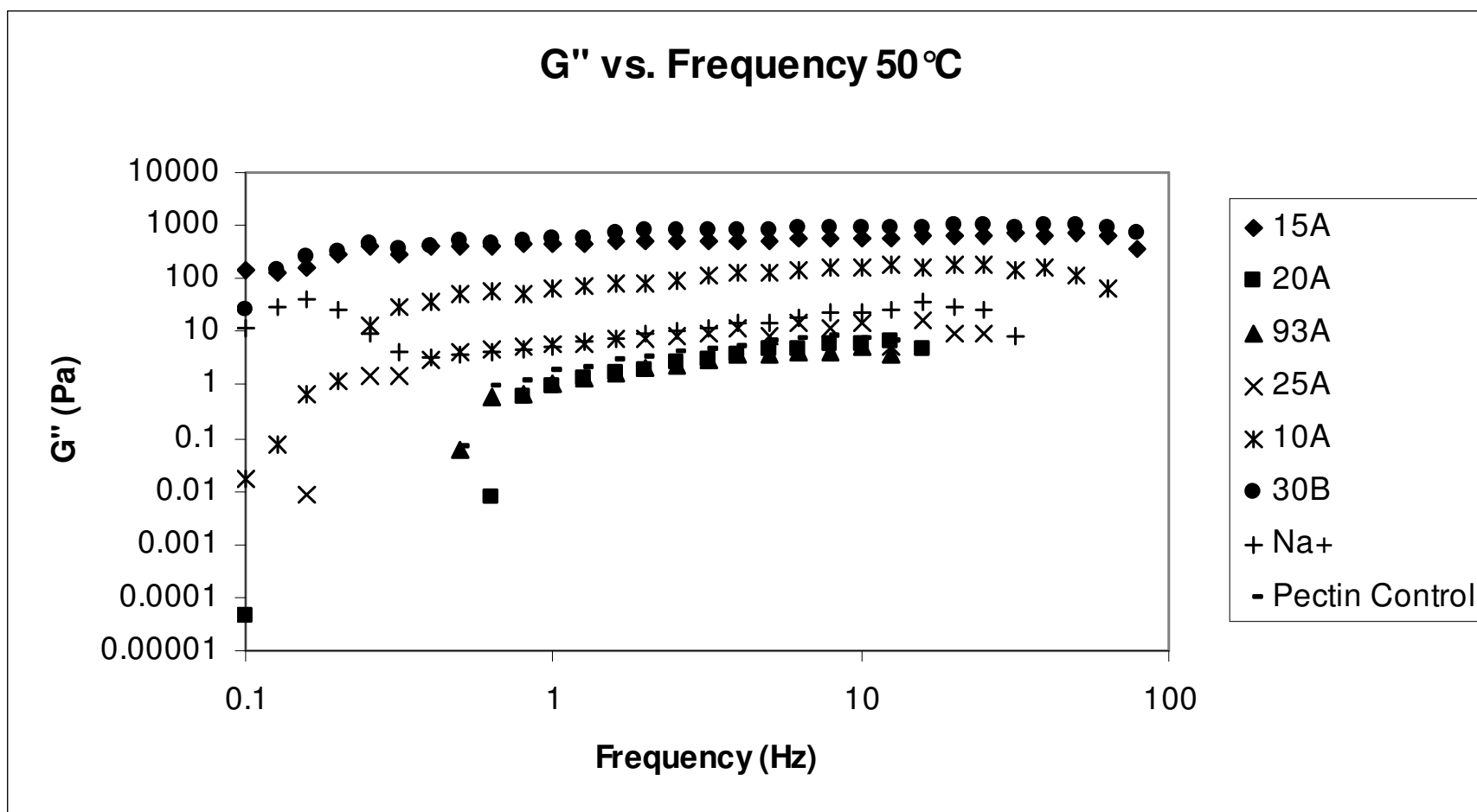


Figure 3.9

G'' pectin/clay dispersions dynamic frequency sweep at 50°C. *G''* vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

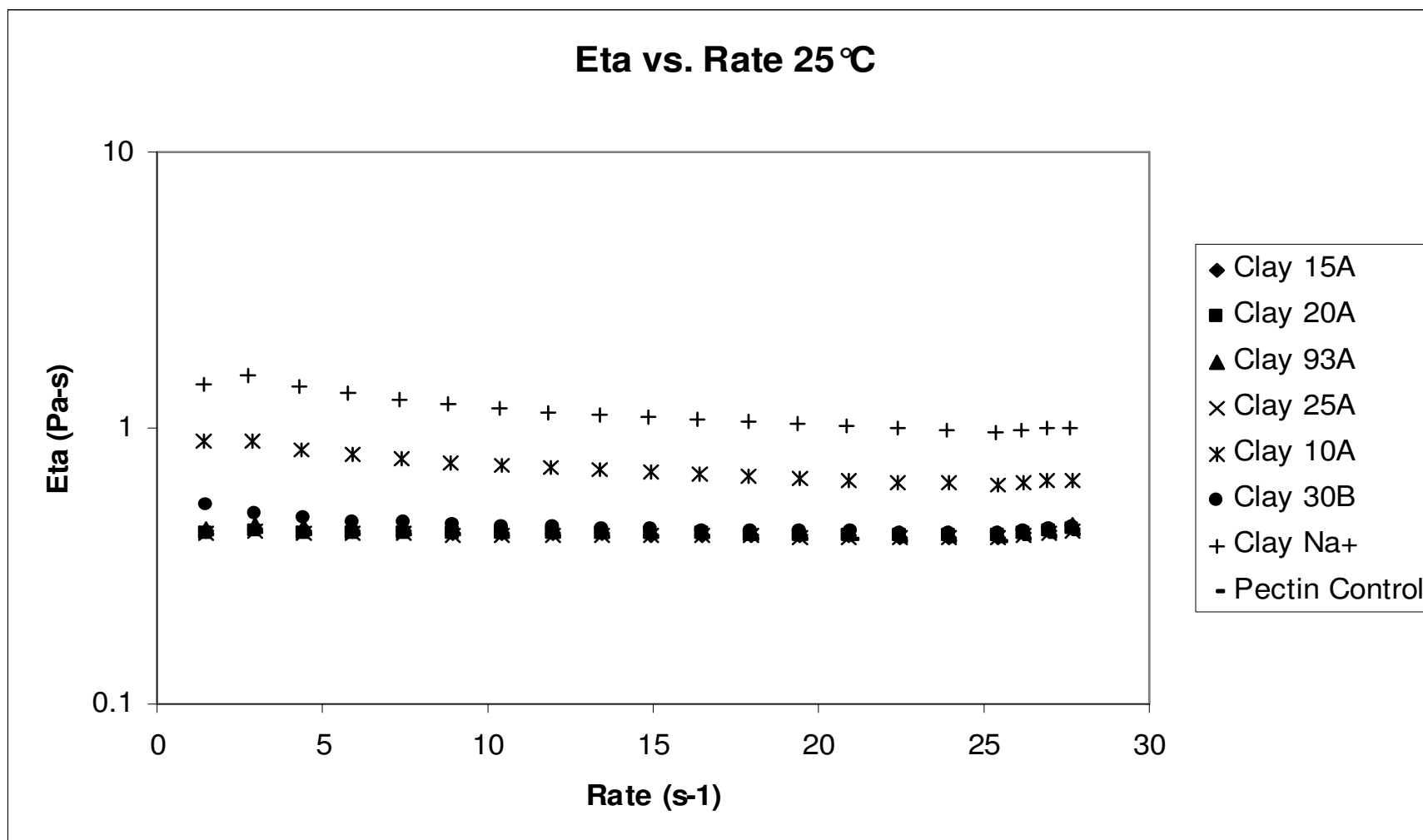


Figure 3.10

Comparison of the viscosity between pectin/clay samples with increasing shear rate at 25°C. Hydrophobicity of clay went from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

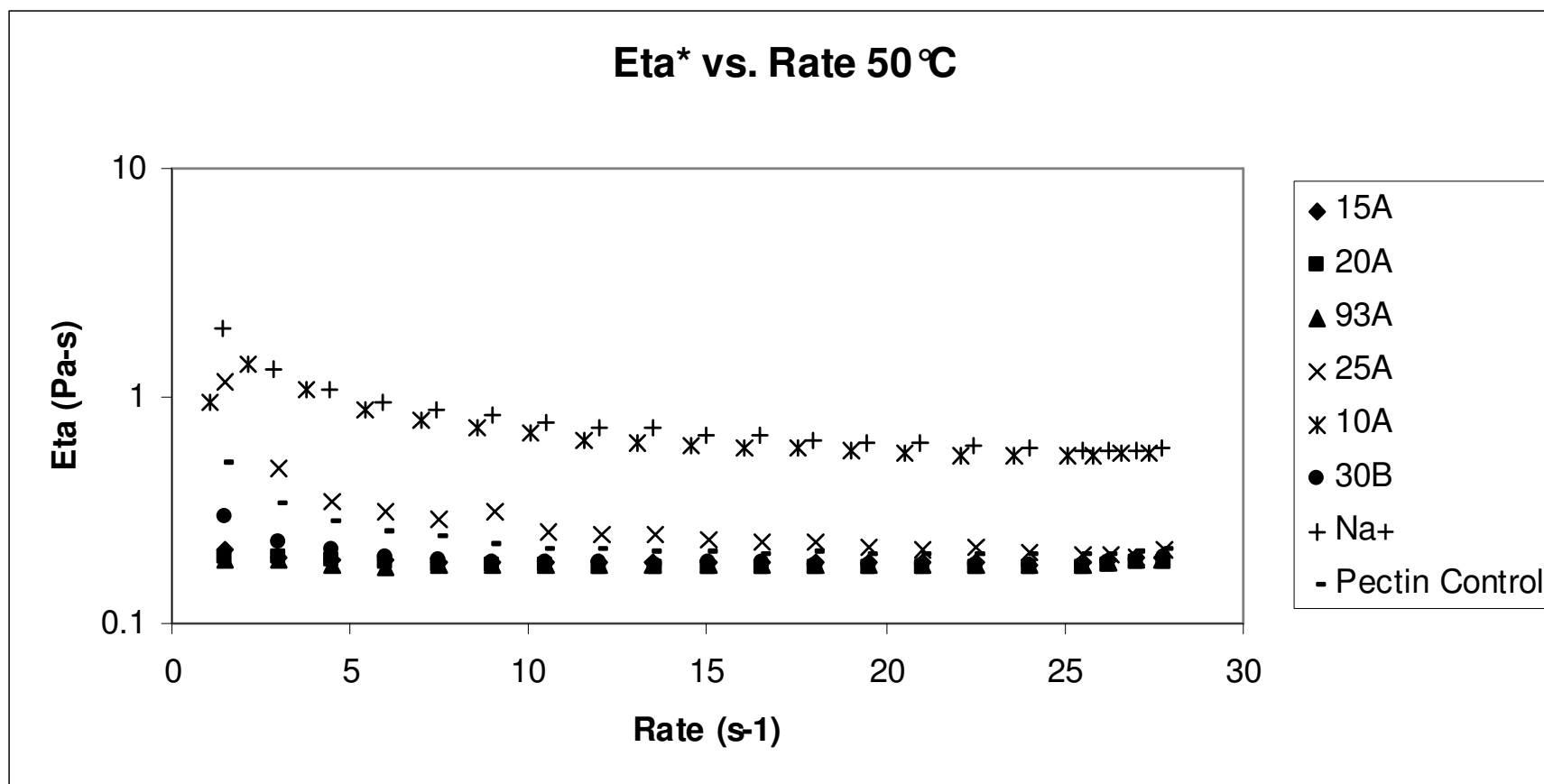


Figure 3.11

Comparison of the viscosity between pectin/clay samples with increasing shear rate at 50°C. Hydrophobicity of clay went from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

	Particle Size (nm)		Zeta Potential (mV)	
	Avg	Std. Dev.	Avg	Std. Dev.
15A	7953	1555.51	-25	1.55
30B	3580	452.79	-24	1.93
Na+	1889	132.54	-26	1.55
Pectin Control	913	44.98	-25	3.22

Table 3.1

ζ-potential of select samples at pH 4 based on hydrophobic and hydrophilic characteristics

	TC (seconds)		Intensity	
	Avg	Std. Dev.	Avg	Std. Dev.
15A	0.2190	0.0070	11352	582
30B	0.1526	0.0084	11289	1583
Na+	0.1401	0.0035	9696	120
Pectin Control	0.8996	0.0335	9902	497

Table 3.2

T2 relaxometry on select samples based on hydrophobic and hydrophilic characteristics

CHAPTER 4

RHEOLOGICAL TESTS TO LOW-METHOXY PECTIN/CLAY NANOPARTICLES

¹ Joshua Liang, Louise Wicker, William Kerr, and Jake H. Mulligan. To be submitted to *Carbohydrate Polymer*.

Abstract

Low-methoxy pectin solutions were blended with modified montmorillonite clay nanoparticles and characterized by strain sweep, frequency sweep, and shear rate ramp test measurements. Solutions were also characterized by particle size analysis, zeta potential, and NMR T2 relaxation tests. Seven clay types with varying levels of hydrophobicity including one hydrophilic (Na^+) were used. Rheological measurements were performed at 25°C and 50°C. No clear differences between samples were observed in strain and frequency sweep data; however, but viscosity measurements showed higher viscosity for Na^+ clay/pectin dispersion at both temperatures. Particle size and T2 relaxation analysis showed Na^+ samples dispersion had the smallest particle size with the fast relaxation rate. This correlated with the rheological data. Zeta potential analysis exhibited a fairly stable colloidal system that is invariant to the type of clay used.

Introduction

Low-methoxy (LM) pectins are complex carbohydrates considered to be heteropolysaccharide, non-repeating monosaccharide chains. Within its complex structure are smooth regions and hairy regions known as homogalacturonan and rhamnogalacturonan I and II (Voragen A.G.J., 1995). Low-methoxy pectins are defined as less than 50%, whereas high-methoxy pectins are greater than 50%. LM pectin used in the food industry can contribute to the manufacturing of such products like jams, jellies, and marmalades (Voragen A.G.J., 1995). Our previous studies on high-methoxy pectin solutions combined with montmorillonite clay nanoparticles showed a higher degree of interaction of the pectin to hydrophilic and less hydrophobic clays. This was observed from rheological measurements that included strain sweep, frequency sweep, and shear rate ramp tests (Liang, Wicker, Kerr & Mulligan, 2008).

Montmorillonite clay particles are of nano size in which the clay sheets are 1 nm thick with exchangeable cations between the sheets that can be modified for nanocomposite formations (Hrachova, Komadel & Fajnor, 2007). In this case, the low-methoxy pectin can be used to interact with the montmorillonite to form nanocomposite where the clay phase of the dispersion is less than 100 nm defining it as a nanocomposite (Chang, An, Cho & Giannelis, 2003; Rao, 2007). This low-methoxy pectin/montmorillonite clay study can be used to characterize and control the rheological properties of the solutions which then can be applied to food industry. LM pectin-clay solutions were characterized by dynamic strain sweeps, dynamic frequency sweeps, and viscosity measurements with increasing shear rate. These tests are then validated by particle size analysis, zeta potential, and NMR T2 spin-spin relaxation tests.

Methodology

Sample Preparation

Non-standardized low-methoxy pectins were donated by CP Kelco Company (Copenhagen, Denmark). Clays were donated from Southern Clay Products, Inc. (Gonzales, Texas). Clay grades used were 15A, 20A, 93A, 25A, 10A, 30B, and Na⁺ in order from most hydrophobic to hydrophilic with Na⁺ being hydrophilic clay. Concentration of clay was 1% wt/volume and LM pectin was 1% wt/volume. Acetate buffer at 0.1 M was used to keep samples around the same pH measured at 3.6-3.7 (Liang, Wicker, Kerr & Mulligan, 2008). Each type of clay was mixed separately in 200 mL of acetate buffer at a high temperature (Fu & Rao, 2001) with the temperature at 50°C. Samples were then stored at 4°C overnight for the purpose of hydration. Samples were then taken out and the low-methoxy pectins were then blended in to make the final dispersion. The samples were stored overnight at 4°C. Samples' pH was measured from range ~3.6-3.7. At 2%, the solution was too thick with aggregation and

coagulation (Liang, Wicker, Kerr & Mulligan, 2008). Therefore, the pectin was reduced to 1% wt/volume instead. Triplicates of samples of pectin/clay, including pectin control samples with no clay, were made.

Strain Sweep

Dynamic strain sweeps were performed on Rheometric Scientific SR-5000 rheometer using Couette flow geometry. Measurements include G' and G'' which helps determine the linear viscoelastic region (Herh, Tkachuck, Wu, Bernzen & Rudolph, 1998; Ramos-Tejada, Arroyo, Perea & Duran, 2001). G' and G'' can help determine the elastic or viscous nature of the solution depending on which modulus dominates the other. The modulus also helps determines the G' and G'' differences within the basic structure of the solution (MRK788-01, 2006). Low-methoxy pectin/clay sample strain sweeps were performed at both 25°C and 50°C. Parameters were set 0.1332% initial strain, 100.0% final strain, and 1 Hz frequency measuring 10 points per decade (Liang, Wicker, Kerr & Mulligan, 2008). All experiments were performed on triplicate dispersions.

Frequency Sweep

Dynamic frequency sweeps measurements were made on the Rheometric Scientific SR-5000 rheometer. Frequency sweeps were performed within the linear viscoelastic region (LVE) by setting the strain within the LVE region (MRK602-01). Parameters were set at 0.1332% strain and 0.1 Hz-79 Hz frequency range. Samples were measured at both 25°C and 50°C. Measurements were measure in triplicate.

Shear Rate

Shear rate experiments were performed in the Rheometric Scientific SR-5000 rheometer. Transient shear rate measured the viscosity and the shear-thickening or shear-thinning properties

of the dispersions along with determination of the type of flow, Newtonian or non-Newtonian (Herh, Tkachuck, Wu, Bernzen & Rudolph, 1998). Pectin/clay dispersions measured viscosity under a final shear rate of 30s^{-1} at zone time of 60 seconds at 25°C and 50°C . Couette flow geometry were used for the dispersion based on low viscosity properties.

Zeta Potential

Samples prepared at 1% low-methoxy pectin and 1% montmorillonite clay were diluted for zeta potential measurements (Tholstrup Sejersen, Salomonsen, Ipsen, Clark, Rolin & Balling Engelsen, 2007). 15A, Na^{+} , and pectin control were used for this experiment. Pectin control samples were diluted from 10 mg/mL to 0.10 mg/mL and pectin/clay samples were diluted from 20 mg/mL to 0.20 mg/mL. Dilution of acetate buffer was from 0.1 M to 0.01 M for each sample to prevent high concentrations of buffer in which the zeta potential analyzer will detect rather than the dispersions themselves. Diluted samples were measured at pH of ~ 4.0 . Particle sizes were measured in the Brookhaven particle size analyzer (Holtville, NY). Particle size and pH were then used to measure the zeta potential in the Brookhaven zeta potential analyzer (Holtville, NY). All samples were performed in average of 5 runs and done in triplicate for both particle size and zeta potential.

T2 Spin-Spin Relaxation

Samples used for T2 spin-spin relaxation were the previously prepared 1% low-methoxy pectin/1% montmorillonite clay dispersions. Dispersions used for NMR included 15A, Na^{+} , and the pectin control sample. 6 mL of samples were used for each tests performed on the Resonance RI NMR software of the Maran NMR instrument (Resonance Instruments, UK). 6 mL of samples were ran over 8 scans on CPMG, Carr-Purcell-Meiboom-Gill, setting (Kerr & Wicker, 2000; Meiboom & Gill, 1958). Relaxation time was determined through the Winfit

software provided. All results were performed on triplicate dispersions.

Results and Discussion

Dynamic Strain Sweep

Figures 4.1- 4.4 show a similar strain sweep trends. There are no clear differences between the different types of pectin/clay dispersions based on. The differences in interaction and stability could not be conceived by comparison among the strain sweeps of all the samples. This is much different from the previous studies with high-methoxy pectins/clay dispersions in which Na^+ and less hydrophobic samples had a higher G' and G'' , showing an increase in interactions (Liang, Wicker, Kerr & Mulligan, 2008). Another difference is G' is greater than G'' initially for both temperatures indicating more elastic characteristics for the low-methoxy pectin/clay dispersions in the linear-viscoelastic region. A result to note is that there is a slightly greater degree of plateau in the plots for 50°C G' and G'' for some of the samples such 30B than at 25°C.

G' and G'' change as the strain increases which gives indication to similar structural changes within each sample. *Figure 4.5* is an example of the 30A sample which shows the change of G' and G'' at both 25°C and 50°C. Both show very similar trends in which the G' eventually starts to decrease into the non-linear viscoelastic region but G'' remains moderately constant. This is an indication of partial breakdown in an elastic network structure within the solution which then may lead to a complete elastic structural breakdown once the G' crosses over the G'' and dominates the G' . Now the 30B sample has a more liquid-like structure than a solid-like characteristic at the non-linear viscoelastic region (Steventon, Parkinson, Fryer & Bottomley, 1990). This is the case for all of the samples showing relatively no structural differences in interactions based on dynamic strain sweep.

Frequency Sweep

Figures 4.6-4.9 shows the results of the dynamic frequency sweeps at 25°C and 50°C. At 25°C, the G' and G'' are very dependent on the frequency. Both modulus increases with increasing frequency indicating a much less stable dispersion for all of the samples. However, at 50°C, some samples, such as 30B, showed a plateau or constant G' at a certain frequency range before becoming dependent with increasing frequency again. This is similar to previous pectin/clay studies with high-methoxy pectins. At a higher temperature of 50°C, the 30B clay/high-methoxy pectin dispersion has almost no dependence with increasing frequency (Liang, Wicker, Kerr & Mulligan, 2008). The hydrophilic sample Na^+ do not show any differences between 25°C and 50°C, which is expected have a greater degree of stability because of the better interactions due to its hydrophilicity. This is also the case with all of the samples which is expected to have different results due to the varying degrees of hydrophobicities of each clay grade. Other studies with polymer/clay nanocomposites, such as poly(propylene carbonate), indicate differences in G' and G'' due to hydrolytic cleavage resistance that gave way to better interaction with a less hydrophobic 30B clay that results in a lower molecular weight of the poly(propylene carbonate) (Zhang, Lee, Lee, Heo & Pittman Jr, 2008). This may be the case in which the low-methoxy pectins do not have the same resistance so all the clay grades may interact to a higher degree. Hydrophobic interaction in the water of the acetate buffer may also provide to the similar results in which the apolar clumping of clay particles in the cage-like water molecule structure may cause the similarities in the readings and the increased temperature to 50°C breaks up some of those structures causing a more stable structure (Steventon, Parkinson, Fryer & Bottomley, 1990).

Shear Rate Measurements

For both temperatures at 25°C and 50°C, the Na⁺ has the highest viscosity measurements indicating that hydrophilic clay interacts with pectin to greater degrees among samples. This is shown in *Figures 4.10-4.11*. At 25°C, all of the samples exhibit a moderately constant viscosity with the increasing shear rate demonstrating a Newtonian flow. At 50°C, all of the samples demonstrate Newtonian flow. However, there is some indication of shear-thinning effect at low shear rates with the sharp decrease in viscosity at the initial shear rates. This is seen in previous studies with high-methoxy pectins and other polymers with increasing temperatures (Liang, Wicker, Kerr & Mulligan, 2008; Yang & Hu, 2006). The Na⁺ hydrophilic clay, with the Na⁺ exchangeable cation between the clay sheets, provides a catalysis of cross-linkage between the low-methoxy pectin and Na⁺ cation. This then leads to a higher viscosity reading (Kjønksen, Hiorth & Nyström, 2005). The higher viscosity in the Na⁺ samples shows a greater degree of interaction between the clay and low-methoxy pectin and therefore a more stable dispersion.

Zeta Potential

Table 4.1 gives the particle size and zeta potential for the most hydrophobic sample 15A, hydrophilic sample Na⁺, and pectin control samples. Particle size is greater in the 15A sample than the Na⁺. This may be due to the hydrophobicity of the 15A clay which prevents the low-methoxy pectin from interacting with the clay nanoparticles that creates a less dispersed system that possesses very high particle size. This lack of homogeneity may also be attributed to a clumping of the clay particles of different sizes that is indicated by the large standard deviation of the 15A particle sizes. Na⁺ has a much smaller particle size and standard deviation because of the better interaction that creates a better dispersion that do not result in clumping to provide a more homogenous dispersion. These results are very similar to past studies performed on high-

methoxy pectin/clays dispersions with 15A having the highest particle size and the Na^+ with the smaller particle size (Liang, Wicker, Kerr & Mulligan, 2008).

Zeta potential is the same for all of the selected samples measured as shown in *Table 4.1*. The result shows the invariance of zeta potential towards particle size, hydrophilicity, and hydrophobicity of samples. However, compared to the high-methoxy pectins, the low-methoxy pectin/clay dispersions has a greater magnitude of zeta potential which then leads a greater degree of stability (Liang, Wicker, Kerr & Mulligan, 2008). This then results in a greater surface charge and more stable pectin solution with low-methoxy pectins at (-25) mV compared to high-methoxy pectins, (-38) mV.

T2 Spin-Spin Relaxation

Table 4.2 shows the results of the NMR T2 relaxometry performed on 15A, Na^+ , and pectin control. The highest relaxation time came from the pectin control followed by 15A and Na^+ . The low-methoxy pectin control relaxation time was higher than high-methoxy pectin control time (Liang, Wicker, Kerr & Mulligan, 2008), which is much different from previous studies at which the high-methoxy time has a higher relaxation time (Kerr & Wicker, 2000). However, the concentration of low-methoxy pectin, in this case, is lower than the previously studied high-methoxy pectin solutions, which then may attribute to a lower relaxation rate because of the higher concentration (Liang, Wicker, Kerr & Mulligan, 2008). The relaxation times correlate with the shear rate viscosity measurements. The Na^+ has a the shortest relaxation time meaning it has the tighter bound system because the greater degree of interaction with intercalation (Nakashima, 2004). The greatly bound system of Na^+ has a more limited mobility of free water compared to the 15A and pectin control which has a more mobility of free water that correlates with a lower viscosity because of a less tightly bound system. These results are

comparable to high-methoxy pectin/clay studies done with the same clay nanoparticles. The 15A clay/pectin dispersions displayed a much higher relaxation rate while the Na^+ exhibited the shortest relaxation time (Liang, Wicker, Kerr & Mulligan, 2008).

Conclusion

The dynamic strain sweep and dynamic frequency sweep do not indicate clear differences in the moduli between the different pectin/clay dispersions. The results show no major differences between samples at 25°C and 50°C. However, at both temperature, there is a critical strain for each sample in which the G' starts to decrease and goes into the non-linear viscoelastic regime. This eventually leads to a partial elastic structural breakdown which results in a more viscous liquid-like characteristic. The frequency sweep, gave indication to a less stable dispersions because of the dependency upon the frequency. Shear rate readings give a better comparison among the different samples in which the hydrophilic Na^+ clay/pectin dispersions has the greatest viscosity. At 50°C, all of the samples have a slight shear-thinning effect before heading into a Newtonian flow. The viscosity measurement is correlated by the particle size and T2 spin-spin relaxation tests. The most hydrophobic sample, 15A, has a longer relaxation rate than the Na^+ /clay dispersion samples likely due to a less tightly bound colloidal system. The particle size indicates the 15A grade clay having a higher average particle size with a larger standard deviation indicating a less stable system providing a greater case of sedimentation.

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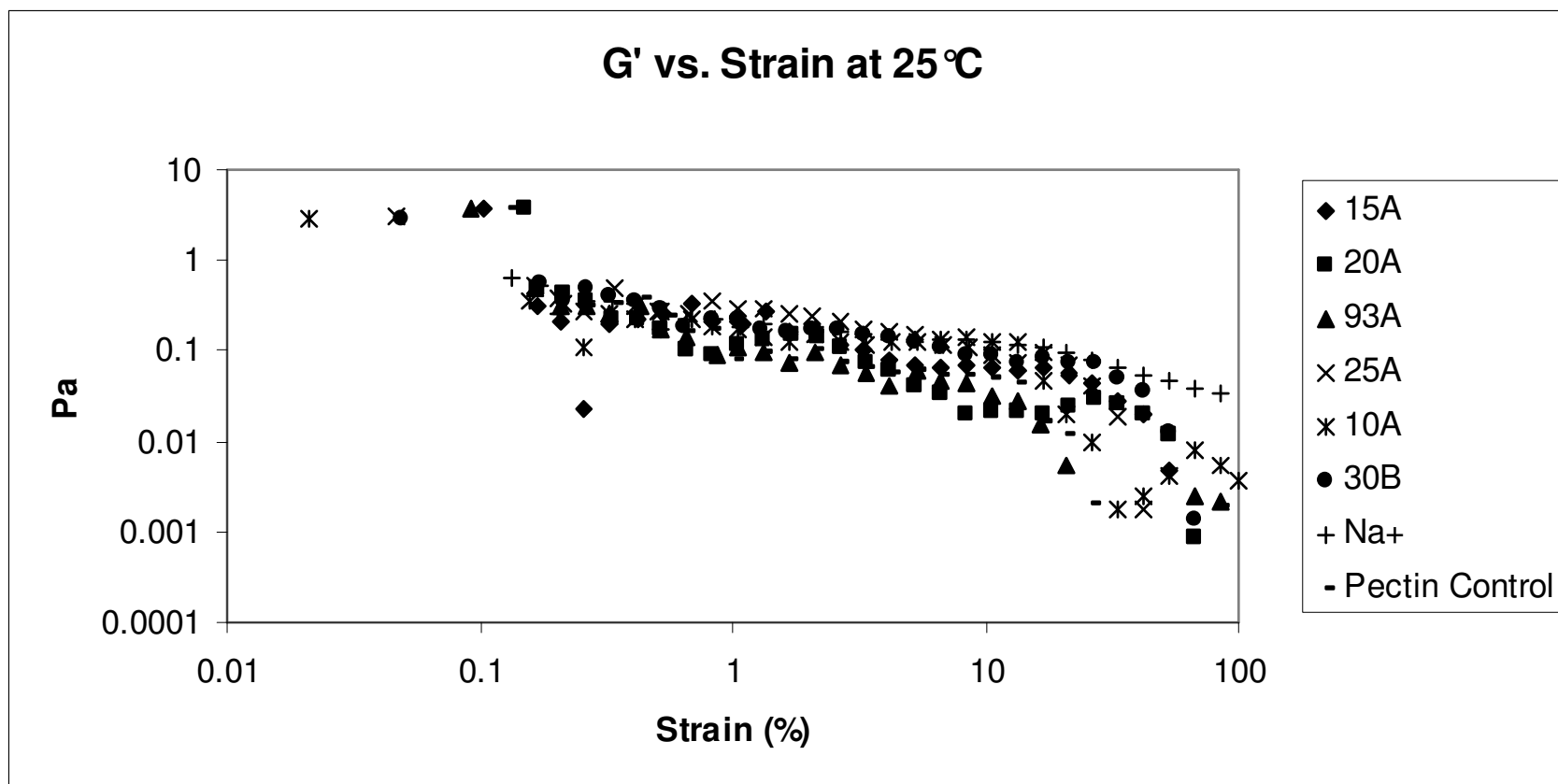


Figure 4.1

G' pectin/clay dispersions dynamic strain sweep at 25°C. G' vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

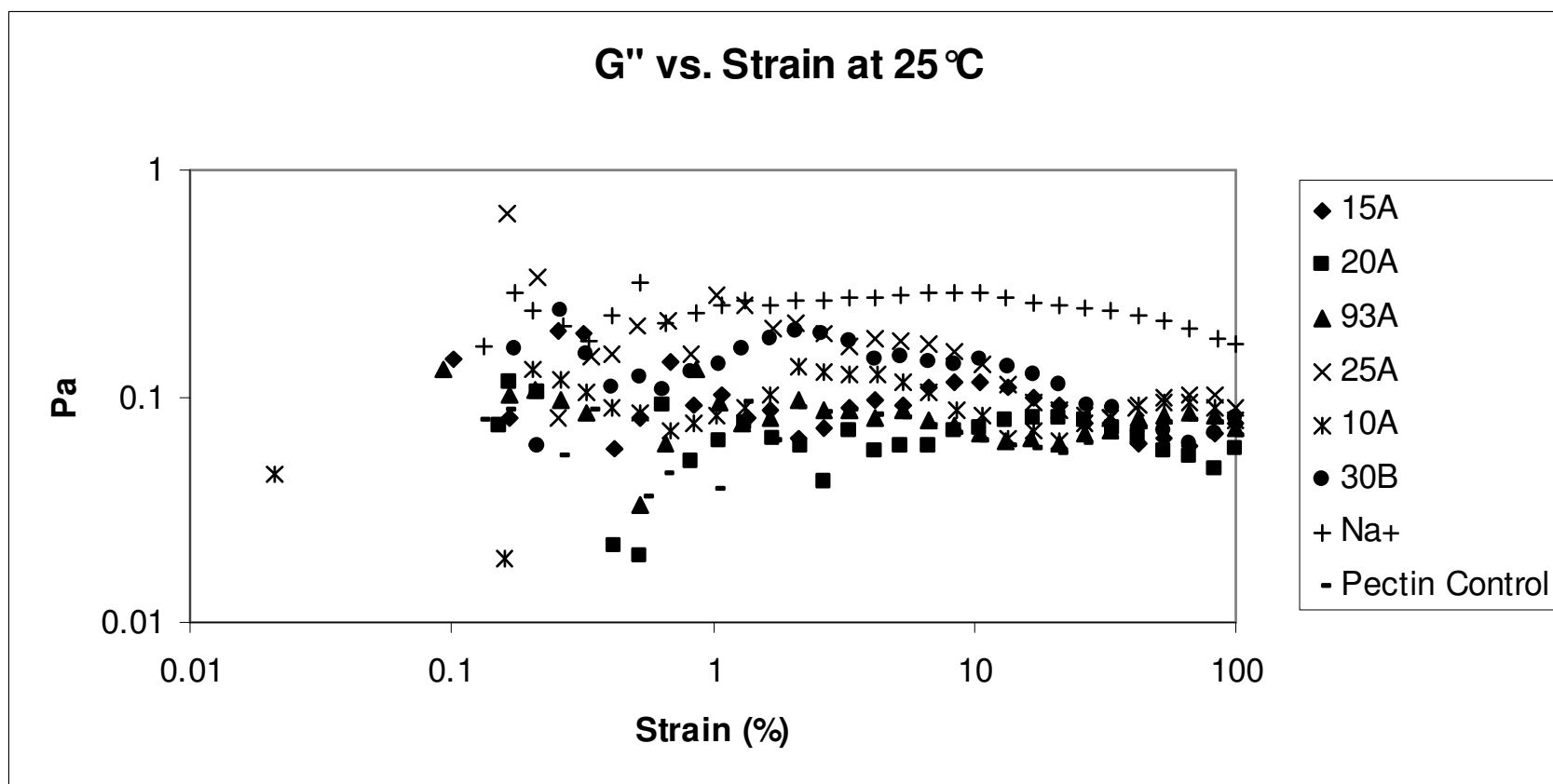


Figure 4.2

G'' pectin/clay dispersions dynamic strain sweep at 25°C. G' vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

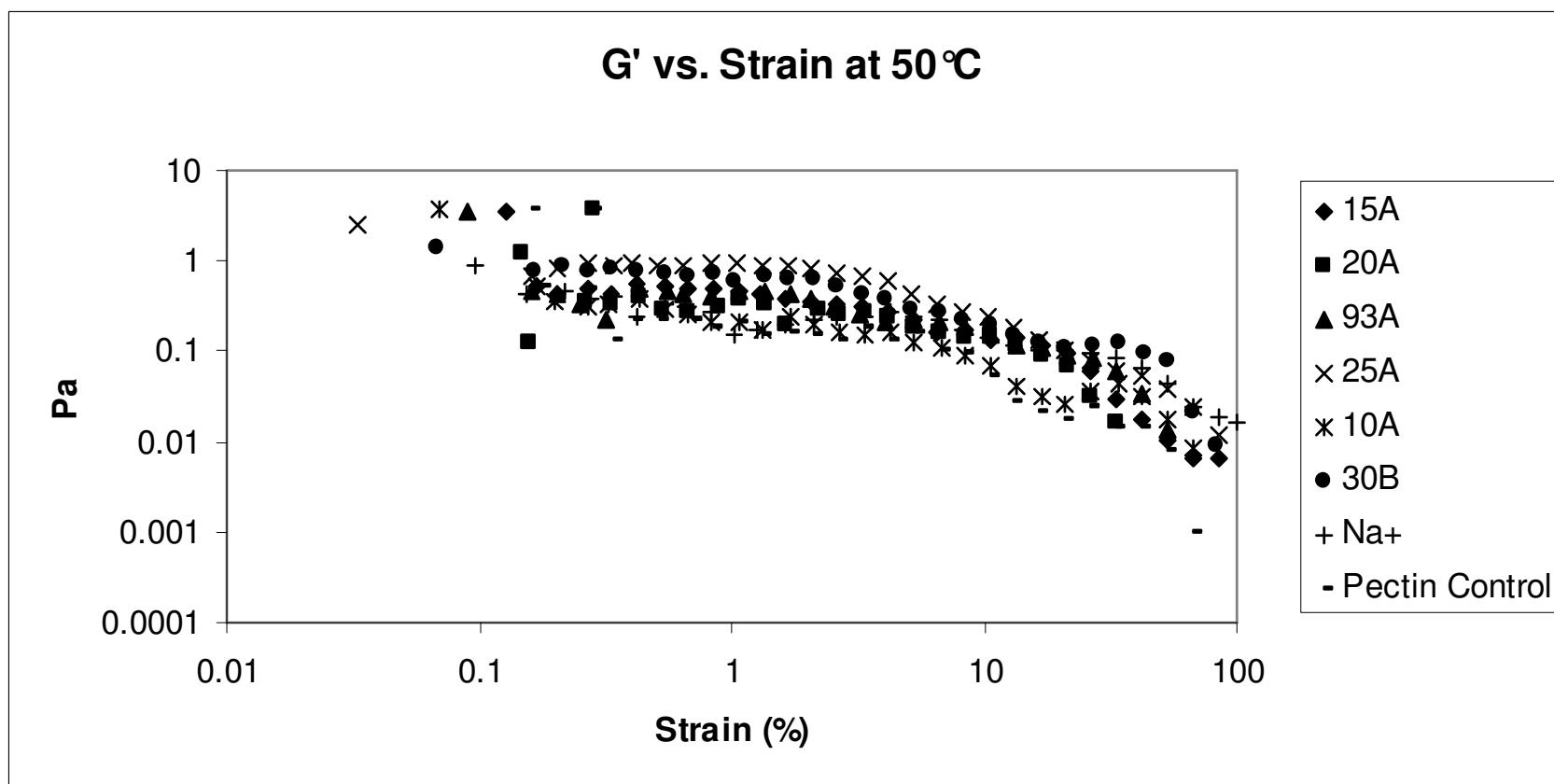


Figure 4.3

G' pectin/clay dispersions dynamic strain sweep at 50°C. G' vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

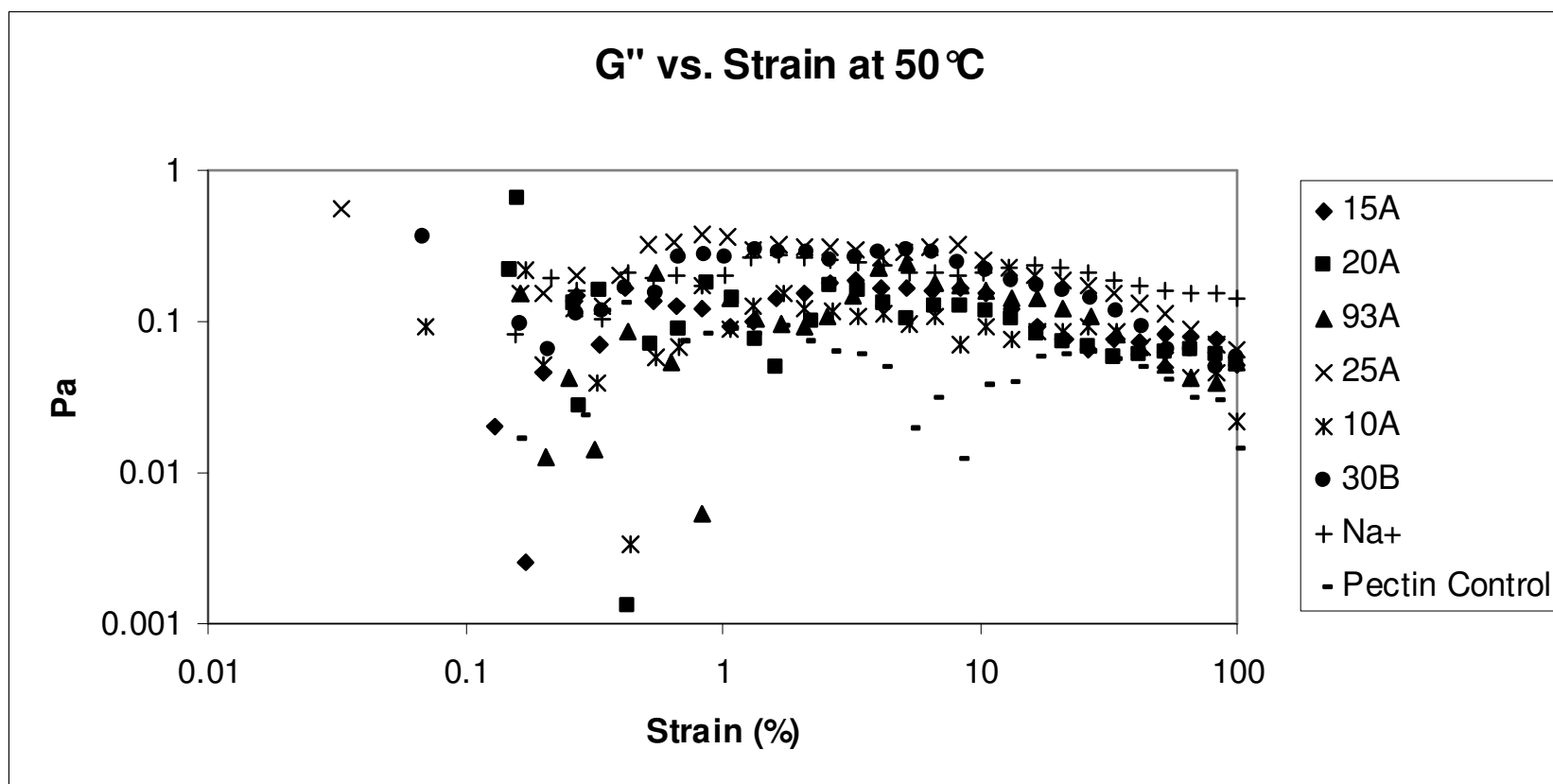


Figure 4.4

G'' pectin/clay dispersions dynamic strain sweep at 50°C. *G''* vs. strain (%) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

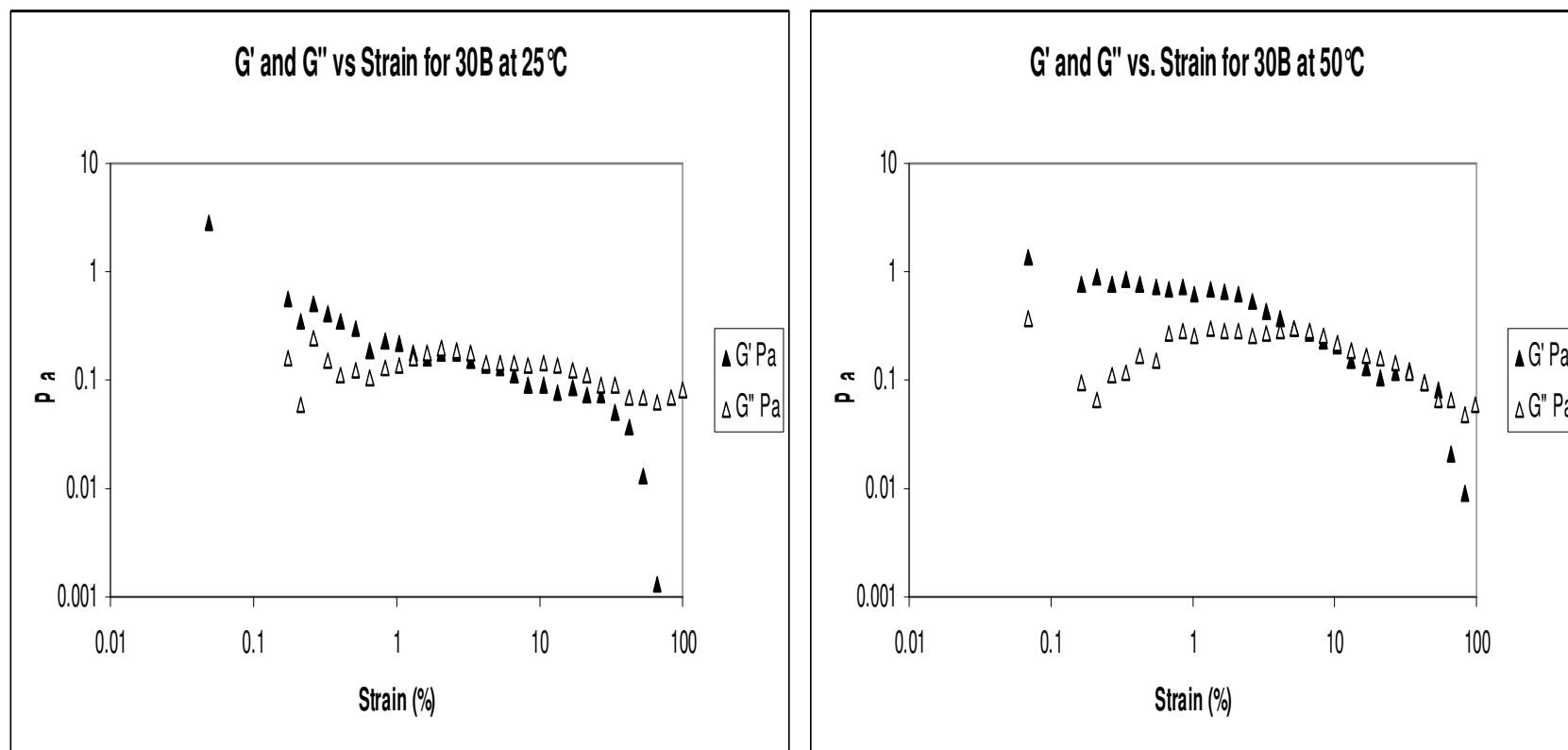


Figure 4.5

Example G' and G'' comparison based on 25°C and 50°C for 30B clay/pectin dispersion. This is an example at which both temperatures exhibit the same partial structural breakdown where G'' eventually dominates G' giving the pectin/clay dispersion a more liquid like characteristic past a certain strain.

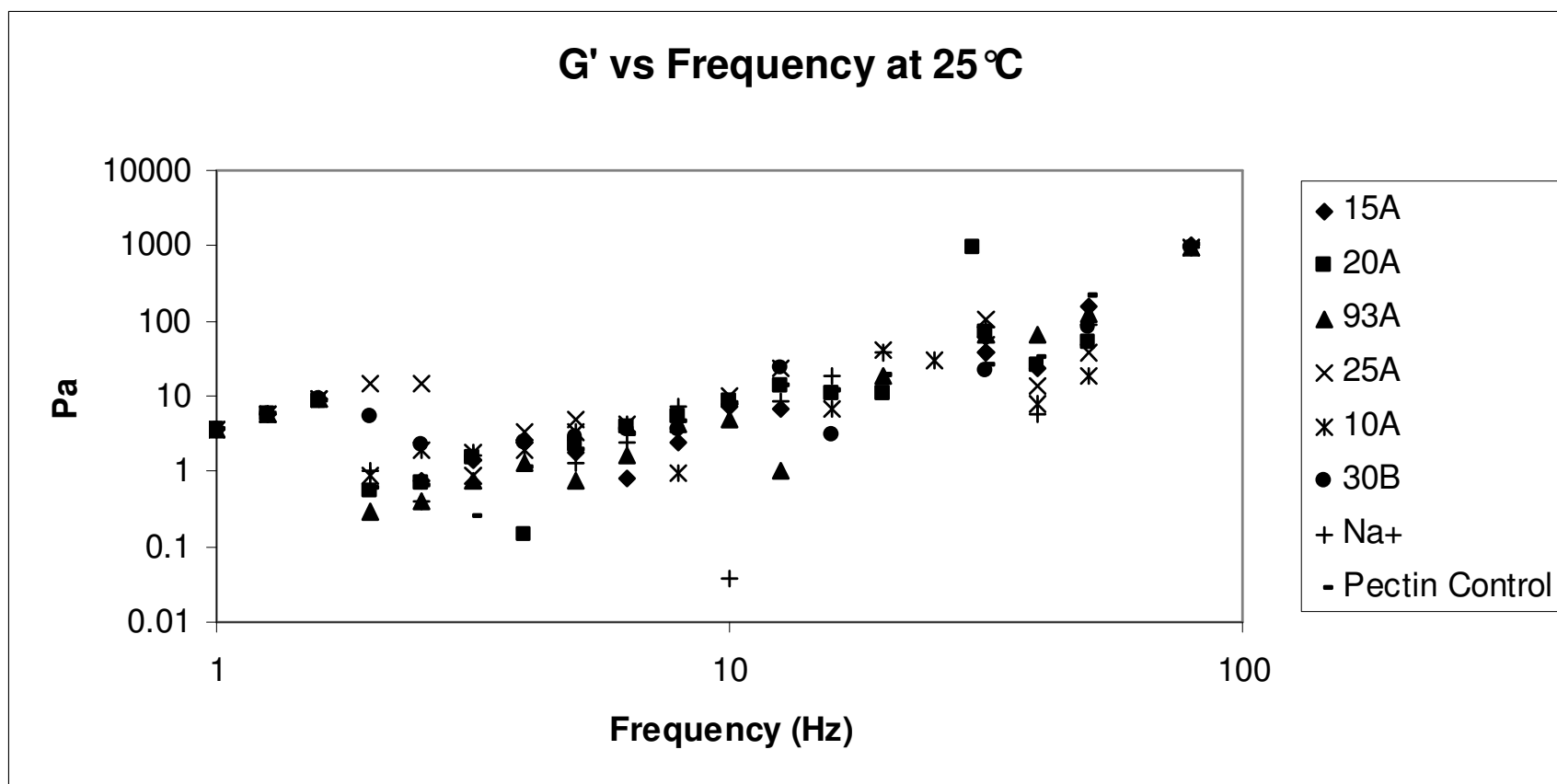


Figure 4.6

G' pectin/clay dispersions dynamic frequency sweep at 25°C. *G'* vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

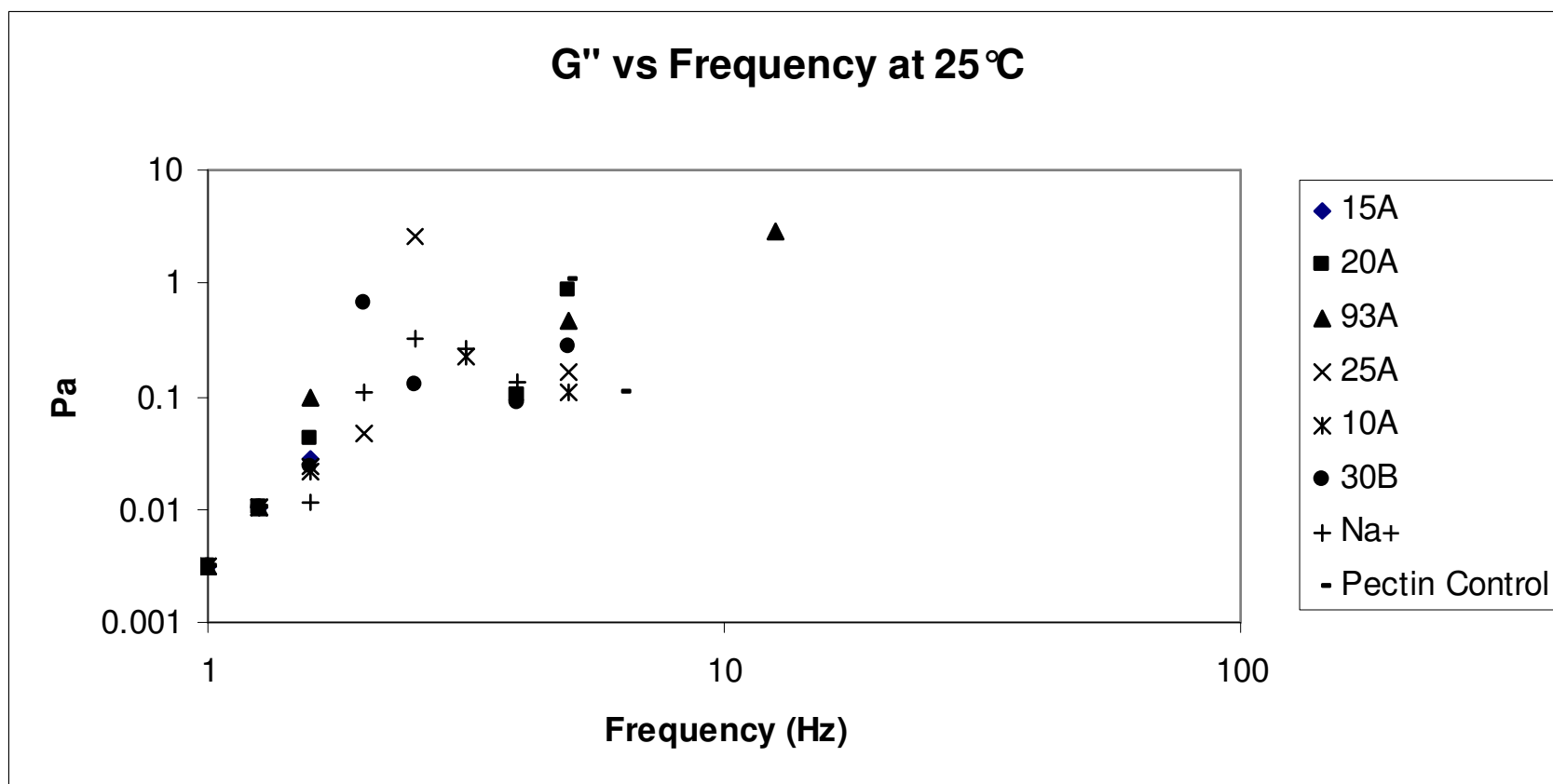


Figure 4.7

G'' pectin/clay dispersions dynamic frequency sweep at 25°C. G'' vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

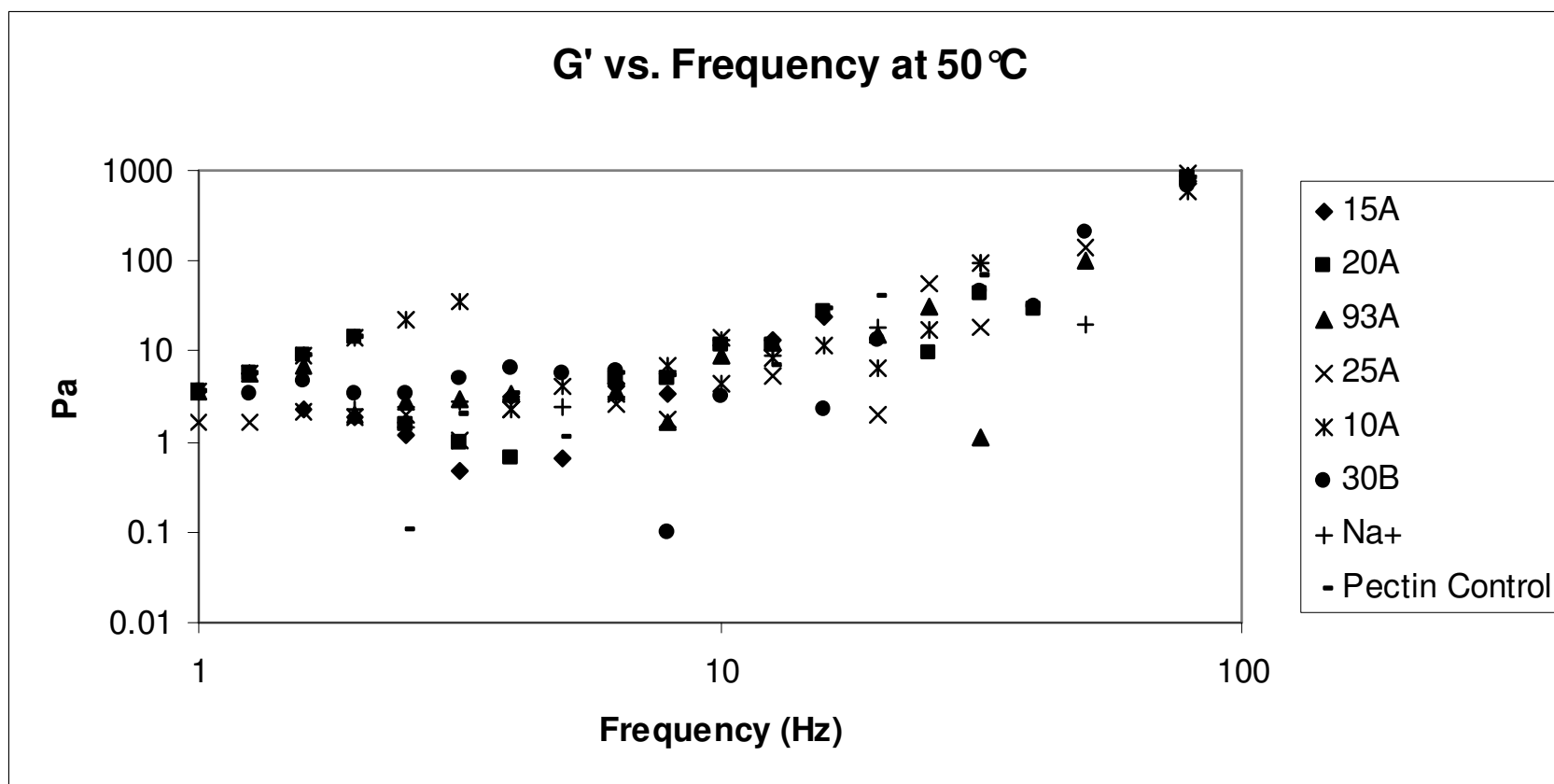


Figure 4.8

G' pectin/clay dispersions dynamic frequency sweep at 50°C. G' vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

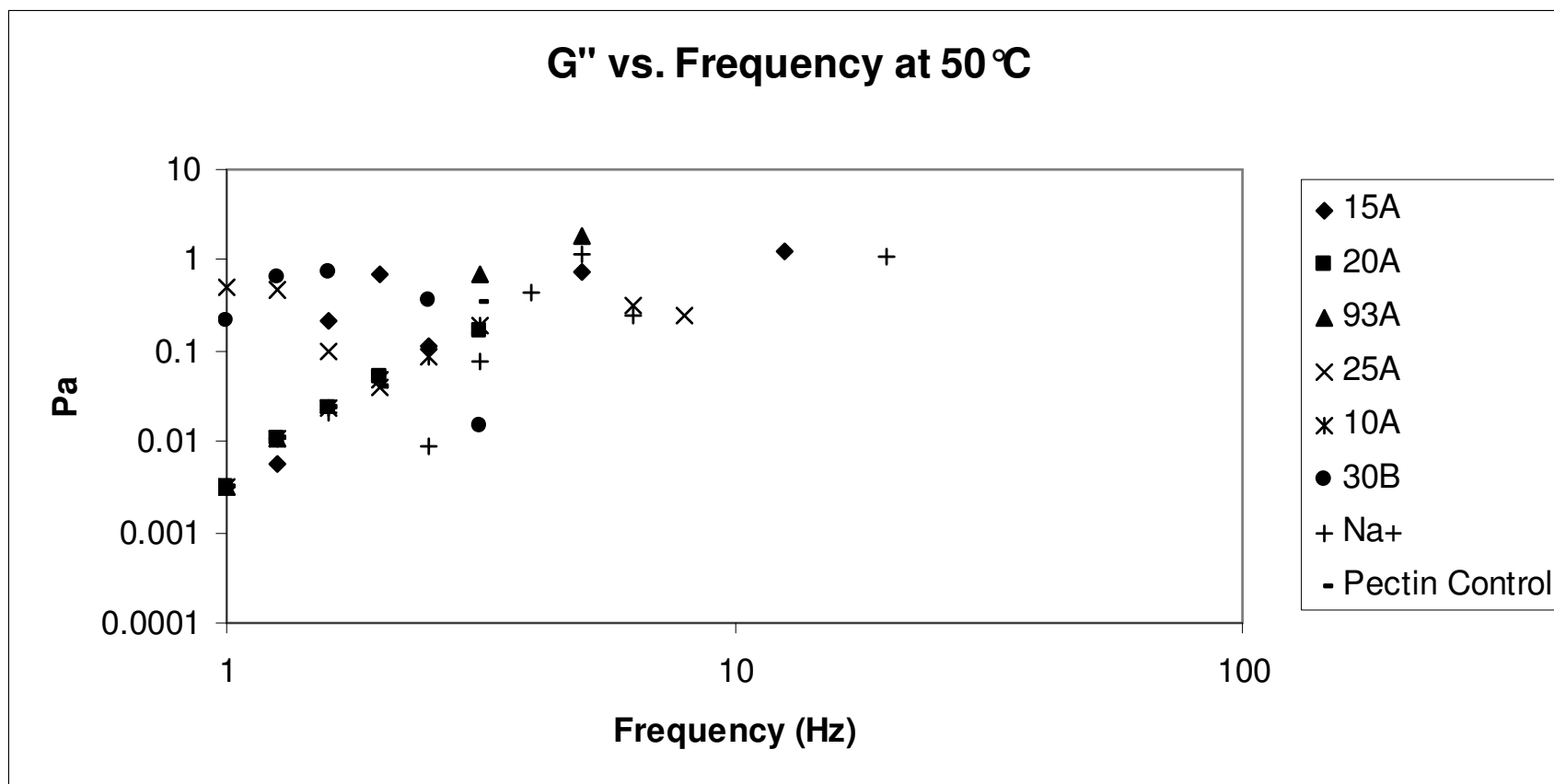


Figure 4.9

G'' pectin/clay dispersions dynamic frequency sweep at 50°C. G'' vs. frequency (Hz) with hydrophobicity of clay from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

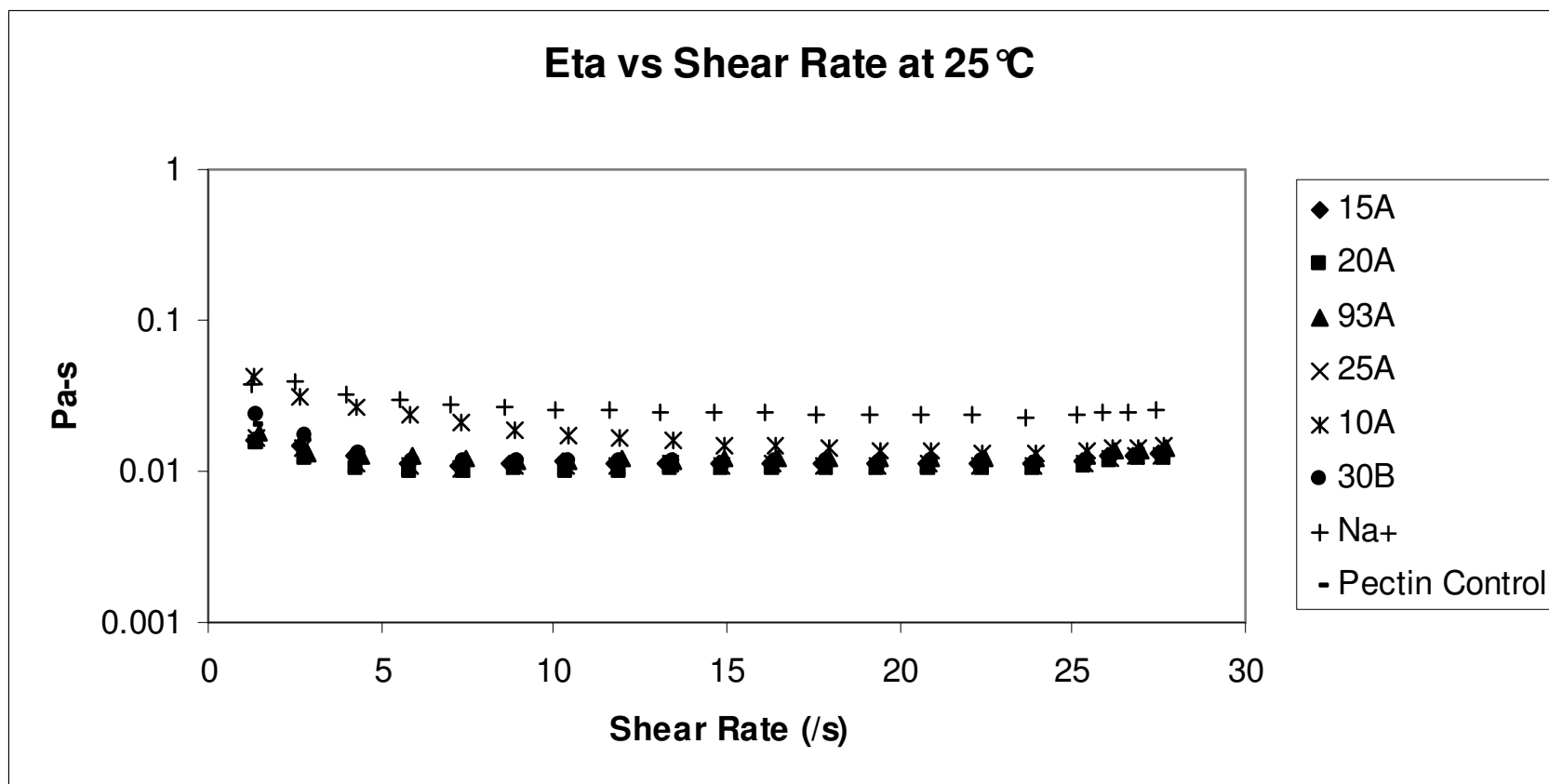


Figure 4.10

Comparison of the viscosity between pectin/clay samples with increasing shear rate at 25°C. Hydrophobicity of clay went from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

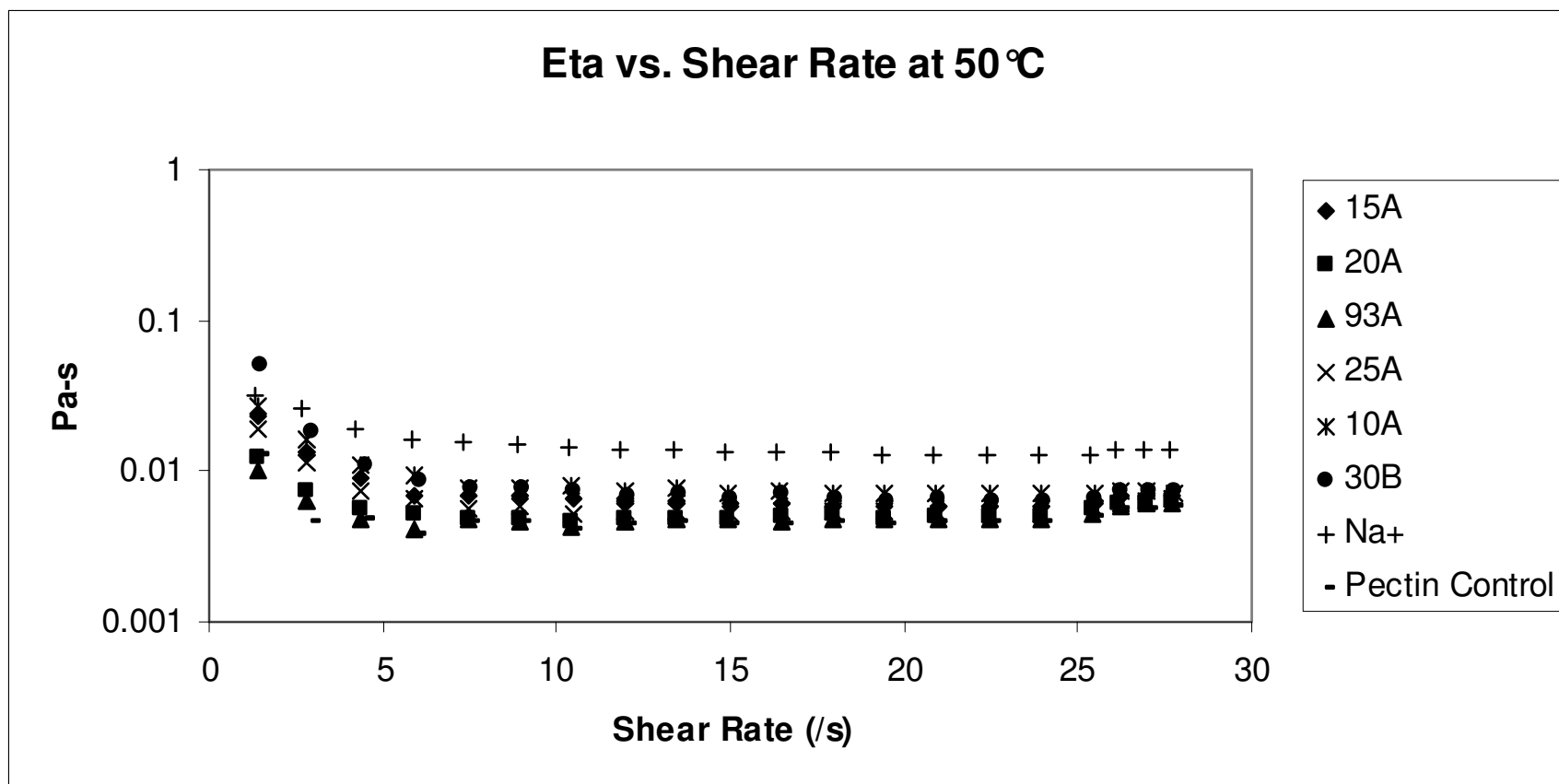


Figure 4.11

Comparison of the viscosity between pectin/clay samples with increasing shear rate at 50°C. Hydrophobicity of clay went from 15A>20A>93A>25A>10A>30B>Na⁺. Na⁺ is a hydrophilic clay sample.

	Particle Size (nm)		Zeta Potential (mV)	
	Avg	Std. Dev.	Avg	Std. Dev.
15A	7493	3697	-37.41	0.42
Na+	1712	447	-36.49	0.47
Pectin Control	984	51	-37.71	0.65

Table 4.1

ζ-potential of select samples at pH 4 based on hydrophobic and hydrophilic characteristics

	TC (seconds)		Intensity	
	Avg	Std. Dev.	Avg	Std. Dev.
15A	0.2823	0.0403	14200	354
Na+	0.1747	0.0041	15844	276
Pectin Control	1.2548	0.0776	12238	132

Table 4.2

T2 spin-spin relaxometry on select samples based on hydrophobic and hydrophilic characteristics.

CHAPTER 5

CONCLUSION

A clear difference existed between high-methoxy and low-methoxy pectin when mixed with montmorillonite clay. However, this is considered a validated difference because of the concentration differences with HM pectin samples being higher than the LM pectin samples. When combined with montmorillonite, the HM pectin/clay dispersion show differences based on different degrees of hydrophobicity and hydrophilicity among the grades of montmorillonite clay. The LM pectin did not show as much of a pronounced difference. That may be due to the lower concentration of pectin used which affected the rheological results of the experiments. However, in both cases, the hydrophilic clay Na^+ exhibited the highest readings for both pectins when compared to the rest of the clays. For the HM pectin samples, the lesser hydrophobic samples had higher strain sweep and shear rate values, showing a trend in which most hydrophobic had the lowest value and the hydrophilic samples had the highest values. This is an indication of higher degree of interaction between the pectin and clay in which the particles are more tightly bound and better dispersed. The particle size analysis and NMR relaxation data for both studies verified this theory. The particle size was the largest in the most hydrophobic sample 15A with a high standard deviation. The Na^+ hydrophilic sample had the lowest particle size and lower standard deviation meaning greater stability, less clumping of the clay with a higher degree of interaction, more homogeneity, and lesser degree of sedimentation. NMR relaxation tests also complimented the particle size with Na^+ having the fastest relaxation times and 15A the slowest. The transverse data for Na^+ showed a more tightly bound particle and longer decay time meaning it takes a longer time for the relaxation back to its original state. This

then was vice versa for the 15A sample. Zeta potential was higher in LM pectin samples than HM pectin samples. However, within each study, the zeta potential was the same showing invariance of the clay grade to the colloidal surface stability of the dispersions.

These two studies can be applied to the study of liquid food rheology in which there have been several studies done on already such as different juices (Gratão, Silveira & Telis-Romero, 2007; Ibarz, Garvin & Costa, 1996; Ibarz & Pagán, 1987). These montmorillonite clay nanoparticles can be used in such applications of fruit juice processing in which the flow property with thermal treatment can be controlled with montmorillonite clay nanoparticles.

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