RHEOLOGY AND GELATION OF PHYSICAL POLYMER GELS

By

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ABSTRACT

The discovery of new potent and water-insoluble drugs has emphasized the need for novel drug-delivery systems and personalized dosification. Biopolymers solutions are an alternative over conventional methods since they can acts as a suspending medium for strong hydrophobic drugs, and have the potential to form gel networks, which can be used to encapsulate and improve long-term stability. Nevertheless, polymer-particle interactions can affect the rheology and gelation process of the system making it unsuitable for the application or affecting the drugs activity. Much more complex interactions are introduced by incorporation of additional components into the system, such as surfactants to improve particle stability or flavorants for taste-masking. The objective of this research work was to determine the effects of fluid formulation parameters on the physical gelation and rheology of biopolymer solutions with suspended solid particles.

In this work, rheological measurements have been used to determine, understand and control the processing conditions which are related to the final product properties. Gelation temperature (T_{gel}) was determined using stress control temperature ramp and the dynamic oscillatory methods and rheology of polymer solution was studied above T_{gel}. The effect of operational and formulation parameter on T_{gel} were also determined.

In Chapter 2 and 3, the effect of particle size and concentration on the gelation temperature of two cold-setting gels, gelatin and sodium alginate (NaAlg), were studied. It was observed that T_{gel} is not affected by stress at applied stress of up to 10 Pa, and decreases with increasing cooling rate. On the other hand, as polymer concentration increases gelation temperature increases. Yet, pH has a very mild effect, as shown in Chapter 2. In Chapter 3, the thermotropic gelation of NaAlg solution was demonstrated using three independent rheological measurements. Both systems were unaffected by the presence of model silica particles ranging from the micron to the nano scale. This was attributed to polymer-particle repulsion due to similar net charges.

In Chapter 4, the effect of an active ingredient and the interaction between type of surfactants and the polymer-particle system was determined by the rheology and gelation temperature measurements. In this case, the model polymer system was the hot-setting hydroxypropylmethyl cellulose (HPMC), an uncharged cellulose derivative. The effect of particle size on solution properties was studied using griseofulvin particles as model drugs. T_{gel} showed complex dependence on particle size, concentration, and surface selectivity. Surfactants with similar net charge as the active ingredient can be either replaced by the polymer on the active's surface or aggregate with the polymer, thus increasing viscosity of the solution and improving gelation due to bridging effects. On the other hand, positively charged and neutral surfactants have minimum polymer-surfactant interactions and are able to effectively stabilize the particles due to the preferential interactions with the active's surface.

Rheological methods were useful to demonstrate physical gelation for a weak gel, propose particles stabilization mechanisms and establish particle size and concentration effects on gelation. This information can be extended to other systems such as characterization of a weak gel, or systems with similar characteristics, where use of surfactant is needed for charge particle stabilization. These data and mechanisms can be correlated to final product properties as drug dissolution, adhesion, or mechanical properties, important in but not limited to films.

RESUMEN

El descubrimiento de nuevas drogas que se caracterizan por ser fuertes e insolubles en agua ha creado la necesidad de nuevos sistemas para entrega de medicamento que garanticen una dosis personalizada. Biopolímeros brindan una solución alternativa en relación a métodos convencionales, debido a que ellos pueden actuar como medio de suspensión para medicamentos fuertes e hidrofóbicos, además tienen la habilidad de formar geles, esta propiedad puede ser usada para encapsular y mejorar la estabilidad a largo plazo. Sin embargo, interacciones polímero-partículas pueden afectar pueden afectar la reología y el proceso de gelación de el sistema haciéndolo inadecuado para la aplicación o afectando la actividad de la droga. Interacciones mucho más complejas suceden cuando se incorpora un componente adicional como lo son los surfactantes o saborizantes, los cuales se utilizan para impartir estabilidad o modificar el sabor original, respectivamente. El objetivo de esta investigación fue determinar los efectos de los parámetros de formulación y operación sobre la gelación física y la reología de de soluciones de biopolímeros con partículas solidas suspendidas.

En este trabajo, mediciones reológicas se usaron para determinar, entender y controlar las condiciones de procesamiento, las cuales están relacionadas a las propiedades del producto final. Temperatura de gelación (T_{gel}) fue determinada usando la prueba de stress controlado y el método dinámico, y la reología de las

soluciones poliméricas se estudio por encima de T_{gel} El efecto de los parámetros de formulación y operación sobre T_{gel} fueron también determinados.

En el Capítulo 2 y 3, el efecto de tamaño y concentración de partículas sobre la temperatura de gelación de dos polímeros que forman geles con disminución de temperatura fueron estudiados. Se observo que T_{gel} no es afectada por el stress a un stress aplicado hasta de 10 Pa, y disminuye con razón de enfriamiento. Por otro lado, cuando la concentración de polímero aumenta, la temperatura de gelación incrementa. Sin embargo, pH no tuvo un efecto significativo sobre T_{gel}. En el Capítulo 3, se demostró la gelación termotrópica de soluciones de NaAlg mediante tres métodos reológicos independientes. Ambos sistemas no fueron afectados por la presencia de partículas de sílica desde micrones a escala nano, debido a repulsiones polímero-partícula debido a una carga neta similar.

En el Capítulo 4, el efecto de un ingrediente activo y la interacción entre tipo de surfactante y el sistema polímero-partícula fue determinado por la reología y mediciones de temperatura de gelación. En este caso, el sistema polimérico modelo fue hidroxipropil metilcelulosa (HPMC), el cual no tiene carga y forma geles con incremento de temperatura. El efecto de tamaño de partículas sobre las propiedades de la solución fue estudiado usando partículas de griseofulvin como una droga modelo. T_{gel} demostró una dependencia compleja en tamaño, concentración y selectividad de la superficie. Surfactantes con carga neta similar al ingrediente activo pueden ser reemplazados por el polímero en la superficie activa o agregarse con el polímero, incrementando la viscosidad de la solución y mejorando la gelación debido

a la formación de puentes. De otro lado, surfactantes neutrales o cargados positivamente tienen mínimas interacciones polímero-surfactante y son capaces de estabilizar las partículas debido a las interacciones preferenciales con la superficie activa.

Métodos reológicos permitieron demostrar gelación física para un gel débil, proponer mecanismos de estabilización de partículas y establecer efecto de tamaño y concentración de partículas en la temperatura de gelación. Esta información puede ser extendida a otros sistemas tales como caracterización de un gel débil, o sistema con características similares, donde el uso de surfactante es necesario para la estabilización de partículas cargadas. Esta data y mecanismos pueden ser correlacionados a propiedades finales de productos como prueba de disolución, adherencia, o propiedades mecánicas, importantes pero no limitada a películas.

To God, the best of my life,
To my parents, my love and my triumph,
To all those who contributed to the accomplishment of my dreams,
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1. INTRODUCTION AND BACKGROUND

The discovery of new drugs has created the need for the development of novel personalized dosages and drug delivery systems when the active ingredient is potent, insoluble in water, and lower concentrations are required. Actually, the use of biodegradable polymers films where an active ingredient is suspended is a good alternative for drug delivery systems [1, 2]. Some advantages of these types of drug delivery methods include fast dissolution, continuous processing, rapid availability, easy dosification, long term stability, and less excipients or ingredient are needed for the formulation [1]. Nevertheless, they have some disadvantages such as: low maximum loading since drug particle size and concentration can affect the mechanical properties of the polymer, and complex particle-polymer interaction which can affect the polymer gelling capacity or particle stabilization.

Additionally, in many of these systems the use of surfactants to stabilize and disperse the solid particles is unavoidable. Yet, interactions between the surfactant and structural gelling material may also affect the dispersion of the particles, promoting agglomerate formation, or decreasing the gelling capacity of the materials, which causes a detrimental effect on the loading capacity of the gel.

In this work, we present an evaluation of the thermotropic gelling ability and steady-state rheology of polysaccharides (sodium alginate and cellulose derivatives) and proteins (gelatin) as structural ingredients in film formulations. Systematic rheological characterizations are required to determine material gelling

capacity, flow behavior, capacity as a suspending medium, and adequate processing unit operations. The relation between rheological properties and thermotropic gelation of an aqueous suspension as a function of particle size, concentration, and particles interaction with other components were studied.

1.1 Natural polymers

Natural biopolymers offer an alternative to synthetic polymers since some of them can be degraded by living cells or by our digestive system. Some examples of common natural biopolymers are polysaccharides (e.g., starch, cellulose, lignin, chitin), proteins (e.g., gelatin, casein, wheat gluten, silk and wool) and lipids (e.g., plant oils including castor oil and animal fats) [3].

Biodegradable polymer gels have a variety of applications in various fields such as the agriculture[4], food [5] and pharmaceutical industries [6, 7]. In the environmental area, natural biopolymers offer an alternative to polymers used conventionally since they can be degraded by living organisms. For example, R. Russo reported advantageous biodegradable polymer films for the solarization process since they do not have to be removed from the soil [4]. In the pharmaceutical industry, biodegradable polymers are also considered as an alternative. They could be used in drug administration to avoid polymer accumulation in the body, especially when they are used for long treatments. Significant advances have been developed in the last years where mechanisms for controlled delivery of drugs and therapeutic agents have been developed. Aminabhvi [6] studied the use of gels as an alternative to

conventional ophthalmologic drops, since their viscous nature provided prolonged corneal contact time. Other applications include the use of polymer microparticles of alginate for the encapsulation and delivery of insulin as reported by C. Pinto [7]. The latter is proposed as a substitute of insulin injections that cause stress and pain.

Biopolymers are ideal candidates as main structural agents, due to their gelling capacity, immobilization capacity in the gel network and inherent flexible processing through existing manufacturing technologies perfected in polymer industries [8]. The inherent thermal processability and the thermotropicity of some of these gels allows for control and flexibility during continuous processing. They have properties that make them attractive for drug delivery applications. They have the ability to form gels which provide the capacity to encapsulation and immobilization of particles. They are: Cheap, FDA approved, from renewable sources and their technologies processing are similar to synthetic polymer. Usually, they form physical gels and the diversity in the network formation is a function of the used polymer.

1.2 Biopolymer gels

Gels are represented as a network of polymer molecules, in which the spaces between molecules are crowded with a solvent. Polymeric gels can be primarily divided into two groups, chemical and physical gels [9].

1.2.1 Chemical gels

Chemical gels form networks through covalent bonds. These types of gels can be formed by polymerization of soluble monomers in water in the presence of multifunctional agents or using soluble polymers in water with chemical organic reactions that involve the functional groups of the polymers [9].

1.2.2 Physical gels

Physical gels are called physical networks or pseudo-gels. These gels are continuous, disordered, and three-dimensional. They are formed by associative forces able to form non-covalent unions, characterized by weak interactions and a reversible potential such as hydrogen bonds, ionic associations, and hydrophobic interactions. The formation of these attachments in the polymer chains are usually induced by modification of thermodynamical parameters of the medium, for example, change in temperature, pH, salt type, ionic strength, or addition of an additional ingredient. Physical gels induced by changes in temperature are called thermotropic gels and they are characterized by the transition of individual chain of polymer of viscous liquids to an elastic gel that occurs abruptly at the gelation point, according to the Flory gelation theory [9, 10].

The interactions that can produce physical gelation are: coil-helix transitions, in which a molecule turns around another; microcrystallites and nodular domains, in which the chains are chemically heterogeneous and the association only

occurs in preferential sites in the chain backbone [10]. The gelation through helix formation [11] is represented in Figure 1.1.

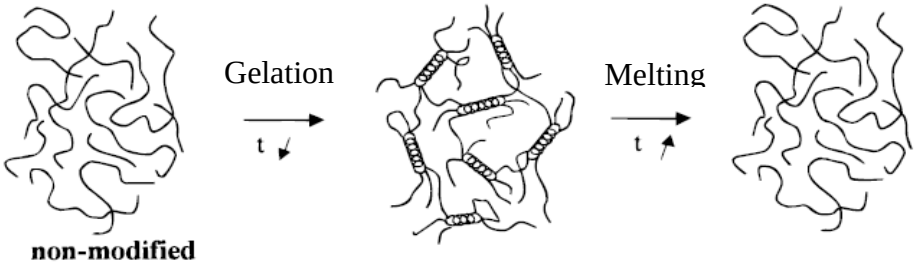

Figure 1.1. Physical gelation and melting of a biopolymer by coil-helix-coil transitions.

Taken from [12]

1.3 Theory of gelation

Flory and Stockmayer developed the classical theory of gelation in the 1940's. This theory considers the growth or chain attachment through random bonds, ignoring loop or circles that can be formed. This process can be compared with the growth of a tree as shown in Figure 1.2.

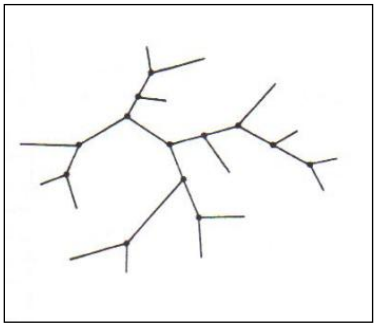

Figure 1.2. Illustration of the gel formation assumed by the classical theory

Taken from reference [10]

Each branch of a tree has many free places for the growth of new ramifications, which follow the process without restrictions due to volume exclusion or circle formation. The gelation point (P_c) in the classical theory is represented by:

$$P_c = \frac{1}{f-1} \quad (1)$$

where f is the coordination number of a tree, that is, the bond number that can be formed in each network site. If the gel is formed through chemical cross-linkers of (B) with the precursor (A), the gelation point depends of the functionalities of f_A and f_B, both of them are shown in equation 2. Where r, is the stoichiometric relation of B to A, and n is the number of moles of A and B, respectively.

$$P_{c,a} = \frac{1}{\sqrt{\frac{(f_A-1)(f_B-1)}{r}}} \quad (2)$$

$$r = \frac{f_B n_B}{f_A n_A} \quad (3)$$

Subsequent developments are based in non-classic nature. However, the majority of the systems are not far from the critical region, for this reason the classical theory is probably adequate [10]. Nevertheless, the available theories, such as the above, fail to describe physical gels due to their random nature.

1.4 Biopolymer solution characterization

The study and understanding of biopolymer solution properties is of great importance at the moment of characterization of biopolymers that form thermo-reversible gels during their processing and developing of new technologies. A fundamental property in the characterization polymer is the determination of the gelation temperature.

Common techniques to determine gelation temperature include optical, thermal, and mechanical (e.g. rheological) methods which relay on abrupt changes observed around the transition. Optical methods offer a nondestructive study but they have some limitations. Examples of optical methods are: turbidimetry [13], near infrared (NIR) spectroscopy [4], and dynamic light scattering (DLS) [14]. Techiques such as NIR require the application of chemometric principles, which requires considerable resources and time. On the other hand, scattering techniques do not provide structural information on individual clusters since scattering during the gelation process is dominated by interference amongst clusters [14, 15]. One of the most simple technique is turbidity measurements but it is subjective [6]. Thermal methods are mainly based on differential scanning calorimetry (DSC) [16, 17] , however, the enthalpy associated with this type of transition is very low, thus requiring the use of highly sensitive instruments such as micro-calorimeters.

The most common methods for the determination of the gelation temperature are through rheological measurements because they are not subjective or

dependent on theoretical approximations. This technique allows monitoring gelation either at nano [18] or macro-scale [12]. At macroscale gelation, two rheological methods are use to determine sol-gel transitions: Constant-stress temperature-ramp (CSTR) viscosity method [12, 19] and the oscillatory dynamic test [11, 20] . The CSTR viscosity method measures the viscosity in a stress-controlled rheometer while cooling the sample and applying a constant stress. The viscosity curve will show a discontinuity, or an overshoot, at the sol–gel transition. Since the gel exerts a higher stress, the applied shear rate tends to zero to maintain the constant stress, while the calculated viscosity tends to infinity. The gelation temperature is predicted as the intercept of the tangents of the viscosity at high temperatures and of the discontinuity as shown in Figure 1.3.

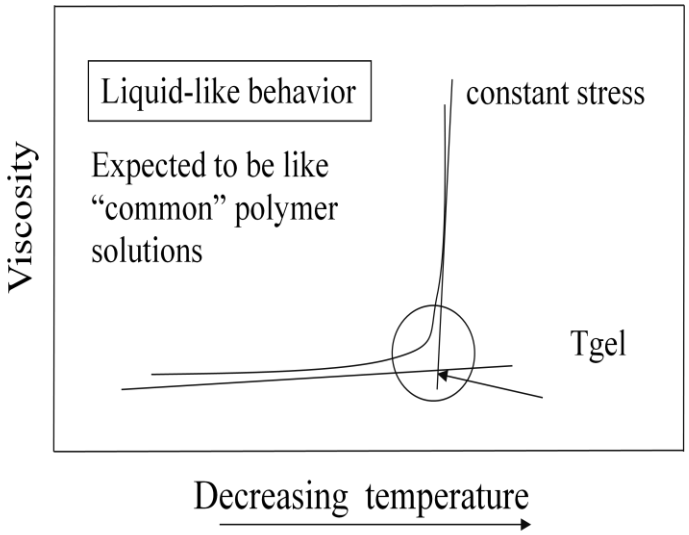

Figure 1.3. Constant stress temperature ramp method to determine Tgel

The second method is the dynamic oscillatory strain control, in which the loss (G") and storage (G') moduli are studied as a function of temperature. This

technique is frequently used because it allows to study gel evolution and it is non destructive. However, *assumptions* are taken at the moment to identify gelation temperature. Some authors define T_{gel} as the temperature at which G′ and G″ are equal [12, 21]. However, it has been shown that for some chemical gels, gelation starts at a lower temperature [22], at the point in which:

i. $G' \sim G'' \sim = \omega^n$

ii. $tan\delta = \tan(\frac{n\pi}{2})$

Both methods are valid to determine gelation temperatures. The first method provides an approximate of the gelation temperature, while with the second method a more precise temperature is obtained, with the disadvantage that long time is needed for study the system over all the frequency range. Gelation temperature in this work, was chosen as the point in which G' = G'' (i.e., solid- and liquid-like contributions are equal) as shown in Figure 1.4

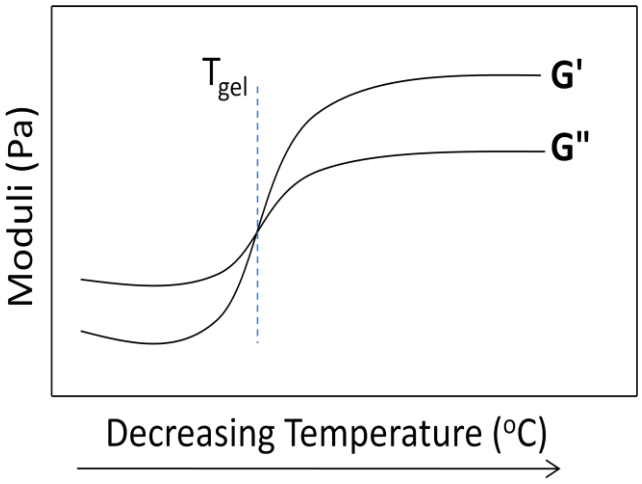

Figure 1.4. Diagram of the dynamic test for gelation temperature determination

1.5 Biopolymers rheological characterization

Rheological methods had been used to characterize biopolymers. In addition to the methods discussed above other rheological properties can also be measured to gather information about the state of the sample. These include: dependence of elastic modulus and viscosity measurements on frequency or time [23, 24], and the shear rate dependence of the viscosity [25]. Some examples of works that used rheological properties to characterize polymers are explained below.

An I Van et al. [11] studied the loss modulus (G") and storage (G') for hydrogels based on gelatin methacrylamide solutions as a function of temperature. The point in which G' = G" was identified as the gelation temperature. This point is characterized by a transition of a primarily viscous solution to an elastic one as shown in Figure 1.5. Additionally, they found that when temperature increases the elastic modulus drops quickly due to the breakage of the physical network.

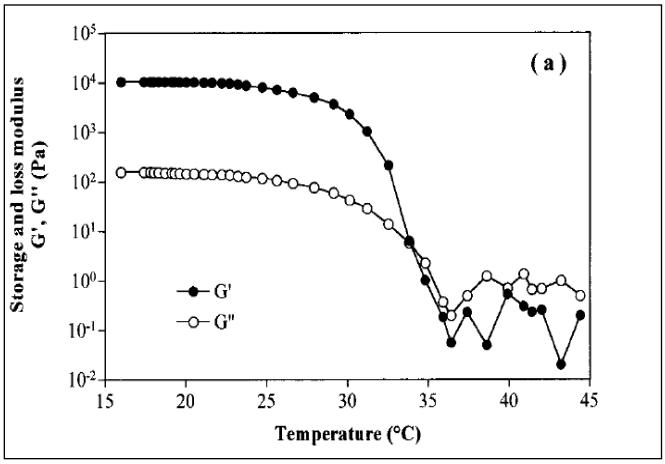

Figure 1.5. Dynamic rheological observations of the physical crosslink of gelatin Taken from reference [11]

While, P. Ding et al. [12] used the same method to determine the temperature of gelation of solutions gelatin/pullulan at different concentrations in the temperature range from 60^{o}C to 20^{o}C. On the other hand, Yun-Seok et al. [19] used the viscosity vs. temperature curve to determine the gelation temperature of a poloxamer solution at different concentrations (i.e, 20, 25 and 30 wt%). Gelation temperature showed dependence on polymer concentration as shown in Figure 1.6. The linear portion of the curve at high temperatures was extrapolated to the temperature axes and this intercept was considered to correspond to T_{gel}. The results using this method showed to be reproducible.

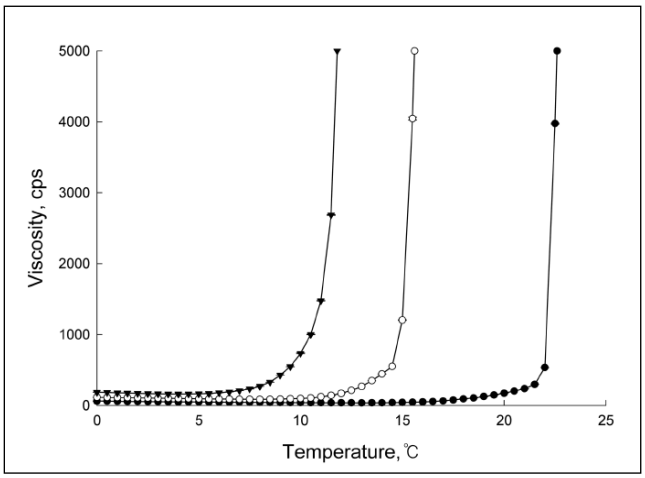

Figure 1.6. Viscosity profiles of aqueous solutions of poloxamer at different concentrations.

The curves correspond to● 20%, ○ 25%, ▼ 30% poloxamer. Taken from reference [19]

A third method to determine T_{gel} was used by Peng Zhou et al [26], in which the fusion of gelatin was studied with sweep experiments, in which $Tm_{3/4}$, $Tm_{1/2}$, $Tm_{1/4}$ and ΔT_m are determined as shown in Figure 1.7. $Tm_{3/4}$, $Tm_{1/2}$, $Tm_{1/4}$

refers to the temperature in which G' is 75, 50 y 25% of the initial value. ΔT_m is the difference between $Tm_{3/4}$ and $Tm_{1/4}$. $Tm_{1/2}$ is used as an approximation for T_{gel}.

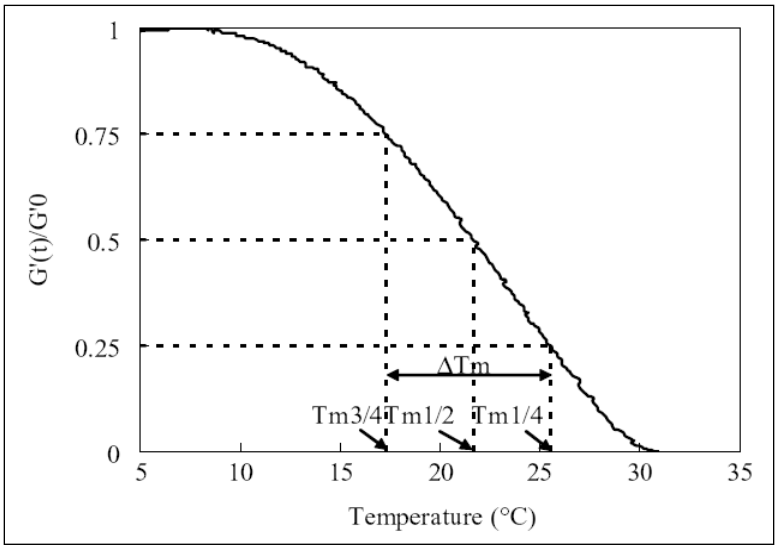

Figure 1.7. Melting curve of gelatin

Taken from reference [26]

For systems containing more than one polymer, its properties were found to be dependent on composition as shown by P. Ding et all [12]. They studied the effect of the temperature and the composition of gelatin and pullulan mixtures at different concentrations, summarized in Table 1. The viscosity and the dynamic modulus were also studied as a function of time at a constant temperature. At 35°C, the viscosity and the storage modulus (G') increased by a small amount with time, and the viscous modulus (G") did not change substantially, while at 32°C both modulus increased notably. In both studies, temperature showed effects on the solution properties.

1.6 References

1. Beth Vondrak, S.B., *Dissolvable Films: Dissolvable Films for Flexible Product Format in Drug Delivery,* in *PHARMACEUTICAL TECHNOLOGY*. 2008.
2. Frey, P., *Film strips and pharmaceuticals.* Pharmaceutical Manufacturer and Packing Sourcer, Winter, 2006: p. 92, 93.
3. Smith, R., *Biodegradable Polymers for Industrial Applications* ed. C. Press. May 2005.
4. Russo, R., M. Malinconico, and P. Romano, *Physical behavior of biodegradable alginate-poly(vinyl alcohol) blend films.* Journal of Polymer Science Part B: Polymer Physics, 2005. **43**(10): p. 1205-1213.
5. Avella, M., J.J. De Vlieger, M.E. Errico, S. Fischer, P. Vacca, and M.G. Volpe, *Biodegradable starch/clay nanocomposite films for food packaging applications.* Food Chemistry, 2005. **93**(3): p. 467-474.
6. Aminabhavi, T.M., A. Sunil, B. Agnihotri, and V.K. Naidu, *Rheological properties and drug release characteristics of pH-responsive hydrogels.* Journal of Applied Polymer Science, 2004. **94**(5): p. 2057-2064.
7. Pinto, C., A. Ronald, and F. Veiga, *Alginate microparticles as novel carrier for oral insulin delivery.* Biotechnology and Bioengineering, 2007. **96**(5): p. 977-989.
8. Osada, Y. and A. Khokhlo, *Polymer Gels and Networks*. 2002, New York: MARCEL DEKKER, INC.
9. Fried, J.R., *Polymer science and technology*, ed. P.H.P.T. Reference. 2003.
10. Larson, R.G., *The Structure and Rheology of Complex Fluids (Topics* ed. O.U. Press. 1999.
11. Van Den Bulcke, A.I., B. Bogdanov, N. De Rooze, E.H. Schacht, M. Cornelissen, and H. Berghmans, *Structural and Rheological Properties of Methacrylamide Modified Gelatin Hydrogels.* Biomacromolecules, 2000. **1**(1): p. 31-38.
12. Ding, P., A.W. Pacek, W.J. Frith, I.T. Norton, and B. Wolf, *The effect of temperature and composition on the interfacial tension and rheology of separated phases in gelatin/pullulan mixtures.* Food Hydrocolloids, 2005. **19**(3): p. 567-574.
13. Karreman, R.J., W.F. Brandt, and G.G. Lindsey, *The yeast Saccharomyces cerevisiae stress response protein Hsp12p decreases the gel strength of agarose used as a model system for the [beta]-glucan layer of the cell wall.* Carbohydrate Polymers, 2005. **60**(2): p. 193-198.
14. Blanco, M.C., D. Leisner, C. Vazquez, and M.A. Lopez-Quintela, *Dynamic Light Scattering in Transient Reversible Gels&#x2020.* Langmuir, 2000. **16**(23): p. 8585-8594.
15. Richter, S., *Recent Gelation Studies on Irreversible and Reversible Systems with Dynamic Light Scattering and Rheology - A Concise Summary.* Macromolecular Chemistry and Physics, 2007. **208**(14): p. 1495-1502.

16. Rodríguez-Hernández, A.I., S. Durand, C. Garnier, A. Tecante, and J.L. Doublier, *Rheology-structure properties of gellan systems: evidence of network formation at low gelan concentrations.* Food Hydrocolloids, 2003. **17**: p. 621-628.
17. Roversi, M. and C. La Mesa, *Rheological properties of protein-surfactant based gels.* Journal of Colloid and Interface Science, 2005. **284**: p. 470476.
18. Barrera, C., V. Florian-Algarin, A. Acevedo, and C. Rinaldi, *Monitoring gelation using magnetic nanoparticles.* Soft Matter, 2010. **6**(15): p. 3662-3668.
19. Y. Seok, Y.H., C. Woong, S. Cheol, E. Seok, *Effect of flavor on the viscosity and gelling point aqueous Poloxamer solution.* . Archives of pharmaceutical research, 2006. **29**: p. 1171-1178.
20. Cho, J., M.-C. Heuzey, A. Bégin, and P.J. Carreau, *Chitosan and glycerophosphate concentration dependence of solution behaviour and gel point using small amplitude oscillatory rheometry.* Food Hydrocolloids, 2006. **20**(6): p. 936-945.
21. Chen, H.-H., C.-H. Lin, and H.-Y. Kang, *Maturation effects in fish gelatin and HPMC composite gels.* Food Hydrocolloids, 2009. **23**(7): p. 1756-1761.
22. Chambon, F. and H.H. Winter, *Linear Viscoelasticity at the Gel Point of a Crosslinking PDMS with Imbalanced Stoichiometry.* Journal of Rheology, 1987. **31**(8): p. 683-697.
23. Mihranyan, A., K. Edsman, and M. Strømme, *Rheological properties of cellulose hydrogels prepared from Cladophora cellulose powder.* Food Hydrocolloids, 2007. **21**(2): p. 267-272.
24. Rodríguez-Hernández, A.I., S. Durand, C. Garnier, A. Tecante, and J.L. Doublier, *Rheology-structure properties of waxy maize starch-gellan mixtures.* Food Hydrocolloids, 2006. **20**(8): p. 1223-1230.
25. *Food Polysaccharides and Their Applications* ed. G.O.P. Alastair M. Stephen. 2006, New York: Marcel Dekker.
26. Zhou, P., S.J. Mulvaney, and J.M. Regenstein, *Properties of Alaska Pollock Skin Gelatin: A Comparison with Tilapia and Pork Skin Gelatins.* Journal of Food Science, 2006. **71**(6): p. C313-C321.

2.THERMOTROPIC GELATION OF GELATIN: EFFECT OF OPERATIONAL PARAMETERS & PARTICLE INCLUSION

2.1 Introduction

Gelatin is a protein obtained by thermal denaturation from collagen isolated from animal skin and bones. It can be extracted from mammalian and marine sources [1-3]. It has a variety of applications in the food [4, 5] and pharmaceutical industries [6, 7]. Gelatin represents about 43 % (Figure 2.1) of the hydrocolloid market [8] due to its unique properties, which are associated to gelling and surface effects. Gel strength, gelling time, melting temperatures and viscosity are properties associated with gelatin gelling. While, properties adhesion, dissolution are related to the surface behavior of gelatin [9]

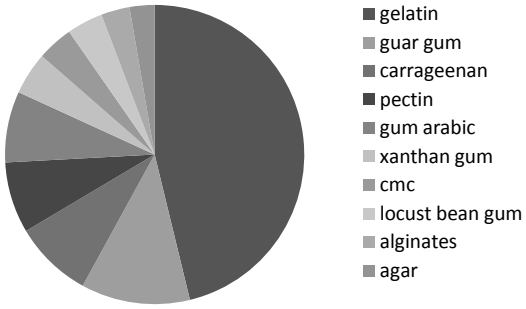

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Figure 2.1. Volume of world market for individual hydrocolloid. Reproduced from reference [8]

Additionally, Gelatin is highly soluble in warm water (>40^{o}C), it is cheap, and it has the intrinsic ability to form thermally reversible gels [10, 11]. When

a gelatin solution is cooled below room temperature, the protein coils form triple helices and progressively a 3D network is formed. This is a thermo reversible transition and the gels melt (helix to coil transition) at temperature above 30 °C [11, 12]. Dynamic rheology has been used to determine gelatin gelation and melting temperature. The heating and cooling curves show hysteresis, which typically is 5^{o}C [9] as shown in Figure 2.2. However, it has been shown that biopolymer gelation temperature is a function of source [13, 14], concentration [15, 16], cooling rate [12], and others.

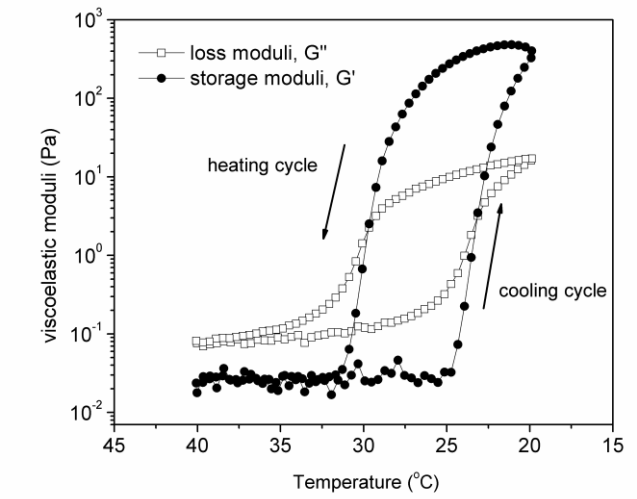


Figure 2.2. Cooling and heating curves of a gelatin solution.

The effect of particle size and concentration on polymer rheological properties is dependent on particle-particle (as electrostatic forces) and particle-polymer interactions (as polymer absorption on the particle) [17-19]. The effect of operational and formulation parameters on gelation temperature of gelatin solutions was studied in this section using stress temperature ramp and dynamic methods.

2.2 Experimental section

2.2.1 Materials

Type B gelatin (batch #035K00011) was obtained from Sigma Aldrich. Aqueous colloidal silica dispersion (50 wt%) LUDOX TM-50 was also purchased from Sigma-Aldrich. The hydrodynamic diameter of the silica particles was determined as 13.1 nm with a polydispersity of 0.234 by dynamic light scattering on a Brookhaven Instruments BI 90-Plus particle size analyzer.

2.2.2 Solution preparation

Gelatin solutions were prepared by mixing Gelatin type B with deionized water. Then the sample was heated in a hot plate at 70^{o}C, while stirring constantly for at least 30 minutes. The silica dispersion was diluted to the desired concentration in deionized water. Silica dispersions of up to 5.0wt% in the specified biopolymer were prepared from a commercial LUDOX silica dispersion.

Gelatin solution and silica particle dispersion were mixed in the appropriate proportions to obtain final desired concentrations. Particle suspension in gelation was kept at the same temperature at it stirring constantly for at least 30 minutes before transfer to the rheometer.

Rheological characterization was performed on a Reologica StressTech HR stress-controlled rheometer equipped with an extended temperature cell (ETC) for temperature control using stainless steel cone-and-plate (d = 30 mm and $\theta < 4^{o}$) and

double-gap Couette (V = 11 mL) fixtures and on an Anton-Paar Physica MCR301 equipped with a Julabo constant temperature water bath control using stainless steel cone-and-plate (d = 50 mm and θ = 0.976º). The rheometer fixture was preheated to the desired temperature, before transferring and loading the hot solution. The sample and fixture temperature were allowed to equilibrate for at least 20 minutes. After temperature reached equilibrium, rheological tests were performed. Gelation temperature was determined using the constant-stress temperature-ramp (CSTR) and dynamic test. The effect of operational parameters such as cooling rate and stress on Tgel was studied in a range of 0.1 to 2 ^{o}C/min and 0.01 to 100 Pa, respectively. The dynamic viscoelastic moduli were measured at a frequency of 1 Hz and a strain of 1% to guarantee measurements in the linear viscoelastic regime while CSTR was measured at 1 Pa. The temperature was decreased from 40 to either 20 or 15 ^{o}C at a cooling rate of 2 ^{o}C/min, after that the sample was heated at the same rate.

2.3 Results and discussion

2.3.1 Effect of operational parameter on T_{gel}

The gelation temperature for a 10 wt% gelatin solution showed dependence on stress and cooling rate. The stress effect on T_{gel} was evaluated from 0.01 to 100 Pa. Results are shown in Figure 2.3. Stress from 0.01 to 1 Pa did not affect significantly T_{gel}, but a lower T_{gel} was obtained for a stress of 100 Pa. This can be attributed to breakup, favored at higher stresses.

On other hand, cooling rate had a strong impact on gelation as shown in Figure 2.4. Cooling rate effect was studied in a range from 2 to 0.1 ^{o}C/min. When the solution is cooled faster a lower gelation temperature is obtained because polymeric solution has shorter relaxation time. A difference of 10 ^{o}C was observed between results obtained at 2 and 0.1 ^{o}C/min.

2.3.2 Effect of formulation parameters

The effect of concentration was studied in a range from 2 to 10 wt%. Concentration had a strong impact on gelation temperature. It increases when gelatin concentration increase due to there is more junction sites available (attachment numbers are elevated) to form the gel. These results are shown in Figure 2.5.

Figure 2.6 shows the effect of pH on T_{gel}. pH adjust using a NaOH solution were done to studied its effect in a range from 5 to 8. Basic groups block the active "amino acids" of the gelatin. Thus, it lowers the concentration of active "junction sites". However, the effect is mild, only two degrees with a difference of 3 in pH.

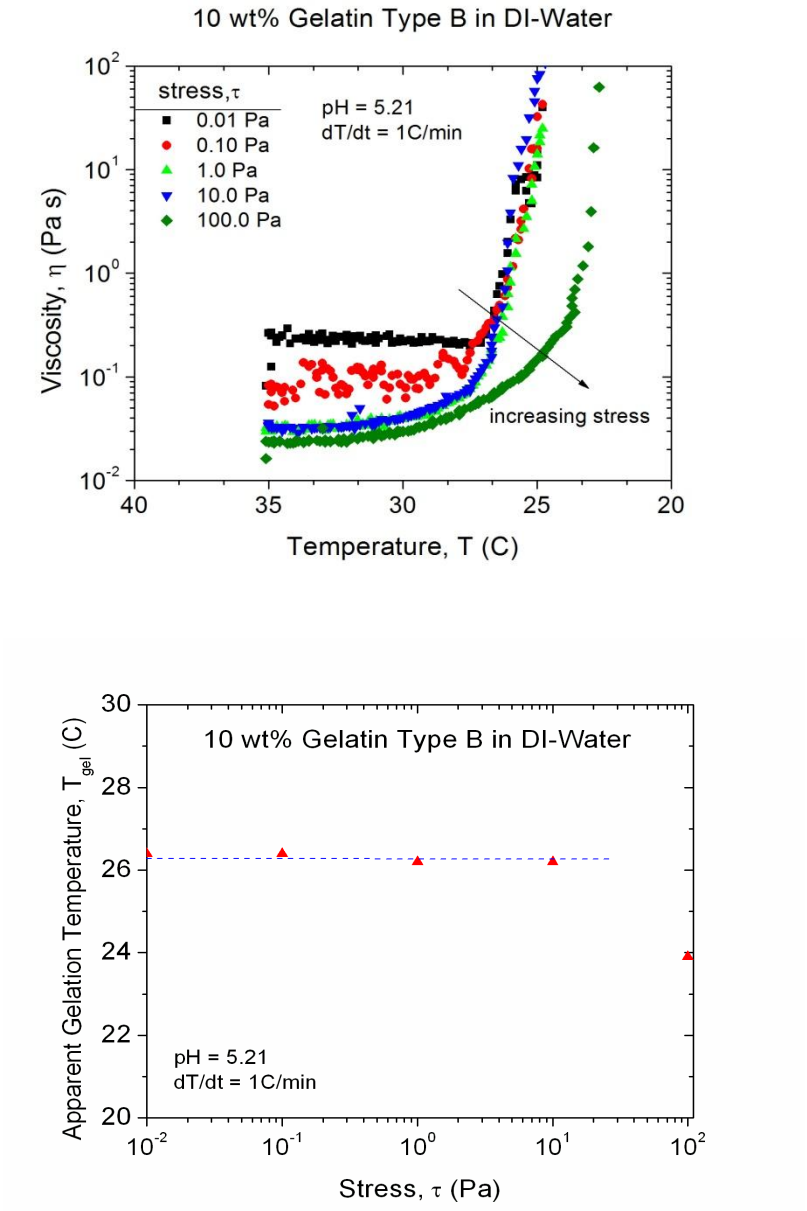


Figure 2.3 Effect of applied shear stress on the gelation temperature 10 wt% gelatin solution by constant stress temperature ramp at 1 ^{o}C/min and a pH = 5.21 viscosity data (top) and gelation temperature summary (bottom)

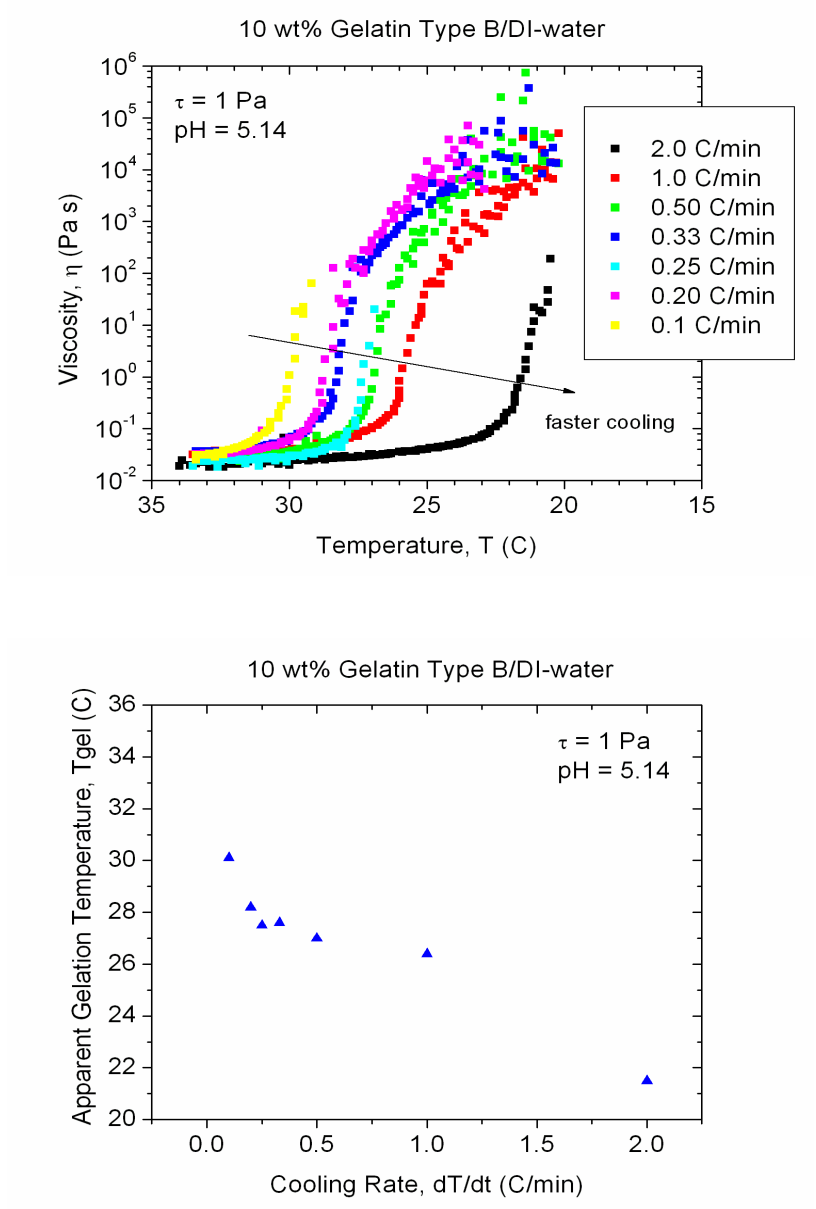


Figure 2.4. Effect of cooling rate on the gelation temperature 10 wt% gelatin solution by constant stress temperature ramp at 1 Pa and a pH = 5.14 Viscosity data (top) and gelation temperature summary (bottom)

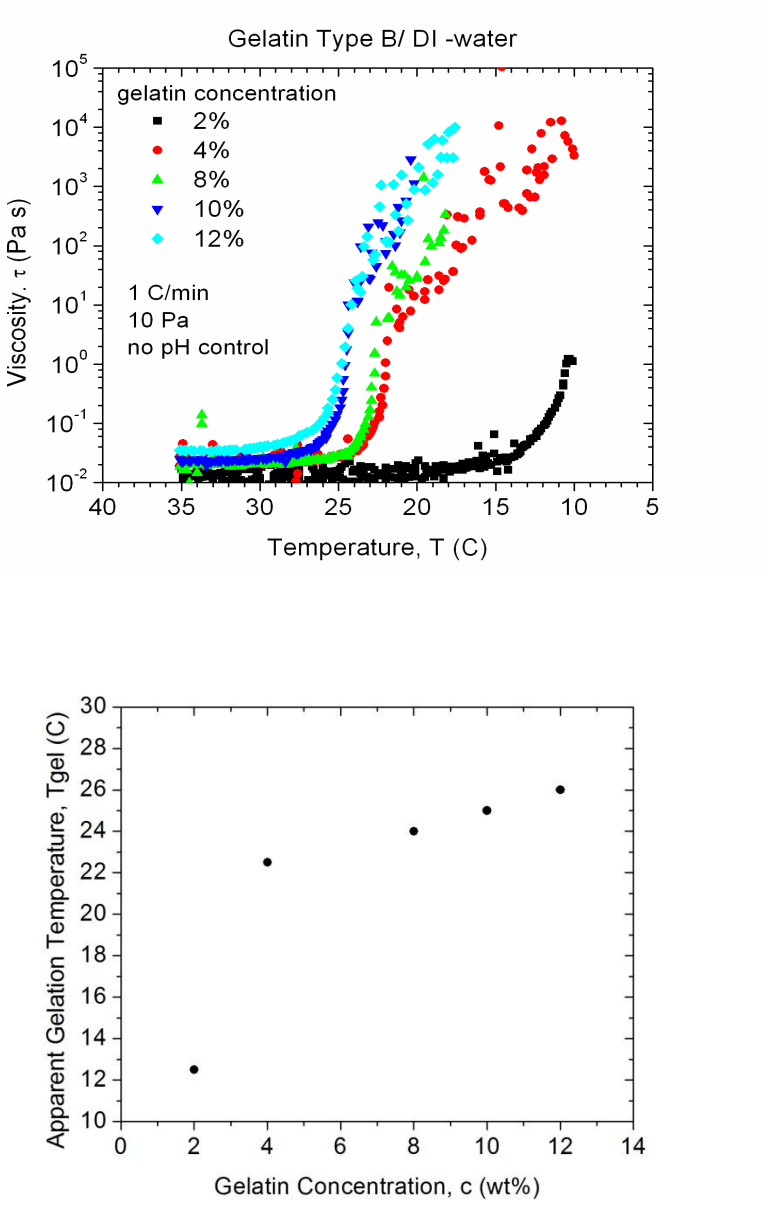


Figure 2.5. pH effect on the gelation temperature

10 wt% gelatin solution by constant stress temperature ramp at 1 ^{o}C/min and 1 Pa Viscosity data (top) and gelation temperature summary (bottom)

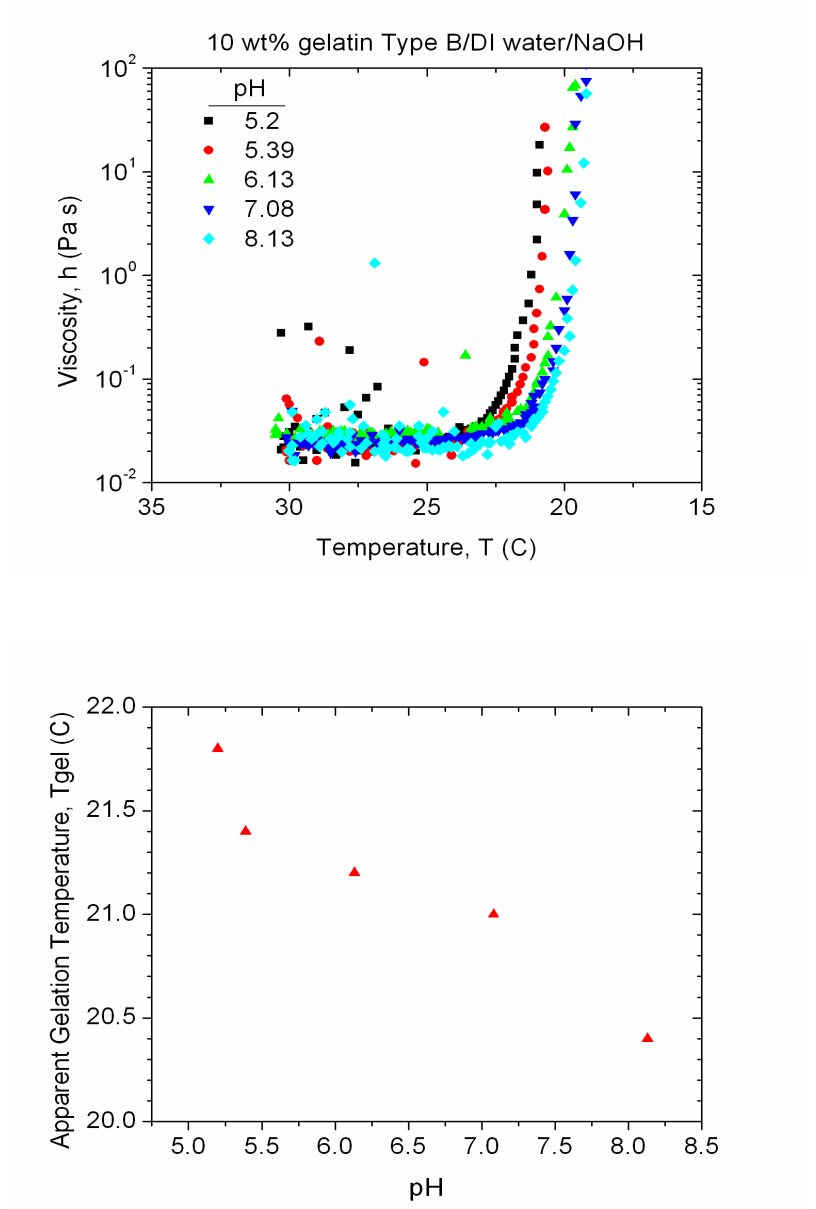


Figure 2.6. Gelatin concentration effect on the gelation temperature

10 wt% gelatin solution by constant stress temperature ramp at 1°C/min and 1Pa Viscosity data (top) and gelation temperature summary (bottom)

2.3.3 Effect of particle inclusions

The gelation temperature for 10 wt% gelatin solutions was previously measured using the constant-stress temperature ramp method at a constant cooling rate of 2 ^{o}C/min and stress of 1 Pa. The gelation temperature was identified as 21.5 ^{o}C. Figure 2.2 shows the diagram for the gelatin temperature transitions using the dynamic viscoelastic moduli method. The gelation temperature was identified as 22.3 ^{o}C while the melting temperature was 30.47 ^{o}C. No pH adjust were done.

Figure 2.7 shows the dynamic response when a 0.3 wt% of silica is dispersed in the gelation solutions. In the cooling cycle, the storage moduli, G′, showed significant scattering due to the low viscosity and the Newtonian behavior of gelatin at high temperature. Neither gelation nor melting temperature were affected by the presence of the silica particles at 0.3wt%. In fact, no significant effect was observed for loadings up to 0.5 wt%, as summarized in Figure 2.8. Thus, low concentrations of silica do not perturb the formation of helical structures. A gelation temperature close to room temperature, 23.6 ± 1.1 ^{o}C was observed within this range. The melting temperature for the systems was 6.3 ± 0.2 ^{o}C above T_{gel}.

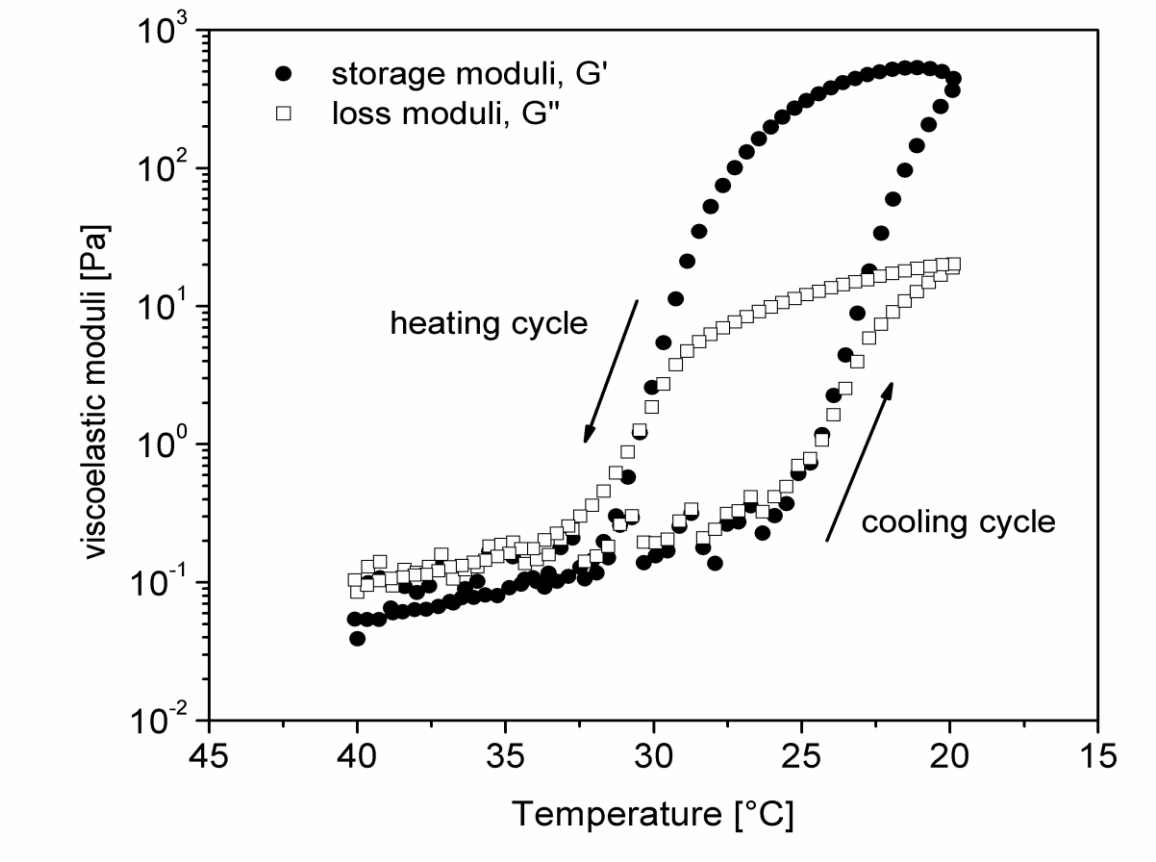


Figure 2.7. Dynamic viscoelastic moduli as a function of temperature for a 0.3 wt% silica dispersion in a 10 wt% aqueous gelatin solution.

Cooling rate: 2^{o}C/min

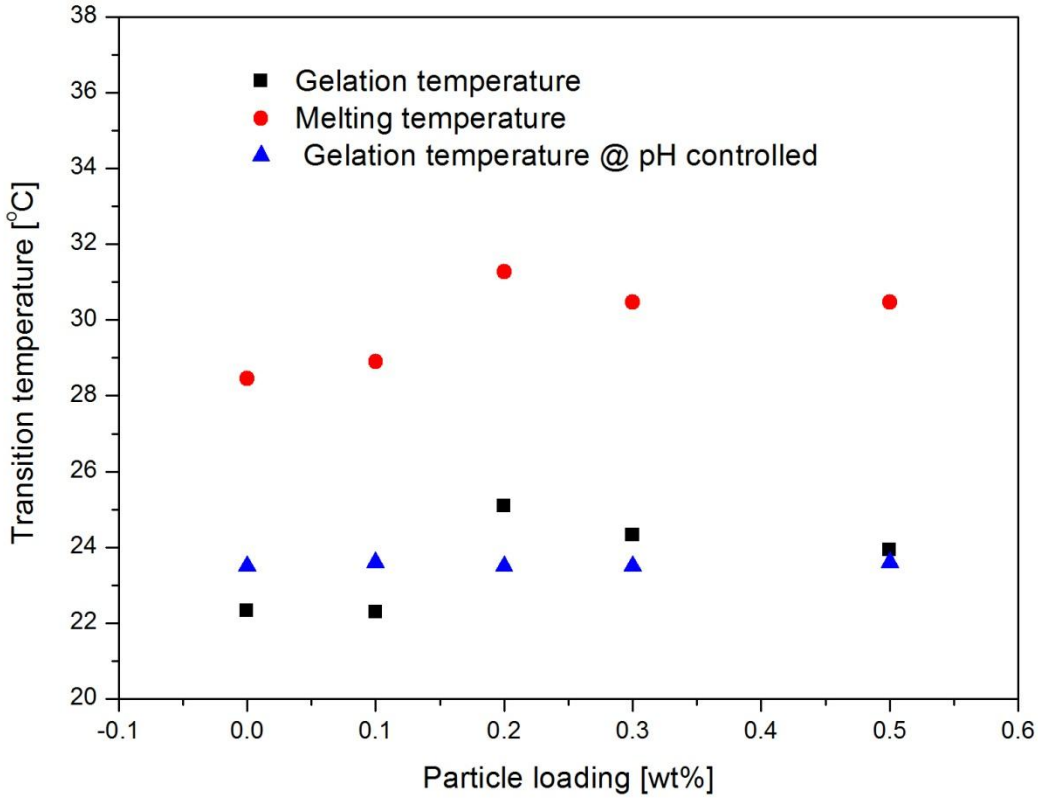


Figure 2.8. Effect of nano-silica loading on the gelation temperature and melting temperatures of a 10 wt% aqueous gelatin solution

Nevertheless, at higher particle loadings (up to 5 wt%), the gelation temperature for 10 wt% gelatin solution was observed to increase with silica loadings. Thus, addition of silica particles has a positive effect on the gelation transition. Figure 2.9 shows the results for 3.0 wt% silica in gelatin dispersion. In this case, gelation temperature was measured to be 28.5 ^{o}C, at least six degrees higher than for the gelatin solution without silica at the same conditions. The melting point was outside the experimental windows due to experiments were set at temperatures between 40 and 20^{o}C. Thus, the hysteresis in the transition temperature, present in this sample, would be greater than 12 ^{o}C, more than double of the observed at lower loadings.

The positive deviation on the gelation temperature caused by high concentrations of silica particles may be associated with a synergistic effect on the network formation. It is possible that the silica particles are acting as additional nodes for the network due to adsorbed gelatin. This may have been caused by a loss of the stabilizing layer of the commercial particles, since pH was not adjusted in our experiments and this solution were diluted.

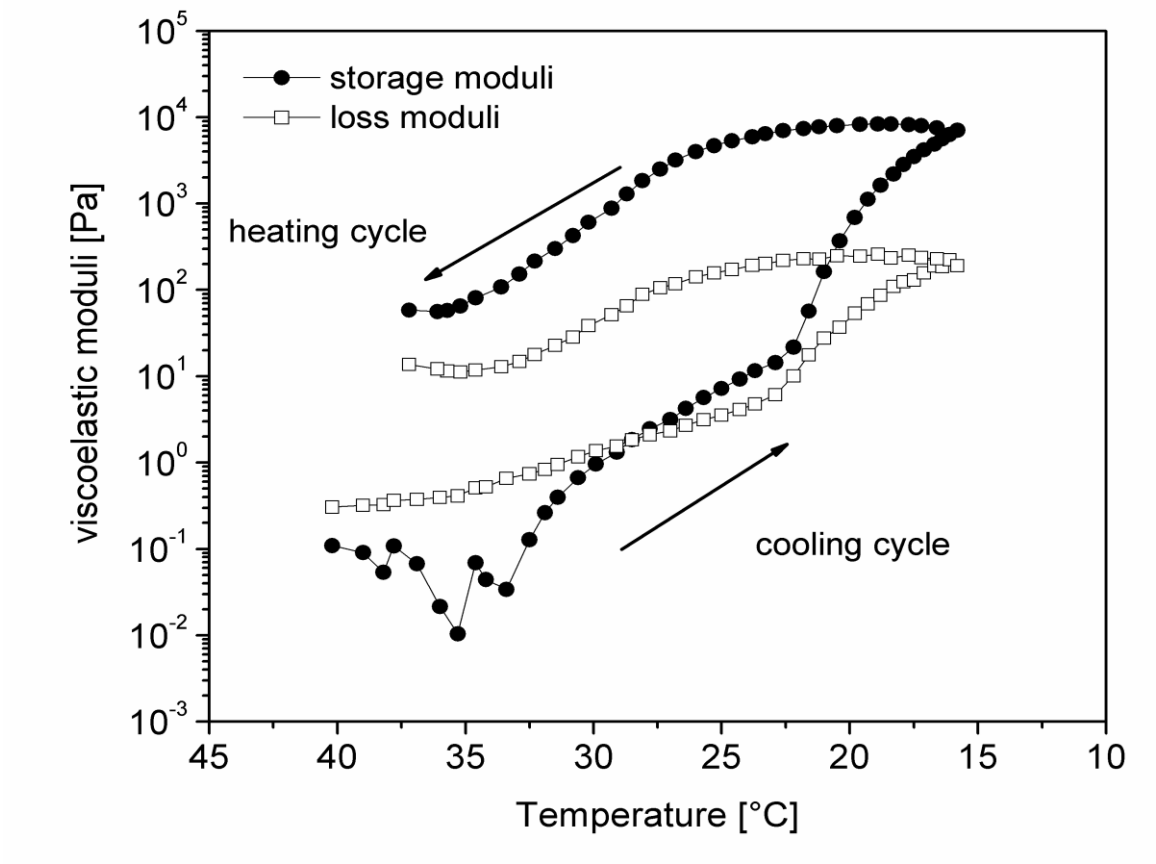


Figure 2.9. Dynamic viscoelastic moduli as a function of temperature for 3 wt% silica dispersion in a 10 wt% aqueous gelatin solution.

Cooling rate: 2 ^{o}C/min

2.3.4 Effect of particle concentration at a controlled pH

Gelatin solutions were prepared using a NaCl stock solution 0.01 M. These experiments involved keeping the gelatin and salt concentrations approximately constant to avoid ionic changes [20]. pH was measured for each silica concentration. Results are showed in Table 1. Gelation temperatures for concentration between 0 to 0.5 wt% showed variations around 0.1 ^{o}C, while that particles at the same concentration but without pH control showed variation en T_{gel} of 1.2 ^{o}C.

Table 2-1. Concentration effect on T_{gel} at pH = 4.97 ± 0.13

Silica concentration (wt%)	0	0.2	0.3	0.4	0.5	1	5
pH	4.91	4.87	4.93	4.97	4.95	4.95	5.26
Gelation Temperature [^{o}C]	23.5	23.6	23.5	23.5	23.6	23.6	23.6

Silica concentration in a range from 0.2 to 5 wt% did not show an effect on T_{gel} and the pH was constant at 4.97 ± 0.13. These results suggest that variation on T_{gel} (without pH adjusts); can be associated to a particle destabilization due to changes in pH.

Since the components of the silica dispersion are unknown, discerning the mechanics and interactions of the gelation in the presence of particles is difficult. Furthermore determination of the effect of formulation parameters is desired, but this system is ineffective for these purposes. Therefore, in the next chapter, silica particles used were synthesized and characterized in our lab. Additionally, NaAlg a polymer from brown algae and less bio-variability chosen as main structural component.

2.4 Conclusion

Apparent gelation temperature increases when shear stress, cooling rate or pH decreases, and when gelatin concentration increases. A stress of 1 Pa was selected or future experiments. It is low enough to avoid network destruction but high enough to avoid sensitivity issues with the torque measurements at low solution concentrations. Cooling rate affects significantly T_{gel}, 1 or 2 ^{o}C/min was chosen due to its similarity to industrial process. pH does not affect significantly gelation temperature for gelatin, however, it was found important to guarantee particle dispersion due to can affect ionic strength and effect particles/particle and particle/polymer interactions.

Note:

Sections of this chapter are reproduced verbatim from our publications:

- ✓ Florián Algarín, V. and A. Acevedo, *Effect of Silica Nanoparticles on Rheological Properties and Gelation Temperature of Biodegradable Polymer Gels.* Nanotech 2009. **1**: p. 198-200.
- ✓ Florián-Algarín, V. and A. Acevedo-Rullan, *Rheology and Gelation Temperature of Aqueous Gelatin and Sodium Alginate Solutions.* AIP Conference Proceedings, 2008. **1027**(1): p. 618-620.

2.5 Reference

1. Gómez-Guillén, M.C., M. Bifani, A. Silva, and P. Montero, *Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (Ugni molinae Turcz)* Food Hydrocolloids, 2007. **21**(7): p. 1133-1143.
2. Gilsenan, P.M. and S.B. Ross-Murphy, *Rheological characterisation of gelatins from mammalian and marine sources.* Food Hydrocolloids, 2000. **14**(3): p. 191-195.
3. Zhou, P., S.J. Mulvaney, and J.M. Regenstein, *Properties of Alaska Pollock Skin Gelatin: A Comparison with Tilapia and Pork Skin Gelatins.* Journal of Food Science, 2006. **71**(6): p. C313-C321.
4. F. Gibbs, S.K., Inteaz Alli, Catherine N. Mulligan, Bernard, *Encapsulation in the food industry: a review.* International Journal of Food Sciences and Nutrition, 1999. **50**(3): p. 213-224.
5. Karim, A.A. and R. Bhat, *Gelatin alternatives for the food industry: recent developments, challenges and prospects.* Trends in Food Science & Technology, 2008. **19**(12): p. 644-656.
6. Li, J.K., N. Wang, and X.S. Wu, *A novel biodegradable system based on gelatin nanoparticles and poly(lactic-co-glycolic acid) microspheres for protein and peptide drug delivery.* Journal of Pharmaceutical Sciences, 1997. **86**(8): p. 891-895.
7. Li, D.X., Y.-K. Oh, S.-J. Lim, J.O. Kim, H.J. Yang, J.H. Sung, C.S. Yong, and H.-G. Choi, *Novel gelatin microcapsule with bioavailability enhancement of ibuprofen using spray-drying technique.* International Journal of Pharmaceutics, 2008. **355**(1-2): p. 277-284.
8. Glyn O. Phillips and P.A. Williams, *Handbook of hydrocolloids*. 2000, Boca Raton, FL: CRC press LLC.
9. Gareis, H. and R. Schrieber, *Gelatine Handbook: Theory and Industrial Practice* 2007 WILEY.
10. Smith, R., *Biodegradable Polymers for Industrial Applications* ed. C. Press. May 2005.
11. Joly-Duhamel, C., D. Hellio, and M. Djabourov, *All Gelatin Networks: 1. Biodiversity and Physical Chemistry.* Langmuir 2002. **18**: p. 7208-7217.
12. Ding, P., A.W. Pacek, W.J. Frith, I.T. Norton, and B. Wolf, *The effect of temperature and composition on the interfacial tension and rheology of separated phases in gelatin/pullulan mixtures.* Food Hydrocolloids, 2005. **19**(3): p. 567-574.
13. Gilsenan, P. and S. Ross-Murphy, *Rheological characterisation of gelatins from mammalian and marine sources.* . Food Hydrocolloids, 2000. **14**: p. 191–195
14. Yoshimura, K., M. Terashima, D. Hozan, T. Ebato, Y. Nomura, Y. Ishii, and K. Shirai, *Physical Properties of Shark Gelatin Compared with Pig Gelatin.* Journal of Agricultural and Food Chemistry, 2000. **48**(6): p. 2023-2027.

15. Florián-Algarín, V. and A. Acevedo, *Rheology and Thermotropic Gelation of Aqueous Sodium Alginate Solutions.* Journal of Pharmaceutical Innovation, 2010. **5**(1): p. 37-44.
16. Susan, M.T. and G.M. Alejandro, *Determination of the maximum gelation temperature in gelatin gels.* Applied Physics Letters, 2004. **84**(21): p. 4242-4244.
17. Zmievski, V., M. Grmela, and M. Bousmina, *Suspensions of rigid spherical particles in polymeric solutions.* Physica A: Statistical Mechanics and its Applications, 2007. **376**: p. 51-74.
18. Hone, J.H.E. and A.M. Howe, *Viscosity of Colloidal Suspensions in Aqueous Gelatin.* Journal of Colloid and Interface Science, 2002. **251**(1): p. 193-199.
19. Kawaguchi, M. and Y. Ryo, *Rheological properties of silica suspensions in aqueous cellulose derivative solutions.* Chemical Engineering Science, 1993. **48**(2): p. 393-400.
20. Kawaguchi, M., *Rheological properties of silica suspensions in polymer solutions.* Advances in Colloid and Interface Science, 1994. **53**: p. 103-127.

3.RHEOLOGY AND THERMOTROPIC GELATION OF AQUEOUS SODIUM ALGINATE SOLUTION

3.1 Introduction

Sodium alginate is a natural biopolymer extracted from the calcium, magnesium, and sodium salts of alginic acid in brown algae's cell walls [1]. Alginate is a linear unbranched copolymer consisting of alternating blocks of D-mannuronic (M) and L-guluronic (G) acid residues at different ratios, according to the source algae. Alginates compose around three percent of the approximately 4.4 billion dollar global market of hydrocolloids and polysaccharides [2]. It is mainly used in the food and pharmaceutical industry as a thickener, immobilization agent, gelling agent, and to produce films and coatings [1, 3]. Additionally, until now there are no safety concerns with the use and consumption of sodium alginate and new applications and increasing demand will not contend with the world's food supplies.

Sodium alginate gels ionotropically (i.e. upon addition of an ion) at constant temperature upon addition of divalent cations, such as Ca^{2+}, Sr^{2+}, Cu^{2+} [4-7]. Ionotropic gelation follows the egg-box model - interchain associations of G-block sequences of 20 or more residues with arrays of site bound ions sandwiched between the participating chains [8, 9]. The gels are formed by exposing a NaAlg film or droplet to a solution containing an excess of the divalent cations. Ion-induced alginate gels have been used to immobilize cells [10-12], enzymes and proteins [13, 14],

microorganisms [15, 16], pharmaceuticals [17, 18], and inorganic solids [19-21]. Since the ionotropic gelation is very fast and the ability to control and modify the structure of the gel is limited, chemical modification [22, 23] and blending with other materials [24-26] has been performed to overcome these disadvantages. Blends are normally gelled thermotropically, which allows better control of the resulting structure. However, NaAlg is usually the additive, i.e. small amounts are used, to promote synergism [27, 28]. In the literature no reports were found of a thermally-induced gel transition using only NaAlg.

In this chapter, the evaluation of the thermotropic gelling ability and steady-state rheology of sodium alginate (NaAlg), a candidate main structural agent for the development of biodegradable film strips, is presented. Additionally, the effect of particle size and concentration on the gelation of NaAlg solutions was determined for model silica particles.

3.2 Experimental section

3.2.1 Materials

An ISP Kelcosol sodium alginate (NaAlg) sample (Lot# 05GV283) was graciously donated by Mutchler Inc Pharmaceutical Ingredients. Silica nano-particles were synthesized by Mariel Santiago at professor Aldo Acevedo's lab at UPRM using the Stöber method [29]. Ethanol, ammonium hydroxide solution and tetraorthosilicate (TEOS) were purchased from Sigma Aldrich. In a 1000L Erlenmeyer, an amount of ethanol was added followed by the ammonium hydroxide solution and a portion of de-

ionized water. The appropriate amounts were calculated using the molarities provided by the Stöber method. After these components were placed in a magnetic stirrer, the TEOS was added drop-wise into the stirred solution, and stirred until reaction was completed.

3.2.2 **Polymer characterization**

The molecular weight distribution was determined in a Waters gel permeation chromatography system equipped with a PL aquagel–OH mixed 8 mm column and a Brookhaven Instruments BiDNDC differential refractometer. Samples were analyzed at 30°C with reference to PEG-PEO standards (Varian, Inc). Results are shown in Figure 3.1. From the distribution shown in the number average (Mn), weight average (Mw), and z-average (Mz) molecular weights were calculated as 36.8, 291, and 1,380 kDa, respectively.

Thermogravimetric analysis (TGA) was performed in a TA Instruments TGA 2950 under an inert nitrogen atmosphere at a 2 ºC/min temperature ramp. Results are shown in Figure 3.2. A weight decrease of 14.6% from 20 to 110 ºC which was associated with water loss was observed. Differential scanning calorimetry (DSC) in a TA Instruments Q20 at a heating rate of 10 ºC/min showed a broad peak from 210 to 265 ºC, which confirmed the TGA results. This result is shown in Figure 3.3. The degradation peak of sodium alginate has been previously reported in the literature in this temperature range [30].

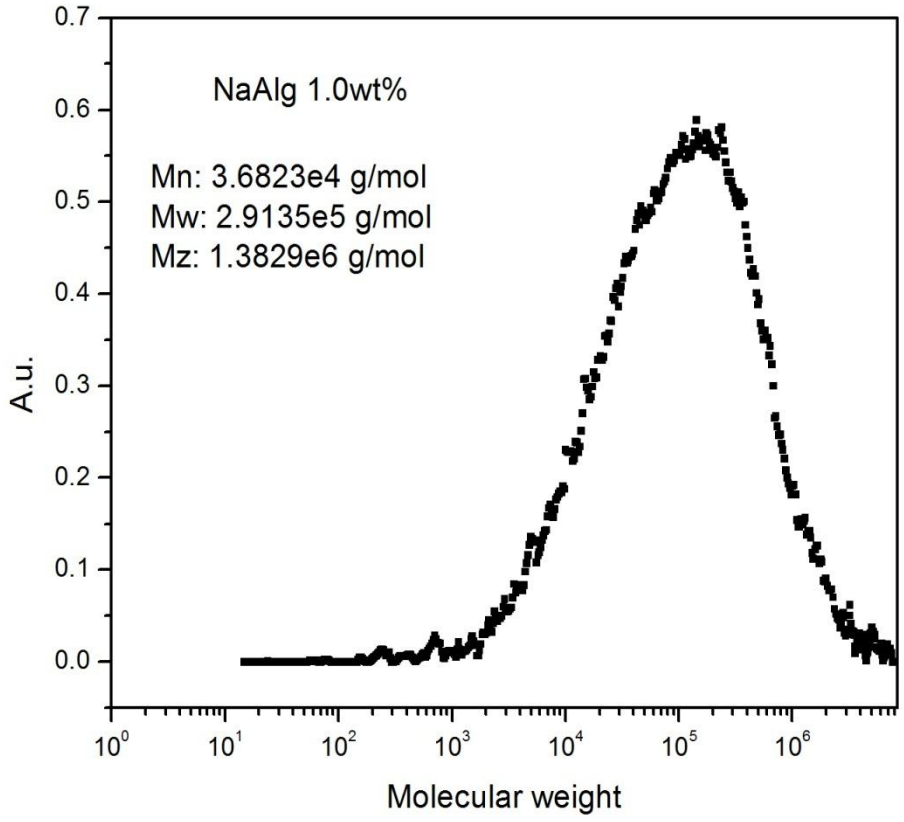


Figure 3.1. Molecular weight distribution for sodium alginate using gel permeation chromatography technique

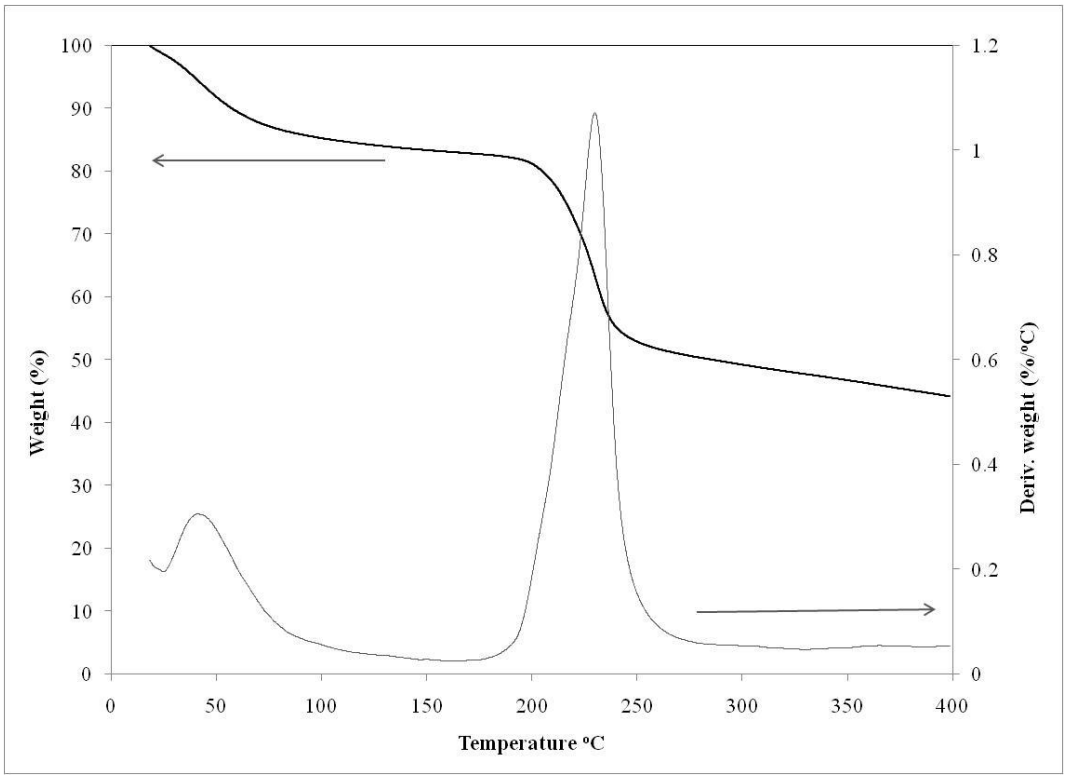


Figure 3.2. Weight loss for NaAlg using TGA

FTIR spectra measured in a Varian 800 were used to determine the M/G ratio through characteristic absorbance peaks at 1,030 and 1,080 cm−1 [32]. The average absorbance ratio (A1030/A1080) for NaAlg without pretreatment and dried at 40°C in vacuum overnight was 1.00 ± 0.01. Results for four samples are summarized in Table 1. From the absorbance relationship for the calcium salt of alginate reported by Sakugawa [32], the mannuronic content was predicted to be approximately 39 ± 4%.

Table 3-1. NaAlg composition determined by absorbance ratio between A_{1030}/A_{1080}

NaAlg	Sample	(A_{1030}/A_{1080})	Average	STDEV
without treatment	1	0.992	0.99428	0.0028
	2	0.9916		
	3	0.9968		
	4	0.9967		
40C/Vacuum - Over Night	1	1.0097	1.00145	0.0055
	2	0.9992		
	3	0.9984		
	4	0.9985		

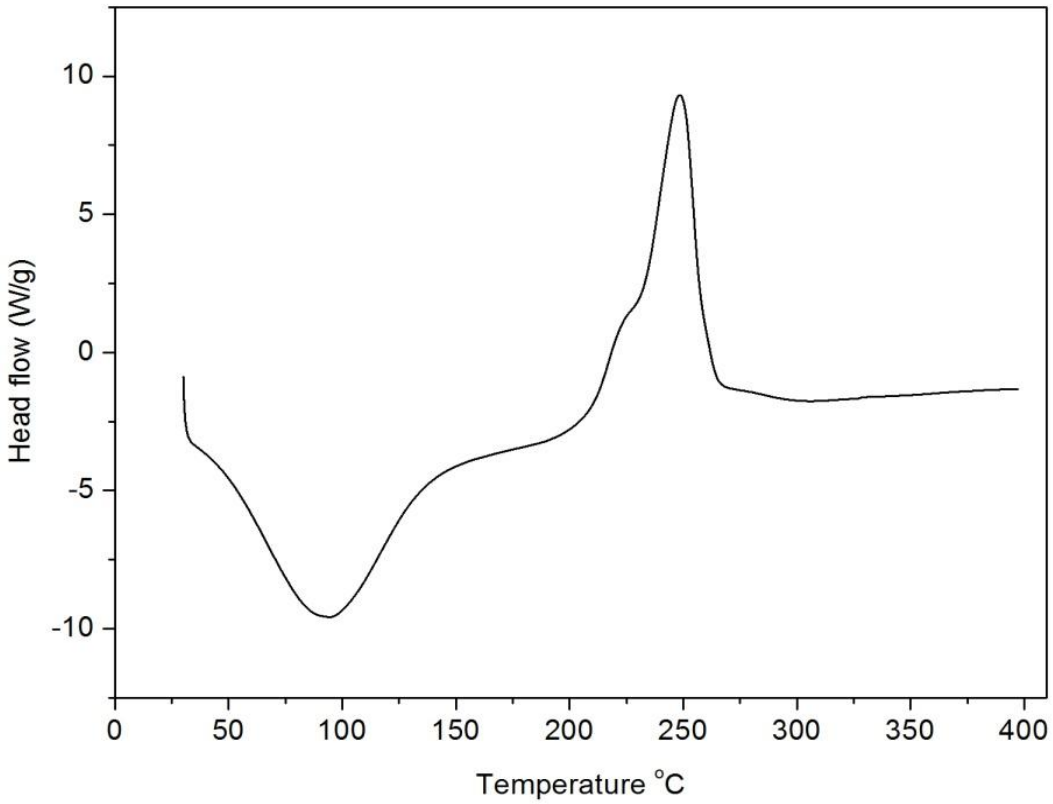


Figure 3.3. Thermogram for NaAlg determined by differential scanning calorimetry

3.2.3 Silica particle characterization

Zeta potential and particle size were measured in a Brookhaven Instruments 90Plus particle size analyzer at 40 ºC. Silica particles were negative in the pH range from 3 to 10. Particles were monodisperse as evidenced by the polydispersity values. A summary of the diameter and zeta potential for the three batches considered in this work is presented in Table 3.2.

Table 3-2. Particle size distribution and zeta potential for silica

Silica batch	Effective diameter	ζ potential (mV) @ pH = 7
1	784.9 ± 0.04	-57.4
2	216 ± 0.044	-20
3	73.3 ± 0.005	-30.7

Silica particles synthesized were negative in all the analyzed pH range as is shown in Table 3.3. However, silica particles with diameter of 0.22 µm were stable <-30 mV> in pH 9, particles of 0.78 µm were stable in at pH range from 5 to 10, while particle of 0.07 µm were stable at pH from 3.5 to 10.

Table 3-3. ζ potential for silica particles at different sizes in a pH range

Silica 0.22 µm			Silica 0.78 µm			Silica 0.07 µm		
pH	ζ Potential (mV)	STDEV	pH	ζ Potential (mV)	STDEV	pH	ζ Potential (mV)	STDEV
9.97	-19.89	4.73	10.01	-56.1	4.45	9.88	-46.18	2.14
9.03	-32.84	4.66	8.99	-60.11	2.94	9.35	-63.97	6.74
7.97	-19.96	3.75	7.97	-64.7	4.88	8.02	-63.55	5.99
7.04	-9.97	2.57	6.94	-57.44	4.82	6.92	-30.01	4.55
5.99	-4.44	2.05	5.96	-48.76	5.92	5.98	-58.82	7.89
4.99	-41.38	4.15	4.97	-32.83	2.39	5.01	-56.89	8.97
4.03	-17.17	4.62	3.95	-22.5	3.11	4.07	-47.37	5.81
2.97	-11.46	1.05	2.99	-26	3.43	2.96	-18.66	5.64
1.98	-3.28	1.19	2.05	4.41	1.59	2.08	-13.45	6.68

3.2.4 Solution preparation

NaAlg allows for preparation of aqueous solutions only at low concentrations, usually less than 2.5%. Solutions were prepared by mixing the appropriate amount of sodium alginate with deionized water. Then, the mixture was heated in a hot-plate at 70 ºC, while stirring constantly for at least 30 minutes. No adjustments of pH were made. The measured pHs of the solutions were between 5.2 and 5.4. pH for NaAlg solution at 1.5 wt% was 9, while the pH for the NaAlg solution containing silica was around 7. Concentrations used in the following sections of this work were not corrected for water content.

3.2.5 Rheology

Rheological characterization was performed on a Reologica StressTech HR stress-controlled rheometer equipped with an extended temperature cell (ETC) for temperature control using stainless steel cone-and-plate ($d = 30$ mm and $\theta < 4º$) and double-gap Couette ($V = 11$ mL) fixtures and on an Anton-Paar Physica MCR301 equipped with a Julabo constant temperature water bath control using stainless steel cone-and-plate fixture ($d = 50$ mm and $\theta = 0.976º$).

The rheometer fixture was pre-heated to the desired temperature, before transferring and loading the hot solution at 70 ºC. The sample and fixture temperature were allowed to equilibrate for at least 20 minutes. After temperature reached equilibrium, rheological tests were performed.

Gelation temperature of NaAlg was determined using the constant-stress temperature-ramp (CSTR) viscosity test and dynamic tests. In the first one, the viscosity was measured at a constant stress of 1 Pa from 50 to 10 ºC at a cooling rate of 2 ºC/min. The cooling rate was chosen to emulate typical processing conditions. In the dynamic tests, storage and loss moduli were measured at a frequency of 1 Hz and a strain of 1% to guarantee measurement in the linear viscoelastic regime, the temperature ramps were similar to those used in the CSTR.

Thixotropy tests were performed by measuring the steady-state viscosity at constant temperature as a function of the shear rate in an upward sweep from 0.1 to 100 s^{-1} immediately followed by a downward sweep from 100 to 0.1 s^{-1}. Steady-state viscosity was also measured at constant 10 ºC increments above gelation temperature as a function of the shear rate from 0.1 to 100 s^{-1}.

3.3 Results and discussion

3.3.1 Gelation temperature of NaAlg solutions

The viscosity curves measured by the CSTR tests from NaAlg solutions ranging from 0.5 to 2.5 wt% are presented in Figure 3.4. The discontinuity in the viscosity associated to network formation, as explained in previous sections, was observed for solutions above 1.0 wt% (perceived on a linear viscosity scale). The gelation temperature (T_{gel}) was determined as the intercept of the tangent line of the viscosity at high temperature and that of the discontinuous viscosity overshoot, as illustrated by the dashed lines over the 1.25 wt% solution in Figure 3.4.

The early stage of an overshoot was observed around 10 ºC on the 1.0 wt % solution, which suggest a gel transition below our lowest experimentally accessible temperature. Thus, for solutions below 0.75 wt%, no gelation was observed within our experimental window. These CSTR viscosity tests confirm the existence of a thermotropic gel transition in aqueous NaAlg systems. Nevertheless, the transition occurs below room temperature, as evidenced by the values of T_{gel} (Table 2). Additionally, the gelation temperature is also observed to increase with polymer concentration. Reproducibility tests performed on various solutions with a concentration of 1.5 wt% yielded an average value of T_{gel} of 15.0 ºC with a standard deviation of 1.9 (n = 5).

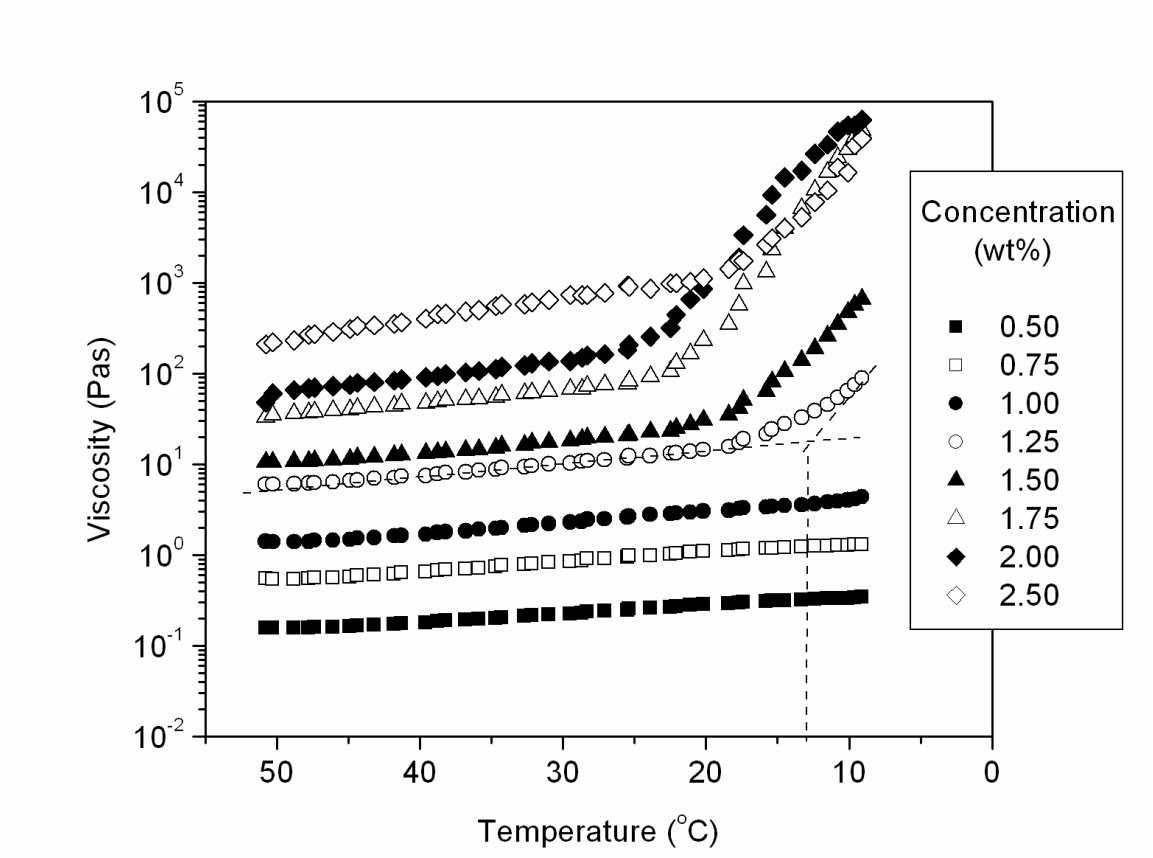


Figure 3.4. Constant-stress temperature-ramp (CSTR) test for various aqueous sodium-alginate solutions

Experimental conditions were constant stress of 1 Pa and cooling rate of 2 ºC/min.

The CSTR viscosity test describes the gelation process under conditions similar to a processing environment. Nevertheless, it is a more destructive method than the small amplitude oscillatory shear test (i.e. determination of viscoelastic moduli). Thus, we applied this method independently to determine and compare values of the gelation temperature obtained from the two methods. As an additional benefit, the sample can be heated continuously after the cooling ramp without a lag time. The latter allows assessing of the reversibility of the transition. [31]

Table 3.2. Summary of transition temperatures for aqueous sodium alginate solutions determined by the constant-stress temperature-ramp (CSTR) viscosity and dynamic linear viscoelastic moduli (DLVEM) methods.

Concentration (wt%)	T_{gel} (°C)		T_{melt} (°C)
	CSTR	DLVEM	DLVEM
0.5	n.o.[a]	--	--
0.75	n.o.	n.o.	n.o.
1	13.5[b]	n.o.	n.o.
1.25	13	--	--
1.5	15.0 ± 1.9[c]	16.7 [b]	28.2 [b]
1.75	18	22	35.7
2	20.5	--	--
2.5	19	--	--

[a] not observed, [b] based on n = 2, [c] error based on n = 5, -- not determined

The evolution of the storage and loss moduli during cooling and heating for a 1.5 wt% solution is shown in Figure 3.5. The gelation temperature was chosen as the equilibrium point where the storage modulus (G′) equals the loss modulus (G″) during cooling of the sample. The average gelation temperature obtained from two dynamic experiments was 16.7, measured with the CSTR method. Furthermore, upon heating an additional crossover of the moduli was observed, which demonstrates the thermoreversible nature of the aqueous NaAlg solutions. The crossover of the heating curves is identified as the melting temperature. Values for various concentrations are summarized in Table 3.2. A hysteresis between the gelling and melting temperatures of the NaAlg solutions was observed. The NaAlg gels melted at least 7 ºC above the gelling point. Similar behavior has been reported for gelatin [31] and agarose [32] solutions. The thermal hysteresis can be attributed to gel domains that remain stable at much higher temperatures than those at which the molecular interactions caused gel formation.

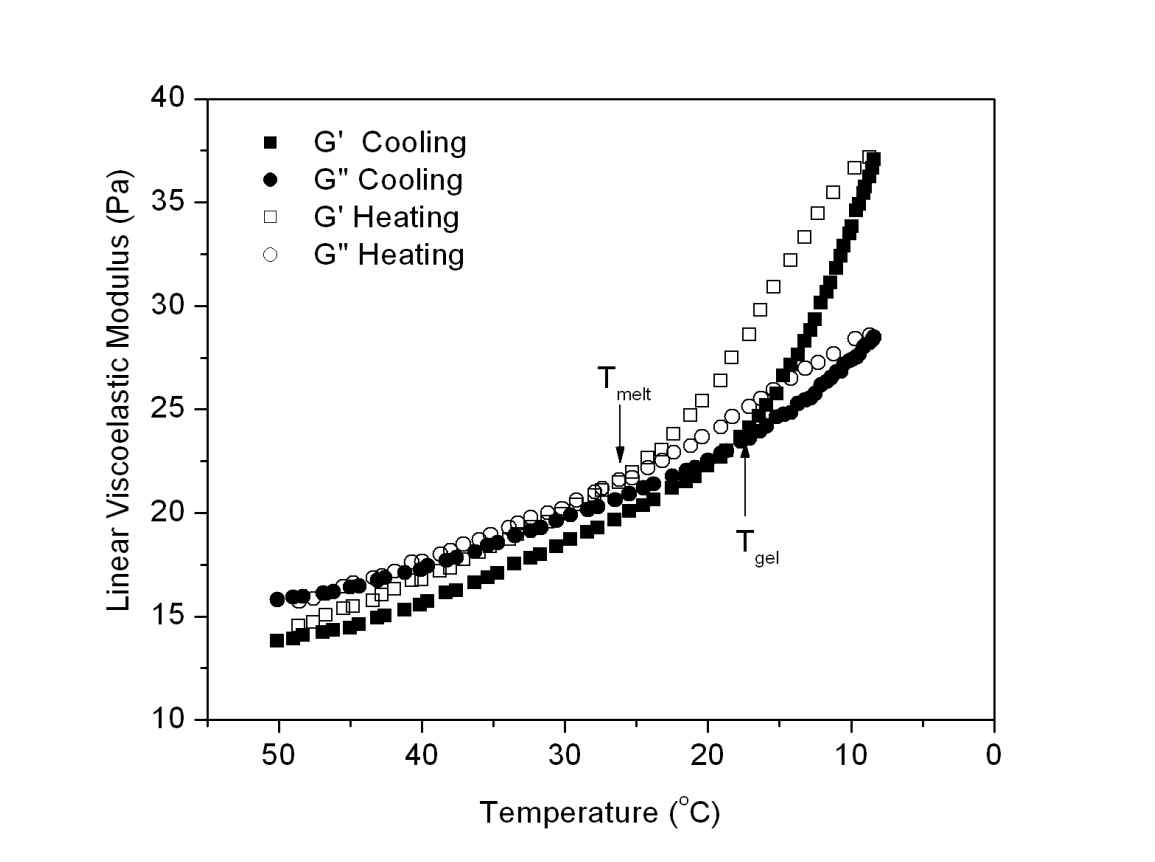


Figure 3.5. Storage (G') and loss modulus (G") as a function of temperature for a 1.5 wt% aqueous sodium alginate solution.

Constant frequency of 1 s^{-1}, strain of 1% and cooling rate of 2 ºC/min were used.

Visual inspection of the gels and the magnitude of G' indicates that NaAlg gel networks are much weaker than those obtained for gelatin. Thus, thixotropy tests were perfomed to further demostrate the formation of internal structure. Figure 3.6 shows thixotropy tests for a 1.5 wt% NaAlg solution at various temperatures below and above T_{gel} (15.0 & 16.7 ºC), where the open and closed symbols indicate upward and downward shear rate sweeps, respectively. The presence of a thixotropic envelope (or hysteresis) between the upward and downward shear rate sweeps demonstrate the destruction of internal structures, in this case, of a physical gel network. The thixotropic envelope was observed for temperatures 10, 15, 20 and 25 ºC. The hysteresis observed at 20 and 25 ºC is somewhat unexpected since the temperature is higher than the gelation temperature measured by the CSTR and dynamic tests.

Nevertheless, upon close inspection of the data curve for the 1.5 wt% NaAlg solution in Figure 3.4, it can be seen that deviations from linear behavior begin around 23 ºC. These deviations suggest that network formation has begun at this temperature. However, we believe that at these temperatures the network does not extend throughout the whole system. This experiment shows further evidence of the thermotropic physical gelation of sodium alginate. It also confirms that the physical gel is very weak, as evidenced by the small area of the hysteresis envelopes.

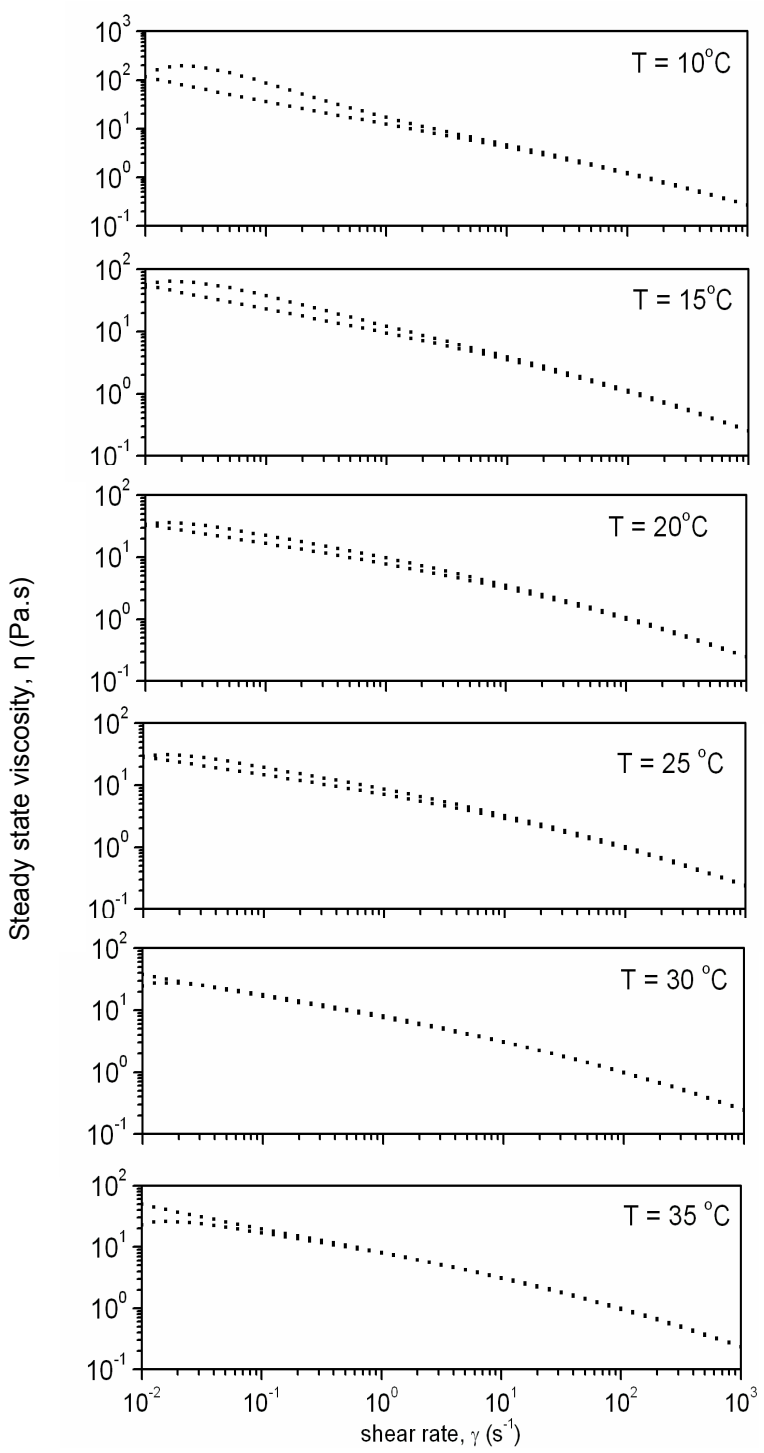


Figure 3.6. Thixotropy tests for a 1.5 wt% sodium alginate solution in water at constant temperatures.

The steady-state rheology of the aqueous sodium alginate solutions above T_{gel} was also characterized as a function of shear rate, temperature, and concentration. Figure 3.7 shows the temperature dependence of the steady-state rheology of a 1.0 wt % solution. The solutions in all cases exhibited shear-thinning behavior with similar scaling over the studied shear rate range, i.e. at all temperatures and all concentrations as is shown in Figure 3.7. Hence, reduction of all the data to a viscosity master curve was suggested.

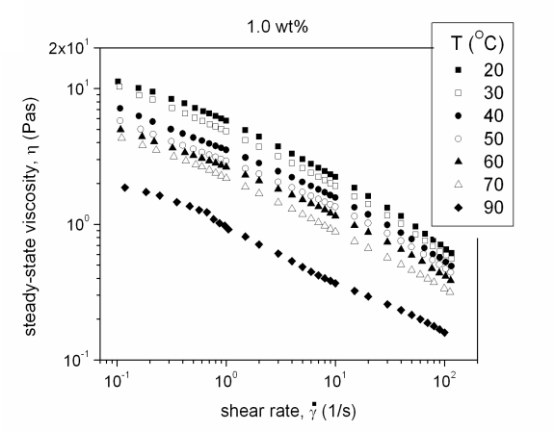
Time-temperature superposition (TTS) based on the Rouse dynamics model was applied [33], where the relationship for reduced shear rate and reduced viscosity are given, respectively by:

$$\dot{\gamma}_{r,T} = a_T \dot{\gamma} \tag{1}$$

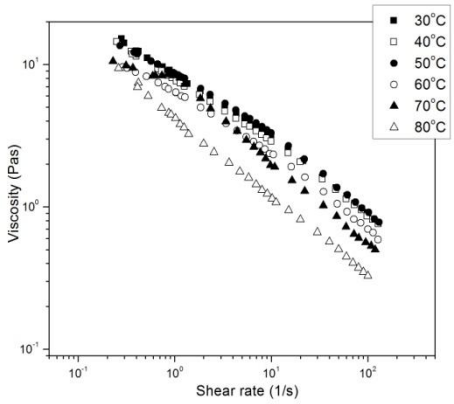
$$\eta_{r,T}\left(a_T \dot{\gamma}\right) = \frac{\eta(T) T_{ref} \rho_{ref}}{a_T T \rho} \tag{2}$$

where, a_T is the empirical temperature shifting parameter, T is the temperature, ρ is the density, and the subscript *ref* refers to the properties of the chosen reference solution. Tabulated water densities where used in Eq. 2 to predict variations in density instead [34].

(a)



(b)



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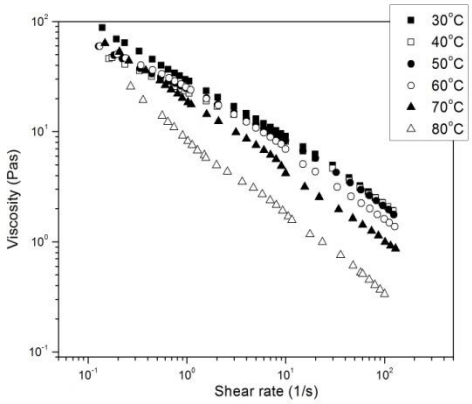

Figure 3.7. Steady-state viscosity of a 1.0 wt% (a), 1.5 wt% (b), 2.5 wt% (c) aqueous sodium alginate solution as a function of temperature.

Figure 3.8 and 3.9 show the temperature-shifted data for all studied concentrations. Good agreement was observed at all concentrations below 70 ºC and at low shear rates, deviations are present for 1.5 and 2.5 wt.% solutions. Deviations due to degradation, poor solubility, or textural stresses are not consistent with the observed higher shear rate scaling of the viscosity at lower shear rates and higher temperatures. A more extended chain configuration at higher temperatures may reduce the effective number of entanglements which may allow the molecules to move freely around each other. Molecules are thus more susceptible to shear forces and to shear-induced orientation even at low shear rates. However, this would be expected to happen at all concentrations, yet the 1.0 wt% NaAlg solution did not show deviations. In situ structural probes such as small-angle X-ray or light scattering techniques may provide additional information on the effect of temperature and flow on the conformation of NaAlg molecules.

The temperature shift parameters are plotted as a function of temperature in Fig. 3.10. The temperature shift parameters of flexible polymers usually show Arrhenius dependence. Fitting of an Arrhenius equation to our data yields values of $-\Delta H/R\times10^{-3}$ for the 1.0, 1.5, and 2.5 wt.% NaAlg solutions of 4.62 ± 0.32, 3.67 ± 0.65, and 3.66 ± 0.43, respectively, with corresponding correlation coefficients (R^2) of 0.948, 0.748, and 0.866. The prediction for $-\Delta H/R\times10^{-3}$ equal to 4 is shown as solid line in Fig. 3.10 shifted one vertical unit down for reference. The activation energies for flow ($-\Delta H/R$) are close to each other, but are independent of concentration.

Correlation factors for the Arrhenius equation show moderately good to very good agreement. Other dependencies, such as the WLF equation, were tested with poor agreement and no correlation. No theoretical basis for the temperature shift parameters was identified. Although TTS has a theoretical basis, it is widely used for systems where there is no theoretical justification [33] . Nevertheless, the advantages of demonstrating the applicability of TTS lie in the prediction of viscosity and other rheological properties with high certainty through widely used semi-empirical models.

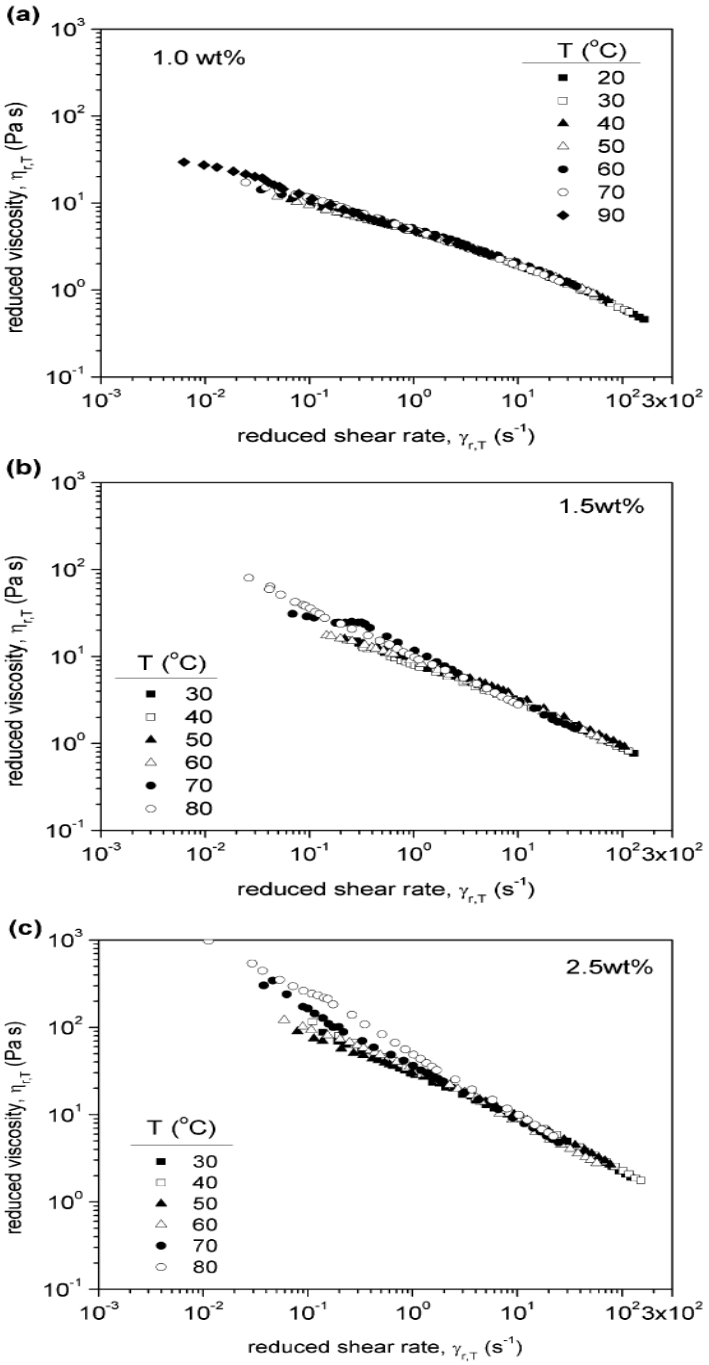

Figure 3.8. Temperature-shifted steady-state viscosity for 1.0 wt % (a), 1.5 wt% (b), and 2.5 wt% (c) aqueous sodium alginate solutions. Reference temperature was 30°C

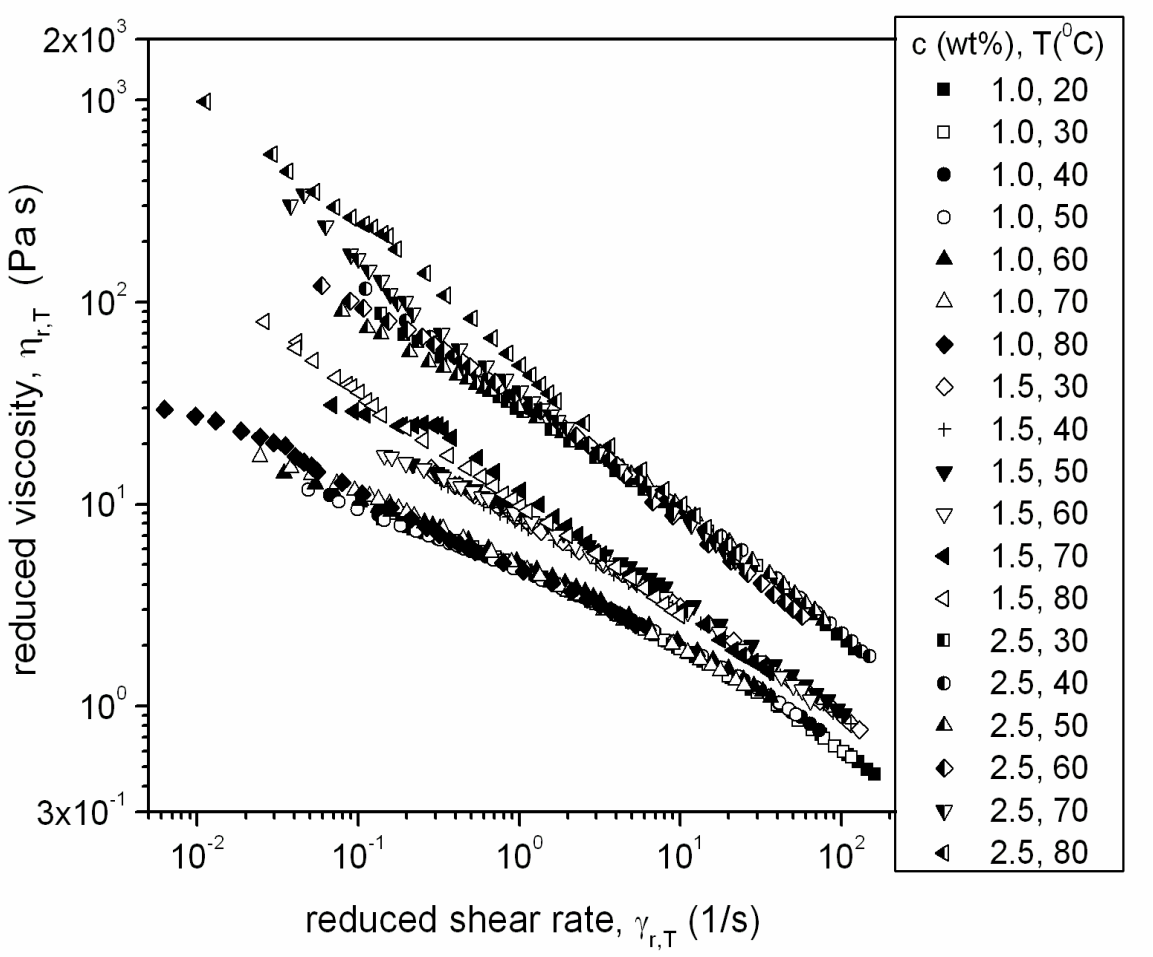

Figure 3.9. Temperature-shifted steady-state viscosity for various concentrations of sodium alginate in water solutions.

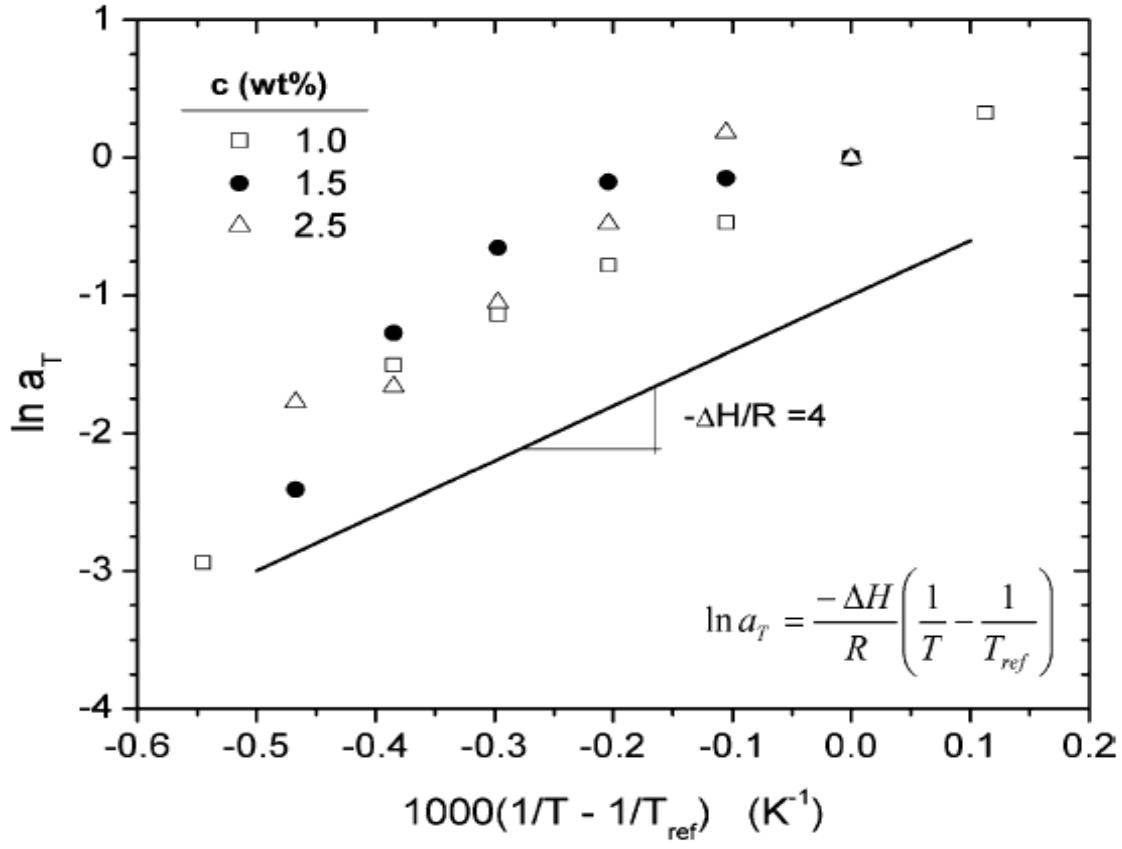

Figure 3.10. Temperature-dependence of the temperature-shifting parameters for sodium alginate.

Solid line represents the theoretical prediction of an Arrhenius equation with a −ΔH/R=4 k shifted one vertical unit down for clarity

Kokini and Surmay (1994) reported the construction of concentration master curves for various polysaccharides, including sodium alginate, yet, their approach was completely empirical [35]. The temperature-shifted data from Fig 8 were reduced by applying the shift factor to the viscosity and shear rate as:

$$\eta_{r,c} = a_c^{-1}\eta \qquad (3)$$

$$\dot{\gamma}_{r,c} = a_c\dot{\gamma} \qquad (4)$$

The concentration shift factor was treated as an adjustable parameter. Figure 3.11 shows the data from Fig. 3.8 concentration-shifted using the approach discussed above. It is observed that the concentration shifting allows for the collapsing of all the temperature-shifted data into a single master curve. Furthermore, it was determined that the concentration shift parameter scales with the dimensional concentration as:

$$a_c = \left(\frac{c}{c_{ref}}\right)^b \qquad (5)$$

where c is the concentration and the subscript denotes the reference value. By applying a linear fit to the adjusted a_c, the exponent b was found to be equal to 3.31 ± 0.33 (R^2 = 0.981). The viscosity for suspensions of rigid polymers and particles in the semidilute regime has been experimentally demonstrated to scale with the cube of concentration in agreement with predictions [36]. It has also been reported that the specific viscosity of concentrated solutions of sodium alginate scales with the b^{th} power of concentration, where b ranges from 3.2 to a 4.1 power [37, 38]. Similar

behavior has been reported for many industrial polysaccharides [39]. Thus, a semi-empirical approach can be applied where the concentration shift parameter can be predicted by Eq. 5 with a cubic-power scaling.

The semi-empirical power-law fluid viscosity model ($\eta = k\dot{\gamma}^n$) was adjusted to the viscosity master curve. Data sets above 70 ºC, which showed deviations from the master curve, were neglected for the fitting of the model. Good agreement was obtained with k = 4.75 and n = -0.446 ± 0.003 with a correlation coefficient R^2 = 0.979. As shown in the plot above, the viscosity above 70 ºC shows a stronger scaling with shear rate (i.e. closer to -0.5). The deviations at higher temperatures may be due to an increase in flexibility and thus, extension of the chains. This in part might increase entanglements of the coils and thus the viscosity. A conformational study of the sodium alginate solutions at different temperature is required in order to elucidate the mechanism of gel formation and the cause of the deviations at higher temperatures.

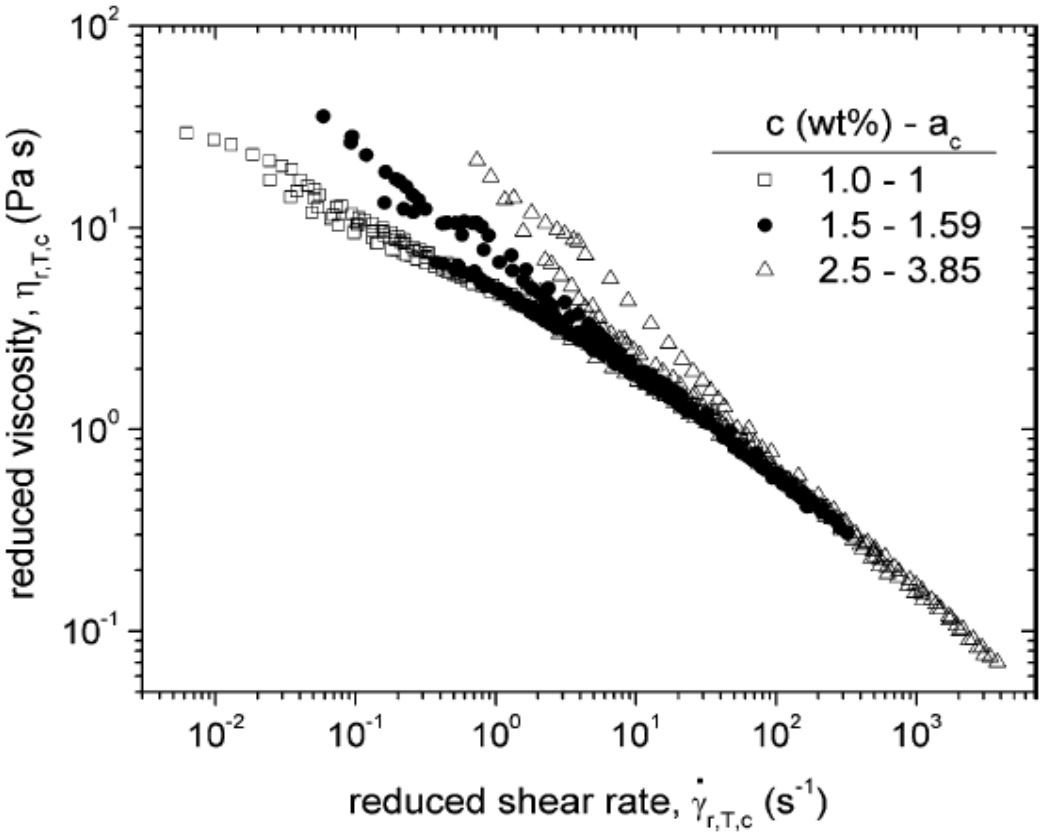

Figure 3.11. Steady-state viscosity temperature–concentration-shifted master curve for aqueous sodium alginate solutions.

Reference temperature and concentration are 30°C and 1.0 wt %, respectively

3.3.3 Effect of particle size and concentration on the gelation temperature

Figure 3.12 shows the effect of particle size and concentration on gelation temperature of a 1.5 wt% NaAlg solution. Each value corresponds to the average of at least three measurements plus or minus their corresponding standard deviation. Gelation temperatures for a NaAlg solution at 1.5 wt% were not affected by the size or concentration at low silica concentration (< 1.4). Stata Software was used for statisticals analysis; results are shown in Table 3.4.

Table 3-4. Statisticals analysis of the effect of particle concentration on gelation temperature of 1.5 wt% NaAlg solution

Silica	**0.07um**			**0.22um**			**0.78um**		
(wt%)	**T_{gel} (^oC)**	**STDEV**	**P value**	**T_{gel} (^oC)**	**STDEV**	**P value**	**T_{gel} (^oC)**	**STDEV**	**P value**
0	**13.63**	0.36		**13.63**	0.36		**13.61**	0.35	
0.2	11.82	0.69	0.02	13.69	1.03	0.93	14.17	0.60	0.24
0.4	12.12	1.06	0.08	13.55	0.44	0.84	13.70	0.96	0.88
0.6	13.00	1.26	0.45	13.78	0.69	0.74	13.83	1.44	0.80
0.8	13.79	1.01	0.81	13.85	1.35	0.79	14.27	1.80	0.57
1	13.20	1.46	0.65	13.63	1.08	1.00	13.73	0.71	0.79
1.2	13.48	1.98	0.91	14.50	0.52	0.07	13.63	0.75	0.96
1.4	12.59	0.85	0.12	15.05	0.71	0.04	14.93	0.97	0.09
1.5	11.54	0.48	0.00	14.82	0.98	0.11	14.30	1.01	0.33

This can be attributed to electrostatic repulsions between NaAlg and silica. Sodium alginate is a negative polyelectrolyte [40] and silica was found to have a negatively charged surface. No adsorption of the polymer to the silica surface is expected. Da Silva et al. studied the effect of silica on the anionic polymer k-carrageen [41]. They found that carrageen gelation was a function of silica particle

load and size. The effect of particle size was studied using particles with 118, 444, 563 and 856 nm in diameter, concentrations from 5 to 50 wt% relative to the polymer, and particle charges around -5 to -20 mV. These particles size and concentration are within our studied range. However, our particles are more negatively charged (-20 to -60 at the solution pH) resulting in a higher repulsion and the gelation mechanism for the polymers are different. K-carrageenan forms physical gels by a coil-helix mechanism similar to gelatin [42]. Da Silva and coworkers argue that particles affect gelation due to an increased effective concentration. We disagree since polymer and particle concentrations are low, thus the inter-particle length scales must be much larger that the polymer length scale. Polymer concentration was 1.5 wt% and the higher silica concentration studied was 1.5 wt%. When silica is present in the system at this concentration causes a maximum increase of 0.02 wt% in polymer concentration and not significant changes are expected for T_{gel}. Thus, confinement effect and polymer depletion should be negligible in the studied range.

Figure 3.12 shows a comparison between gelation temperatures obtained for NaAlg solutions containing silica with particle size 0.07 and 0.22 μm. The corresponding P values are reported in Table 3.4. Gelation temperature for silica-loaded polymer was only significantly affected ($P < 0.03$) with particle size at high silica concentrations ($\geq$ 1.4 wt%). Similar results were obtained by a comparative analysis between gelation temperature for NaAlg containing silica particles with diameters of 0.07 and 0.78 μm, shown in Figure 3.13. Corresponding P values are summarized in Table 3.4.

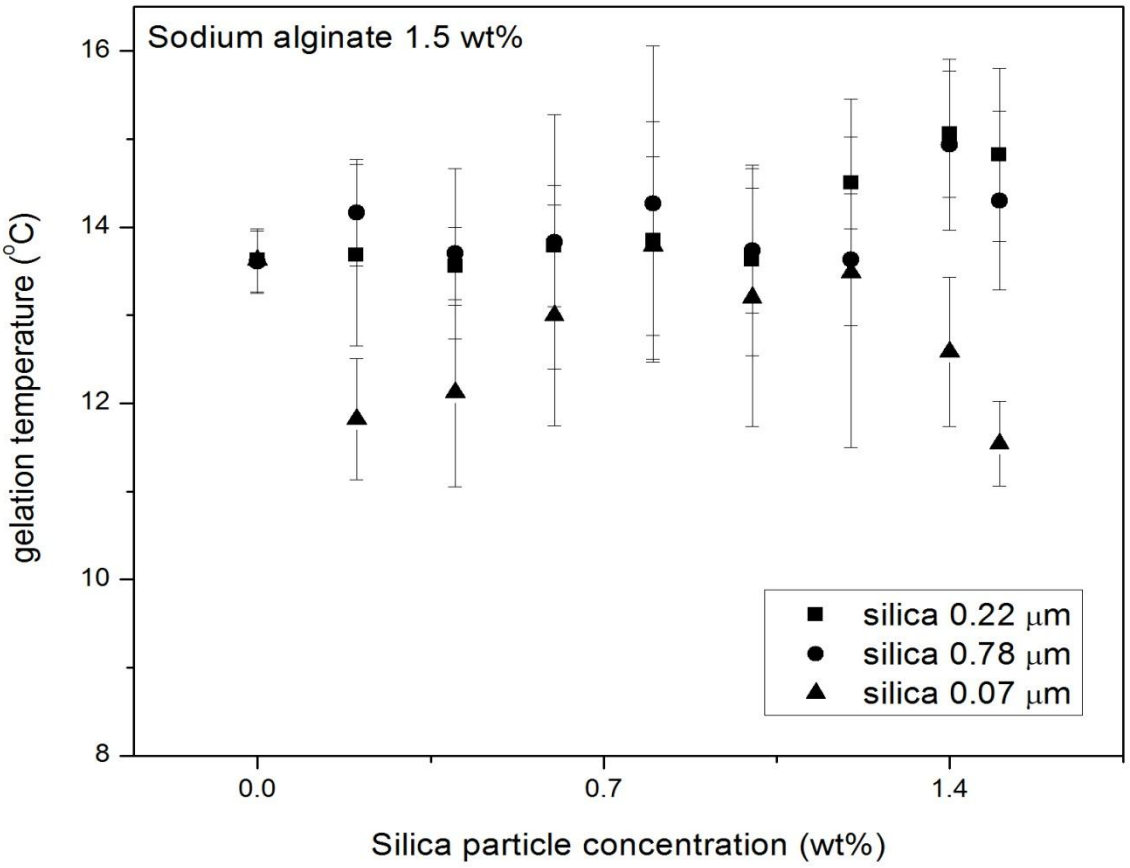

Figure 3.12. Effect of silica size and concentration on gelation temperature of a 1.5 wt% NaAlg solution

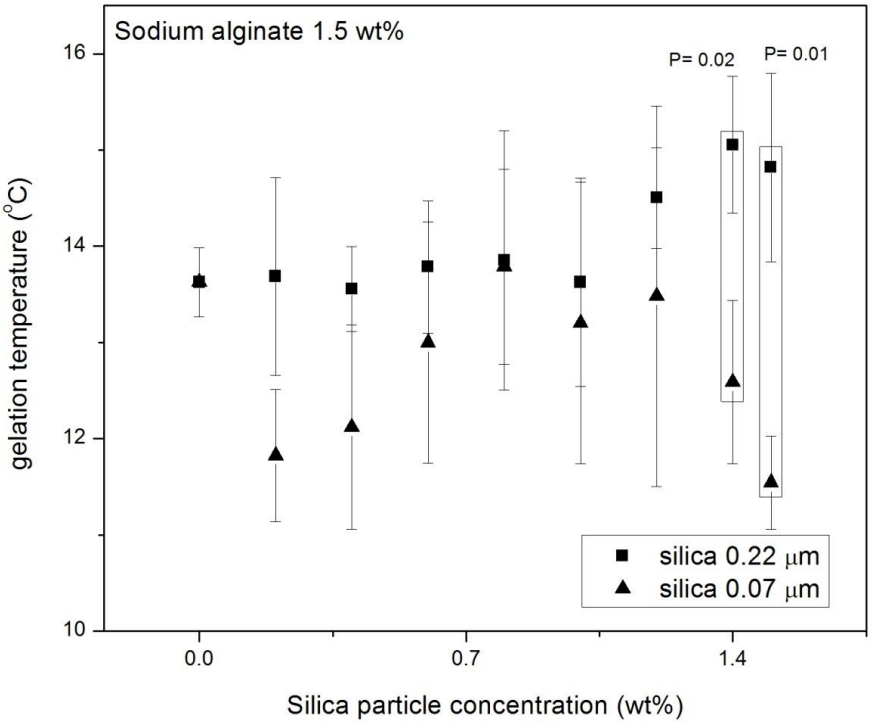

Figure 3.13. Effect of silica size (0.07 and 0.22 µm) and concentration on gelation temperature of a 1.5 wt% NaAlg solution

Table 3-5. Silica size effect (0.07 and 0.22 µm) on gelation temperature

Sil wt%	0.22 µm		0.07 µm		**P**
	T_{gel}(°C)	STDEV	T_{gel}(°C)	STDEV	
0	13.63	0.36	13.63	0.36	1.00
0.2	13.69	1.03	11.82	0.69	0.06
0.4	13.55	0.44	12.12	1.06	0.10
0.6	13.78	0.69	13.00	1.26	0.40
0.8	13.85	1.35	13.79	1.01	0.95
1	13.63	1.08	13.20	1.46	0.71
1.2	14.50	0.52	13.48	1.98	0.43
1.4	15.05	0.71	12.59	0.85	0.02
1.5	14.82	0.98	11.54	0.48	0.01

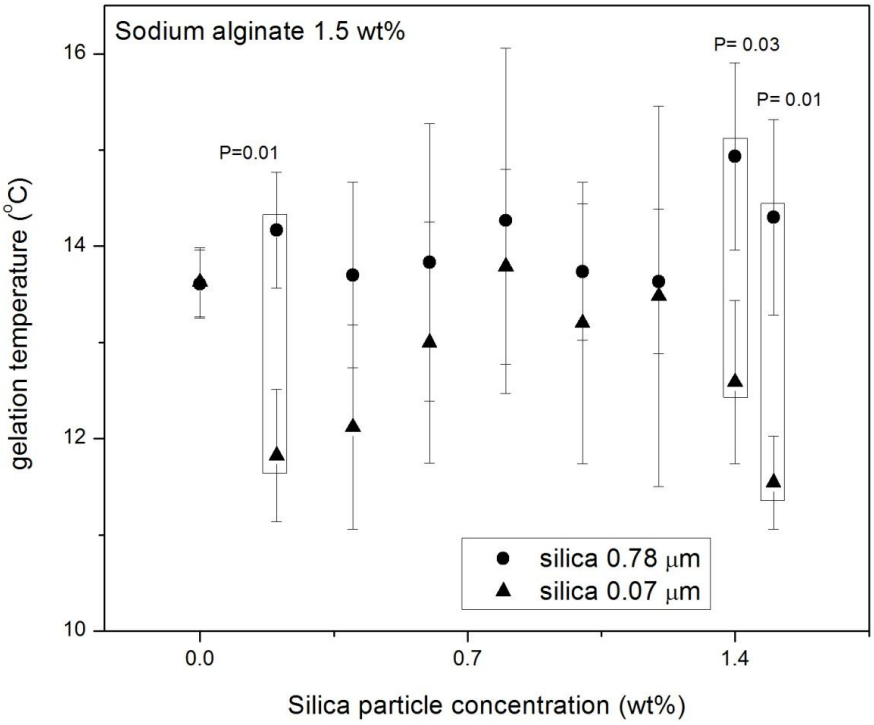

Figure 3.14. Effect of silica size (0.07 and 0.78 µm) and concentration on gelation temperature of a 1.5 wt% NaAlg solution

Table 3-6. Silica size effect (0.07 and 0.78 µm) on gelation temperature

Sil wt%	0.07 µm		0.78 µm		**P**
	T_{gel}(°C)	STDEV	T_{gel}(°C)	STDEV	
0	13.63	0.36	13.61	0.35	0.95
0.2	11.82	0.69	14.17	0.60	0.01
0.4	12.12	1.06	13.70	0.96	0.14
0.6	13.00	1.26	13.83	1.44	0.49
0.8	13.79	1.01	14.27	1.80	0.71
1	13.20	1.46	13.73	0.71	0.60
1.2	13.48	1.98	13.63	0.75	0.91
1.4	12.59	0.85	14.93	0.97	0.03
1.5	11.54	0.48	14.30	1.01	0.01

3.4 Conclusions

In this chapter, the existence of a thermotropic gelation in sodium alginate solutions was demonstrated through three independent rheological tests: the constant-stress temperature-ramp (CSTR), dynamic viscoelastic moduli, and thixotropy test. Oscillatory dynamic tests also showed the thermoreversibility of the gels. The gel structure in this system is very weak as evidenced by the viscosity drop in the thixotropy test and the magnitude of the storage moduli. Aditionally, the gelation occurs below room temperature. Thus, the direct use of the gelled NaAlg as the main structural component in film-forming applications and/or suspending medium for solid particles may be limited.

It was also demonstrated that the steady-state rheology of aqueous NaAlg solutions above T_{gel} in a temperature range from 20 to 60 ºC, concentrations between 1.0 to 2.5 wt% and shear rates from 0.1 to 100 s^{-1} may be collapsed into a single master curve using time-temperature superposition and a semi-empirical approach for the concentration shifting. Since shear-thinning behavior was observed in all cases, a power-law fluid model was fitted to the master curve with good agreement. The power-law model and shifting parameters may be used to predict *a priori* steady-state rheology.

In was also found that for our similarly charged polymer-particle system, gelation temperature was unaffected by low particle loadings (up to 1.5 wt %)

independently of particle diameter. This is attributed to the nature of our solutions, both in polymer and particle concentrations. At much higher particle concentration particle-particle interactions and polymer confinement may affect the T_{gel}. Nevertheless, that particle range was outside of the scope of this work.

Note:

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3.5 References

1. McHugh, D.J., *A Guide to the Seaweed Industry*. FAO Fisheries Technical Paper, No 441. 2003, Rome.

2. Phillips, G.O. and P.A. Williams, eds. *Handbook of Hydrocolloids*. 2000, Woodhead Publishing Limited/CRC Press LLC.

3. Braun, D.B. and M.R. Rosen, *Rheology Modifiers Handbook: Practical Use and Application*. 2000, New York: William Andrew Publishing.

4. Wang, Z.Y., Q.Z. Zhang, M. Konno, and S. Saito, *Sol-Gel Transition of Alginate Solution by the Addition of Various Divalent-Cations - a Rheological Study.* Biopolymers, 1994. **34**(6): p. 737-746.

5. Liu, X.X., L.Y. Qian, T. Shu, and Z. Tong, *Rheology characterization of sol-gel transition in aqueous alginate solutions induced by calcium cations through in situ release.* Polymer, 2003. **44**(2): p. 407-412.

6. Lu, L., X.X. Liu, L.Y. Qian, and Z. Tong, *Sol-gel transition in aqueous alginate solutions induced by cupric cations observed with viscoelasticity.* Polymer Journal, 2003. **35**(10): p. 804-809.

7. de Kerchove, A.J. and M. Elimelech, *Formation of polysaccharide gel layers in the presence of Ca2+ and K+ ions: Measurements and mechanisms.* Biomacromolecules, 2007. **8**(1): p. 113-121.

8. Grant, G.T., E. Morris, D.A. Rees, P.J.C. Smith, and D. Thom, *Biological interactions between polysaccharides and divalent cations: The egg-box model.* FEBS Letters, 1973. **32**: p. 195-198.

9. Braccini, I. and S. Perez, *Molecular basis of C2+-induced gelation in alginates and pectins: The egg-box model revisited.* Biomacromolecules, 2001. **2**: p. 1089-1096.

10. Abbah, S.A., W.W. Lu, D. Chan, K.M.C. Cheung, W.G. Liu, F. Zhao, Z.Y. Li, J.C.Y. Leong, and K.D.K. Luk, *Osteogenic behavior of alginate encapsulated bone marrow stromal cells: An in vitro study.* Journal of Materials Science-Materials in Medicine, 2008. **19**(5): p. 2113-2119.

11. Kost, J. and R. Goldbart, *Natural and Modified Polysaccharides*, in *Handbook of Biodegradable Polymers*, A.J. Domb, J. Kost, and D.M. Wiseman, Editors. 1997, CRC Press: Boca Raton, FL.

12. Rao, C.S., S.S. Madhavendra, R.S. Rao, P.J. Hobbs, and R.S. Prakasham, *Studies on improving the immobilized bead reusability and alkaline protease production by isolated immobilized Bacillus circulans (MTCC 6811) using*

overall evaluation criteria. Applied Biochemistry and Biotechnology, 2008. **150**(1): p. 65-83.

13. Wells, L.A. and H. Sheardown, *Extended release of high pI proteins from alginate microspheres via a novel encapsulation technique.* European Journal of Pharmaceutics and Biopharmaceutics, 2007. **65**(3): p. 329-335.

14. Li, T.P., N. Wang, S.H. Li, Q.C. Zhao, M. Guo, and C.Y. Zhang, *Optimization of covalent immobilization of pectinase on sodium alginate support.* Biotechnology Letters, 2007. **29**(9): p. 1413-1416.

15. Yabur, R., Y. Bashan, and G. Hernandez-Carmona, *Alginate from the macroalgae Sargassum sinicola as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion.* Journal of Applied Phycology, 2007. **19**(1): p. 43-53.

16. Potumarthi, R., C. Subhakar, A. Pavani, and A. Jetty, *Evaluation of various parameters of calcium-alginate immobilization method for enhanced alkaline protease production by Bacillus licheniformis NCIM-2042 using statistical methods.* Bioresource Technology, 2008. **99**(6): p. 1776-1786.

17. Mladenovska, K., O. Cruaud, P. Richomme, E. Belamie, R.S. Raicki, M.C. Venier-Julienne, E. Popovski, J.P. Benoit, and K. Goracinova, *5-ASA loaded chitosan-Ca-alginate microparticles: Preparation and physicochemical characterization.* International Journal of Pharmaceutics, 2007. **345**: p. 59-69.

18. Reis, C.P., A.J. Ribeiro, F. Veiga, R.J. Neufeld, and C. Damge, *Polyelectrolyte biomaterial interactions provide nanoparticulate carrier for oral insulin delivery.* Drug Delivery, 2008. **15**(2): p. 127-139.

19. Mitamura, K., T. Imae, N. Saito, and O. Takai, *Fabrication and structure of alginate gel incorporating gold nanorods.* Journal of Physical Chemistry C, 2008. **112**(2): p. 416-422.

20. Bhat, S.D. and T.M. Aminabhavi, *Zeolite K-LTL-loaded sodium alginate mixed matrix membranes for pervaporation dehydration of aqueous-organic mixtures.* Journal of Membrane Science, 2007. **306**(1-2): p. 173-185.

21. Patil, M.B., R.S. Veerapur, S.A. Patil, C.D. Madhusoodana, and T.M. Aminabhavi, *Preparation and characterization of filled matrix membranes of sodium alginate incorporated with aluminum-containing mesoporous silica for pervaporation dehydration of alcohols.* Separation and Purification Technology, 2007. **54**(1): p. 34-43.

22. Chan, A.W., R.A. Whitney, and R.J. Neufeld, *Kinetic Controlled Synthesis of pH-Responsive Network Alginate.* Biomacromolecules, 2008. **9**(9): p. 2536-2545.

23. Mørch, Ã.A., I. Donati, B.L. Strand, and G. Skjåk-Bræk, *Molecular Engineering as an Approach to Design New Functional Properties of Alginate.* Biomacromolecules, 2007. **8**(9): p. 2809-2814.

24. Aminabhavi, T.M., S.A. Agnihotri, and B.V.K. Naidu, *Rheological properties and drug release characteristics of pH-responsive hydrogels.* Journal of Applied Polymer Science, 2004. **94**(5): p. 2057-2064.

25. Rao, K., M.C.S. Subha, B.V.K. Naidu, M. Sairam, N.N. Mallikarjuna, and T.M. Aminabhavi, *Controlled release of diclofenac sodium and ibuprofen through beads of sodium alginate and hydroxy ethyl cellulose blends.* Journal of Applied Polymer Science, 2006. **102**(6): p. 5708-5718.

26. George, M. and T.E. Abraham, *Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review.* Journal of Controlled Release, 2006. **114**(1): p. 1-14.

27. Voron'ko, N.G., S.R. Derkach, and V.N. Izmailova, *Rheological properties of gels of gelatin with sodium alginate.* Russian Journal of Applied Chemistry, 2002. **75**(5): p. 790-794.

28. Xiao, C.B., H.J. Liu, Y.S. Lu, and L. Zhang, *Blend films from sodium alginate and gelatin solutions.* Journal of Macromolecular Science-Pure and Applied Chemistry, 2001. **38**(3): p. 317-328.

29. Stöber, W., A. Fink, and E. Bohn, *Controlled growth of monodisperse silica spheres in the micron size range.* Journal of Colloid and Interface Science, 1968. **26**(1): p. 62-69.

30. Soares, J.P., J.E. Santos, G.O. Chierice, and E.T.G. Cavalheiro, *Thermal behavior of alginic acid and its sodium salt.* Ecletica Quimica, 2004. **29**(2): p. 57-63.

31. Schrieber, R. and H. Gareis, *Gelatine Handbook: Theory and Industrial Practice*. 2007, Weinheim: Wiley-VCH Verlag GmbH & Co.

32. Fernandez, E., D. Lopez, C. Mijangos, M. Duskova-Smrckova, M. Ilavsky, and K. Dusek, *Rheological and thermal properties of agarose aqueous solutions and hydrogels.* Journal of Polymer Science Part B-Polymer Physics, 2008. **46**(3): p. 322-328.

33. Morrison, F., *Understanding rheology*. 2001, New York: Oxford University Press.

34. Perry, R.H. and D.W. Green, eds. *Perry's Chemical Engineer's Handbook*. 7th ed. 1997, McGraw-Hill.

35. Kokini, J.L. and K. Surmay, *Steady shear viscosity first normal stress difference and recoverable strain in carboxymethyl cellulose, sodium alginate and guar gum.* Carbohydrate Polymers, 1994. **23**: p. 27-33.

36. Larson , R.G., *Structure and Rheology of Complex Fluids*. 1999, New York: Oxford University Press.

37. Morris, E.R., A.N. Cutler, S.B. Ross-Murphy, D.A. Rees, and J. Price, *Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions.* Carbohydrate Polymers, 1981. **1**(1): p. 5-21.

38. Seale, R., E.R. Morris, and D.A. Rees, *Inteactions of alginates with univalent cations.* Carbohydrate Research, 1982. **110**(1): p. 101-112.

39. Lapasin, R. and S. Pricl, *Rheology of Industrial Polysaccharides: Theory and Applications*. A Chapman and Hall Food Science Book. 1999, Maryland: Aspen Publishers, Inc.

40. Tharwat, T., *Colloids in agrochemicals*, WILEY, Editor. 2009.

41. Da-Silva, D.-A.L., F. Pinto, J.A. Lopes-da-Silva, T. Trindade, B.J. Goodfellow, and A.M. Gil, *Rheological behavior of thermoreversible [kappa]-carrageenan/nanosilica gels.* Journal of Colloid and Interface Science, 2008. **320**(2): p. 575-581.

42. Joly-Duhamel, C., D. Hellio, and M. Djabourov, *All Gelatin Networks: 1. Biodiversity and Physical Chemistry.* Langmuir 2002. **18**: p. 7208-7217.

4.SURFACTANT EFFECT ON THE RHEOLOGY AND GELATION OF HPMC SOLUTIONS LOADED WITH A MODEL DRUG

4.1 Introduction

Surfactants play an important role as dispersing agents improving the separation of particles, and preventing clumping and settling. In many systems the use of surfactants is necessary to stabilize and disperse solid particles is unavoidable [1]. The latter are extremely important properties in the development of reproducible homogeneous and stable pharmaceutical products. In this section the effect of surfactant on the rheology and gelation temperature of HPMC solution with and without active ingredient was studied.

Surfactants are classified as anionic, cationic, nonionic, or amphoteric depending on their head group charge. Anionic surfactants represent approximately 60% of surfactant production in the United State of America [2]. Sulfates are the most common anionic surfactant, thus sodium dodecyl sulfate was studied as a model of this group. Cetylpyridinium chloride (CPC) was the cationic surfactant chosen due to its applications [3]. While, lecithin was the studied amphoteric surfactant, since it is a common surfactant used in the food and pharmaceutical industries [4].

When a surfactant and a non-ionic polymer are mixed two competing phenomena may take place, either self-aggregation of surfactant molecules to form micelles or interaction of individual surfactant molecules with the monomer units of

the polymer chain [5]. Self-aggregation into micelles in aqueous media occurs at a characteristic concentration for each surfactant denoted the critical micelle concentration (CMC) [2]. Surfactants can interact with polymers, more so if the polymer contains both polar and non-polar functional groups. On the other hand when the surfactant is mixed with the polymer, if they interact complexes may be formed. Interactions between non-ionic polymers and surfactants are affected by surfactant head group charge and tail length [6].

If the surface coverage per molecule of the surfactant is high and the concentration exceeds a characteristic value, polymer-surfactant micelles are produced. This concentration is denoted the critical aggregation concentration (CAC) and it is lower than the CMC [7].

The presence of a surfactant can affect the physicochemical properties of the product, such as surface tension, conductivity, rheological, and optical properties. Consequently, the performance properties, such as release rate and diffusion through the polymer, are also affected. To guarantee particle dispersion, the identification of the interactions of surfactants and polymers is needed, in the absence and presence of active pharmaceutical ingredients, and their effect on the gelation process. In this chapter, the effect of surfactant net charge and concentration on the gelation temperature and viscosity of an aqueous non-ionic polymer solution are evaluated by rheological measurements, with and without the water insoluble drug (griseofulvin). The well-studied hydroxypropyl methylcellulose (HPMC) system was chosen as the model non-ionic polymer.

HPMC is a biopolymer derived from cellulose in which the hydroxyl groups are replaced by hydroxypropyl and methyl groups. It has the ability to form gels upon heating due to the hydrophobic interactions between the methoxy groups present along its backbone [8, 9]. The gelation temperature (T_{gel}) for HPMC has been studied by others through optical rotation [10], differential scanning calorimetry (DSC) [11] and dynamic rheometry [12, 13], among other methods. Two steps in the gelation process for HPMC have been identified [14-16]. In the first stage, hydrophobic interactions and formation of aggregates take place while, at the second stage is characterized by a phase separation where clusters grow to form crystallites [12]. As HPMC molecules are heated, their mobility increases exposing their hydrophobic regions. Intermolecular hydrophobic interactions occur followed by formation of aggregates to form a network.

4.2 Experimental section

4.2.1 Materials

A 86 kDa hydroxypropyl methylcellulose (Cat # 423203, Batch# MKBB0393/MKBC7873) was obtained from Sigma-Aldrich. The sample was used as purchased. The surfactants SDS and CPC were obtained from Sigma Aldrich and lecithin from MP Biomedical. The critical micelle concentration (CMC) of SDS and CPC have been reported in the literature as ~8.08 [17] and ~1.01 mM [18], respectively. The CMC for the refined soy lecithin was taken as ~15 mM (collaborative work Paul Takhistov's lab). The model drug chosen was griseofulvin.

It is a commercial antifungal and low water solubility drug [19]. Micronized griseofulvin was donated by Johnson & Johnson Company. A schematic representation of the polymer, surfactants and particles is shown in Figure 4.1.

4.2.2 Griseofulvin particle characterization

Griseofulvin particle size distribution is in the 10 – 25 µm range using a hammer mill. While, griseofulvin nanoparticles suspensions in lecithin have a size distribution as is shown in Figure 4.1 with a particle size 0.15 µm.

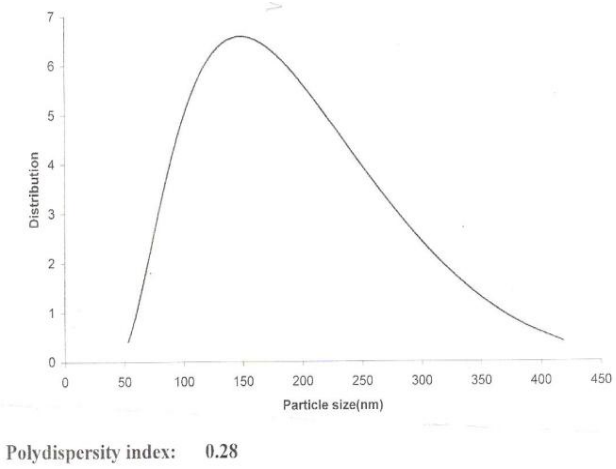

Figure 4.1. Particle size distribution for griseofulvin suspension using lecithin as surfactant at a ratio 5:1 (Collaborative work Paul Takhistov's Lab)

4.2.3 Sample preparation

Aqueous ternary mixtures of surfactant, polymer and drug were prepared by mixing stock solutions of each to obtain the required final studied compositions. Surfactant solutions were prepared by mixing with deionized water and stirring for at

least 30 min. While griseofulvin solutions were sonicated at least for a half hour using a Branson 450W high power sonicator to avoid particle agglomeration. Solutions were magnetically stirred at 25ºC until analysis. HPMC was dissolved in hot water at 50ºC and stirred overnight at room temperature. Surfactant concentrations were studied up to their CMC, while HPMC and griseofulvin concentration were held constant for both at 1 wt%. The effect of size and concentration of griseofulvin particles on the gelation temperature of a HPMC solution at 1 wt% was determined using dynamic tests. Two different sizes were studied, 0.15 and 15 μm. Griseofulvin micro particles and nano griseofulvin suspension in lecithin were donated by the Takhistov lab at Rutgers University. Both particles were suspended in aqueous media using a ratio of 5:1 of lecithin to particles. Then, a HPMC solution was added to the suspension and stirred overnight.

4.2.4 Rheology

Rheological characterization was performed on a Rheologica stress-controlled rheometer equipped with an extended temperature cell for temperature control using a double gap Coutte (V = 11 mL) fixture. The rheometer geometry was set to the desired temperature before loading the solution. Solutions were left at rest for at least five minutes to relax and equilibrate.

Dynamic rheological measurements were used to determine gelation temperatures of biopolymers during heating ramps [13, 16]. The storage and loss moduli were measured during a heating ramp from 25 to 75ºC at a rate of 0.5^{o}C/min.

Frequency and strain were fixed at 1Hz and 1%, respectively. Steady state viscosity was determined at 25 ºC at shear rates from 0.1 to 100 s^{-1}. Viscosity can provide information about conformational effects due to polymer-surfactant interactions [20]. Reported values of T_{gel} and viscosities correspond to the average of at least three measurements plus or minus their respective standard deviation.

4.3 Results and discussion

4.3.1 Sodium dodecyl sulfate effect on HPMC T_{gel}

Figure 4.2 shows the gelation temperature, T_{gel}, of HPMC systems as a function of SDS concentration below their CMC with (closed symbols) and without (open symbols) griseofulvin. Gelation temperature for pure HPMC was found to be 57.4 ± 1.5 ºC. At low SDS concentrations no significant change in T_{gel} is observed without griseofulvin ($P > 0.2$). Around 0.6CMC (~5mM) a minimum in T_{gel} is observed, which represents a drop of 5.6 ºC from that of the HPMC only solution ($P = 0.005$). Afterwards, at SDS concentration of 0.7 CMC, T_{gel} increases surpassing the value for HPMC by 3 ºC. Then, T_{gel} remain similar at concentrations of 0.7, 0.8 and 1 times CMC, with P values larger than 0.6. A complete statistical analysis for effect of particle and surfactant is shown at the end of this chapter as an appendix.

These results are in agreement with gelation temperature results reported by other authors but determined by calorimetric techniques [21]. Su and coworkers [21] reported that low SDS concentrations (i.e 2 and 4 mM) do not have a significant effect on the gelation temperature (T_{gel} ~ 55 ºC) of a 1 wt% 86 kDa HPMC solution.

Sunil and Bin [22] attribute the minimum and sudden increase in gelation temperature to adsorption of SDS molecules to the hydrophobic sites of the polymer followed by aggregate formation. The onset of aggregate formation is called the critical aggregation concentration (CAC). A CAC of 6 mM for SDS in HPMC was extracted from calorimetric measurements. Similarly, the increase of about 8 °C for HPMC solutions with SDS concentration above 6 mM, with reference to pure HPMC, was attributed to the higher energy that was necessary to displace aggregates and break water cages.

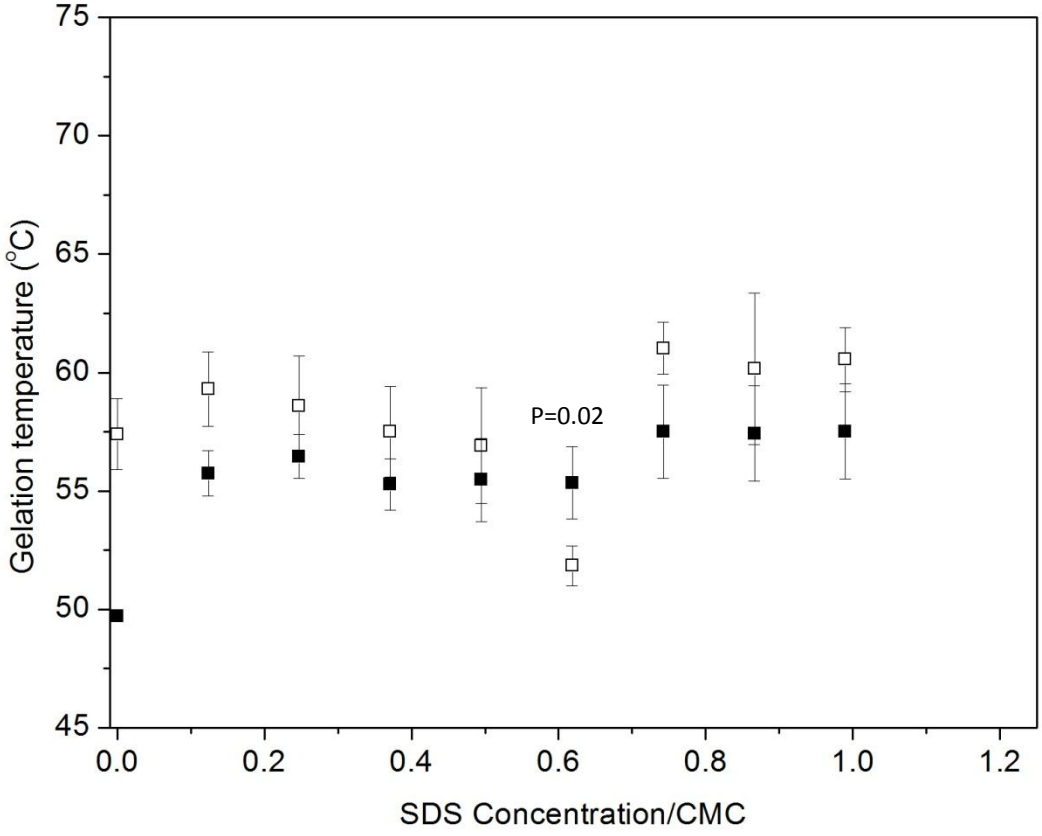

Figure 4.2. Gelation temperature of 1 wt% aqueous hydroxypropyl methylcellulose solutions with sodium dodecyl sulfate with (■) and without (□) 1 wt% griseofulvin

The steady-state viscosity of aqueous HPMC solutions showed a Newtonian plateau at low shear rates ($\dot{\gamma} <$ 10Pa·s) followed by a shear-thinning regime at higher shear rates, which is a characteristic behavior for most polymer solutions [23]. No effect on the onset of shear thinning with the addition of SDS was observed. Reported values correspond to the Newtonian plateau or equivalently the zero-shear viscosity. This regime provides more information about the component interactions, since it is assumed that they are not disturbed by shear.

The effect of SDS concentration on the magnitude of the zero-shear steady-state viscosity of HPMC solutions at 25 ºC is shown as open symbols in Figure 4.3. Low SDS concentrations did not show a significant effect on the viscosity ($P > 0.09$). Above 0.6 CMC, the viscosity increases by up to a factor of 2, as it reaches the CMC. A significant increase in T_{gel} was found for SDS at 0.87 CMC ($P < 0.038$). An increase in the viscosity supports the proposed mechanism that higher order structures, such as aggregates, are being formed. Additional evidence is provided by the fact the onset of the increase in viscosity is observed at the same concentration as the minimum in T_{gel}, which was attributed to formation of aggregates.

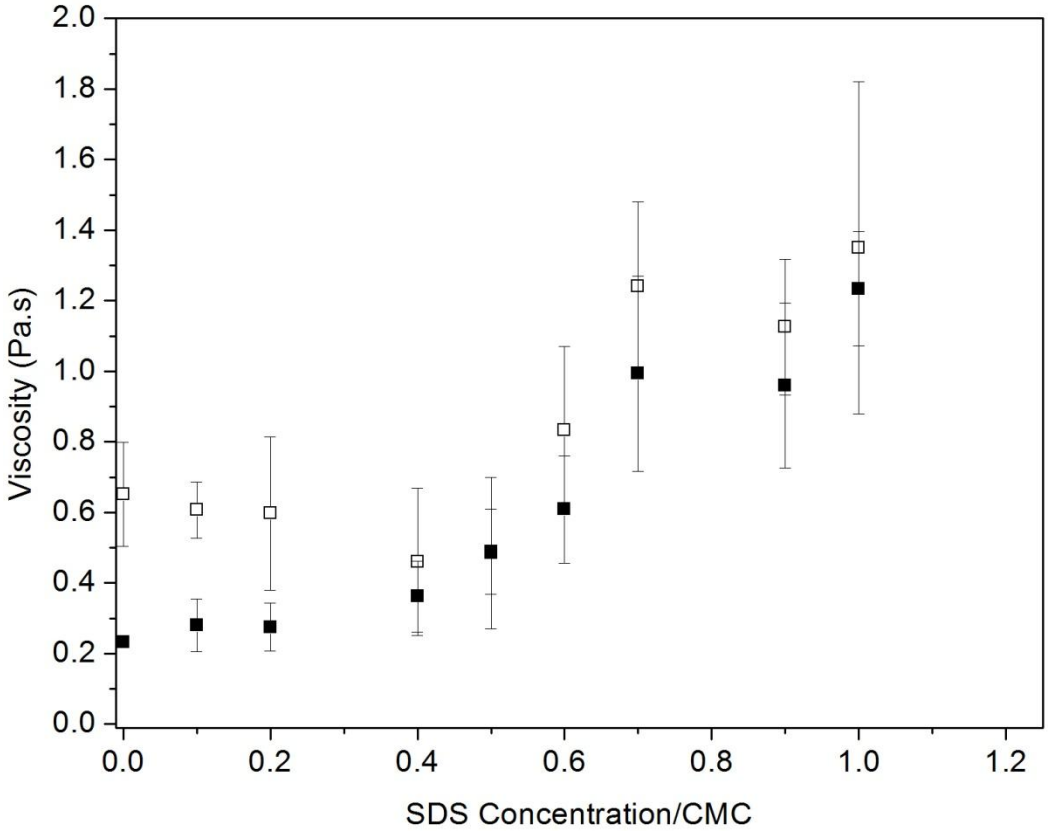

Figure 4.3. Zero-shear steady state viscosity of aqueous hydroxypropyl methylcellulose solutions at 1% with sodium dodecyl sulfate with (■) and without (□) griseofulvin.

When griseofulvin was present in the system an overall decrease in T_{gel} was initially obtained. This decrease was more pronounced in the absence of surfactant, which was 7.5 ºC lower than pure HPMC (P = 0.0004). The decrease in gelation temperature suggests co-adsorption of the polymer and surfactant on the active's surface. The particle then bridges hydrophobic HPMC bundles or crystallites, thus promoting gelation at lower temperatures. The schematic mechanism for HPMC gelation in the presence of SDS and griseofulvin is shown in Figure 4.4. The decrease in viscosity, Figure 4.3, also supports the hypothesis of polymer adsorption on the particles surface, which causes a reduction in the effective viscosity of the medium.

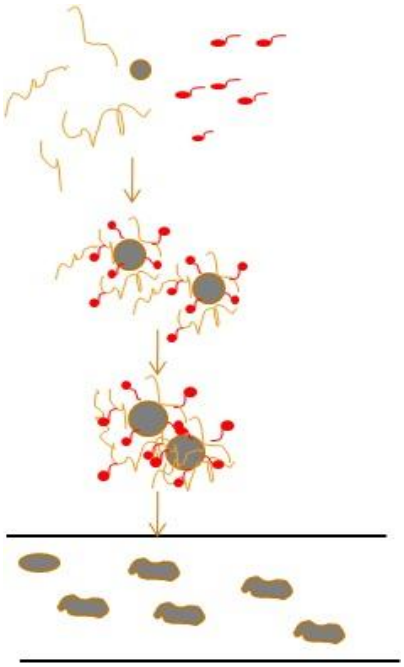

Figure 4.4. Schematic mechanism for HPMC gelation containing SDS and Griseofuvin

As SDS at 0.1 CMC is added to the HPMC-griseofulvin mixtures T_{gel} increases by 5.7 ºC above HPMC-griseofulvin solution (P = 0.0005). Upon increasing the SDS concentration (up to 0.6CMC), T_{gel} does not show a significant change with

reference to 0.1 SDS concentration/CMC ($P > 0.06$). A slight increase, without going through a minimum, of about ~1.6 ºC is observed at higher SDS concentrations. Nevertheless, the difference is very close to the experimental error and much lower than for their HPMC-SDS counterparts. The absence of the minimum suggests that there is no or little aggregation of SDS and HPMC. There is no effect of SDS concentration on T_{gel} when griseofulvin is present in the system for SDS concentration from 0.1 to 1 times CMC ($P > 0.11$). These values are similar to the T_{gel} for HPMC solutions ($P > 0.11$).

Griseofulvin has a negative surface [24] and negative SDS preferentially interact with the polymer. Then, gelation temperature is the results of two factors: griseofulvin acting as binding agent with the polymer and SDS forming aggregates with the HPMC hydrophobic group. The first one causes a decrease in T_{gel}, while the second has the opposite effect, it inhibits the interactions between the hydrophobic sites of HPMC. These results were agreed with the adsorption data reported by Berglund et coworkers for the hmHEC/SDS/silica systems. The coadsorption of a hydrophobic polymer and on a negatively charged surface reported by Berglund and coworkers [25]. It was demonstrated that SDS was able to bind to hmHEC (hydrophobically modified hydroxyethyl cellulose) at concentration as low as 0.1 mM. The viscosity shows a similar trend to the HPMC-SDDS viscosity without active ingredient, no change up to 0.6 CMC followed by an increase without going through a minimum. The viscosity at higher SDS concentrations is also similar to that of the HPMC without griseofulvin ($P > 0.26$), which also agrees with a replacement mechanism of HPMC from the particles surface by the surfactant.

4.3.2 Cethylpiridium chloride effect on HPMC T_{gel}

The effect of CPC concentration on gelation of HPMC solutions is shown in Figure 4.5. Gelation temperature obtained for CPC concentration from 0.1 to 1.1 CMC was significantly different to T_{gel} for HPMC pure with a $P < 0.04$. Upon addition of 0.1 CMC of CPC to an HPMC solution (i.e. open squares in Fig 5), the gelation temperature is inhibited (i.e. increased) by 8 ºC ($P = 0.002$). Increasing the CPC concentration, up to 0.4 CMC, decreases T_{gel}. For concentration above 0.4 CMC no significant change in gelation temperature were observed ($P > 0.26$). Yet, the gelation temperature remains at least 2.6 ºC above that for the HPMC solution.

Formation of micelles or higher order structures should produce an increase in viscosity as that observed for SDS. However, the zero-shear viscosity is mostly unaffected with increasing surfactant concentration (open squares in Figure 4.6). Cationic surfactants typically have larger head groups than anionic ones. The surfactant still interacts with the polymer, as evidenced by the halving of the viscosity upon addition of CPC, which suggests a reduction of the hydrodynamic fraction of the polymer. Yet, fewer surfactant molecules interact per hydrophobic site, in contrast with SDS molecules. Steric hindrance due to the large size of the cationic head group may prevent CPC molecules to aggregate around the hydrophobic sites. Yet they are able to shield them preventing them from interacting and causing a displacement of gelation to higher temperatures.

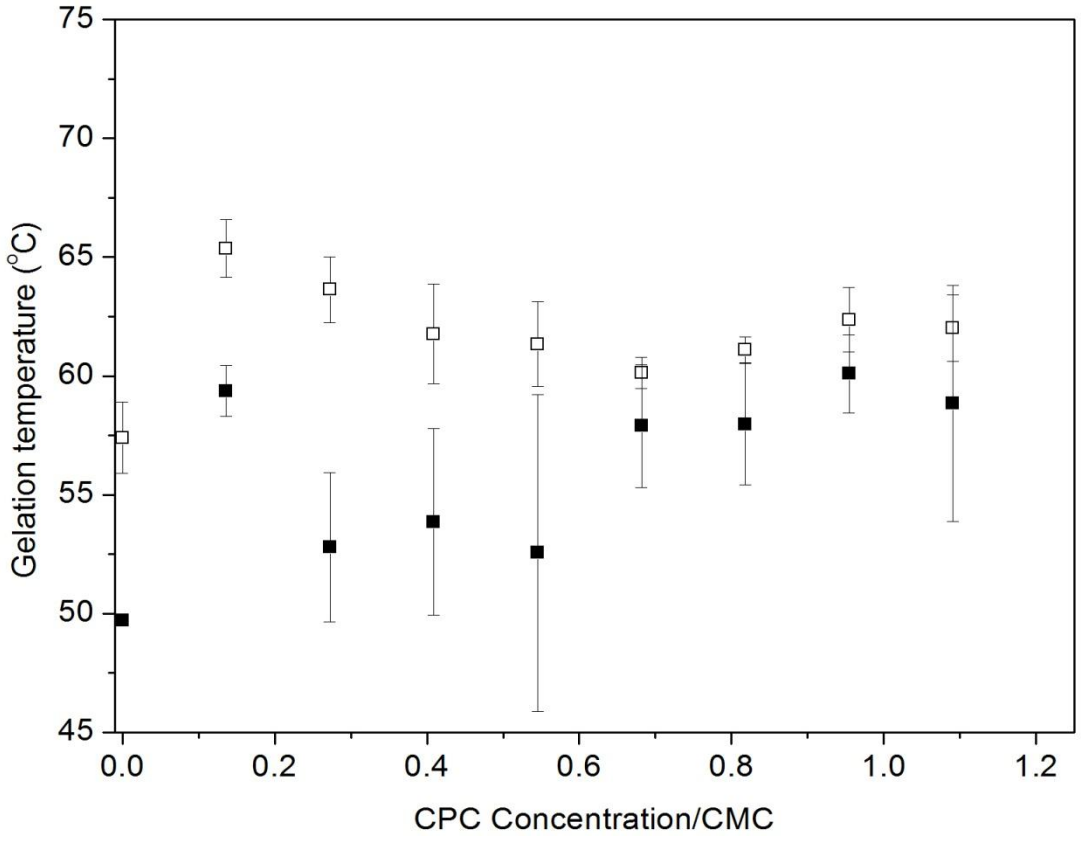

Figure 4.5. Gelation temperature of aqueous hydroxypropyl methylcellulose solutions at 1% with cetylpyridinium chloride monohydrate with (■) and without (□) griseofulvin

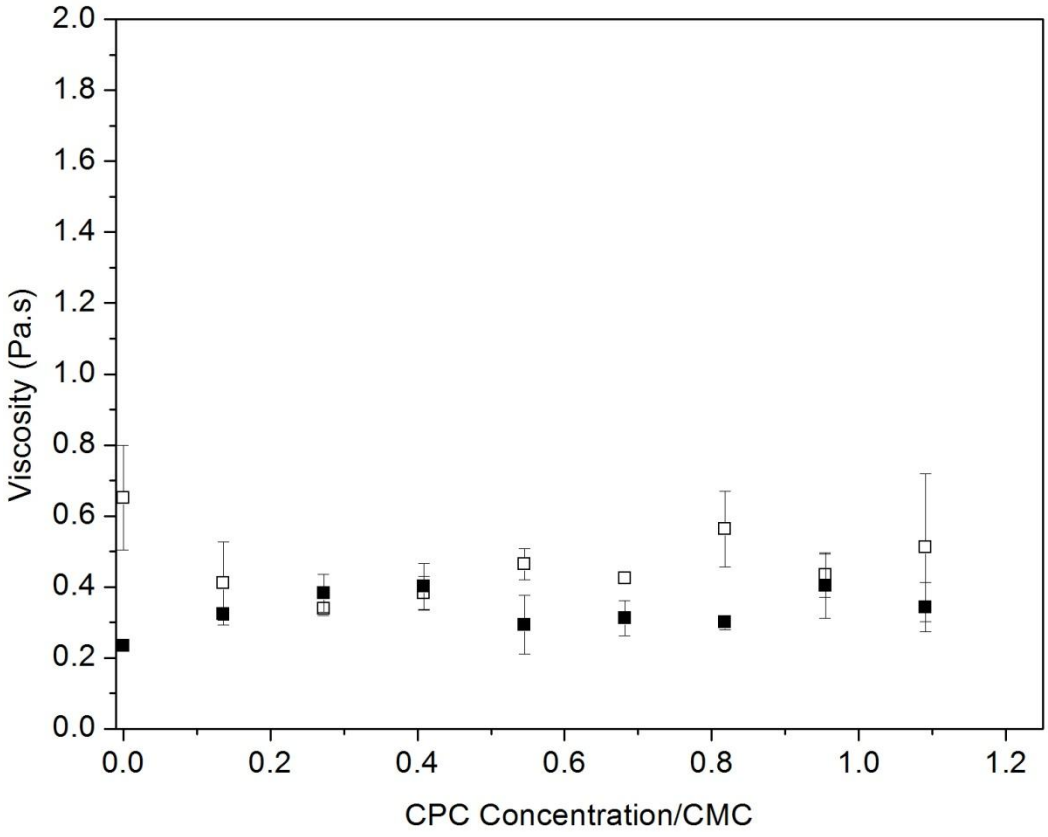

Figure 4.6. Zero-shear steady state viscosity of aqueous hydroxypropyl methylcellulose solutions at 1% with cetylpyridinium chloride monohydrate with (■) and without (□) griseofulvin

As discussed previously, T_{gel} is promoted by the addition of griseofulvin to HPMC, since the particle effectively bridges the HPMC crystallites. The schematic gelation of HPMC in presence of CPC and griseofulvin is shown in Figure 4.7. Gelation temperature for CPC concentrations from 0.1 to 1 containing griseofulvin was not affected significantly with reference to HPMC pure.

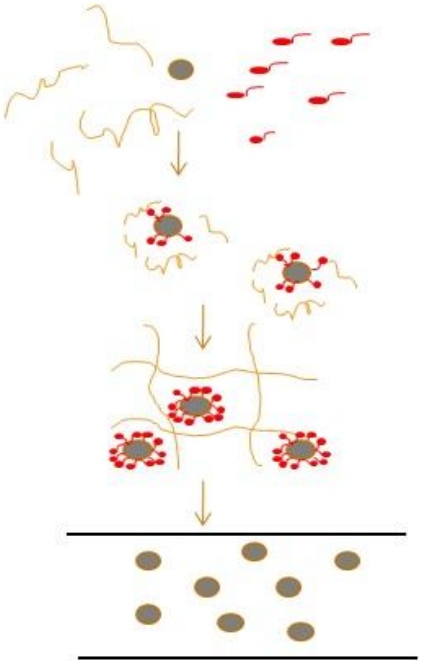

Figure 4.7. Schematic mechanism for HPMC gelation containing CPC and Griseofulvin

The average values are higher by at least 8 ºC, but within experimental error of each other. At higher concentrations T_{gel} reaches a value close to the gel point for HPMC and is mostly unaffected by further increases in CPC. The viscosity of the HPMC-griseofulvin (closed squares in Fig. 46) is mostly unaffected by addition or

increase of CPC concentration ($P > 0.2$). Thus, no evidence of aggregate formation was observed in the presence of griseofulvin.

At low CPC concentrations, the surfactant and polymer adsorbs onto the griseofulvin surface. The surfactant's surface coverage is low. Thus, the bridging effect of the griseofulvin is only slightly diminished, increasing gelation temperature. As the CPC concentration increases, the CPC replaces the HPMC on the surface of the active, stabilizing it. The larger head groups prevent HPMC to adsorb back onto the particle's surface. Only a small amount of CPC is expected to be free to interact with the polymer, as evidenced by the minuscule changes in viscosity. Consequently, the gelation temperature eventually approaches that of pure HPMC.

4.3.3 Lecithin effect on HPMC T_{gel}

Adding lecithin to an HPMC solution displaces the gelation temperature toward higher values at all concentrations by at least 7 ºC as shown in Figure 4.8. A significant difference in T_{gel} was obtained through the range of lecithin concentration studied ($P < 0.004$). Upon addition of 0.1 CMC lecithin, the gelation increases to 67.7 ºC. From 0.2 to 1 times the CMC, T_{gel} remains within experimental error of each other ($P > 0.67$). The latter resembles the behavior observed for the CPC-HPMC system. However, Figure 4.9 shows that only the initial addition of lecithin affects the solution viscosity, but increasing the concentration of surfactant does not have an effect ($P > 0.06$). Analogous to the CPC system, evidenced of polymer-surfactation

was observed. There is no difference between T_{gel} for HPMC-lecithin with and without griseofulvin ($P > 0.05$), with the exception of 0.8CMC lecithin ($P = 0.01$).

The hydrophobic head group of lecithin is also much larger than for anionic surfactants, which suggests that it will have the same effect as the cationic surfactants such as CPC. Larger head groups will interact with the polymer at much lower surfactant molecules per hydrophobic site, which restricts the formation of aggregates. Nevertheless, the surfactant is still capable of stabilizing the sites, which prevents them from participating in network formation.

The gelation temperature of the HPMC-griseofulvin solutions increases with increasing lecithin concentration until 0.3 CMC, as shown in Figure 4.8. When the concentration approaches CMC, T_{gel} in the presence of griseofulvin approaches that of the system without drug. The linear the viscosity of the HPMC-griseofulvin solution is also unaffected by changes in the concentration of lecithin. The drug stabilization mechanism in the presence of HPMC seems to be similar for the non-ionic lecithin to that of the cationic CPC. The surfactant adsorbs onto the griseofulvin's surface removing and blocking HPMC from it. Thus, the bridging effect caused by the HPMC-griseofulvin bridging effect is diminished until the particle is completely covered by lecithin.

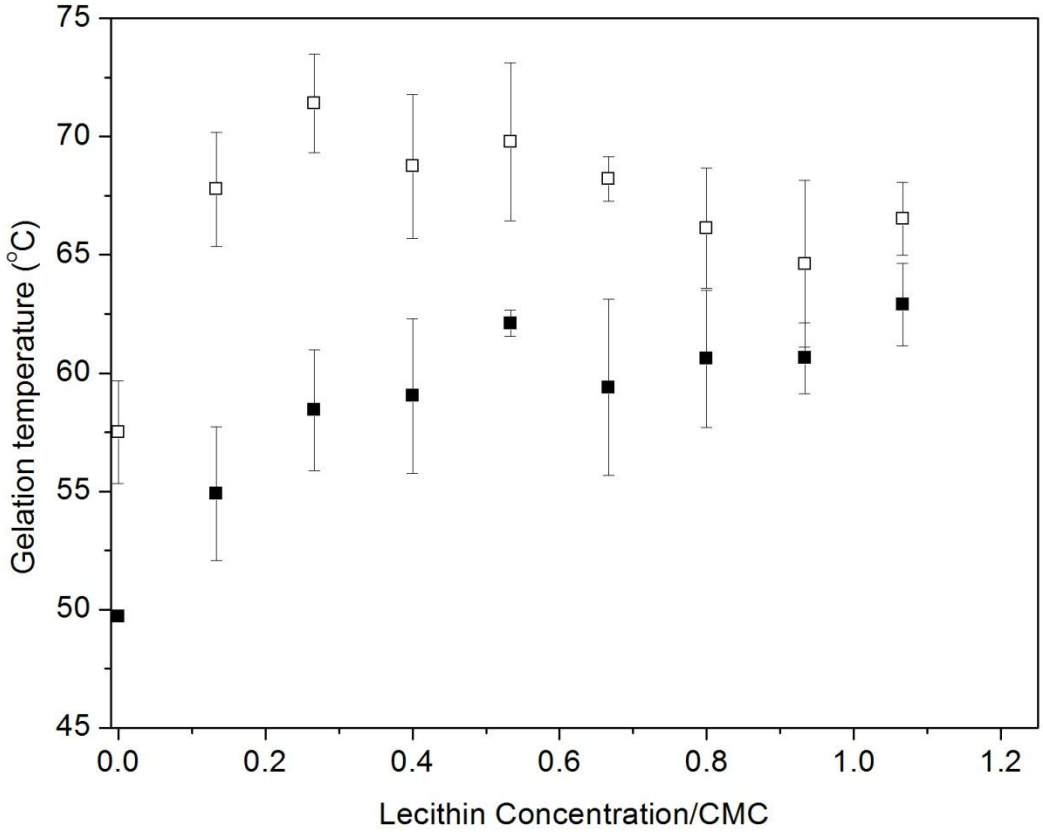

Figure 4.8. Gelation temperature of aqueous hydroxypropyl methylcellulose solutions at 1% with lecithin with (■) and without (□) griseofulvin

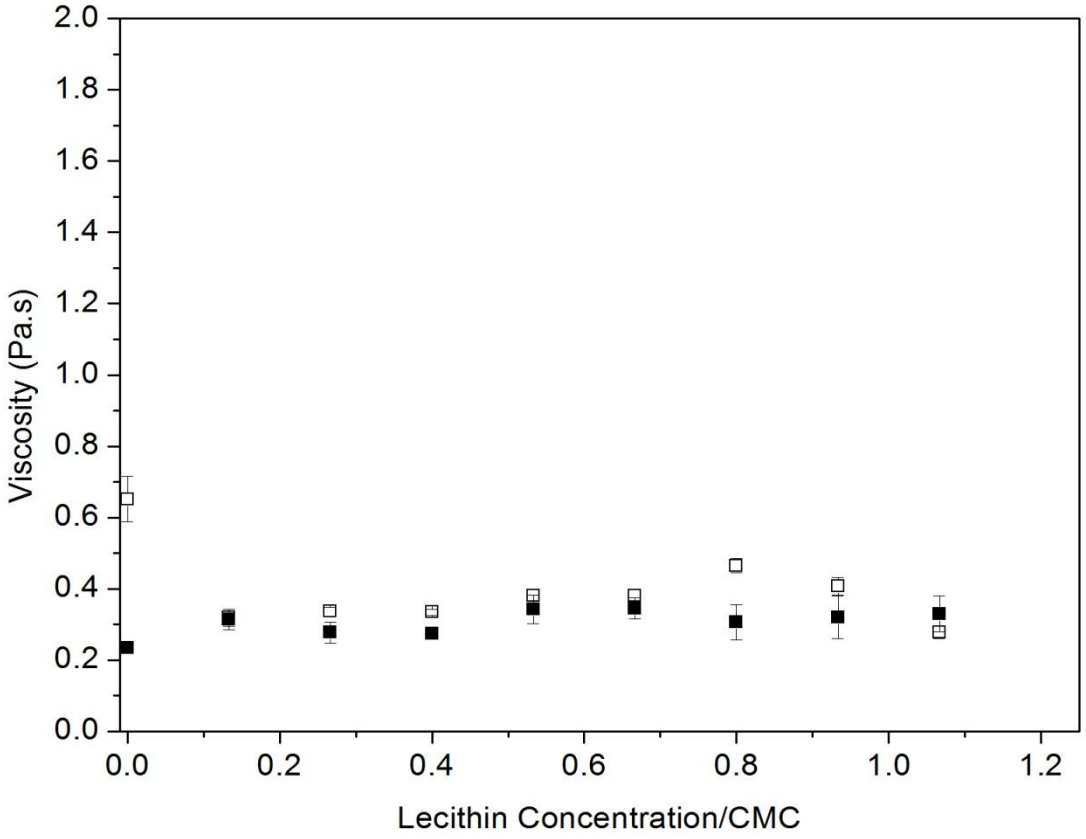

Figure 4.9. Zero-shear steady state viscosity of aqueous hydroxypropyl methylcellulose solutions at 1% with lecithin with (■) and without (□) griseofulvin

4.3.4 Effect of size and concentration of griseofulvin

Viscosity of HPMC solutions was not affected by adding nano or micro particles as shown in Figure 4.11 ($P > 0.1$), as it was explained before, in this system no aggregates are being formed thus no changes in viscosity were observed for particle loading of up to 0.01wt%.

The gelation temperature for the HPMC system at 1 wt% was 55.6 ± 1.3 ºC. When griseofulvin suspension with 0.15 nm of particle size was added to HPMC solution an increase of at least 4 ^{o}C in the gelation temperature was observed at all lecithin concentrations in the range studied ($P < 0.03$). However, gelation temperature was almost the same (~60.9 ± 1 ^{o}C) within experimental error. Results are shown in Figure 4.10. This increase in gelation temperature is due to griseofulvin is acting as a steric barrier blocking the HPMC gelation.

However, gelation temperature was not affected when micro particles suspension were added to the system ($P > 0.07$). However, the observation may suggest that griseofulvin agglomerates, which does not affect viscosity since it is not a function of particle size but it is function of volume fraction. Yet, since lecithin concentration are low particle coverage is ineffective, thus lecithin can prefer to interact with polymer to decrease surface energy.

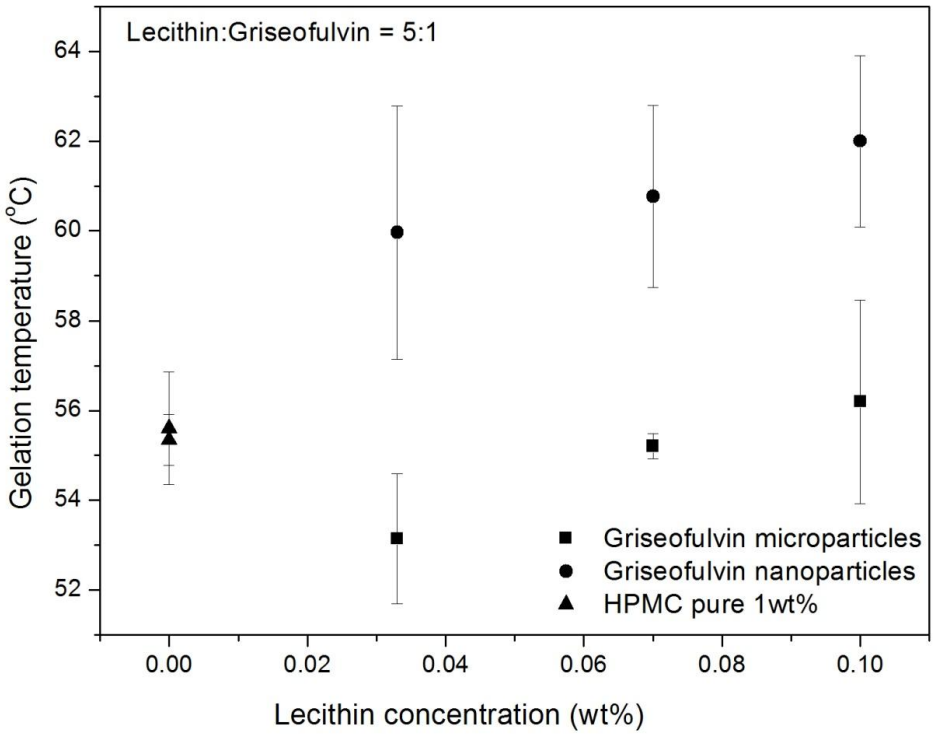

Figure 4.10. Particle size effect on gelation temperature of a 1 wt% HPMC solution

Table 4-1. Effect of Nano and micro particles on T_{gel}

Lecithin : G = 5 : 1	Nanoparticles		Microparticles		P
Lecithin wt%	Tgel (°C)	STDEV	Tgel (°C)	STDEV	
0.033	53.14	1.45	59.97	2.82	0.02
0.07	55.21	0.29	60.77	2.03	0.01
0.1	56.19	2.26	62.00	1.91	0.03

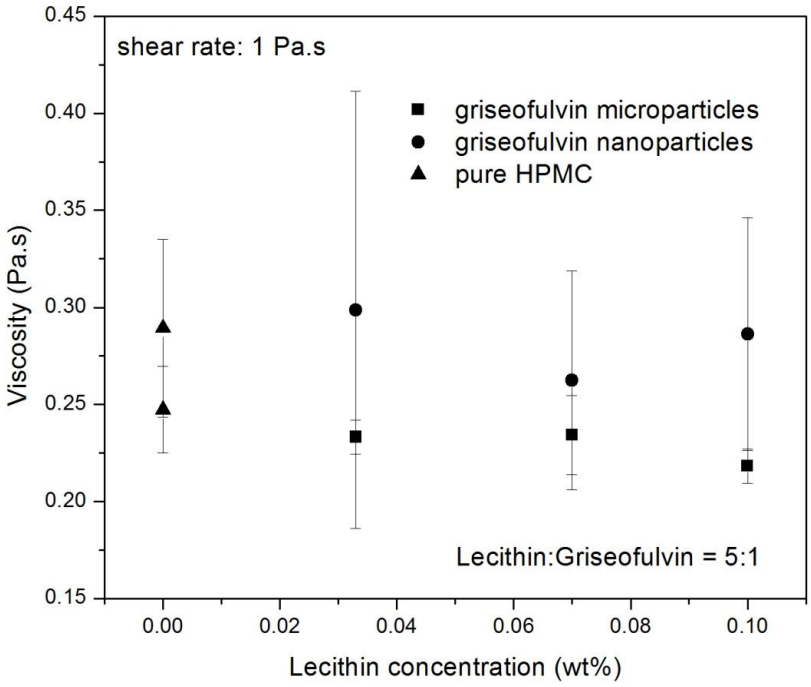

Figure 4.11. Particle size effect on steady-state viscosity of a 1 wt% HPMC solution at a shear rate of 1 s^{-1}

Table 4-2. Effect of Nano and micro particles on η

Lecithin : G = 5:1	Nanoparticles		Microparticles		
Lecithin wt%	η(Pa.s)	STDEV	η(Pa.s)	STDEV	P
0.033	0.17	0.00	0.18	0.04	0.69
0.07	0.17	0.01	0.17	0.02	1.00
0.1	0.16	0.01	0.18	0.02	0.20

4.4 Conclusion

HPMC gelation temperature showed dependence on concentration and type of surfactant. Surfactants inhibit HPMC gelation temperature as a result of their interaction with the hydrophobic sites of the polymer. The effect of type of surfactant on T_{gel} was mostly affected by the relative size of the hydrophilic headgroup, which are somewhat related to the net charge. When griseofulvin is present in the system, HPMC gelation temperature decreased drastically due to adsorption of the polymer on hydrophobic surface of the active ingredient, thus effectively acting as a cross linking agent. However, surfactant-particle interaction showed dependence with surfactant charge. SDS surfactant is not able to properly stabilize griseofulvin due to the repulsion amongst the negatively charged surface. SDS preferentially forms aggregates (or complexes) with HPMC. CPC and lecithin are able to bind to the griseofulvin particles therefore less particles are acting as bridges between hydrophobic sites of HPMC. At a specific concentration, surfactant is able to stabilize particles completely. Thus, gelation temperature for the systems approximates that of pure HPMC. Viscosity studies showed the formation of aggregates for system with anionic surfactant (SDS), while no aggregates were observed for systems containing cationic (cetylpyridinium chloride) or non ionic (lecithin). Viscosity with the addition of active ingredient was unaffected for the system with CPC and lecithin. However, lower viscosities were obtained for the system containing SDS due to the adsorption of the polymer on the particle surface. Additionally, gelation temperature showed be dependent on particle size.

4.5 References

1. Tadros, T.F., *Applied surfactants: principles and applications*. 2005: Wiley-VCH, Weinheim.
2. Miller, C.A., *Interfacial Phenomena: Equilibrium and Dynamic Effects,* . Second Edition ed, ed. L. Taylor & Francis Group. 2008.
3. Mehta, S.K., S. Chaudhary, and K.K. Bhasin, *Self-assembly of cetylpyridinium chloride in water-DMF binary mixtures: A spectroscopic and physicochemical approach.* Journal of Colloid and Interface Science, 2008. **321**(2): p. 426-433.
4. Wu, Y. and T. Wang, *Soybean lecithin fractionation and functionality.* Journal of the American Oil Chemists' Society, 2003. **80**(4): p. 319-326.
5. Osada, Y., R. Gangopadhyay, and J. Ping Gong, *Cooperative Binding in Surfactant-Polymer Association,* in *Reflexive Polymers and Hydrogels*. 2004, CRC Press.
6. Brackman, J.C. and J.B.F.N. Engberts, *The effect of surfactant headgroup charge on polymer--micelle interaction.* Journal of Colloid and Interface Science, 1989. **132**(1): p. 250-255.
7. Griffiths, P.C. and A.Y.F. Cheung, *Interaction between surfactants and gelatin in aqueous solutions.* Materials Science and Technology, 2002. **18**: p. 591-599.
8. Sarkar, N., *Thermal gelation properties of methyl and hydroxypropyl methylcellulose.* Journal of Applied Polymer Science, 1979. **24**(4): p. 1073-1087.
9. Chen, H.-H., C.-H. Lin, and H.-Y. Kang, *Maturation effects in fish gelatin and HPMC composite gels.* Food Hydrocolloids, 2009. **23**(7): p. 1756-1761.
10. Joly-Duhamel, C., D. Hellio, and M. Djabourov, *All Gelatin Networks: 1. Biodiversity and Physical Chemistry.* Langmuir 2002. **18**: p. 7208-7217.
11. Roversi, M. and C. La Mesa, *Rheological properties of protein-surfactant based gels.* Journal of Colloid and Interface Science, 2005. **284**(2): p. 470-476.
12. Pérez, O.E., V. Wargon, and A. M.R. Pilosof, *Gelation and structural characteristics of incompatible whey proteins/hydroxypropylmethylcellulose mixtures.* Food Hydrocolloids, 2006. **20**(7): p. 966-974.
13. Ding, P., A.W. Pacek, W.J. Frith, I.T. Norton, and B. Wolf, *The effect of temperature and composition on the interfacial tension and rheology of separated phases in gelatin/pullulan mixtures.* Food Hydrocolloids, 2005. **19**(3): p. 567-574.
14. Silva, S.M.C., F.V. Pinto, F.E. Antunes, M.G. Miguel, J.J.S. Sousa, and A.A.C.C. Pais, *Aggregation and gelation in hydroxypropylmethyl cellulose aqueous solutions.* Journal of Colloid and Interface Science, 2008. **327**(2): p. 333-340.
15. Haque, A., R.K. Richardson, E.R. Morris, M.J. Gidley, and D.C. Caswell, *Thermogelation of methylcellulose. Part II: effect of hydroxypropyl substituents.* Carbohydrate Polymers, 1993. **22**(3): p. 175-186.
16. Bajwa, G.S., C. Sammon, P. Timmins, and C.D. Melia, *Molecular and mechanical properties of hydroxypropyl methylcellulose solutions during the sol:gel transition.* Polymer, 2009. **50**(19): p. 4571-4576.

17. Fuguet, E., C. Rafols, M. Roses, and E. Bosch, *Critical micelle concentration of surfactants in aqueous buffered and unbuffered systems.* Analytica Chimica Acta, 2005. **548**(1-2): p. 95-100.
18. García-Río, L., J.R. Leis, J.C. Mejuto, V. Mosquera, and P. Rodríguez-Dafonte, *Stability of mixed micelles of cetylpyridinium chloride and linear primary alkylamines.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2007. **309**(1-3): p. 216-223.
19. Boullata, J.I. and V.T. Armenti, *Handbook of drug-nutrient interactions*. 2010, New York, NY: Humana Press.
20. Goddard, E.D., *Polymer--surfactant interaction Part I. uncharged water-soluble polymers and charged surfactants.* Colloids and Surfaces, 1986. **19**(2-3): p. 255-300.
21. Su, J., S. Liu, J. Sunil, and Y. Lam, *Effect of SDS on the gelation of hydroxypropylmethylcellulose hydrogels.* Journal of Thermal Analysis & Calorimetry, 2008. **93**(2): p. 495-501.
22. Sunil, C.J. and C. Bin, *Influence of surfactant properties on thermal behavior and sol-gel transitions in surfactant-HPMC mixtures.* Journal of Applied Polymer Science, 2009. **113**(5): p. 2887-2893.
23. *Food Polysaccharides and Their Applications* ed. G.O.P. Alastair M. Stephen. 2006, New York: Marcel Dekker.
24. Florey, K., *Analytical profiles of drug substances*, ed. A.o.P. Sciences. Vol. 8. 1979.
25. Berglund, K.D., T.M. Przybycien, and R.D. Tilton, *Coadsorption of sodium dodecyl sulfate with hydrophobically modified nonionic cellulose polymers. 1. Role of polymer hydrophobic modification.* Langmuir, 2003. **19**(7): p. 2705-2713.

4.6 APPENDIX 4

Appendix 4. 1 Statistical analysis of the effect of SDS concentration on gelation temperature of HPMC solution with and without griseofulvin at 1 wt%

[SDS]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%		
	Tgel(^{o}C)	STDEV	P	Tgel(^{o}C)	STDEV	P
0	**57.40**	1.51		**49.73**	0.7	
0.12	59.30	1.56	0.20	57.45	1.0	0.00
0.25	58.60	2.11	0.47	56.45	0.9	0.00
0.37	57.49	1.91	0.95	55.27	1.1	0.00
0.50	56.91	2.44	0.78	55.46	1.8	0.01
0.62	51.84	0.85	0.01	55.33	1.5	0.00
0.74	61.03	1.11	0.03	57.50	2.0	0.00
0.87	60.15	3.21	0.25	57.42	2.0	0.00
0.99	60.55	1.36	0.05	57.51	1.9	0.00

Appendix 4.2 Statistical analysis of the effect of CPC concentration on gelation temperature of HPMC solution with and without griseofulvin at 1 wt%

[CPC]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%		
	Tgel(^{o}C)	STDEV	P	Tgel(^{o}C)	STDEV	P
0	**57.40**	1.51		**49.73**	0.7	
0.15	65.37	1.21	0.00	59.01	0.6	0.00
0.3	63.63	1.38	0.01	52.79	3.1	0.17
0.45	61.77	2.10	0.04	55.64	3.7	0.05
0.6	61.33	1.79	0.04	52.55	6.7	0.51
0.75	60.13	0.67	0.05	57.89	2.6	0.01
0.9	61.10	0.53	0.02	57.97	2.6	0.01
1.05	62.37	1.37	0.01	60.09	1.6	0.00
1.2	62.00	1.40	0.02	58.83	5.0	0.04

Appendix 4.3 Statistical analysis of the effect of Lecithin concentration on gelation temperature of HPMC solution with and without griseofulvin at 1 wt%

[Lecithin]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%		
	Tgel(°C)	STDEV	P	Tgel(°C)	STDEV	P
0.0	**57.40**	1.5		**49.73**	0.7	
0.1	67.77	2.4	0.00	54.90	2.8	0.04
0.3	71.40	2.1	0.00	58.43	2.6	0.01
0.4	68.74	3.0	0.00	59.03	3.3	0.01
0.5	69.78	3.3	0.00	62.10	0.6	0.00
0.7	68.21	0.9	0.00	59.40	3.7	0.01
0.8	66.80	3.1	0.01	60.60	2.9	0.00
0.9	68.81	3.5	0.01	60.63	1.5	0.00
1.1	66.53	1.5	0.00	62.90	1.7	0.00

Appendix 4.4 Statistical analysis of the effect of SDS concentration on viscosity of HPMC solution with and without griseofulvin at 1 wt%

[SDS]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%			HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P
0.00	**0.76**	**0.19**					**0.26**	**0.09**	
0.12	0.69	0.09	0.60	0.29	0.09	0.02	0.29	0.09	0.70
0.25	0.68	0.27	0.70	0.29	0.09	0.04	0.29	0.09	0.70
0.37	0.51	0.25	0.24	0.39	0.11	0.04	0.39	0.11	0.18
0.50	0.54	0.25	0.29	0.54	0.15	0.19	0.54	0.15	0.05
0.62	0.98	0.32	0.36	0.70	0.19	0.72	0.70	0.19	0.02
0.74	2.04	1.08	0.11	1.36	0.53	0.14	1.36	0.53	0.02
0.87	1.37	0.25	0.03	1.19	0.36	0.14	1.19	0.36	0.01
0.99	1.89	0.82	0.08	1.58	0.25	0.01	1.58	0.25	0.01

Appendix 4.5 Statistical analysis of the effect of CPC concentration on viscosity of HPMC solution with and without griseofulvin at 1 wt%

[CPC]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%			HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P
0	**0.76**	**0.19**					**0.26**	**0.09**	
0.15	0.46	0.16	0.10	0.34	0.01	0.03	0.34	0.01	0.20
0.3	0.36	0.03	0.02	0.42	0.06	0.04	0.42	0.06	0.06
0.45	0.41	0.05	0.04	0.44	0.08	0.06	0.44	0.08	0.06
0.6	0.52	0.07	0.12	0.31	0.10	0.02	0.31	0.10	0.55
0.75	0.46	0.00	0.05	0.22	0.20	0.03	0.22	0.20	0.77
0.9	0.53	0.19	0.22	0.32	0.02	0.02	0.32	0.02	0.32
1.05	0.47	0.08	0.08	0.44	0.10	0.06	0.44	0.10	0.08
1.2	0.81	0.68	0.91	0.36	0.08	0.03	0.36	0.08	0.22

Appendix 4.6 Statistical analysis of the effect of Lecithin concentration on viscosity of HPMC solution with and without griseofulvin at 1 wt%

[Lecithin]/CMC	HPMC 1 wt%			HPMC 1 wt%+ G wt%			HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P	η (Pa.s)	STDEV	P
0.0	0.76	0.19					**0.26**	**0.09**	
0.1	0.34	0.03	0.04	0.35	0.02	0.04	0.35	0.02	0.17
0.3	0.37	0.01	0.02	0.29	0.04	0.02	0.29	0.04	0.63
0.4	0.38	0.03	0.03	0.29	0.02	0.01	0.29	0.02	0.60
0.5	0.43	0.10	0.06	0.38	0.05	0.03	0.38	0.05	0.11
0.7	0.44	0.07	0.05	0.39	0.04	0.03	0.39	0.04	0.08
0.8	0.61	0.09	0.01	0.36	0.06	0.03	0.36	0.06	0.18
0.9	0.52	0.09	0.12	0.40	0.08	0.04	0.40	0.08	0.11
1.1	0.54	0.04	0.12	0.42	0.06	0.04	0.42	0.06	0.06

Appendix 4.7 Comparison between T_{gel} for HPMC-SDS with and without G

[SDS]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	Tgel(^{o}C)	STDEV	Tgel(^{o}C)	STDEV	P
0	57.40	1.51	49.73	0.7	0.00
0.12	59.30	1.56	57.45	1.0	0.13
0.25	58.60	2.11	56.45	0.9	0.18
0.37	57.49	1.91	55.27	1.1	0.19
0.50	56.91	2.44	55.46	1.8	0.45
0.62	51.84	0.85	55.33	1.5	0.02
0.74	61.03	1.11	57.50	2.0	0.06
0.87	60.15	3.21	57.42	2.0	0.28
0.99	60.55	1.36	57.51	1.9	0.09

Appendix 4. 8 Comparison between T_{gel} for HPMC-CPC with and without G

[CPC]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	Tgel(^{o}C)	STDEV	Tgel(^{o}C)	STDEV	P
0	57.40	1.51	49.73	0.7	
0.15	65.37	1.21	59.01	0.6	0.01
0.3	63.63	1.38	52.79	3.1	0.01
0.45	61.77	2.10	55.64	3.7	0.07
0.6	61.33	1.79	52.55	6.7	0.09
0.75	60.13	0.67	57.89	2.6	0.22
0.9	61.10	0.53	57.97	2.6	0.11
1.05	62.37	1.37	60.09	1.6	0.13
1.2	62.00	1.40	58.83	5.0	0.35

Appendix 4. 9 Comparison between T_{gel} for HPMC-Lec with and without G

[Lecithin]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	Tgel(°C)	STDEV	Tgel(°C)	STDEV	P
0.0	57.40	1.5	49.73	0.7	
0.1	67.77	2.4	54.90	2.8	0.00
0.3	71.40	2.1	58.43	2.6	0.00
0.4	68.74	3.0	59.03	3.3	0.02
0.5	69.78	3.3	62.10	0.6	0.01
0.7	68.21	0.9	59.40	3.7	0.02
0.8	66.80	3.1	60.60	2.9	0.06
0.9	68.81	3.5	60.63	1.5	0.02
1.1	66.53	1.5	62.90	1.7	0.05

Appendix 4. 10 Comparison between η for HPMC-SDS with and without G

[SDS]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	η (Pa.s)	STDEV	P
0.00	0.76	0.19	0.26	0.09	0.01
0.12	0.69	0.09	0.29	0.09	0.01
0.25	0.68	0.27	0.29	0.09	0.08
0.37	0.51	0.25	0.39	0.11	0.49
0.50	0.54	0.25	0.54	0.15	1.00
0.62	0.98	0.32	0.70	0.19	0.26
0.74	2.04	1.08	1.36	0.53	0.34
0.87	1.37	0.25	1.19	0.36	0.52
0.99	1.89	0.62	1.58	0.25	0.56

Appendix 4. 11 Comparison between η for HPMC-CPC with and without G

[CPC]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	η (Pa.s)	STDEV	P
0	0.76	0.19	0.26	0.09	0.01
0.15	0.46	0.16	0.34	0.01	0.26
0.3	0.36	0.03	0.42	0.06	0.20
0.45	0.41	0.05	0.44	0.08	0.61
0.6	0.52	0.07	0.31	0.10	0.04
0.75	0.46	0.00	0.22	0.20	0.11
0.9	0.53	0.19	0.32	0.02	0.13
1.05	0.47	0.08	0.44	0.10	0.71
1.2	0.81	0.68	0.36	0.08	0.32

Appendix 4. 12 Comparison between η for HPMC-Lec with and without G

[Lecithin]/CMC	HPMC 1 wt%		HPMC 1 wt%+ G wt%		
	η (Pa.s)	STDEV	η (Pa.s)	STDEV	P
0.0	0.76	0.19	0.26	0.09	0.01
0.1	0.34	0.03	0.35	0.02	0.66
0.3	0.37	0.01	0.29	0.04	0.03
0.4	0.38	0.03	0.29	0.02	0.01
0.5	0.43	0.10	0.38	0.05	0.48
0.7	0.44	0.07	0.39	0.04	0.34
0.8	0.61	0.09	0.36	0.06	0.02
0.9	0.52	0.09	0.40	0.08	0.16
1.1	0.54	0.04	0.42	0.06	0.04

5.CONCLUDING REMARKS

A film containing a strong and non-soluble active ingredient which is homogenously dispersed can be an alternative for stabilization and accurate dosification of the drug. A polymer or gel network is preferable to provide encapsulation of the drug, which improves long-term stability. Gelation temperature is a critical processing parameter that needs to be determined in order to set an adequate processing temperature range and to establish the final product microstructure. On the other hand, rheological properties are necessary to understand the effect of the processing variables on final product performance.

In this work, it was shown that polymer characterization can be studied using rheological techniques. Rheological properties help to understand the physical process and interactions. They can be also related to the formation and final film properties required such as particles dispersion and drug release. Additionally, phenomenological models to determine rheological properties, for example viscosity as a function of temperature and concentration, such as we developed for NaAlg, can be generated and extended to others weak gels.

In some cases, the use of surfactants to stabilize particles is necessary. Consequently, interactions between surfactant, particles and polymer can occur changing suspension and gel properties. It was demonstrated that these interactions are dependent on type of surfactant, particle properties and polymer nature. The latter are extremely important in the development of reproducible homogeneous and stable pharmaceutical products, which can be extrapolated to systems with similar

characteristic as the one studied. In this work, a non-charged polymer, with hydrophobic and hydrophobic functional groups, a negatively charged particle and surfactants with different charges were studied. For the studied system, at high enough concentrations all surfactants are able to stabilize the drug; nevertheless, they affect the rheological properties due to differing microstructure. Yet, gelation temperature increases with anionic and neutral surfactants, while viscosity remains constant. The opposite behavior was observed for systems containing cationic surfactant. Results showed that the effectively of the surfactant to stabilize charged particles in presence of a non ionic polymer is related to its head group charge. Charged particles can be suspended successfully using an opposite charged surfactants due to no repulsion are present and polymer is no able to replace surfactant at the particle surface. It is expected that these interaction do not cause a drastic change in T_{gel} and viscosity will remain unaffected. While, if particles and surfactant have the same charge, repulsion are expected and adsorption of the polymer on the surface is favorable causing aggregate formation and changes in T_{gel} and viscosity.

This information can be used to develop and process drug-loaded films with *a priori* understanding of the complex interactions in these systems. Similarly, the effect of particle size and concentration on the gelation process of model systems showed to be related to the properties of the system chosen.

Additionally, he effect of charged particles on the gelation temperature of a charged biopolymer was studied using silica particles and NaAlg solution Silica is a model of negatively and hydrophobic particles. Results showed that silica with

particle size from nano to submicron and at loading of up to at concentration below 1.4 wt% did not have an effect on the T_{gel} of the negatively charged 1.5 wt% NaAlg solution. In this system polymer is no able to absord in the particle surface due to repulsion and not change in viscosity and T_{gel} are expecting Pharmaceutical particles with properties similar to silica interacting with polymer as NaAlg, facilitate film processing due to no additional considerations will be necessaries.

For future work, development of a model for the rheology above T_{gel} for different cold-setting gels or below T_{gel} for hot-setting gels can be useful to predict solution viscosity as a function of formulation and processing parameters. Additionally, different methods as casting or rolling can be used to form films and their physical properties as texture, color, softness, hardness and drug release, among others, can be related to the suspension properties and to determine if additives, such as colorants or plasticizers are needed.