RHEOLOGICAL CHARACTERIZATION OF PHARMACEUTICAL GEL FORMULATIONS

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ABSTRACT

The aim of this research work was to study the rheological properties of an industrially relevant gelatin-based pharmaceutical formulation, since the rheology of the product provides information regarding the structure of the fluid. Moreover, it is desired to have better control over its final viscosity to meet product specifications stipulated by regulatory agencies. Accordingly, the rheological behavior of the formulation was studied under steady and dynamic flow conditions, typical of manufacturing processes. The biovariability of the main structural material can produce significant changes on the rheology of the final formulation. Thus, molecular weight distribution (MWD), water content, and chemical structure of various gelatin drum cuts were determined. Although variations on the distributions were observed, these were found to correlate poorly with the steady-state viscosity and gelation transition. Additionally, the excipients influence on the steady-state rheology was determined by a sensitivity analysis. Gelatin and xanthan were identified as the most important components of the formulation. By solution viscosity and gel strength measurements, poor or no gelation was observed at a gelatin to xanthan ratio of 20, 13, and 12.5 for gelatin 1, 1.3, 2.5 wt% mixtures, respectively. These suggest that under these conditions the formation of complexes prevails, as shown by zeta potential measurements. Addition of salts produced a reduction or vanishing of the interactions between gelatin and xanthan, attributed to the stabilization of xanthan carboxilic group charges. Xanthan is a polyanion and gelatin is an ampholyte, hence, it is possible that the opposed charges have a detrimental effect on the viscosity.

RESUMEN

El propósito de esté trabajo de investigación es estudiar las propiedades reológicas de una formulación farmacéutica industrial la cual es basada en gelatina, ya que la reología del producto provee información vital sobre su estructura. Ademas se desea obtener un mejor control sobre la viscosidad final del producto para que este pueda cumplir las especificaciones estipuladas por agencias reguladoras. De acuerdo a lo mencionado anteriormente, el comportamiento reológico de una formulación farmacéutica ha sido estudiada bajo condiciones de flujo estacionarias y dinámicas, siendo estas típicas de procesos de manufactura. La biovariabilidad del material estructural principal puede producir cambios significativos en la reología final de la formulación. Por lo tanto distribuciones de peso molecular, contenido de agua, y estructura química de varias muestras de gelatina de un mismo lote fueron determinadas. Aunque se observaron variaciones en la distribución, se encontró que estas reflejaron un pobre ajuste con la viscosidad en estado estacionario y con la transición de gelación. En adición, la influencia de los excipientes en la reología en estado estacionario fue determinada con un análisis de sensitividad. Se identifico a gelatina y xantan como los componentes mas influyentes en la formulación. Con medidas de viscosidad de solución y dureza del gel, poca o ninguna gelación fue observada bajo una razon de gelatina a xantan de 20, 13, y 12.5 para mezclas de gelatina de 1, 1.3, 2.5 wt%, respectivamente. Esto sugiere que bajo estas condiciones existen formación de complejos, mostrado por mediciones de potencial zeta. La adición de sales produjo una reducción o desaparición de interacciones entre gelatina y xantan, atribuido a la estabilización de las cargas de los grupos carboxílicos de xantan. Xanthan es un polianion y gelatina es anfoterica, por lo tanto es posible que las cargas opuestas muestren un efecto perjudicial en la viscosidad.

To baby...

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Abbreviations and Symbols

API	active pharmaceutical ingredient
T_{gel}	gelation temperature
T_{melt}	melting temperature
BP	biopolymer
FDDT	fast dissolving/ disintegrating tablets
SAOS	small amplitude oscillatory shear
pI	isoelectric point
SDR	structure development rate
WHC	water holding capacity
G'	storage or elastic modulus
G"	loss or viscous modulus
CSTR	constant stress temperature ramp
sol	gel liquid precursor
gel	solid formed from the sol
tan δ	loss tangent angle, equal to G"/G'

HM	high methoxy
NIRS	near infra-red spectroscopy
iPS	isotactic poly(styrene)
DSC	differential scanning calorimetry
DoE	design of experiment
Ω	conductivity
ρ	density
M_w	weight-average molecular weight
Da	Dalton
FDA	Food Drug Administration
FFD	fractional factorial design
FT-IR	Fourier transform infra-red
GPC	gel permeation chromatography
MWD	molecular weight distribution
M_n	number-average molecular weight
M_z	z-average molecular weight
TGA	thermal gravimetric analysis
DG	double gap
IEF	isoelectric focusing
V	volts
GDC	gelatin drum cut
η	viscosity
R^2	correlation factor
PEG	polyethylene glycol

1 INTRODUCTION

1.1 Motivation

Biopolymers (BP's) are polymers derived from living organisms and plant sources. Unlike most synthetic polymers, BP's are biodegradable. BP's are part of innovating products and also are changing the way of manufacturing many products in various areas such as pharmaceutical, food, cosmetic, agriculture, textiles, chemicals, health care, plastics, and medicine. For example, plastics can be manufactured with BP's such as polylactic acid (PLA)[1] and poly-3-hydroxybutyrate[2] as an alternative for petroleum derived plastics. Other biopolymers form thermoreversible biocompatible and biodegradable physical gels (See section 2.3 for details), which are attractive for the fabrication of edible products. For example, gelatin gels are temperature-sensitive and for this reason they have gained considerable attention in the pharmaceutical field due to the easy manipulation of their phase transition as a result of temperature changes[3]. Moreover, biopolymers are used in edible products such as gel caps (e.g. Tylenol™ rapid release), films (e.g. Listerine™ pocketpacks), tablets (e.g. Advil™ caplets), marshmallows (e.g. Kraft™ jet-puffed marshmallows), and salad dressings (e.g. Wish-bone™). Additionally, the combination of biopolymers can lead to economical benefits by offering multiple functionalities in structural (e.g. gel formation) and mechanical aspects (e.g. elongation at break), among others. In some cases, biopolymer combinations can provide flexibility in designable properties. The properties of a system can be designed by choosing the appropriate biopolymers and additives. It is important to understand the rheology, effect of excipients, and also the synergistic or antagonistic effects in biopolymer mixtures in order to design and optimize products with consistent specifications.

1.2 Objectives

The main goal of this study was to determine the rheological properties of a gelatin-based formulation produced by a local industry. In order to achieve this goal, three specific objectives were followed. First, the main structural component of the formulation (i.e. gelatin) was analyzed to determine the effect of biovariability in its properties, if any, which could affect the pharmaceutical product specifications. Second, a sensitivity analysis was conducted based on design of experiments (DoE) to determine the most influential factors (i.e. excipients) on the final viscosity of the placebo formulation. Then, the molecular interactions between the two most influential factors were investigated. Additionally, the effect of a drug model (salt) on the interactions between the influential factors was analyzed.

Rheology changes attributed to changes of excipients concentration and processing conditions (e.g. temperature, cooling rate, stress) should be known to design and optimize the manufacture of the drug product successfully. It is important to study the rheological properties of the formulation in order to have a better control over the resulting final product viscosity and avoid the waste of lots.

1.3 Overview of the following chapters

Chapter 2 focuses on the theoretical background regarding pharmaceutical formulations, excipient categories and functions, gels, biopolymer mixtures, rheology of physical gels, and methods for gelation point determination. Moreover, the case study of this research work is presented. Chapter 3 describes the excipients comprising the case study formulation, experimental methods, and characterization techniques. Chapter 4 presents results on gelatin

biovariability assessed by various characterization techniques. Moreover, discussion and conclusions are made based on results. Chapter 5 deals with the determination of formulation rheology assessed by constant stress temperature ramp (CSTR), small-amplitude oscillatory shear (SAOS), and steady-state viscosity tests. In addition, an analysis of the design of experiments (DoE) was performed to determine the most influential factors on the final viscosity of the formulation. Finally, Chapter 6 focuses on the rheology and molecular interactions of biopolymer mixtures comprised of the formulation most influential factors: gelatin and xanthan.

2 BACKGROUND

Pharmaceutical formulations fundamentals and theory are discussed in this chapter. Moreover, common pharmaceutical formulation component categories, their functions, examples, and the importance of systematically characterizing them are discussed. Biopolymer systems were reviewed from single to multi-biopolymer systems along with additives. Sol-gel transitions are a key characteristic in gel systems and their applications. Thus, methods for gelation point determination are reviewed. Finally, the case study of this research work is briefly presented.

2.1 Pharmaceutical Formulation

A formulation is the product of developing or preparing something according to a formula or recipe. This is a common practice to achieve desired and consistent product properties (i.e. quality). Formulations are prepared every day in every home and every industry in either solid or fluid states. This work is focused on fluid pharmaceutical formulations. Among fluid formulations are identified suspensions, gels, aerosols, and emulsions, to name a few. Pharmaceutical formulation components can be classified as active pharmaceutical ingredients (APIs) and excipients. A placebo formulation is the combination of all the excipients without the API. The APIs are intended to cause pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of a disease or to affect the structure and function of the body[4]. Excipients are substances that have a specific function in the formulation preparation or final product, such as preventing formation of foams, changing the color, consistency, or flavor, such that the general population accepts it. Final product properties are dependent on the combination of excipients used, their concentration, and their interactions[5].

The most common pharmaceutical formulations are tablet (i.e. solid) formulations. Nevertheless, there are many other types of formulations such as the fluid formulations mentioned above. In particular, pharmaceutical gel formulations have received increasing attention in the past decades. A gel formulation generally consists of one or more liquid excipients, a gelling agent, and API. Numerous excipients exist for different types of formulations. Common pharmaceutical excipient categories and functions are discussed in the following section, including relevant categories for this research work.

2.2 Excipients categories and functions

As a general rule, the formulations should only contain the necessary excipients (i.e. keep it simple) because the addition of unnecessary components adds more variables to the system. Simple formulations generally reduce manufacturing problems due to the easier understanding of a system[6]. Some excipient categories, functions, and examples are described from Table 2.1 to 2.5. Process modifying agents improve processing and they are described in Table 2.1. Structural modifying agents promote the formation of complex structures between molecules or particles and they are described in Table 2.2. Perception modifying agents enhance sensory properties during product consumption and they are described in Table 2.3. Drug release modifying agents manipulate the release profile of a drug and they are described in Table 2.4. Protection modifying agents prevent changes on the final product properties enhancing stability and they are described in Table 2.5.

Table 2.1 Classification of process modifying agents*

Type	Function	Example
fillers	give volume to tablets that have low drug dosages and make the production process easier	lactose
dry and wet binders	aid the formation of granules and tablets	microcrystalline cellulose and povidone
lubricants	reduce friction between powder and die walls	magnesium stearate
antiadherants	reduce stickiness between powder and die walls	talc
glidants	improve powder flowability	calcium silicate
defomers	prevent the formation of foam or gases	simethicone

Table 2.2 Classification of structural modifying agents*

Type	Function	Example
Gelling	Provoke a change of state (sol-gel transition)	gelatin
Crosslinkers	Aid the interactions between particles or molecules	genipin

Table 2.3 Classification of perception modifying agents*

Type	Function	Example
Flavorants	Enhance product flavor and mouthfeel sensation	peppermint, aspartame, glycine
Colorants	Enhance product appearance	red ferric oxide
Texturizers/viscosity imparting	Manipulate solution thickness	xanthan gum
Taste masking	Disguises the bitter taste of some drugs	resins

Table 2.4 Classification of drug release modifying agents*

Type	Function	Example
Dissolution modifiers	Manipulate drug dissolutions profiles	HPMC
Disintegrants	Expand, break tablet and dissolve when in contact with water	starch

Table 2.5 Classification of protection modifying agents*

Type	Function	Example
Coatings	Protect the integrity of the tablet or capsule	polyethylene glycol
Preservatives	Prevents degradation of tablet components	vitamin E

* Refer to reference [5]

It is important to mention that excipients can have multiple functions depending on their physical properties (e.g. molecular weight), surface chemistry (e.g. ionic groups), concentration, and interactions with other components. In fluid formulations, the excipients that affect the rheology (e.g. viscosity and physical state) are of great importance because they may also affect processing, structural, perception, and drug release properties. Moreover, the combination of these excipients (i.e. gelatin-xanthan) also affects the rheology. Furthermore, these complex systems could affect (positively or negatively) the structure, rheology and state changes (e.g. sol-gel transition) of a formulation. It is important to systematically characterize the combination of these excipients to improve or optimize formulations and to troubleshoot manufacturing production lines. Of particular interest are gel properties, the focus of this work, which is described in the next section.

2.3 Gels

"Gelation is the conversion of a solution to a disordered network by formation of chemical or physical bonds between the particles or molecules composing the liquid."[7] Gelation point is also called the sol-gel transition. Gels are complex fluids, which are classified as chemical or physical gel. A network formed by chemical bonds between the particles or molecules is known as a chemical gel, whereas a network formed by physical intermolecular associations is known as a physical gel. Chemical gels are generally related to synthetic polymers and strong thermo-irreversible gels, whereas, physical gels are related to biopolymers and thermo-reversible gels. In general, gel networks (structures) are capable of entrapping solvent[8], particles[9, 10] or other components[11-13]. Gels show viscoelastic behavior, which means that they have a combination of solid- and liquid-like material properties. The physical

characteristics of gels may vary with temperature changes (i.e. thermotropic), pH, concentration, or ionic strength. When subjecting a sample to a temperature change (either heating or cooling), the temperature where the transition from solution to gel occurs is known as the gelation temperature (T_{gel}), while, the transition from gel to solution is known as the melting temperature (T_{melt}). The measurement of T_{gel} is commonly used to characterize the sol-gel behavior. The unique properties of gels make them very attractive for the pharmaceutical and the food industry, nevertheless correct characterization of the transition is required to optimize processing and formulation of products.

Physical gelation is possible through intramolecular, intermolecular and molecule-solvent interactions. Helical structures[14], microcrystallites[15, 16], or nodular domains[17] are interactions that can lead to physical gelation. Intermolecular associations can be either van der Waals forces, hydrophobic interactions, electrostatic attractions, or hydrogen bonds[7]. A hydrogen bond has weak association energy, but a sequence of hydrogen bonds (a molecular 'zipper') has enough energy to hold molecular strands together[15]. Network formation of physical gels is random, thus it cannot be easily described mathematically hindering the developments of concrete theories. Many gelation mechanisms for gel systems have been proposed to explain the particle and molecular associations, for example the helix-coil of gelatin, the eggbox model of sodium alginate, or the microcrystalline regions of κ-carrageenan, amongst others[16]. Some gelation mechanisms are widely accepted yet they differ from system to system, which makes it difficult to obtain concrete generalized models or theories.

The formation of covalent bonds between the polymer gel and the API is not desirable because there are risks of losing API activity, stability, also being easily digested or increasing toxicity. For example, if you try to eat a chemical gel strip it would not dissolve thus it is out of

question as an oral drug delivery vehicle. For this reason, among others, chemical gels are not used as part of edible products. Chemical gels are outside the scope of this research, thus no further discussion is provided afterwards in this document other than to differentiate with physical gels.

Physical gels are of interest for pharmaceutical applications mainly because they are thermoreversible around body and room temperature and do not form covalent bonds with the API. Physical gels thermoreversibility means that the transition between solution and gel states is reversible with changes in temperature. The thermoreversibility is a result of the low energy interactions that stabilize the gel network[18]. Thermoreversibility is by far, the most technologically important characteristic of physical gels[19]. This property is very useful when processing sol-gel fluids. For example, a sol-gel formulation can be processed as a solution in critical stages (e.g. mixing or pumping) and as a gel at other stages (e.g. suspending particles or filling molds).

Biopolymers generally form thermoreversible physical gels, which are attractive for the fabrication of edible products. Gel formulations are of great use for patients who do not like to take pills or have difficulty swallowing pills. Moreover, their characteristics make them simple to process. In the next section, relevant gelatin-based systems and additives are discussed in detail.

2.4 Biopolymers and gelatin

Biopolymers (BPs) are polymers from biological origin such as starch (botanical), gelatin (animal), and xanthan gum (microbial). Unlike most synthetic polymers, BPs are biodegradable. As mentioned earlier, some BPs can form thermoreversible gels, which is a very attractive

property exploited in industrial applications. They were discovered years ago but it was not until the 1970s that their development and manufacture increased[20]. The initiative of using biological sources for polymer manufacturing was a measure taken by some industries to avoid or decrease future dependence on petroleum-derived products. Indeed, BPs are widely produced, making them highly available for manufacturing processes. All these characteristics favor them over synthetic polymers in many applications, especially for body contact (e.g. wound dressings) and consumption. Refer to Table A.1 in the Appendix for details about common biopolymer properties. Thus, BPs are part of innovative products and have had a fast integration in many areas such as the pharmaceutical, food, cosmetic, agriculture, textiles, chemicals, health care, plastics, and medicine fields. Figure 2.1 shows a schematic of the volume production on the world market for individual biopolymers[21].

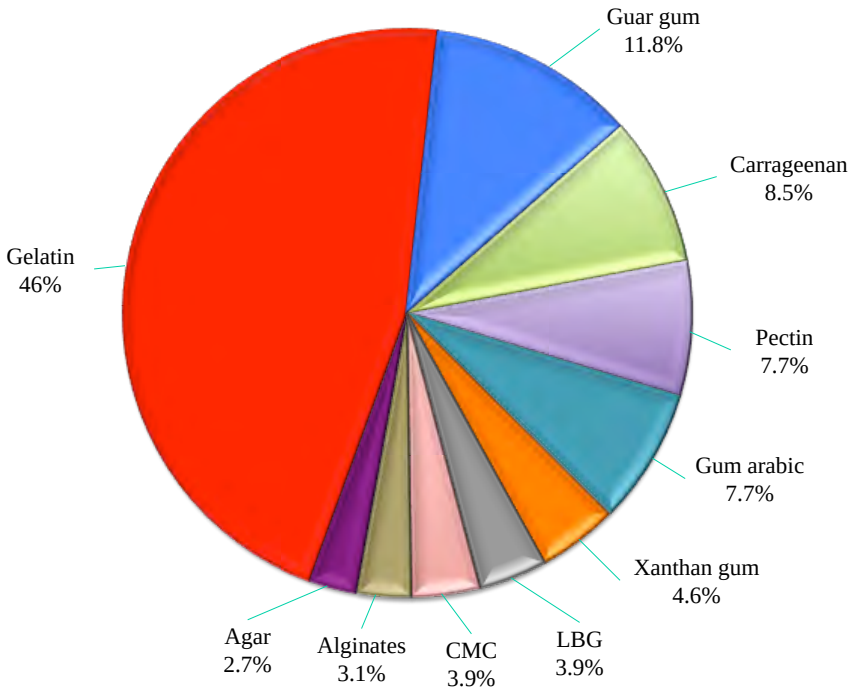


Figure 2.1 Volume on world market (260 ktons) for individual biopolymers
Reproduced from reference[21]

BP gels are very useful for drug delivery in many human body locations, as depicted in Figure 2.2. The porosity of BP gels also permits loading of drugs into the gel matrix and subsequently releasing a drug at a rate dependent on the diffusion coefficient of a small molecule or macromolecule through the gel network[22]. In addition, degradable implanted gel carriers eliminate the need of recovery with additional surgeries[23]. BP gels are very attractive to the pharmaceutical industry for the manufacturing of gel caps, formulations, and many other products. Fast dissolving or disintegrating tablets (FDDTs) may be prepared from BP gel formulations, as is the case of the case study of this research work. FDDTs are a perfect dosage form for children, schizophrenic patients, and nauseous people[24].

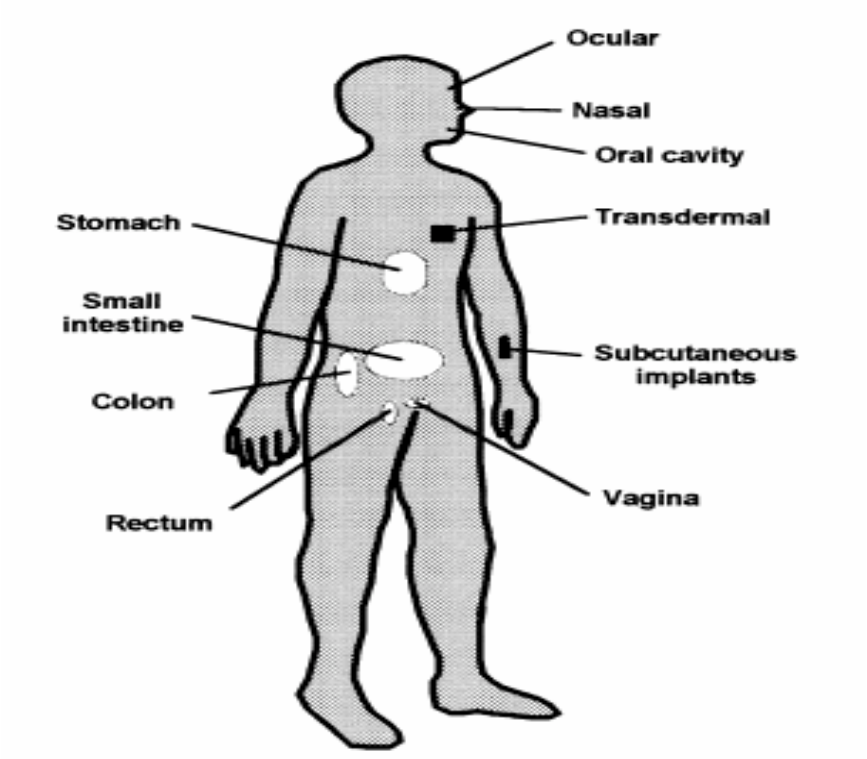


Figure 2.2 Locations applicable for gel-base drug delivery systems
Reproduced from reference[3]

Extensive research has been done on BP systems, but here the focus was be on relevant non-modified gelatin-based systems. Moreover, additives can also be part of these systems. Single gelatin-based biopolymer systems have been studied with numerous additives, which can be used to alter system properties (e.g. performance) or perspective. Cortesi et al.[25] proposed the use of oxidized mono- and di-saccharides (D-glucose, D-fructose and D-sucrose) as biocompatible crosslinkers for gelatin-based pharmaceutical devices (microspheres and disks) to slow the swelling and dissolution of gelatin. The objective of their studies was to have a drug controlled or sustained release. As a result, the addition of biocompatible crosslinkers reduced the dissolution of gelatin. As an advantage, the authors concluded that the use of saccharides provides an alternative for chemical crosslinkers (e.g. formaldehyde, glutaraldehyde), which had toxicity side effects. Alternatively, Ciper et al.[26] prepared capsule-based fast disintegrating dosage forms (Fastcaps) for the oral cavity incorporating low bloom strength gelatin with xylitol or PEG. The hydrophilic additives substantially improved capsules disintegration time (9-13 seconds). Additionally, enzymes such as tyrosinase and laccase[27], and transglutaminase[28] are used in food applications as gelatin crosslinking tools. In biomedical applications, genipin[29-34], proanthocyanidin[35, 36], and pyrroloquinoline quinone[31] are used as gelatin crosslinking agents.

In the last decades, the combination of two or more BPs has become a common practice to achieve product properties that could not be obtained using single gelatin formulations. The most commonly used combination incorporates two BPs. Of particular importance in this context are protein-polysaccharide combinations, which have received high attention in recent years. Polysaccharide-polysaccharide and gelatin-protein combinations have similar importance but are outside the scope of this work. Figure 2.3 illustrates a schematic of the possible interaction between two BPs and the resulting systems. Interactions can proceed by association

of biopolymers resulting in precipitation or gelation. Precipitation (two phase) occurs as a result of biopolymers forming neutral charge complexes that have poor or no interaction with the liquid excipient. Gelation (single phase) proceeds as explained before in the previous section. Interactions can also lead to no association of biopolymers resulting in a single phase or separated phase system. The no association effect can be attributed to the incompatibility of molecules or opposite charge effects causing molecules to repel each other.

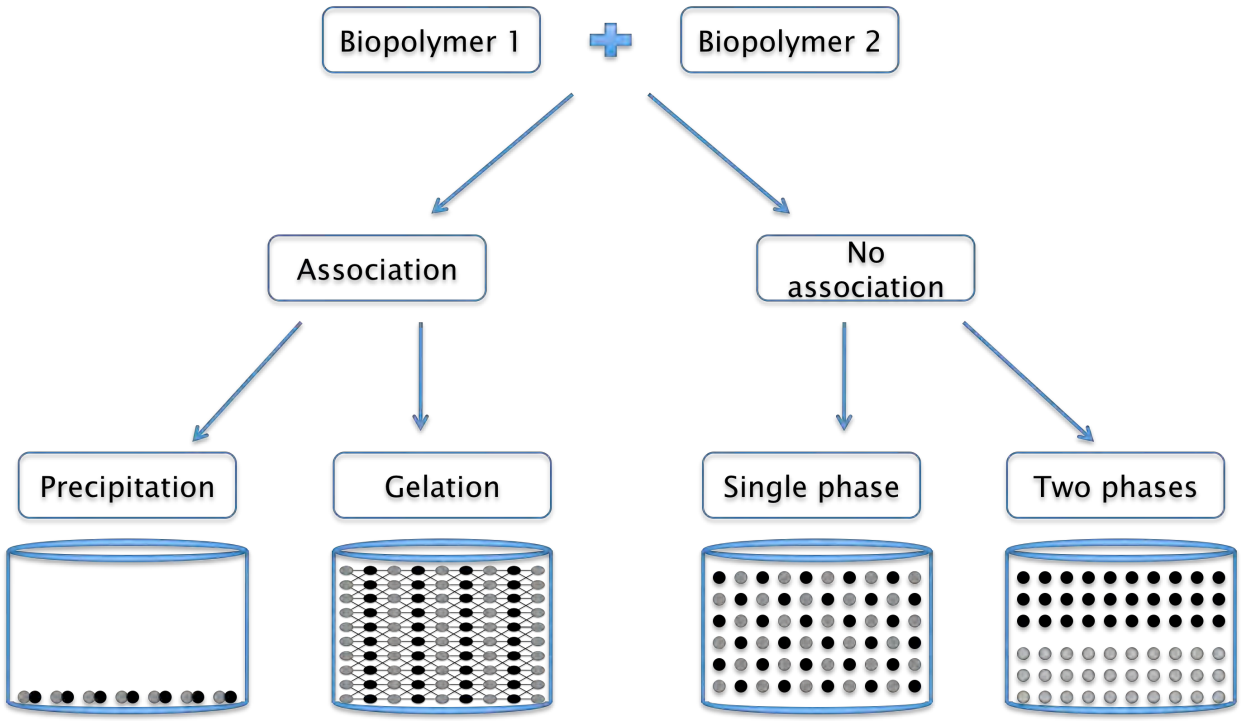


Figure 2.3 Possible interactions between two biopolymers and resulting systems
Reproduced from reference [21]

Lau et al.[37] studied gelatin (type B, 0–1.4% w/v) - gellan (1.6–0.2% w/v) systems at different polymer ratios and calcium (0-30 mM) concentrations. Gellan is a microbial anionic polysaccharide, which as xanthan gum and other polysaccharides forms gels in the presence of mono- and di- valent cations (e.g. Na^{+}, Ca^{+2})[38]. Its gels can be soft or brittle depending on the

acyl quantity. Figure 2.4 illustrates a schematic comparison of gellan gel strength with other biopolymers[21]. Lau et al.[37] observed that T_{gel} increased with increasing gellan and Ca^{+2} concentration, as depicted in Figure 2.5. The proposed gelling mechanism for this system is helix-coil transition, similar to gelatin. Gelation temperatures were determined with small-amplitude oscillatory shear (SAOS) test. As a result, the mixture gelation behavior was similar only to the one of gellan. In other words, the system T_{gel} was similar to gellan-only T_{gel}.

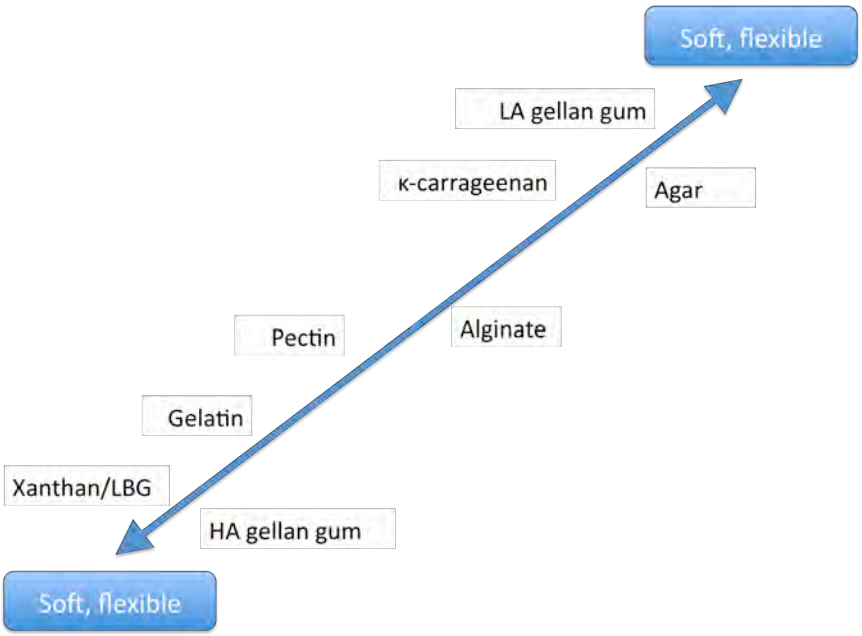


Figure 2.4 Schematic comparison of gellan gel strength to other biopolymers gels
Reproduced from reference [21]

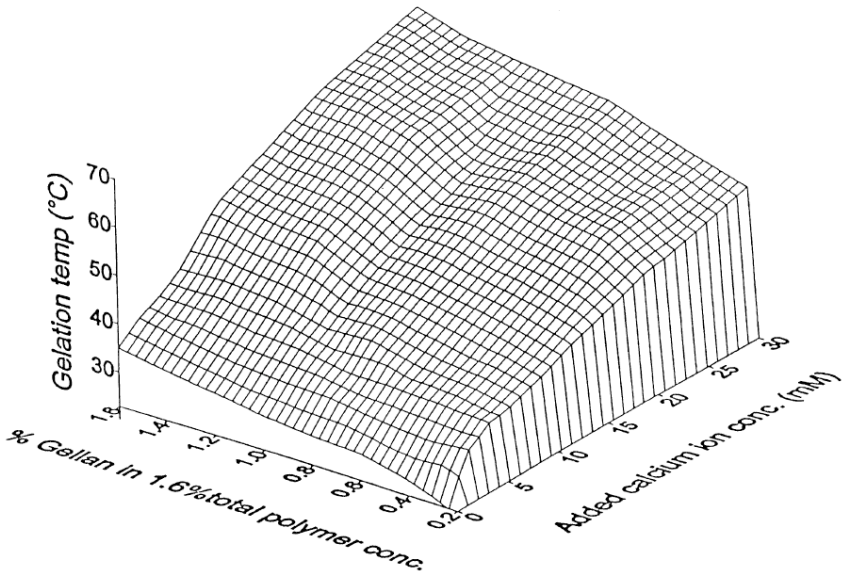


Figure 2.5 Three dimensional contour of T_{gel} versus % gellan in 1.6% total polymer concentration and added calcium ion concentration
Reproduced from reference [37]

On the other hand, Lee et al.[8] studied 1% total polymer systems of gelatin (type A)-gellan with sodium chloride (up to 300 mmol/L). The authors determined that flow behavior and texture were dependent on polymer ratio and salt concentration. Also, shear-thinning (See Section 2.5 for definition) behavior decreased with increase in gelatin concentration, temperature, and salt concentration.

Voron'ko et al.[39] studied gelatin (type B, 1%) - sodium alginate (0.0001-0.15%) systems at acidic pH from 5.3 to 6.0. Sodium alginate is an algal anionic polysaccharide. At those concentrations, sodium alginate does not form gels. After six days, the authors observed no macroscopic separation. Voron'ko et al.[39] concluded that the formation of complexes between these charged biopolymers proceeded by hydrophobic interactions, H-bonds, and salt-like interactions. Aggregation was prevented because at the pH conditions used the gelatin has a high

quantity of negatively charged groups. Synergistic interactions between these biopolymers were observed at pH < pI. Moreover, rheological behavior was dependent on polymer ratio.

Dong et al.[40] prepared gelatin-alginate films crosslinked with Ca^{+2} ions and added a drug model called ciprofloxacin hydrochloride. Addition of alginate to gelatin systems is very attractive for drug-controlled release due to the fact that the swelling property can be manipulated depending on the concentration of Ca^{+2} added (i.e. crosslinking degree). The reduction of swelling is desired when reduction of the permeability of solutes is needed. The films developed can be successfully used for localized (e.g. surgery) and oral cavity drug delivery.

Zasypkin et al.[41] studied gelatin-dextran systems and determined that small quantities of dextran (< 0.5% w/w) provide a single-phase gelatin gel and increase greatly the rate of gelatin gelation. As an advantage, the amount of gelling agent used can be reduced. Also, the authors studied gelatin-sodium alginate gels and determined that the melting temperature was higher than for gelatin gel alone. The authors concluded that the thermo-mechanical properties of complex gels like this one are quite stable for a long period.

Ternary biopolymer systems are not common. Nevertheless, the resulting properties of the combination of more than two biopolymers could be surprisingly useful. Hee et al.[42] formulated gels with similar texture to Camember and Coulommiers cheeses for equipment calibration and sensory panelist training. This task came from the fact that single gelatin systems could not immitate the texture of these cheeses. The authors made combinations of biopolymers such as gelatin type A, guar, karaya gum, xanthan gum, maltodextrin, and starch. As a result, gelatin-maltodextrin-starch combination matched the texture of these cheeses.

The results of many studies showed that gel structural, mechanical and textural properties can be tailored by choosing the appropriate BPs and additives to obtain a desired set of properties. Among the properties that can be manipulated are identified T_{gel}[37, 43, 44], gelation or structure development rate (SDR)[37, 41, 44], T_{melt}[37, 44], visco-elasticity (G' & G")[8], texture profile (e.g. hardness, brittleness, cohesiveness)[8, 37, 44, 45], water holding capacity (WHC)[8, 45], swelling[40], dissolution[25, 26], drug controlled or sustained release[25, 40], stability at room temperature[8, 37], tensile strength[40], elongation at break[40], and drug load[40]. Nevertheless, due to the complexity of biopolymer combinations along with additives, these systems should be systematically studied to improve or develop novel products. In the next section, common rheological behaviors of physical gels (e.g. biopolymers) are discussed.

2.5 Rheology of Physical Gel

Shear thinning, shear thickening, and thixotropy are encountered behaviors of these materials when subjected to external deformation. Rheological data for biopolymers systems can be fitted with semi empirical models such as Bingham or Newtonian[46], Ostwald de Waele[47], Herschel-Bulkley[48], and Casson[49].

Shear thinning (i.e. pseudoplasticity) is the decrease of viscosity of a fluid when it is subjected to an increase in shear rate. Gelatin and xanthan gels are an example of shear thinning fluids[50, 51]. For example, gelatin network is fractioned over time by the shear rate applied to the sample hence the viscosity decreases.

Shear thickening is the increase of viscosity of a fluid when it is subjected to an increase in shear rate. Starch solutions are one example of the few molecular rather than particulate systems that exhibit shear-thickening behavior[52, 53]. Some polymers solutions show this behavior

and it is due to the molecules or particles alignment or aggregation. May be observed if measurements are conducted while the gel network formation is progressing and the shear rates do not break it.

Thixotropy is a kind of viscoelasticity, but with a very long relaxation time[7]. In other words, the viscosity of a thixotropic fluid decreases with applied shear rate through time and then viscosity recovers to its initial state when the shear rate is removed[54, 55]. When an increasing shear rate is applied, the bonds of the sample structure break, while, the structure re-forms when decreasing the shear rate. Some examples of food products that exhibit thixotropic behavior are ketchup, mayonnaise, and mustard[56]. Such behavior is typical of these products when you squeeze the product bottle (i.e. apply shear rate) and when the product is out of the bottle at rest it recovers its initial viscosity.

2.6 Methods for gelation point determination

It is highly important to determine the sol-gel transition point to understand the behavior of gels at specific conditions and to optimize processing parameters. Rheological, visual, spectroscopy, and thermal methods are the most common tests and are discussed and compared below.

2.6.1 Rheological methods

Constant stress temperature ramp (CSTR) is a rheological test used to determine the gelation point or temperature. The test consists in performing a cooling or heating temperature ramp while applying a constant stress to a sample. Gelation point can be identified during gelation process as the point where the shear rate tends to zero and the resistance of the sample to

flow (i.e. viscosity) increases abruptly to infinite. Figure 2.6 illustrates the general behavior of gel solutions. Two straight lines were drawn on the linear regions of the curve, then, going down to the temperature axis from the intersection of the straight lines gives the approximate T_{gel}.

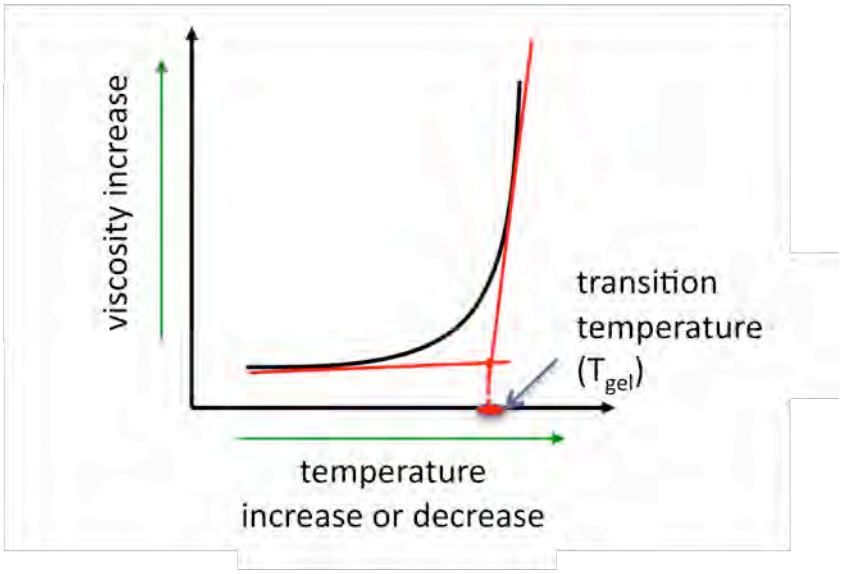


Figure 2.6 Schematic of viscosity versus temperature curve showing the sol-gel transition temperature

Rhee et al.[57] studied poloxamer solutions. Poloxamer (trade name is Pluronic) are triblock copolymers and gelling agents. Poloxamer sol-gel transitions occur due to temperature increase. These gels are being studied because they promise good control in drug delivery. The authors studied the effects of additives on the gelling point and viscosity. Moreover, the T_{gel} was determined from the curves of each solution as depicted in Figure 2.7. Ciper et al.[26] also used this method to determine gelation temperature of gelatin (35 wt%) solutions of different bloom strengths, as depicted in Figure 2.8. Both experiments on Figures 2.7 and 2.8 are constant stress temperature ramps (CSTR) tests.

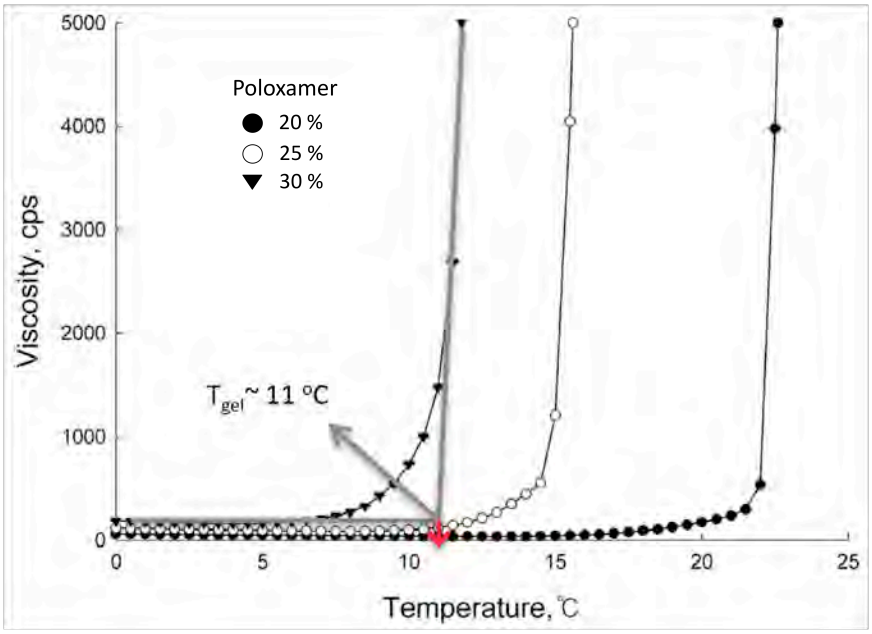


Figure 2.7 Sol-gel transitions for poloxamer solutions due to temperature increase
Reproduced from reference [57]

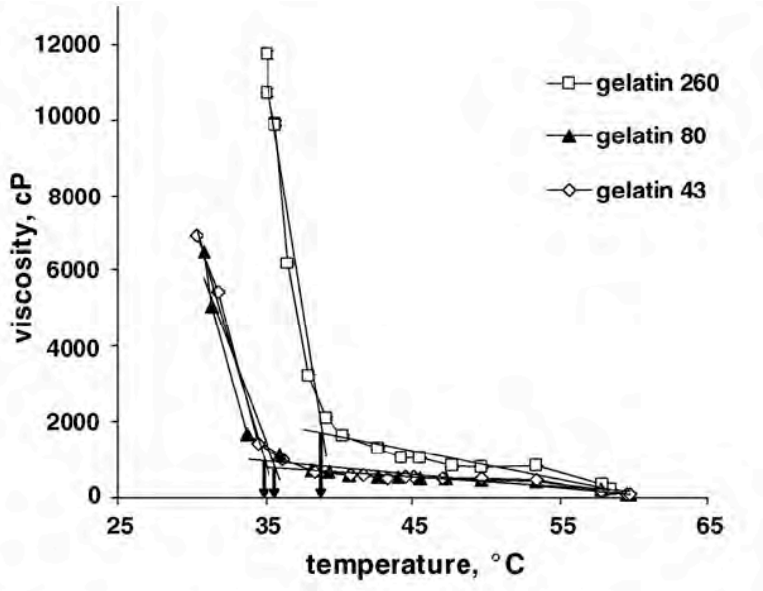


Figure 2.8 Sol-gel transitions for gelatin (35 w/w%) solutions of different bloom strengths due to temperature decrease
Reproduced from reference [26]

Small-amplitude oscillatory shear (SAOS) is another common rheological method used to determine the gelation point or temperature. The material functions for SAOS are the G′ and G″[58]. The test consists in applying a very small strain at a constant frequency to determine the storage or elastic module (G′) and the loss or viscous (G″) moduli as a function of temperature. G′ represents the amount of elastic energy stored by a viscoelastic fluid or gel network. G″ is the amount of energy dissipated by the sample or the viscous component. Loss tangent (tan δ) is the tangent of the phase angle and is equal to G″/G′. If tan δ >>1, the sample behaves like a liquid. On the other hand, the sample behaves like a solid if tan δ <<1. Figures 2.9a and 2.9b show two examples. In these experiments, a solution is cooled, heated or left at constant temperature while monitoring the moduli values. At the beginning, G″ is greater than G′ (tan δ >>1), hence, the behavior is liquid-like. Then, the sol-gel transition is identified at the intersection of the moduli curves. Finally, G′ is greater than G″ (tan δ <<1), hence, the behavior is solid-like.

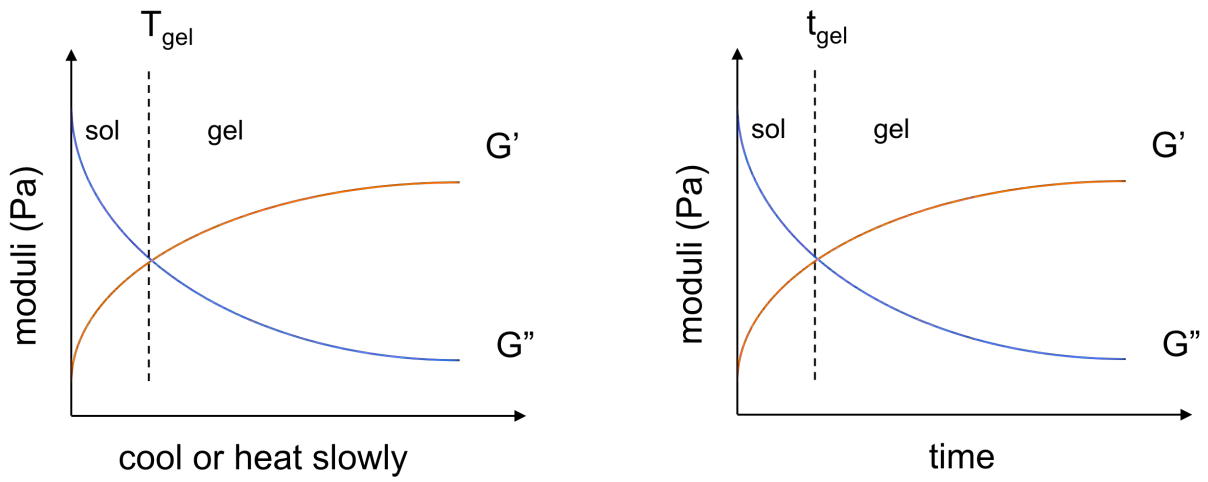


Figure 2.9 Schematic diagram of storage and loss moduli recordings as a function of temperature (a) and time (b).

Ding et al.[59] studied biopolymer mixtures of gelatin-pullulan. Pullulan is a non-charged polysaccharide commonly used in pharmaceutical and food applications. The increasing uses of pullulan lead the authors to study its interactions with gelatin. The gelling and melting points of the samples were investigated using the SAOS test. The hysteresis between the T_{gel} and T_{melt} indicates that molecules need different amounts of energy to group together or separate. The authors determined a T_{gel} value near 28°C based on the criterion of $G' = G''$ (gelation point), as depicted in Figure 2.10. Tosh et al.[18] used two tests for T_{gel} determination. The rheological test used was SAOS test. Both, Tosh et al.[18] and Ding et al.[59] used the $G' = G''$ criterion to define the gelation point.

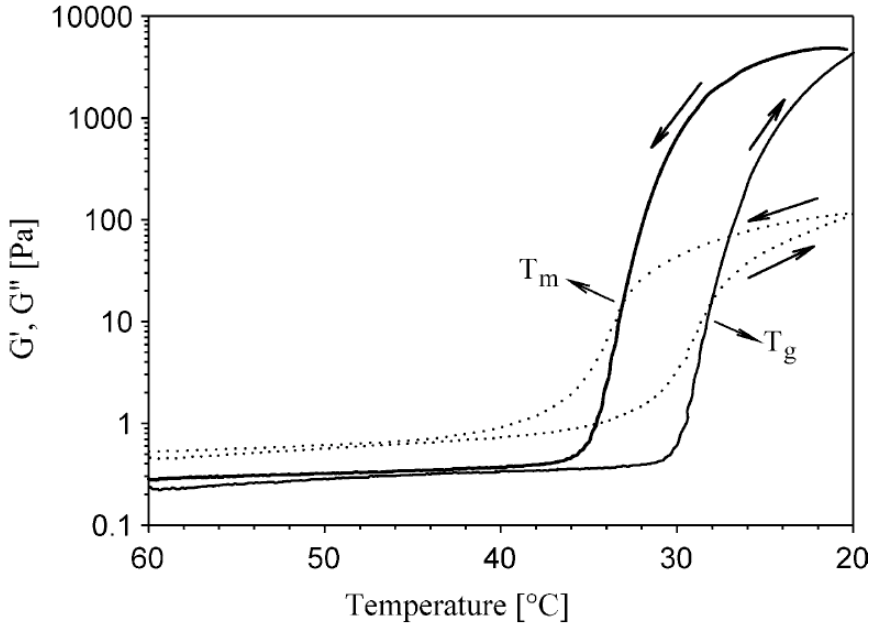


Figure 2.10 Viscoelastic properties of gelatin rich phase in phase separated systems of gelatin-pullulan (1Hz frequency, 0.67 °C/min cooling and heating rates; arrows indicate direction of temperature change)
Reproduced from reference [59]

Lau et al.[37] studied biopolymer mixtures of gelatin-gellan with calcium ions. Gellan is an anionic polysaccharide widely used in many applications. The authors used SAOS test to study sol-gel transitions with a different approach, as depicted in Figure 2.11. A line was drawn through the vertical linear region to intersect the temperature axis as the criterion to determine the T_{gel}. Dahme et al.[60] and Tang et al.[61, 62] also used this linear extrapolation method to study gelling properties of HM pectin and gellan solutions because the gelation temperatures correlated well with the ones measured with complementary techniques such as visible light spectrophotometry.

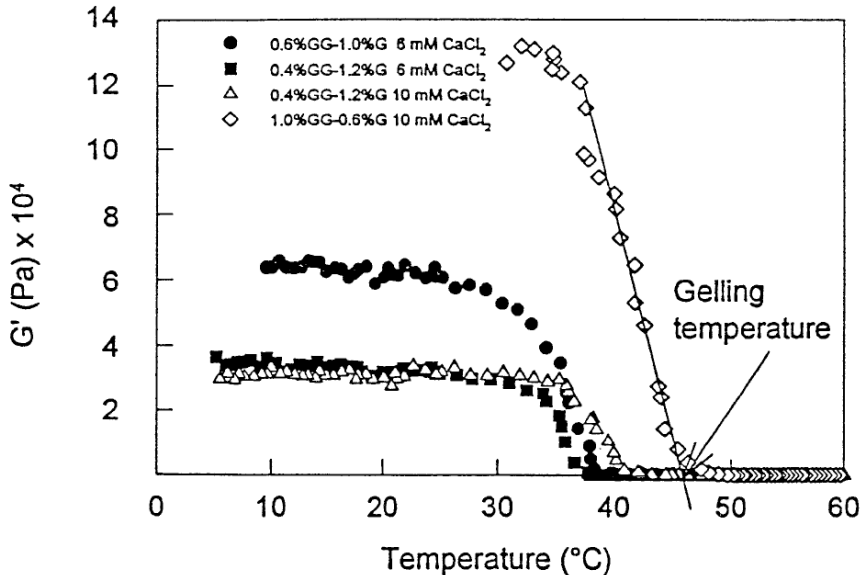


Figure 2.11 Small-amplitude oscillatory shear (SAOS) measurements for gelatin-gellan-calcium ions solutions while decreasing temperature at 0.6 ^{o}C/min
Reproduced from reference [37]

2.6.2 Visual and Optical

Tosh et al.[18] also determined the gelation point by monitoring the degree of turbidity of the sample. The turbidity increases abruptly at the gel point. Figure 2.12 illustrates a schematic

of turbidity during a sol to gel transition. While, Joly-Duhamel et al.[14] determined helix formation of gelatins from different biological origins by optical rotation measurements, as depicted in Figure 2.13. T_{gel} was estimated from the graph. Nevertheless, the authors concluded that they could not locate precisely the start of helices formation.

Tang et al.[61] used a direct visual observation of a mechanical property to determine the gelation point and temperature. The transition of gellan in the prescence of calcium ions was obvious because of an abrupt change on viscosity that was determined as the point when the thermocouple wire tip was difficult to move without bending it. A limitation of this technique was that the T_{gel} values measured deviated up to 3.5 ^{o}C (underestimate) when gelation temperature was higher than 50 ^{o}C.

Tang et al.[61] used visible light spectroscopy to determine the T_{gel} of gellan solutions. Gellan solutions turbidity increases during gelation, hence, this property was used indirectly to measure the T_{gel}. The beginning of gelation was discernible when an abrupt change in light absorbance occurred. The authors concluded that T_{gel} values agreed well with dynamic rheological measurements.

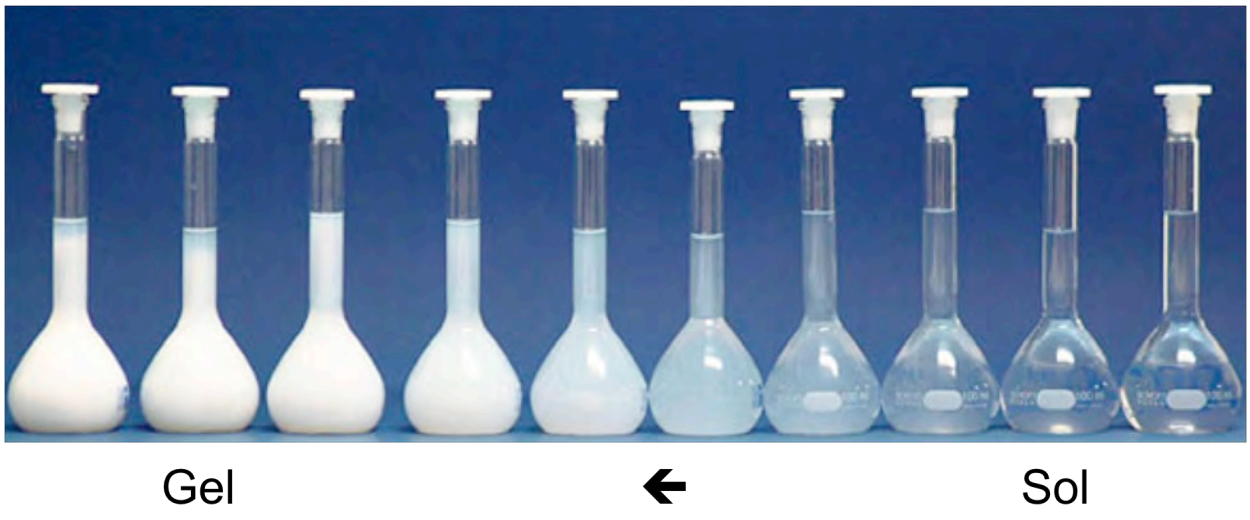


Figure 2.12 Schematic of the turbidity increase during a sol to gel transition

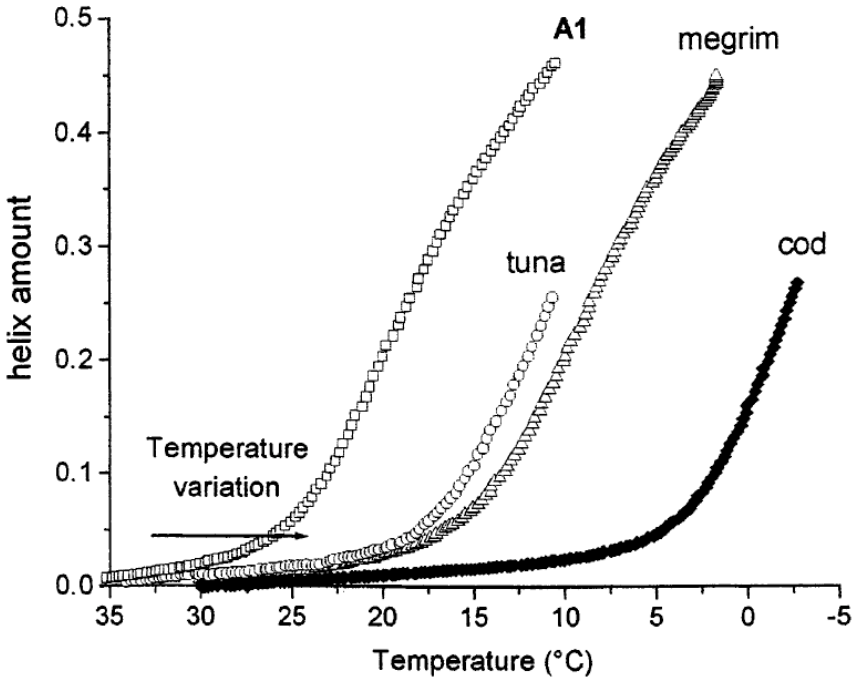


Figure 2.13 Helix formation determined by optical rotation measurements for gelatins of different biological origins as a function of temperature at a cooling rate of 0.5°C/min Reproduced from reference [14]

2.6.3 Spectroscopy

Huang et al.[63] used near infrared spectroscopy (NIRS) to determine the sol-gel transition of gellan and whey protein solutions. The authors concluded that the T_{gels} determined by NIRS were similar to those obtained by dynamic rheological tests and that this technique has great potential to monitor structural changes.

Ng et al.[64] used Brillouin spectroscopy to determine the sol-gel transition of triton X-100-water system. The use of this technique comes from the fact that a parameter (i.e. Brillouin width) of this equipment changes abruptly at the T_{gel}. The limitation of this technique is that the sample has to be transparent to light.

Berghmans et al.[65] used fluorescence spectroscopy to study the thermoreversible gelation and determine the sol-gel transition of isotactic poly(styrene) (iPS) in decalin. Here, the ratio of the emission intensity over the excimer emission intensity changes abruptly at the T_{gel}. The gelation point and rate can be studied with this technique. Similarly, the authors concluded that the measurements provided in this publication do not offer information related to system structure or morphology.

2.6.4 Thermal

Differential scanning calorimetry (DSC) is commonly used to determine sol-gel transitions or as a complementary technique[66-71]. Nevertheless, high resolution is needed and this technique is non-invasive. For example, an exothermic peak when performing a cooling temperature ramp identifies the T_{gel} of a gelatin solution. Figure 2.14 illustrates an example of a thermogram.

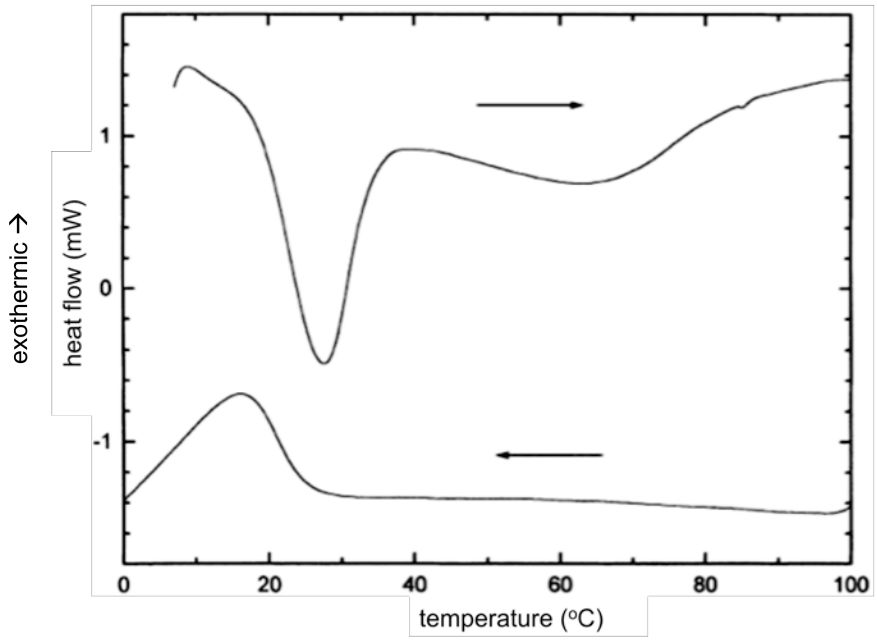


Figure 2.14 Thermogram of a ternary biopolymer system; heating (→) and cooling (←)
Reproduced from reference [68]

2.6.5 Methods Comparison

All the methods have limitations but it is not always easy to be consistent and precise when estimating the T_{gel} by optical methods. Moreover, most spectroscopy methods have to be well synchronized with external mechanical equipment to ensure good results and some of them require the use of transparent samples. Transparency is an essential physical property required to allow light to pass through a sample, hence, it is a disadvantage for some spectroscopy techniques. Furthermore, thermal methods like DSC are noninvasive, which does not allow deforming the sample while monitoring structure changes. A rheometer does not have the limitations of the optical, spectroscopy, and thermal equipments because it has all the necessary accessories to determine T_{gel} integrated (i.e. synchronized) in a single validated setup. Also, it has different geometries that adjust to different liquid viscosities. Rheological methods for T_{gel} determination are a very useful tool because they give accurate and consistent results. Moreover, these methods are highly trusted and accepted by the scientific community. As an advantage, the rheometer uses a small quantity of sample and is able to simulate the conditions of industrial process stages at small scale. The study of the rheological properties of products such as pharmaceutical gel formulations is important for better understanding of the process stages and consumer intake. Indeed, the quality of the final product is measured by its properties.

2.7 Case Study

Throughout this research project a placebo sol-gel formulation composed of eleven excipients was studied and characterized. The excipients are illustrated in Table 2.6 and described in detail in Chapter 3. Janssen Ortho LLC (Gurabo, P.R.) provided the excipients used in this research project. The main structural component of the placebo formulation is a widely

known excipient called gelatin. Gelatin is a well-known biopolymer that is highly used by many industries because of its attractive set of properties including gelling capacity at a wide range of concentrations. Additionally, the effect of parameters was studied.

Table 2.6 Excipients composing the placebo formulation

Excipient	**Function**
gelatin	primary structural
mannitol	structural
xanthan gum	viscosity imparting
carbopol 974P	viscosity imparting
amberlite IRP-64	taste masking
aspartame	flavorant, sweetener
peppermint oil	flavorant
glycine	mouthfeel enhancer
red ferric oxide	colorant
simethicone	defoaming
sodium hydroxide	neutralizing
water	medium

As mentioned earlier, the protein-polysaccharide combination has received high attention during the past decades due to the benefits that it could provide, such as designable properties and economical benefits. The placebo formulation studied in this project has a protein (gelatin)

and a polysaccharide (xanthan) as part of its components. As a specific objective, a biopolymer mixture consisting of a protein-polysaccharide was studied, specifically, a gelatin-xanthan mixture. Gelatin is a multifunctional biopolymer with the advantage that has been well studied for years due to its unique set of properties. Xanthan has a great stability over a wide pH and temperature range[21]. Also, it is well known that addition of salts affect the rheology and molecular conformation of xanthan (See xanthan section for details). Therefore, the effects of additives including salts were determined. Synergistic and antagonistic (competitive) effects are both important to study. Since gelatin and xanthan are polyelectrolytes, the system is expected to be complex. Although gelatin and xanthan have different characteristics, experiments were performed to determine if the combination of these biopolymers results in a gel with improved properties. There is evidence that similar gelatin-polysaccharide complex gels improve setting and melting properties and temperature stability compared to gels prepared using gelatin alone[41, 72]. Yet, this type of system could form complexes that could inhibit gel formation. Nevertheless, there is no systematic study of gelatin-xanthan mixtures. The analysis of results provides an insight on practical industrial applications related to the usage of this combination of biopolymers.

3 Materials and Methods

The materials and methods used in this research work are explained in detail in this chapter. The excipients origins, molecular structure and applications are presented in detail. Additionally, this chapter includes the design of experiments used to determine the most influential components of the placebo formulation. Details on placebo formulations, biopolymer systems and gelatin drum cut solution preparation, processing steps, and test parameters are described. Then, characterization methods used to analyze the main structural component (i.e. gelatin) biovariability are described. Finally, solution properties such as pH, conductivity, and density of the solutions are presented.

3.1 Materials

3.1.1 Gelatin

Gelatin is a protein (biopolymer) derived from collagen. Commercial gelatin can be obtained from bovine[73, 74], porcine[75, 76], and fish[77, 78] sources among others. It can be obtained by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of animal collagen[19]. Figure 3.1 shows an example segment of the molecular structure of gelatin. Generally, average molecular weight (M_w) for gelatins have been reported from 20 to 200 kDa, even though, M_w up to 10^6 order have also been reported[79]. Its abundance and excellent biodegradability and biocompatibility, have attracted great attention. An attractive property of gelatin is its ability to form thermo-reversible physical gels in a wide concentration range, which allows manufacturing of a wide variety of products with different specifications. Figure 3.2 illustrates a schematic of the physical gelation mechanism proposed for gelatin solutions through

coil to helix transitions induced by temperature changes[80]. Gelatin is one of the most studied, produced and commonly used gelling agents. It also has other functions such as coating agent[81, 82] and film-former[40, 83, 84]. Gelatin can be used in quick dissolve products[83, 85], implantable delivery system[86, 87], gelatin capsules[88, 89], microencapsulation of drugs[90, 91], food products[92, 93], and photographic emulsions[94].

Proteins, such as gelatin, are amphoteric "also called ampholyte" polyelectrolytes, which means they have a dual nature, they can be polycation or polyanion. Their net charge depend on pH. If you relate the common structure of a protein (i.e. sequence of amino acids) to the common structure of a single amino acid, it is easier to associate the net charge to the structure, as shown in Figure 3.3. Peptide bonds between amino acids are formed by condensation reaction, therefore, protein ionizable groups left are located in the R groups, N-terminal residue and C-terminal residue. The ionizable groups when protonated are –COOH and $-NH_3^+$. At low pH (e.g. pH=1), the ionizable groups are protonated, therefore, the positive net charge of the proteins. At high pH (e.g. pH=11), the ionizable groups are deprotonated, therefore, the negative charge of proteins. At a pH equal to the isoelectric point (pI), proteins have a net charge of 0 due to opposed charge balance of ionizable groups. Consequently, gelatin is a polycation at a pH < pI and a polyanion at a pH > pI. Reported isoelectric point range for gelatin type A and B are 7-9 and 4.7-5.4, respectively.

In this research work, gelatin is considered the primary structural agent (gelling agent) in the preparation of pharmaceutical gel formulations.

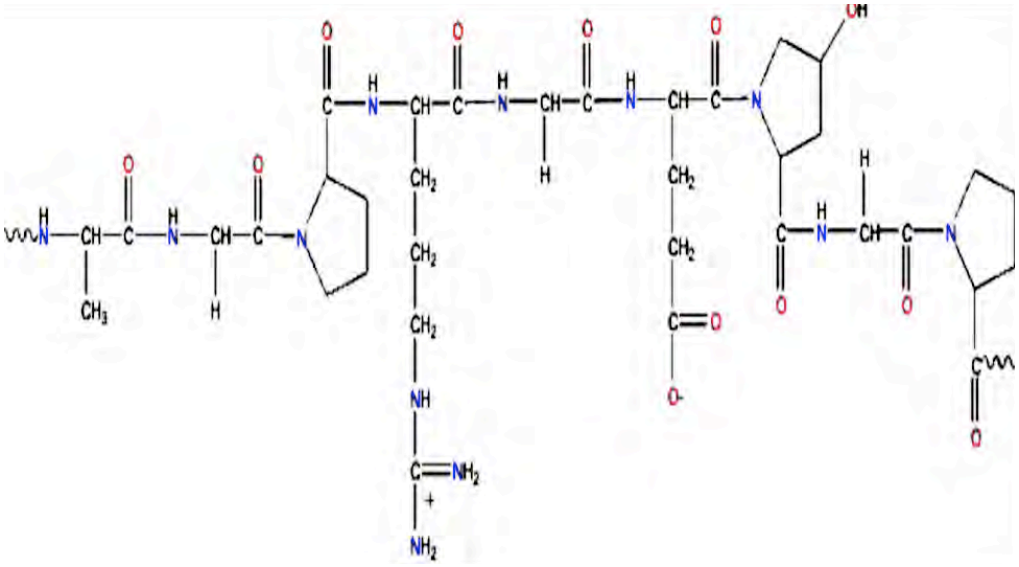


Figure 3.1 Chemical structure of a gelatin segment -Alanine-Glycine-Proline-Arginine-Glycine-Glutamate-4Hyp-Glycine-Proline-

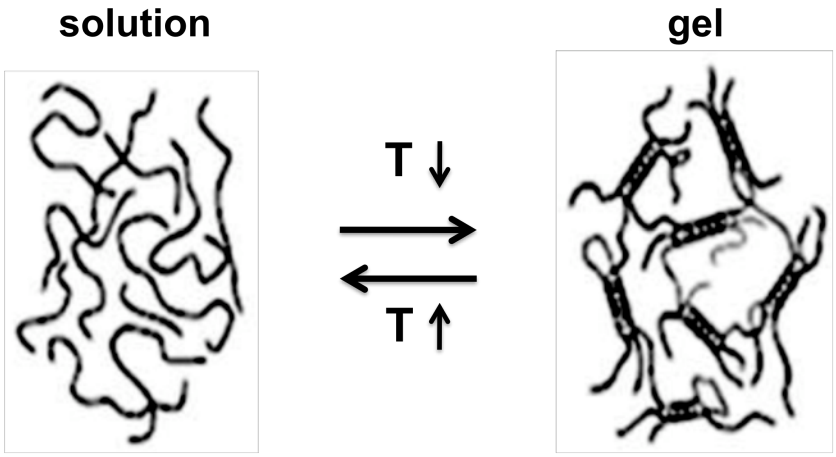


Figure 3.2 Schematic of gelatin proposed gelation mechanism
Reproduced from reference [80]

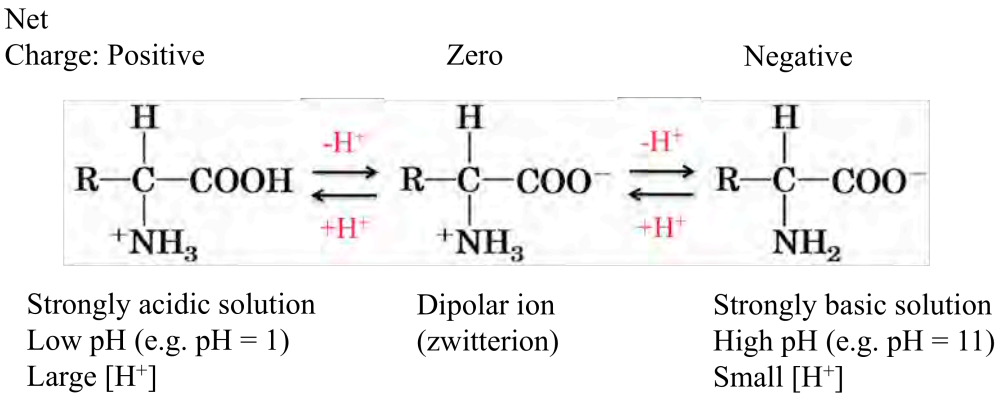


Figure 3.3 Transformation of an amino acid as a function of pH
Reproduced from references [95, 96]

3.1.2 Mannitol

Mannitol is a polyol, sugar alcohol, and sorbitol stereoisomer. Figure 3.4 illustrates the chemical structure of mannitol. It has many functions such as diluent, diluent for lyophilized preparations, sweetening agent, tablet and capsule diluent, and tonicity agent[5]. Mannitol can be used in pharmaceutical formulations, food products, direct compression tablet applications, and granulations[5]. In this work, mannitol is used as a structural agent.

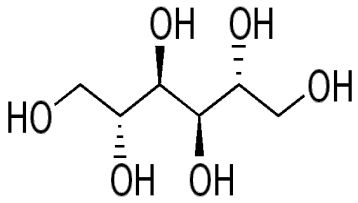


Figure 3.4 Chemical structure of the mannitol molecule

3.1.3 Xanthan gum

Heteropolysaccharides contain two or more different kinds of saccharide monomers[96]. Xanthan gum is a high molecular weight heteropolysaccharide. The average molecular weight has been reported in the order of 10^6 Da[97-99]. Xanthan gum is an anionic polysaccharide consisting of a cellulosic backbone of (1,4)-β-D-glucose residues, and a trisaccharide side chain consisting of β-D-mannose (1,4)- β-D-glucuronic acid-(1,2)- β-D-mannose attached at C-3 to alternate glucose residues of the main chain[100]. Figure 3.5 shows the molecular structure of xanthan gum[21]. It is called an anionic polysaccharide due to the negative charge from the carboxyl groups in its side chains. Viscosity of xanthan gum solutions is relatively insensitive to pH variations[19, 21]. In aqueous solution it does not form gels alone[15] but it does with adition of salts (Na^+, K^+, Ca^{+2}). The gelling mechanism of xanthan gum in the presence of cations[101] is schematically described in Figure 3.6. Figure 3.7 shows a comparison of the flow behavior of xanthan gum and other common hydrocolloids[21]. Xanthan gum has many functions such as viscosity-increasing, suspending, thickening, emulsifying and stabilizing agent[5]. It is used in pharmaceutical formulations[102, 103], food products[104-106], sustained-release matrix tablets[107, 108], and ophthalmic products[109, 110], amongst others. Additionally, the xanthan gum used for this research work has 16.15 wt% water content, as depicted in Figure 3.8. The thermal gravimetric analysis used to determine the water content of xanthan gum was performed from room temperature to 300 ^oC, a heating rate of 2.5 ^oC/min, and using nitrogen as the inert environment.

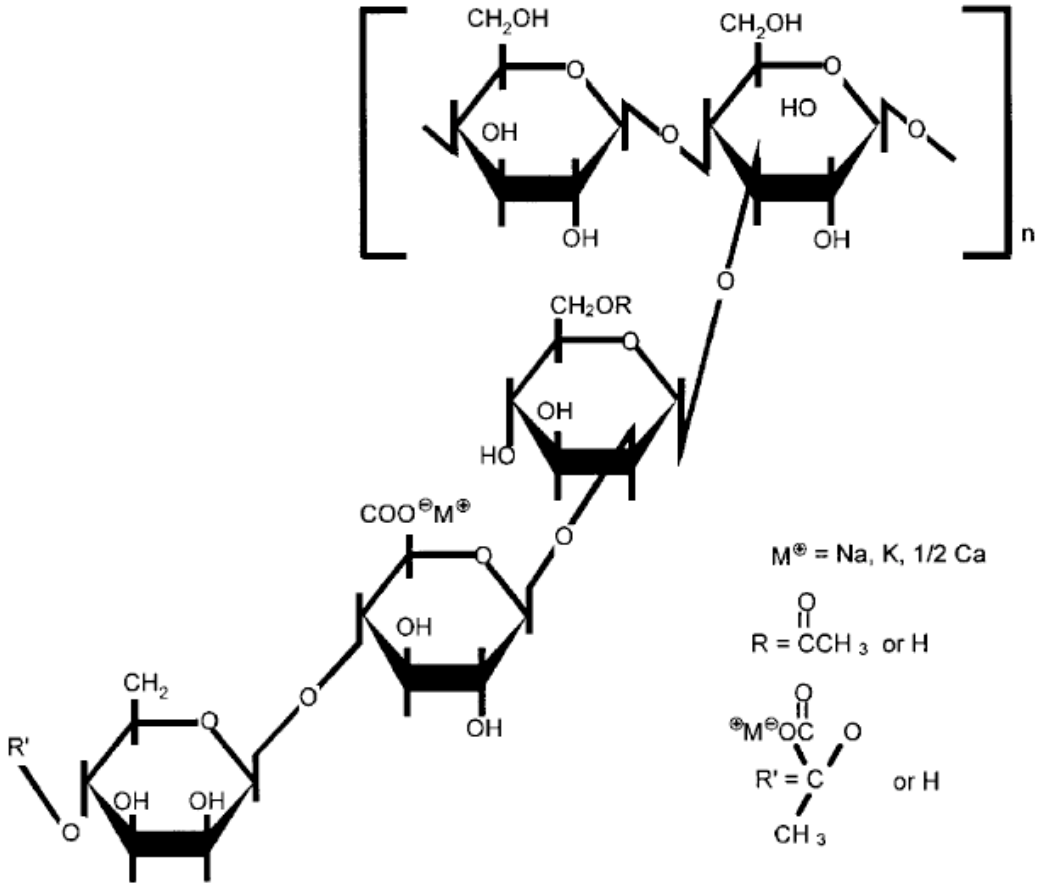


Figure 3.5 Chemical structure of the xanthan gum molecule
Reproduced from reference [21]

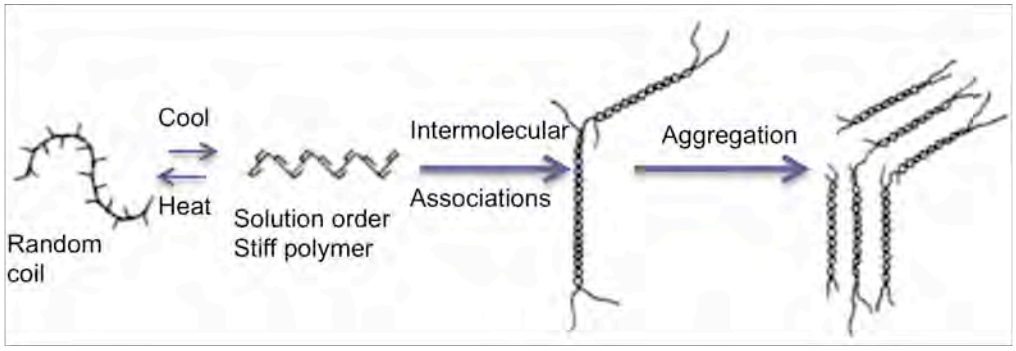


Figure 3.6 Schematic diagram of the proposed gelling mechanism of xanthan gum
Reproduced from reference [101]

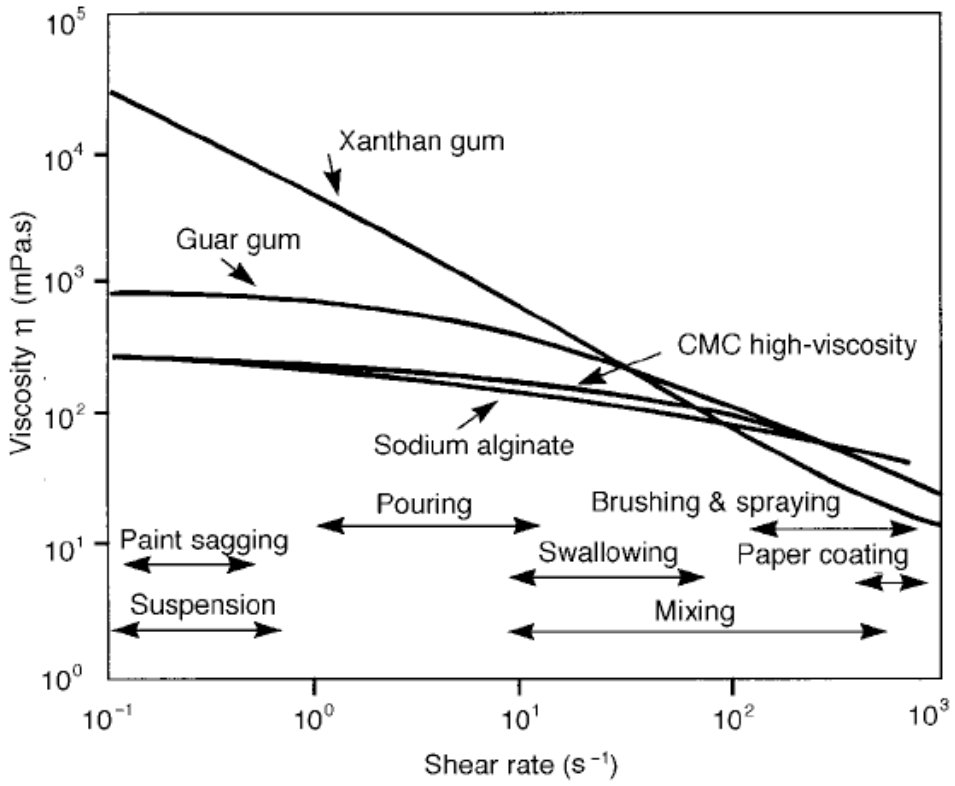


Figure 3.7 Xanthan gum (0.5%) flow behavior compared to other common biopolymer solutions Reproduced from [21]

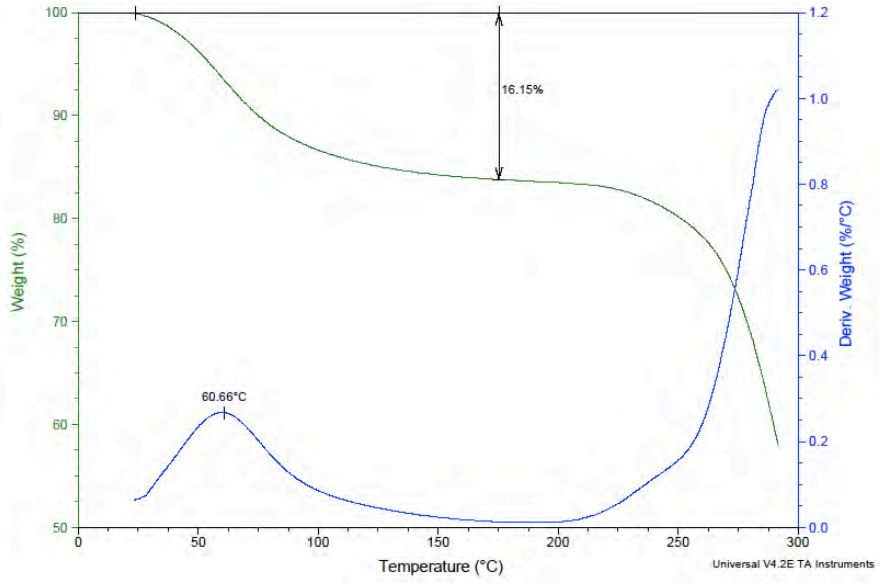


Figure 3.8 Thermal gravimetric analysis of xanthan gum from room temperature to 300 °C at a heating rate of 2.5 °C/min and using nitrogen as the inert environment

3.1.4 Carbopol 974P

Carbopol 974P is a high molecular weight homopolymer of acrylic acid crosslinked with allyl ethers of pentaerythritol[111]. Figure 3.9 illustrates the chemical structure of carbopol 974P. It has many functions such as suspending agent, tablet binder, emulsifying agent, and viscosity-increasing agent. Carbopol 974P is used in pharmaceutical formulations (e.g. gels, creams, ophthalmic, suspensions, tablets, wet granulations) and cosmetics[5]. It is very important to mention that Carbopol 974P normally requires a neutralizing agent or it could have electrostatic interactions with other excipients[111].

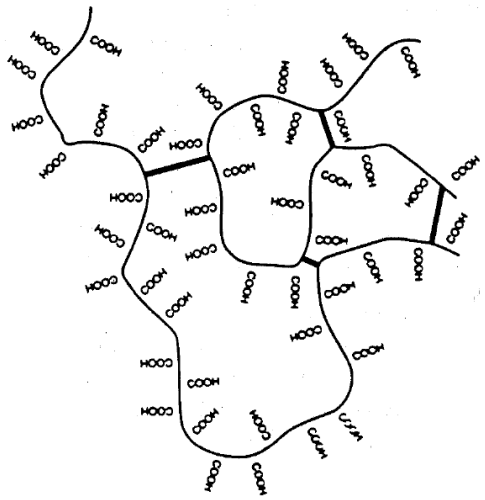


Figure 3.9 Chemical structure of the crosslinked acrylic polymer molecule
Reproduced from reference [111]

3.1.5 Amberlite IRP - 64

Also known as polacrilin resin, it is obtained by a copolymerization of methacrylic acid with divinylbenzene[5]. It is an insoluble, weakly acidic, and hydrogen form cation exchange resin[112]. Moreover, it is used as a cationic drug carrier, taste-masking agent, stabilizing agent,

and for sustained or controlled release[112]. Figure 3.10 illustrates the chemical structure of Amberlite IRP-64.

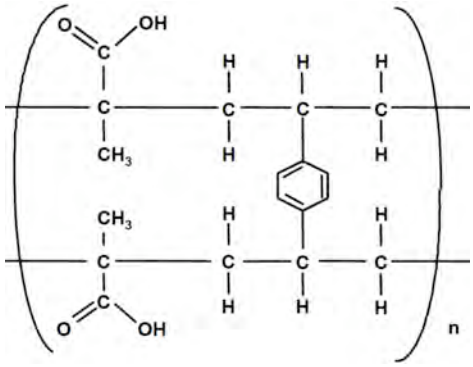


Figure 3.10 Amberlite resin molecular structure
Reproduced from reference [113]

3.1.6 Aspartame

Aspartame is an artificial amino acid and non-saccharide sweetener. It is used in drinks and food products. It is used as a sugar substitute for people with diabetes. Aspartame molecule is illustrated in Figure 3.11b.

3.1.7 Peppermint oil

Peppermint oil is extracted from a plant called peppermint. It has a high menthol concentration and is used for flavoring edible products. Peppermint oil molecule is illustrated in Figure 3.11a.

3.1.8 Glycine

Glycine is the simplest amino acid. It forms approximately a third part of the composition of gelatin[19]. Some industrial uses are sweetener, dietary supplement, and buffering agent[5]. Glycine molecule is illustrated in Figure 3.11d.

3.1.9 Red ferric oxide

Red ferric oxide is a pigment approved by the Food and Drug Administration (FDA).

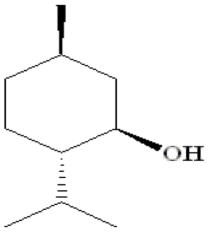
3.1.10 Simethicone

Simethicone is an antifoaming, tablet diluent, and water-repelling agent[5]. It is mostly used in antacid products. Simethicone molecule is illustrated in Figure 3.11c.

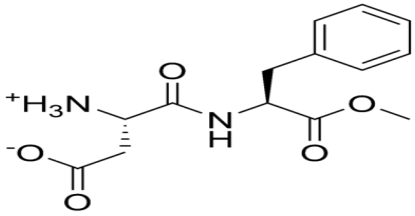
3.1.11 Sodium hydroxide

Sodium hydroxide (NaOH) at room temperature is a white, crystalline, odorless, and hygroscopic solid. A strong base used to control product pH.

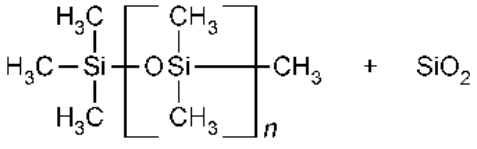
a) Peppermint oil



b) Aspartame



c) Simethicone



d) Glycine

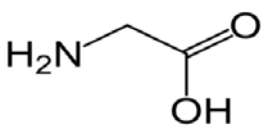


Figure 3.11 Molecular structure of excipients

3.2 Experimental Methods

3.2.1 Design of Experiments (DoE)

One of the main objectives herein was to determine which excipients are the most influential on the final viscosity of the case-study placebo formulation. In order to do this, a design of experiments was developed and analyzed with statistical software.

Two types of designs that study the effects of factors are discussed below. These are typically used to perform screening experiments, product design, process design, and process optimization. A DoE for screening experiments was used. The first design considered was the Full Factorial Design that studies the effect of two or more factors, in this case, excipients. It investigates all possible combinations of factor levels (i.e. low and high). The factor effect is the response change produced by a change of the factor level. Figure 3.12a is an example of a full factorial design for factors A and B. The vertices of the square represent the possible combinations to be studied. Each axis shows the low and high levels for each factor. The plus sign is the high level and the minus sign is the low level. For this example design, four samples have to be prepared and the response variable (e.g. viscosity) measured. The response variable values are analyzed in Minitab® statistical software to determine the effects of each factor. For eleven excipients, the possible combinations that should be studied are 2,048. The quantity of excipients makes the use of this design inappropriate.

Thus, a Fractional Factorial Design (FFD), which studies a fraction of the full factorial design, was considered. Figure 3.12b is an example of a fractional factorial design for factors A, B, and C. The dotted vertices represent the combinations required by the FFD to study the effect of independent parameters, instead of 2^n needed by full factorial design. Thus, in this case, a fractional factorial design of resolution III for eleven factors (1/128 fraction of a full factorial

design) was chosen. The resolution of this design is a good start to identify which factors are significant on the chosen response variable (i.e. final viscosity). This design is appropriate for eleven excipients because it is not time (each set of experiments takes approximately 4 hours plus sample preparation) and material consuming. Thus, in the case of complex fluid formulations, a fractional factorial design is better suited to analyze the sensitivity of components on physical properties. Table 3.1 summarizes the concentrations used for these purposes. Additional details are presented in Chapter 5.

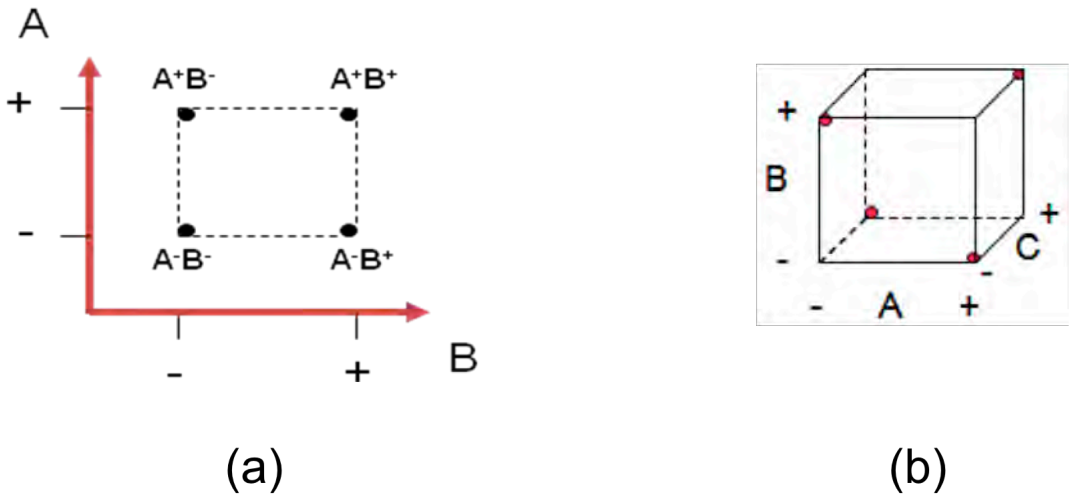


Figure 3.12 Full factorial design for factors A and B (a) and Fractional Factorial Design for factors A, B, and C (b)

Table 3.1 DoE combinations on a 100g basis*

Formulation #	red ferric oxide (10^{-3})	simethicone (10^{-3})	xatham gum (10^{-3})	carbopol 974P (10^{-2})	peppermint (10^{-2})	aspartame (10^{-1})	glycine	amberlite IRP-64	gelatin	mannitol	NaOH (10^{-2})
1	0.625	2.0	2.5	1.5	3.0	0.5	0.6	0.6	1.95	2.4	3
2	1.88	2.0	2.5	1.5	9.0	0.5	1.8	3.6	0.65	0.8	0.78
3	0.625	6.0	2.5	1.5	9.0	2.25	0.6	3.6	0.65	0.8	3
4	1.88	6.0	2.5	1.5	3.0	2.25	1.8	0.6	1.95	2.4	0.78
5	0.625	2.0	7.5	1.5	9.0	2.25	1.8	0.6	0.65	2.4	0.78
6	1.88	2.0	7.5	1.5	3.0	2.25	0.6	3.6	1.95	0.8	3
7	0.625	6.0	7.5	1.5	3.0	0.5	1.8	3.6	1.95	0.8	0.78
8	1.88	6.0	7.5	1.5	9.0	0.5	0.6	0.6	0.65	2.4	3
9	0.625	2.0	2.5	4.5	3.0	2.25	1.8	3.6	0.65	2.4	3
10	1.88	2.0	2.5	4.5	9.0	2.25	0.6	0.6	1.95	0.8	0.78
11	0.625	6.0	2.5	4.5	9.0	0.5	1.8	0.6	1.95	0.8	3
12	1.88	6.0	2.5	4.5	3.0	0.5	0.6	3.6	0.65	2.4	0.78
13	0.625	2.0	7.5	4.5	9.0	0.5	0.6	3.6	1.95	0.8	0.78
14	1.88	2.0	7.5	4.5	3.0	0.5	1.8	0.6	0.65	0.8	3
15	0.625	6.0	7.5	4.5	3.0	2.25	0.6	0.6	0.65	2.4	0.78
16	1.88	6.0	7.5	4.5	9.0	2.25	1.8	3.6	1.95	2.4	3
17	1.253	4.0	5.0	3.0	6.0	1.375	1.2	2.1	1.3	1.6	1.89
18	1.253	4.0	5.0	3.0	6.0	1.375	1.2	2.1	1.3	1.6	1.89
19	1.253	4.0	5.0	3.0	6.0	1.375	1.2	2.1	1.3	1.6	1.89

*The sum of the masses on the table plus the mass of water adds up to 100 g.

3.2.2 Placebo formulation preparation and processing

Excipients were weighed on a hundred grams basis, mixed with distilled water and dissolved on a hot plate at 50°C with the aid of mechanical stirring for 40 minutes. Then, 11mL were transferred to the rheometer at 50°C and the rest of the formulation was left stirring without heating. The pH, conductivity, and density of the formulation were measured. A constant stress temperature ramp (CSTR) test was performed from 50 to 7 °C at a cooling rate of 1 °C/min and a stress of 1 Pa. When CSTR test ended, the geometry was cleaned and 11mL were transferred to the rheometer at 22°C. A small-amplitude oscillatory shear (SAOS) test was performed for an hour at a frequency of 1 Hz and a strain of 0.01. When the SAOS test ended, a steady state viscosity test was performed. This procedure was repeated for all DoE combinations.

A schematic of the procedure described above and the parameters used for each rheological test (Section 3.2.3.4) is presented in Figure 3.13. This procedure was developed as a typical procedure used to process a gel formulation. The results offered an insight of how the real formulation behaves under the chosen parameters. Variations on formulation concentrations were also studied. Also, part of this experimental method is used to study and determine the interactions between the most influential components determined by the FFD.

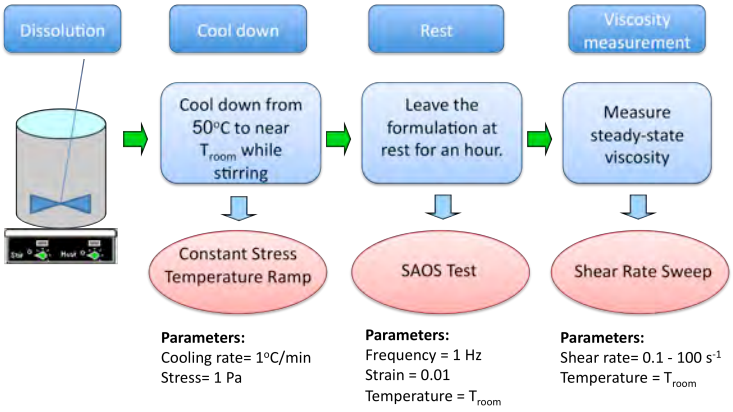


Figure 3.13 Laboratory method for placebo formulation preparation and processing (tests and parameters)

3.2.3 Characterization techniques

Gelatin is the main structural component of the pharmaceutical formulation studied in this research project. This excipient was analyzed by the following techniques to determine any biovariability in its properties, if any, which could affect the pharmaceutical product specifications.

3.2.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR was used to do a structural characterization of the variability of gelatin lot samples. It is a form of absorption spectroscopy. The equipment determines characteristic functional groups of a chemical structure and in that manner identifies materials. The spectra collected were used to corroborate the presence of characteristic bands of gelatin functional groups. Table 3.2 summarizes the characteristic absorption bands for gelatin and the bonds to which they are attributed[40]. This technique is very fast and did not required sample preparation as solid gelatin was analyzed. The measurements were made with a Varian 800 Scimitar Series FT-IR at room temperature.

Table 3.2 Gelatin characteristic absorption bands on FT-IR spectra

Absorption bands (cm^{-1})	**Attributed to**
3,270	O-H and N-H stretching vibration
1,630	Amide I (C=O and C-N stretching vibration)
1,537	Amide II (N-H bending vibration)
1,236	Amide III (C-N stretching vibration)

3.2.3.2 Gel Permeation Chromatography

Gel pemeation chromatography (GPC) was used to determine the molecular weight distribution (MWD) of gelatin lot samples and to verify if there is a significant difference between them. MWD of gelatin is an important and relevant characteristic to evaluate its gelling power[19].

For GPC experiments, gelatin can be dissolved in a buffer[19] or salt[114] solution. Gelatin at a concentration of 10 g/L in a 0.1M NaCl solution was prepared for these experiments. The measurements were made at 30^{o}C with a Waters GPC System equipped with a Varian Column PLaquagel-OH Mixed 8μm (300*7.5mm) and a Brookhaven Instruments molecular weight analyzer (BI-MwA). The BI-MwA detector was calibrated using a dextran standard. M_w, M_n, and M_z were analyzed to identify scaling with the final product steady state viscosity.

3.2.3.3 Thermal Gravimetric Analysis

Thermal gravimetric analysis (TGA) was used to determine the moisture (i.e. water content) of solid gelatin samples. The equipment monitors the change in weight of a sample in a controlled (e.g. air, nitrogen) environment as the temperature increases[115]. This technique did not require sample preparation. Therefore, samples were analyzed as received. The measurements were made with a TA Instruments TGA 2950 Thermogravimetric Analyzer (using platinum pans). Nitrogen was used for the controlled environment. Experiments were performed from 20 to 250^{o}C.

3.2.3.4 Rheology

Constant stress temperature ramp (CSTR), small-amplitude oscillatory shear (SAOS) and steady-state viscosity tests were used to determine rheological behavior of samples. CSTR and

SAOS tests are explained in Section 2.6.1. The parameters used for these tests are detailed in Figure 3.13. Delay time, integration time, and integration periods were chosen such that the sample reaches steady state.

In addition, steady-state viscosity measurements were made for gelatin drum cut samples to determine rheological bio-variability, if any. The samples were subjected to an increasing shear rate while monitoring its steady state viscosity. Aqueous gelatin 2 wt% solutions were prepared for bio-variability experiments. The viscosity measurements were made at 22^{o}C. A CSTR test followed by a SAOS test were performed prior to the steady-state viscosity measurements. The shear rate dependence was determined from 0.01 to 100 s^{-1}.

Rheological tests were performed in a Rheologica StressTech HR stress-control rheometer and CCE temperature controller. Figure 3.14 illustrates the equipment setup while Figure 3.15 illustrates a schematic of the double gap (DG) Couette fixture (volume = 11mL) used to perform the rheological tests. This geometry was used because it is adequate for low to moderate viscous fluids such as the gelatin solutions before the sol-gel transition.

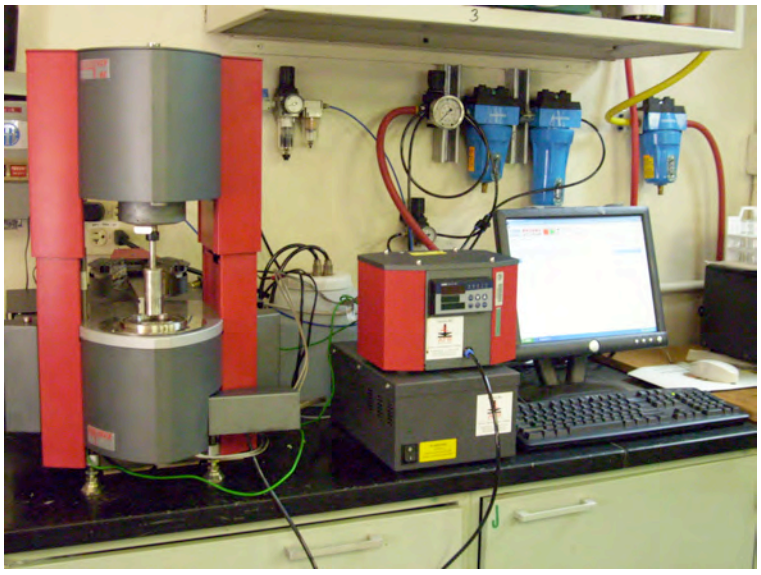


Figure 3.14 Rheometer equipment setup

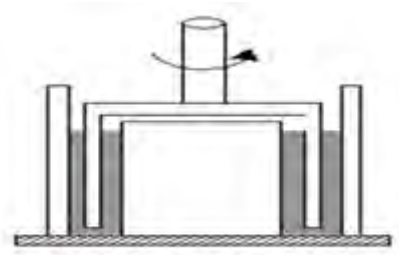


Figure 3.15 Diagram of double gap (DG) couette fixture or concentric cylinders

3.2.3.5 Zeta potential

Zeta potential measurements were used as a complementay technique to analyze molecular interactions between gelatin and xanthan molecules in aqueous solutions. Zeta potential values above zero indicate that the net charge of the biopolymer solution is positive, whereas below zero indicate a negative net charge. Moreover, a zeta potential value close to zero indicates that the solution is neutral. Gelatin-xanthan mixtures were prepared using deionized water without addition of salts. The measurements were made with a Brookhaven Instruments 90 Plus Particle Size Analyzer at 40 ^{o}C.

3.2.3.6 Other solution properties

pH, conductivity (Ω) and density (ρ) were measured for most of the analyzed samples. pH and conductivity measurements were made with an Accumet XL50 dual channel pH/ion/conductivity meter. Density measurements were made with Anton Paar DMA 4100 M. Refer to tables A.2 to A.6 in the Appendix for formulation properties and gelatin-xanthan mixtures properties.

4 Bio-variability of Gelatin

Gelatin drum cut (GDC) samples used for pharmaceutical product manufacturing at the local pharmaceutical were provided for variability verification of its properties. T_{gel}, steady-state viscosity, water content, molecular weight distribution (MWD), functional groups identification, and isoelectric point were evaluated. It is important to determine if variations exist on the properties of the primary structural agent (i.e. gelatin) that could lead to process output changes.

4.1 Molecular Weight Distribution

The molecular weight distribution (MWD) of GDC samples (10 g/L) was determined by GPC. Figure 4.1 illustrates the MWDs results for GDC samples. GDC base was the gelatin drum cut used to prepare placebo formulations and gelatin-xanthan systems. The determined average molecular weight (M_w) range of the GDC samples goes approximately from 20 to 30 kDa. The polydispersity index range goes from 1.170 to 1.715. Gelatin polydispersity index is generally 2 [19]. Table 4.1 presents a summary of the moment distributions. The M_w is related to the resultant viscosity while the M_z is related to the gelation of the sol-gel fluid[19]. The MWDs show some variations that were further analyzed in this chapter with complementary techniques.

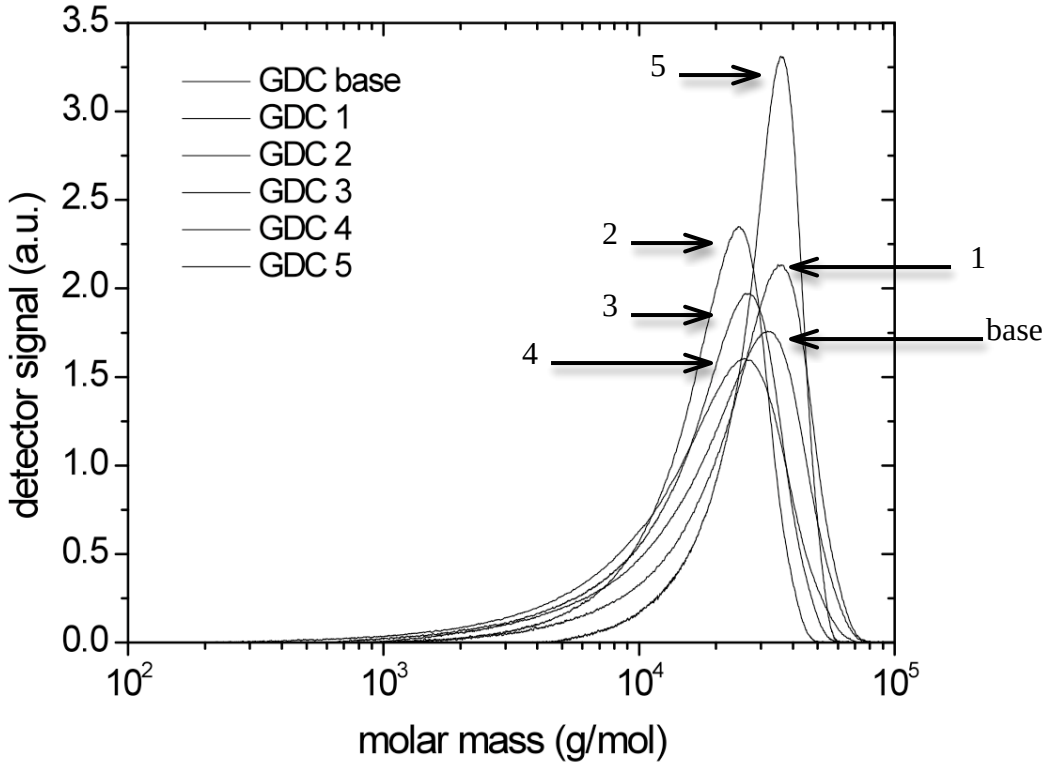


Figure 4.1 Gelatin drum cut samples molecular weight distributions

Table 4.1 Gelatin molecular weight moments and polydispersity

Lightscattering Calibration	**M_w**	**D**	**M_n**	**M_z**
Gelatin base	25,228	1.715	14,710	32,525
Gelatin #1	29,133	1.507	19,323	35,435
Gelatin #2	20,355	1.354	15,030	23,950
Gelatin #3	21,191	1.687	12,559	26,544
Gelatin #4	24,702	1.168	21,141	26,937
Gelatin #5	30,945	1.17	26,432	34,166

4.2 Water Content

Weight loss profiles as a function of temperature were determined by thermal gravimetric analysis (TGA). The TGA shows that GDC samples have small variations in moisture, as depicted in Figure 4.2. Drum cut #0 in Figure 4.2 represents the GDC base. The average water content of the samples was 12.88 wt%. The standard deviation of the water content was 0.70. The water content is near the reported in the literature that is 8 to 12 wt%

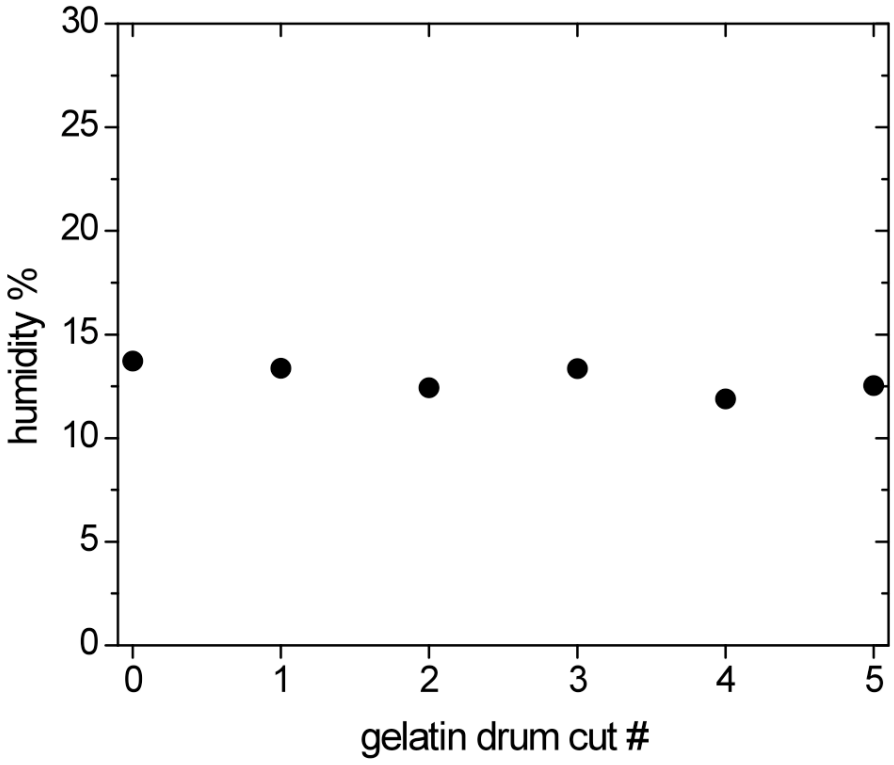


Figure 4.2 Gelatin drum cut samples moisture analysis

4.3 Structural Characterization

Figure 4.3 illustrates the spectra obtained from the FT-IR data of GDC samples. Gelatin characteristic bands on Table 3.2 were identified in Figure 4.3. The analysis confirms the presence of the characteristic bands of gelatin on the GDC samples.

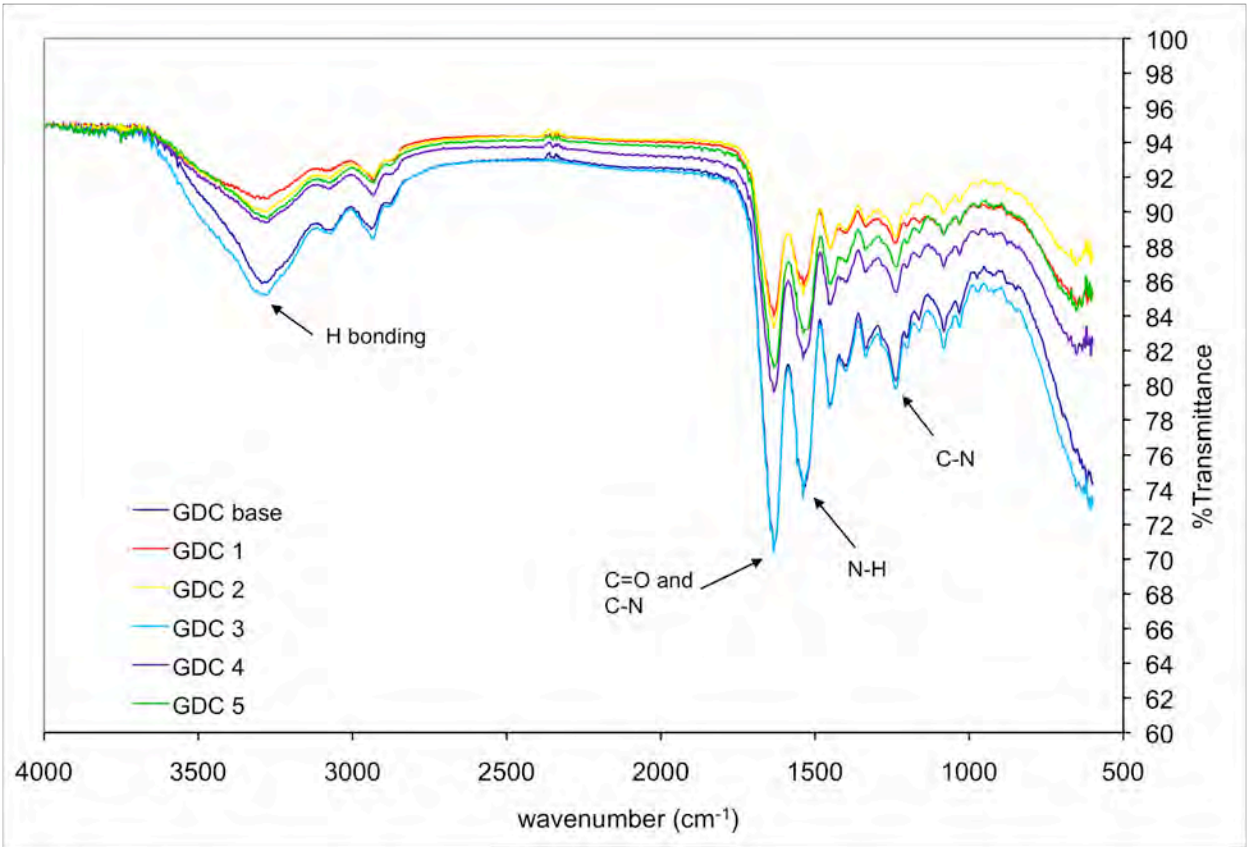


Figure 4.3 FT-IR spectrum for solid gelatin drum cut samples

4.4 Gelation temperature of GDC samples

Sol-gel transitions for GDC were nearly identical, as depicted in Figure 4.4. The gelation temperature is approximately 11 ^{o}C. The gelation process was not affected by variations on M_z.

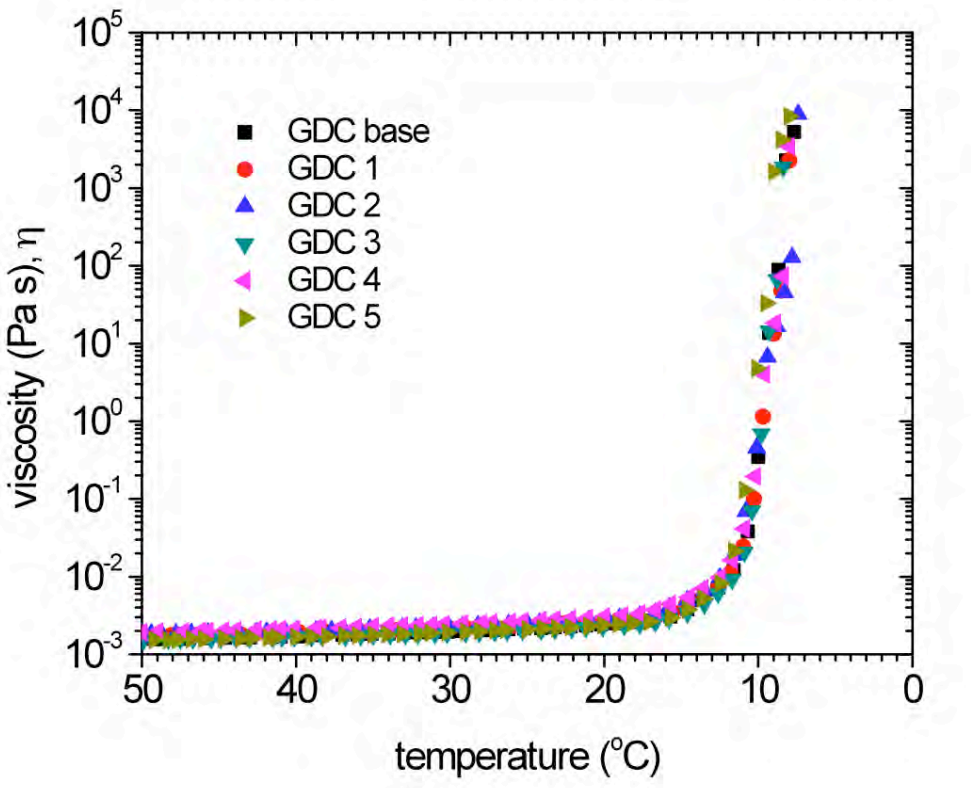


Figure 4.4 Constant stress temperature ramps of gelatin (1.3 wt%) drum cut samples at a cooling rate of 1^{o}C/min and a stress of 1 Pa

4.5 Steady-state viscosity of GDC samples

The steady-state viscosity was determined for each GDC sample. Steady-state viscosity was measured as a function of shear rate (0.1-100 s^{-1}) for a 2 wt% gelatin. As depicted in Figure 4.5, it appears that M_w variability is not causing significant variability on steady-state viscosity behavior. To verify this inference, Figure 4.6 was prepared by combining the steady-state

viscosity data and the M_w of GDC samples illustrated on Table 4.1. For flexible polymers, it is reported in the literature that the viscosity is directly proportional to the molecular weight below the entanglement molecular weight and with a 3.4 power scaling[7]. Figure 4.6 demonstrates that there was no directly proportional correlation between the M_w and the steady-state viscosity within the studied shear rate range. Instead, there was no correlation as evidenced by the extremely low correlation factors, i.e. R^2. This can be attributed to the relatively small variations of the molecular weight of the samples. Similar behavior was observed when the M_n and M_z were analyzed.

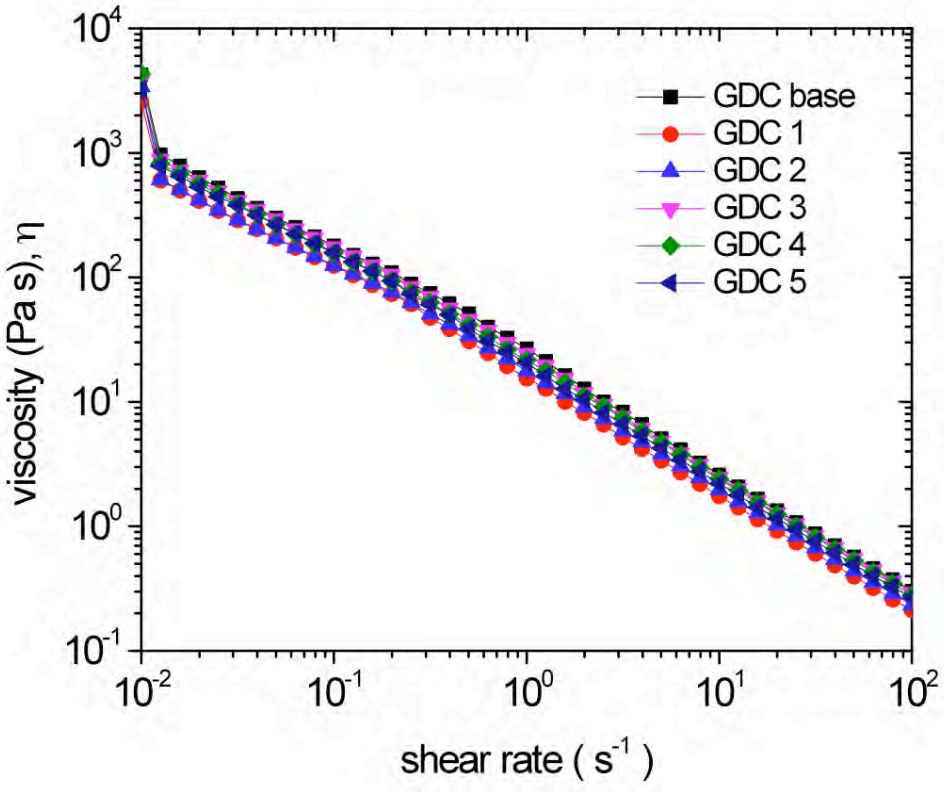


Figure 4.5 Steady-state viscosity of gelatin (2 wt%) drum cut samples

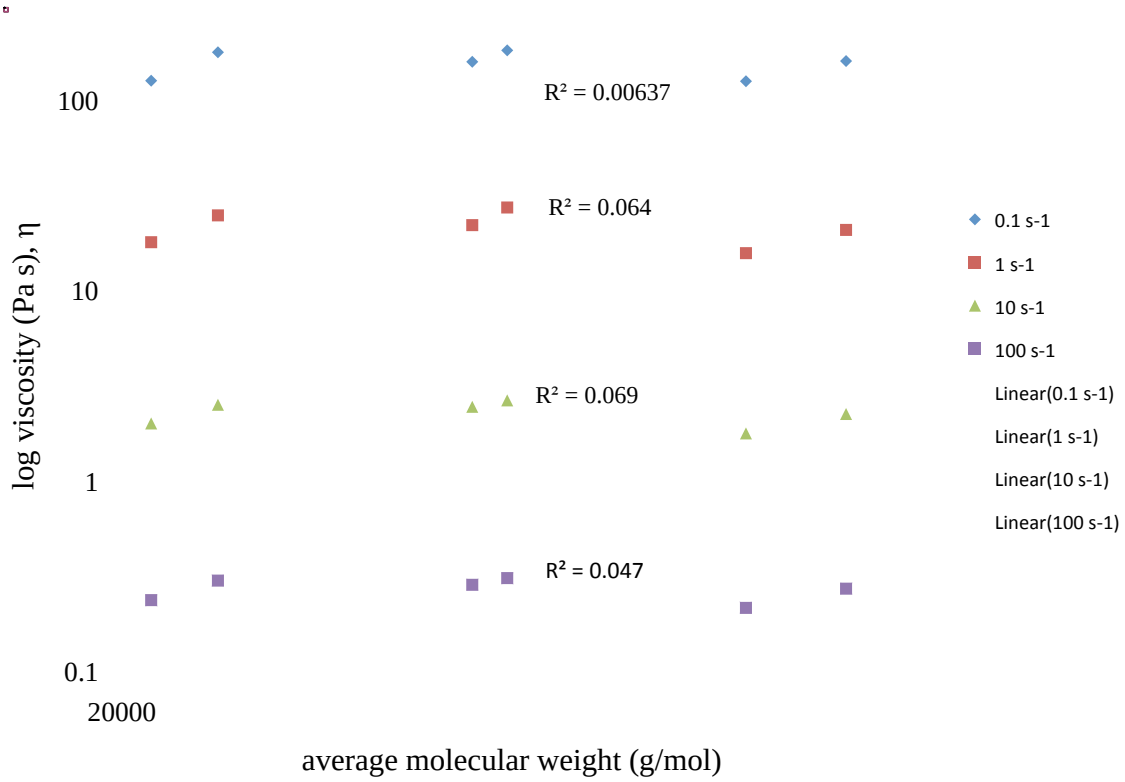


Figure 4.6 Weight average molecular weight effect on steady-state viscosity of gelatin solutions

4.6 Conclusions

Six gelatin drum cuts were analyzed and the results revealed mild variations in the molecular weight distribution. As determined by CSTR test, the gelation transitions of gelatin solutions were not affected by variations of the molecular weight moments. According to the low correlation factors between the M_w and the steady-state viscosity, the M_w variations did not have a significant effect on the steady-state viscosity results. Even though variations on the molecular weight distributions exist, the rheological properties of gelatin solutions were barely affected by these variations. Additionally, water content variations were small and agreed with the reported in the literature. In conclusion, variations on gelatin physical properties did not were responsible for variations of the formulation viscosity specifications.

5 Formulation Rheology

The effect of factors (i.e. excipients) composition and processing conditions (temperature, cooling rate, shear rate) on rheology should be known to successfully design, manufacture, and optimize the drug product. Details on gelation temperature, gel network development, steady-state viscosity, and design of experiment (DoE) analysis are described. The goal is to study the rheological properties of the formulation in order to understand the process and to have control over the resulting final viscosity.

5.1 Gelation temperature

A constant stress temperature ramp (CSTR) test was performed in the rheometer after dissolving the excipients in distilled water. The samples were cooled from 50^{o}C to approximately 10^{o}C while applying a 1 Pa stress (stirring) as shown in Figure 5.1. A small number of the formulations reached gel state at these conditions. Not reaching the gel state at the conditions of this step does not mean that something is wrong; this is just the first processing step. Gelation temperature of formulation #1 and #4 are 16^{o}C and 15.5^{o}C, respectively. Gelation transition of formulation #11 is not clear because the gelation point is at a lower temperature. For other formulations, a sol-gel transition did not occurred. Results are summarized in Figure 5.2.

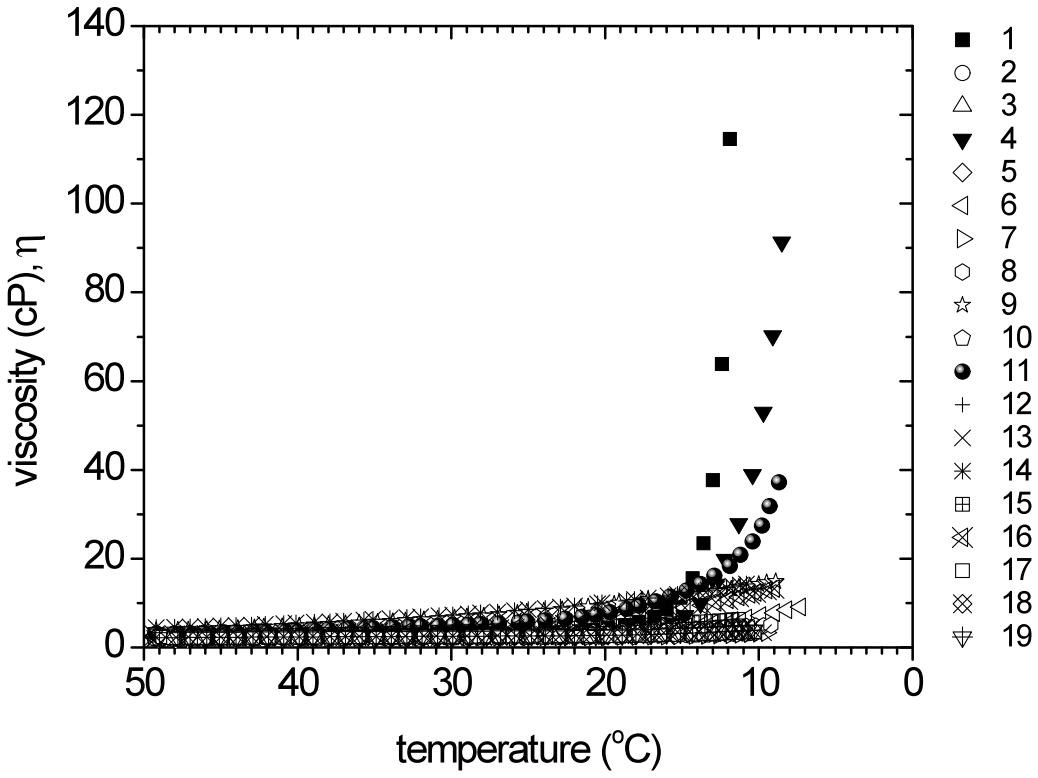


Figure 5.1 Placebo formulations constant stress temperature ramps from 50 to 10°C at a stress of 1Pa and a cooling rate of 1°C/min

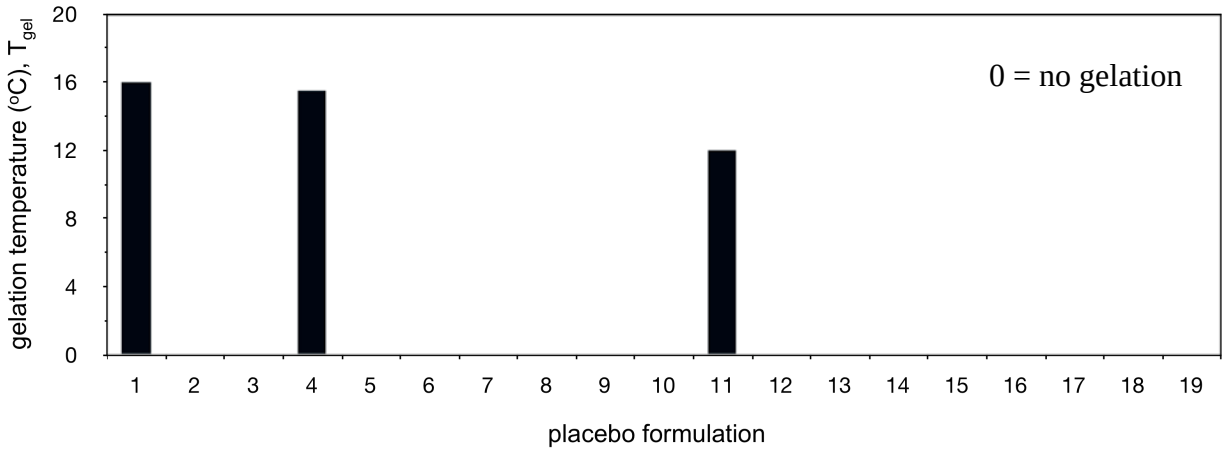


Figure 5.2 Gelation temperature of placebo formulations

5.2 Gel network study

After CSTR test, the geometry was cleaned and new sample was loaded to the rheometer. Then, a SAOS test was performed at 22°C. A small deformation at a small frequency was applied to the sample in order to determine G', G", phase angle, and complex viscosity without interfering the gel network development. The increase in complex viscosity of placebo formulations in Figure 5.3 was interpreted as physical gel network development. For example, formulations #7 and #13 had a fast gel network development. Another example, formulations #1 and #4 had a slow gel network development. It was observed that formulations with slower gel network development, reached the higher final viscosities, which is a result of a more organized structure. Also, there was no observation of gel network development for some formulations. Complex viscosity values that did not start at a low viscosity was because all formulations were equilibrated in the rheometer 5 minutes before beginning the test and those formulations had a fast gel network development.

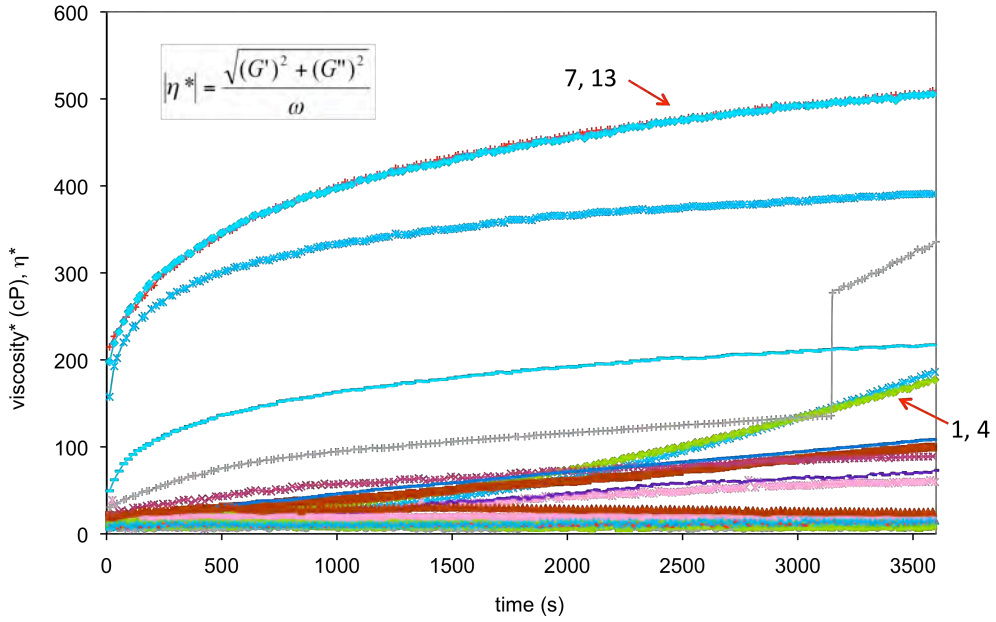


Figure 5.3 Complex viscosity of placebo formulation versus time measured at a strain of 1%, frequency of 1 Hz, and constant temperature of 22 °C

Using the T_{gel} definition used by Ding and co-workers[59], the tan δ (G"/G') value indicates if the sample is behaving as liquid-like (tan δ>>1) or solid-like (tan δ<<1). Figure 5.4 shows the loss tangent values for placebo formulations that reached the gel state at these under these conditions. At the end of the SAOS test, the elastic modulus (G') is larger than the viscous modulus (G"). Therefore, formulations 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 13, and 16 passed through a sol-gel transition.

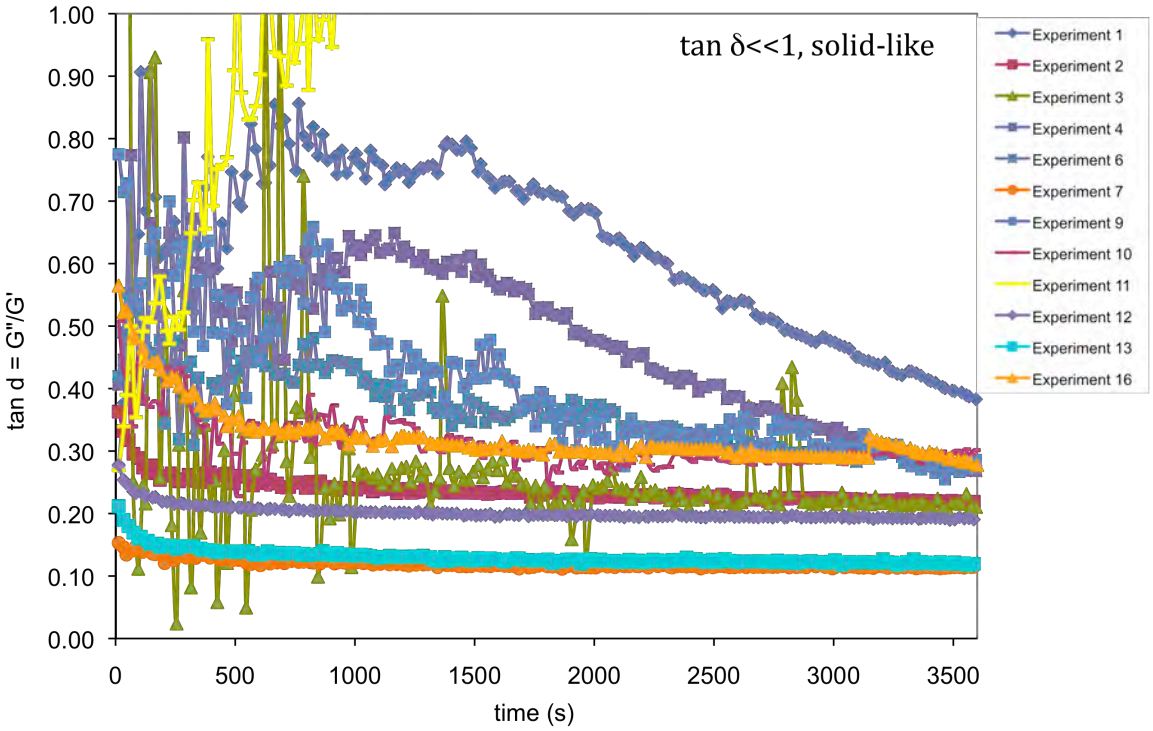


Figure 5.4 Loss tangent results for selected placebo formulations at 22°C

5.3 Steady-state viscosity of gel formulations

Steady-state viscosity as a function of shear rate was measured for all formulations after SAOS test. The steady-state viscosities at three shear rates (1, 10, and 100 s^{-1}) are illustrated for each formulation in Figure 5.5. A dotted line marks the minimum acceptance criteria for viscosity (100 cP) required by the pharmaceutical formulation. Interestingly, the formulations that meet the acceptance criteria were the formulations that reached the gel state in the CSTR (Section 5.1) and SAOS (Section 5.2) tests. Therefore, the acceptance criterion of the formulation was to reach the gel state at the end of processing steps. These results were used in the next section to determine which excipients were the most influential on the final viscosity.

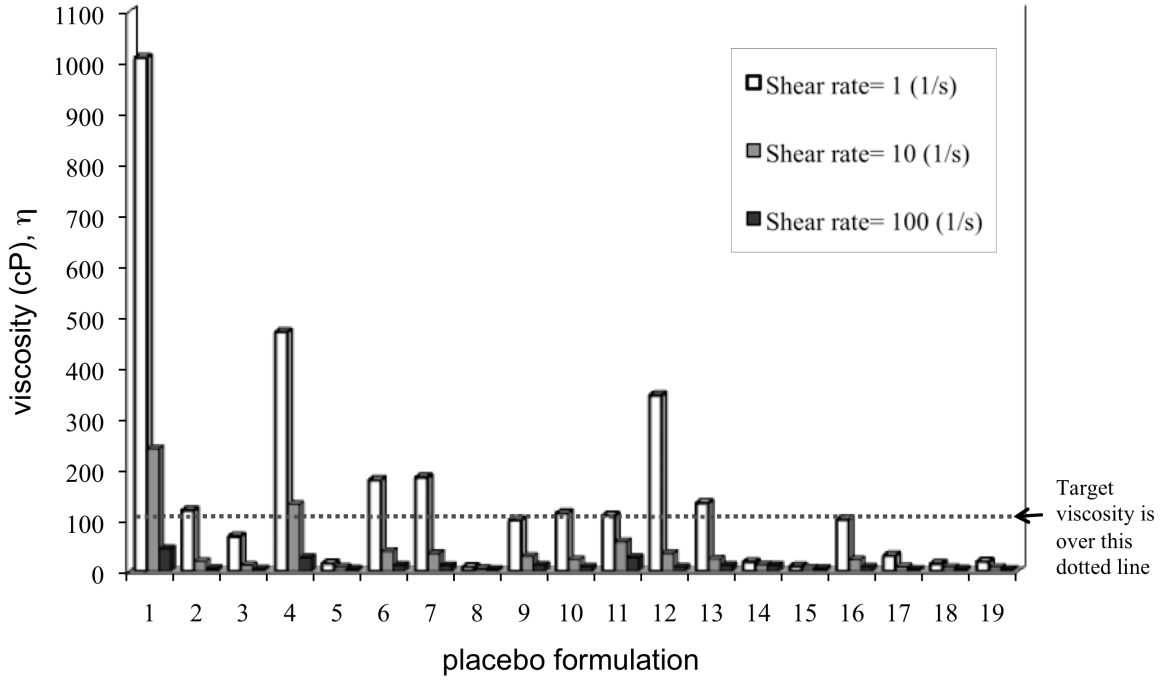
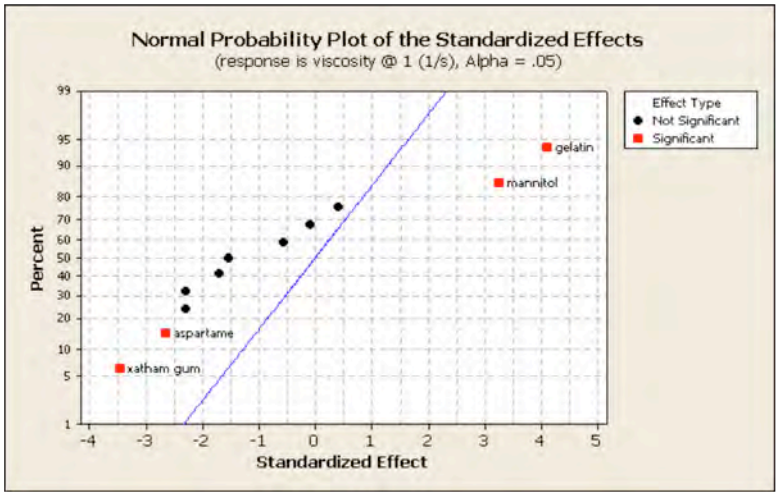


Figure 5.5 Finalized product viscosity of formulations at a shear rate of 1, 10, and 100 s^{-1}

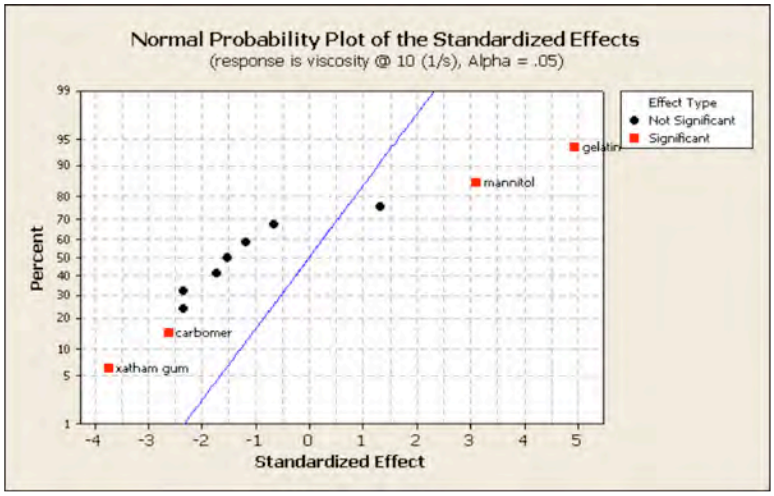
5.4 DoE analysis

Figure 5.6 illustrates the influential factors on the placebo formulations final viscosity determined with normal probability plots of the standardized effects at shear rates of 1, 10, and 100 s^{-1}. These results are summarized in Table 5.1. "Although many ingredients can alter the structure of the gels, it is generally agreed that proteins and polysaccharides are the most important constructional material"[42]. Gelatin and xanthan showed the more pronounced effect on the final viscosity of the placebo formulation. As described earlier, gelatin behaves as a polycation at the pH used and it is a structural agent that forms gels, therefore, the positive effect on viscosity. Xanthan is a polyanion that can have electrostatic interactions with gelatin and other positively charged molecules. If xanthan forms electrostatic complexes with gelatin, it reduces the gelatin available to form gel, thus, the negative effect on the final viscosity. Other effects such as aiding the gelatin gel formation by providing more hydrogen bonds (e.g. mannitol) or affect the functionality of gelatin by having electrostatic interactions (e.g. carbopol) are observed in a lower extent and their not consistent at all shear rates. Therefore, further experiments are focused on gelatin and xanthan effects. The resulting effects are valid for the system studied and excipients concentrations used.

a)



b)



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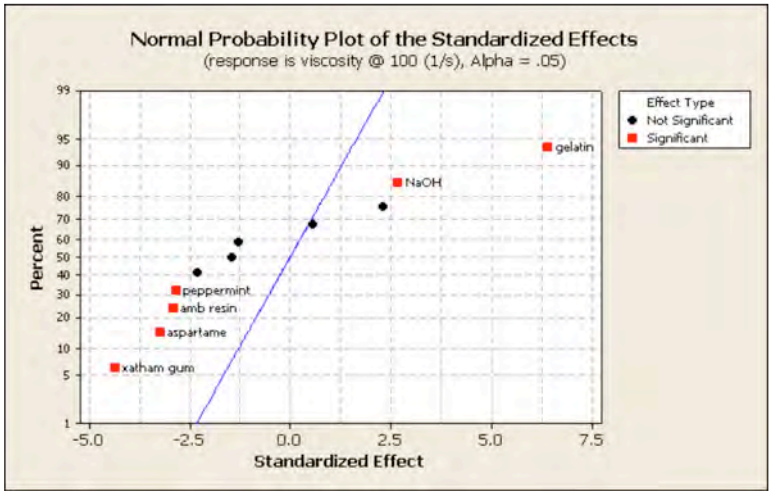

Figure 5.6 Placebo formulations normal probability plots of 1 (a), 10 (b), and 100 (c) s^{-1} shear rates

Table 5.1 Influential components on the final viscosity (η) of placebo formulation

shear rate = 1 s^{-1}	Positive: gelatin, mannitol
	Negative: xanthan gum, aspartame
shear rate = 10 s^{-1}	Positive: gelatin, mannitol
	Negative: xanthan gum, carbomer
shear rate = 100 s^{-1}	Positive: gelatin, sodium hydroxide
	Negative: xanthan gum, aspartame, amberlite resin, peppermint

The components are listed in magnitude of decreasing effect.
Positive - increases viscosity
Negative - decreases viscosity

5.5 Conclusions

Nineteen formulations were prepared following a fractional factorial experimental design. The rheological behavior of these formulations was analyzed under flow conditions typical of manufacturing processes. The results revealed that formulations that met the acceptance criteria for viscosity (>100 cP) also were the same that reached the gel state. Thus, gelation is required to meet product specifications. As determined by the DoE analysis, gelatin and xanthan gum showed the more pronounced effect on the final viscosity of the formulation. This can be attributed to the polyelectrolyte nature and gelling capacity of these excipients.

6 Gelatin-Xanthan mixtures

Results from the previous section lead to study the interactions of the most influential formulation components (i.e. gelatin and xanthan) on the gelation temperature (T_{gel}) and gel strength. To the best of our knowledge, there is no systematic study reported in the literature for gelatin-xanthan mixtures. Gelatin (1-5 wt%)- xanthan (0- 0.5 wt%) interactions were studied with and without salt and/or mannitol. Finally, conclusions are made based on results obtained from zeta potential measurements.

6.1 Xanthan addition to gelatin solutions

Figures 6.1 to 6.4 illustrate the xanthan addition effect to gelatin 1, 1.3, 2.5, and 5 wt% solutions, respectively. There are definite variations in the rheological behavior of these mixtures. Mixtures comprised of gelatin 1 and 1.3 wt% (Figures 6.1 and 6.2) show a gelatin-like behavior at xanthan concentrations equal or below 0.005 wt%. At xanthan concentrations from 0.05 to 0.1 wt% there was no transition (i.e. discontinuity) observed on the viscosity. These results suggest the formation of electrostatic complexes between gelatin and xanthan molecules. In Figure 6.1, gelatin (1 wt%) - xanthan (0.1 wt%) mixture shows a slight minimum in the viscosity curve that may be attributed to the movement of gelatin-xanthan complexes in the fluid. At xanthan concentrations equal or higher than 0.2 wt%, transitions are observed again. Also, there is an indication that the initial viscosity increases as xanthan concentration increases. This can be attributed to the rigidity of the xanthan molecule, which causes a bulk viscosity increase.

Mixtures comprised of gelatin 2.5 wt% (Figure 6.3) show a gelatin-like behavior at xanthan concentrations equal or below 0.1 wt%. At xanthan concentrations of 0.2 to 0.3 wt% there was no transition (i.e. discontinuity) observed on the viscosity. In Figure 6.3, gelatin (2.5

wt%) - xanthan (0.1 wt%) show a transition temperature lower (T_{gel} = 14^{o}C) than the T_{gel} of gelatin alone. This may be due to a reduction of the gelatin available for gel formation after complexes are formed between gelatin and xanthan. As an observation, gelatin (2.5 wt%) - xanthan (0.2 wt%) shows a similar behavior as gelatin (1 wt%) - xanthan (0.1 wt%). This can be attributed to the same reason explained previously because gelatin to xanthan ratio was similar to that identified for gelatin 1 wt% mixture. The wet basis gelatin to xanthan ratios were 10 and 12.5 for 1 and 2.5 wt% gelatin mixtures, respectively. At xanthan concentrations equal or higher than 0.4 wt%, transitions are observed on the viscosity again.

Mixtures comprised of gelatin 5 wt% (Figure 6.4) show gelatin-like behaviors at xanthan concentration equal or below 0.2 wt%. Over xanthan concentrations of 0.2 wt% there are transitions but the viscosity is not constant before the transitions. This can be attributed to the same reason of the previously evaluated gelatin to xanthan ratios. Since the wet basis gelatin to xanthan ratios were 16.6, 12.5 and 10 for xanthan concentrations of 0.3, 0.4 and 0.5 wt% respectively.

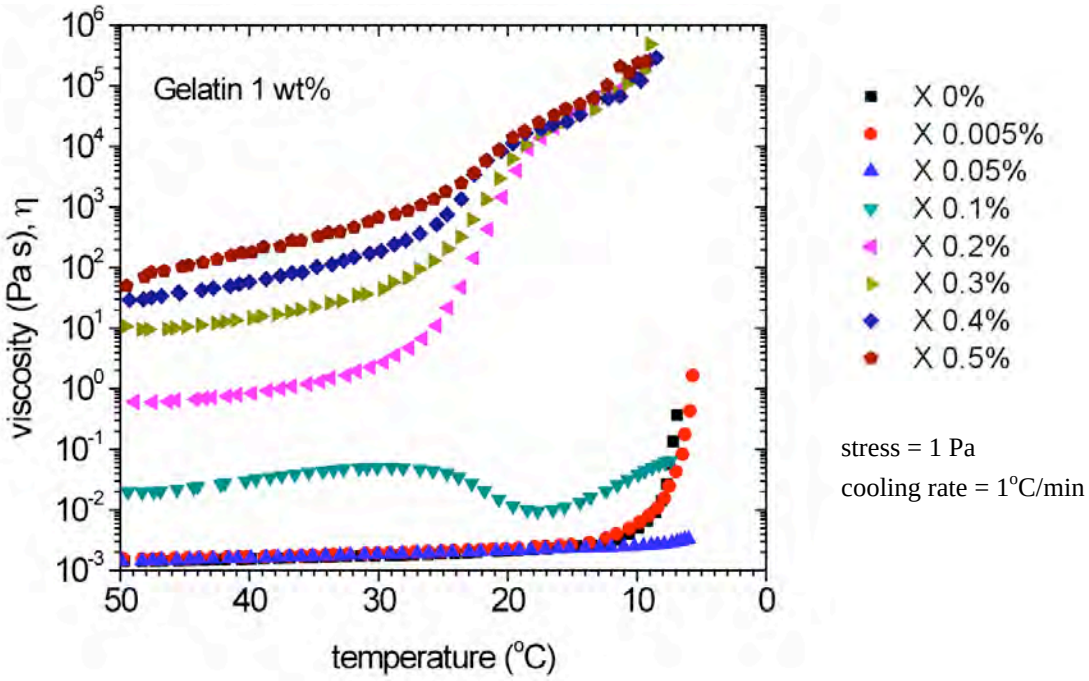

Figure 6.1 Xanthan (X) effect on the viscosity of aqueous 1 wt% gelatin solutions at a constant stress of 1 Pa and a cooling rate of 1 °C/min

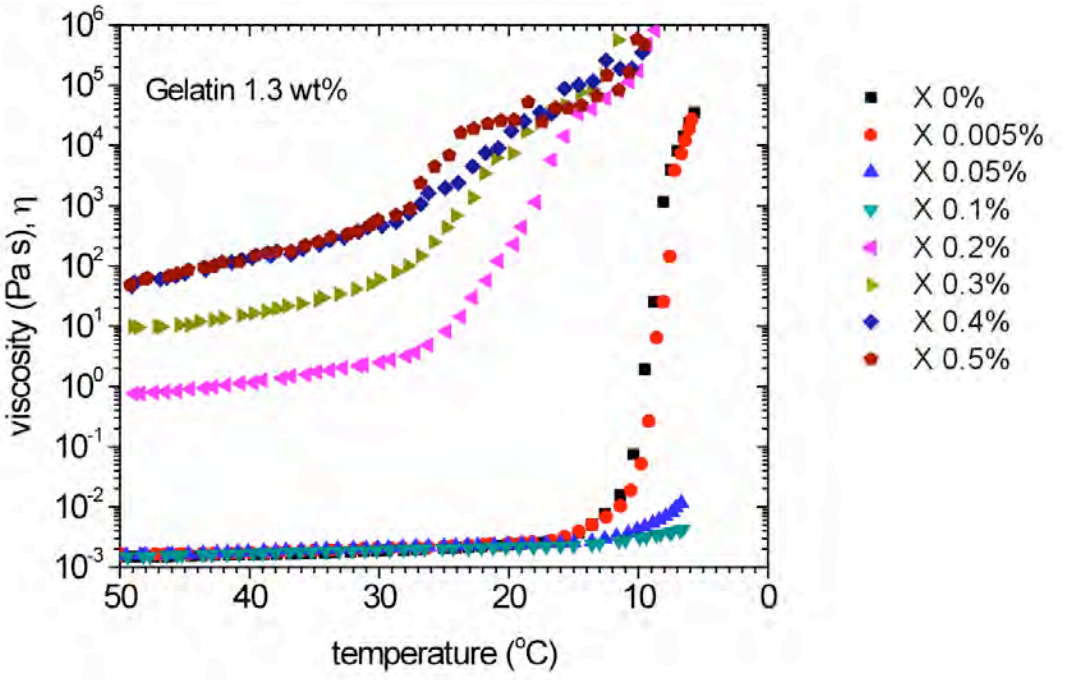

Figure 6.2 Xanthan (X) effect on the viscosity of aqueous 1.3 wt% gelatin solutions at a constant stress of 1 Pa and a cooling rate of 1 °C/min

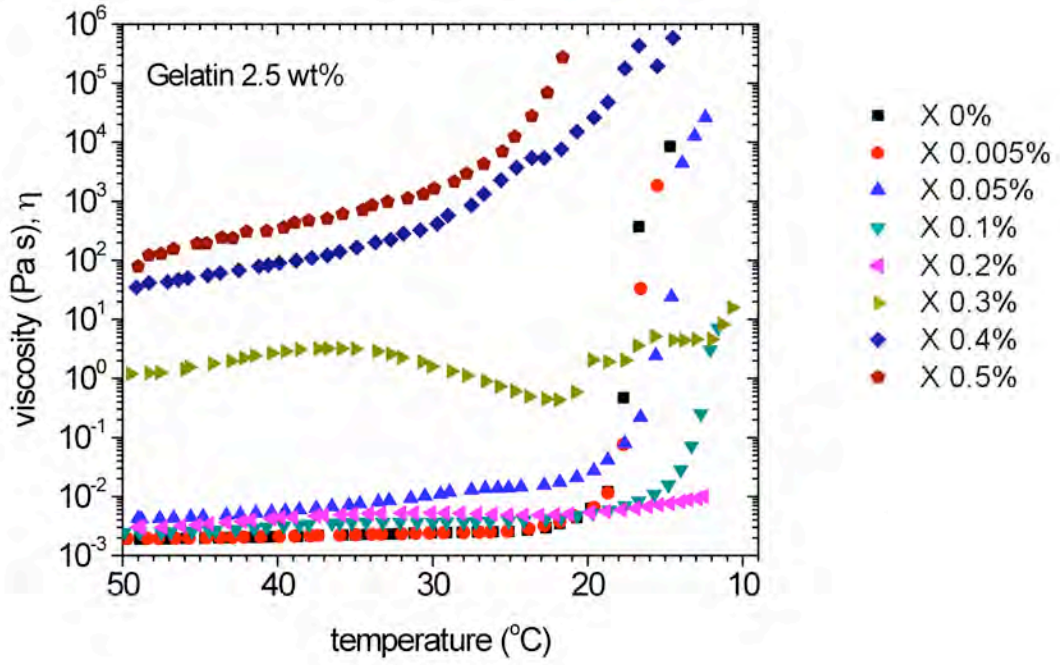

Figure 6.3 Xanthan (X) effect on the viscosity of aqueous 2.5 wt% gelatin solutions at a constant stress of 1 Pa and a cooling rate of 1 °C/min

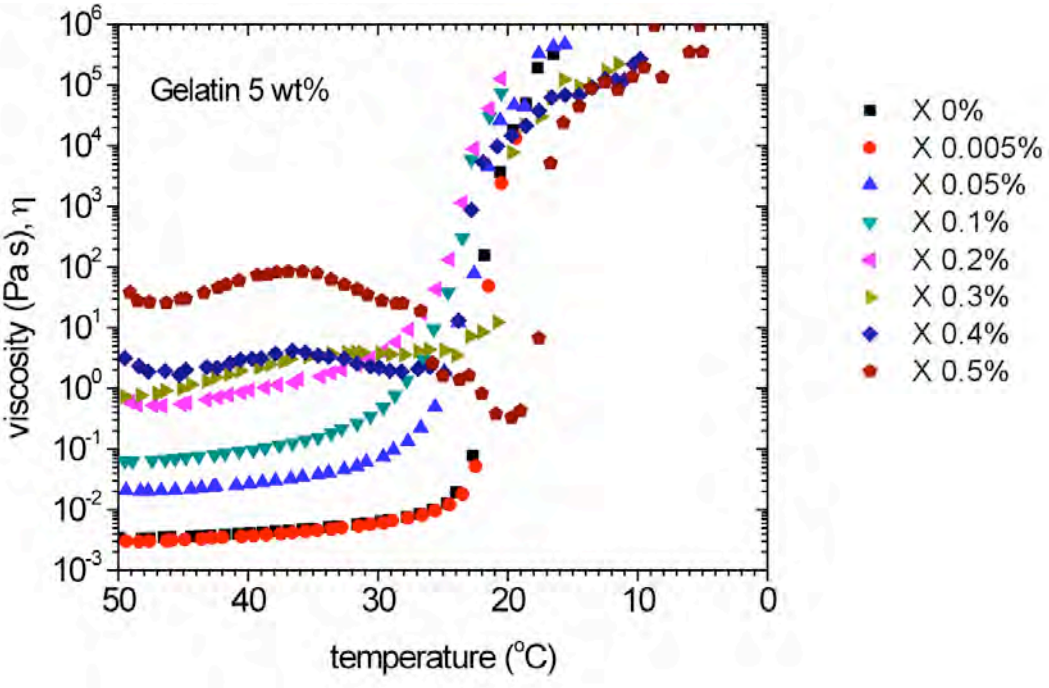

Figure 6.4 Xanthan (X) effect on the viscosity of aqueous 5 wt% gelatin solutions at a constant stress of 1 Pa and a cooling rate of 1 °C/min

6.2 Mannitol addition to gelatin solutions and gelatin-xanthan mixtures

Mannitol is a sugar alcohol of neutral charge that contributes to the formation of hydrogen bonds. It was the third most influential excipient on the final viscosity of the formulation (Section 5.4). Therefore, the effect of 1 and 2 wt% mannitol addition to gelatin solutions was studied, as depicted in Figure 6.5. For each gelatin concentration, the curves look similar. In other words, the viscosity is constant as temperature decreases until it reaches the gel point where it increases drastically. Figure 6.6 presents a summary that demonstrates that mannitol does not have a significant effect on the T_{gel} of gelatin solutions, possibly because mannitol is a small molecule compared to biopolymers and it is non-ionic. Similarly, mannitol addition to gelatin-xanthan mixtures did not have a significant effect on the rheological behavior and gelation of the system (Refer to Figures A.1 to A.3 in the Appendix). Accordingly, mannitol was not investigated further on gelatin-xanthan mixtures.

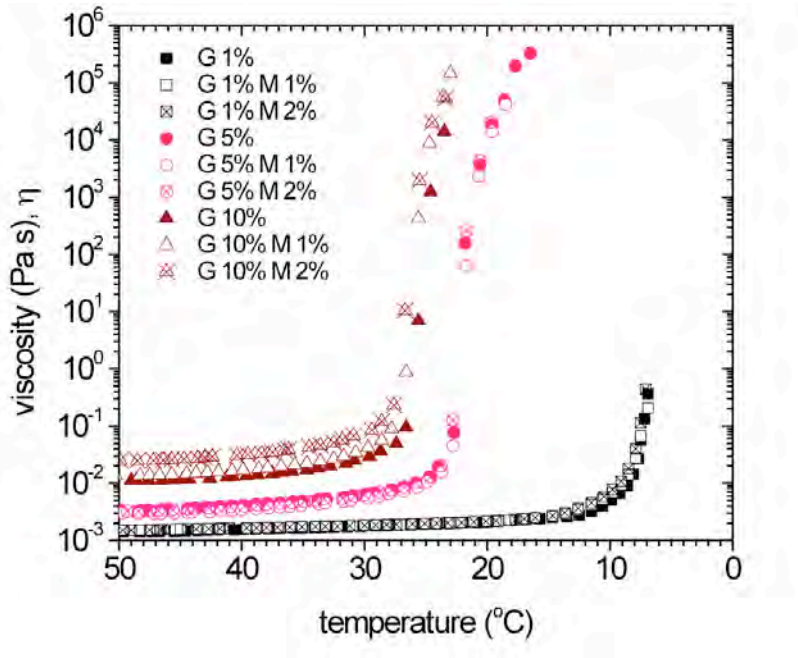

Figure 6.5 Mannitol (M) effect on the viscosity of gelatin (G) solutions at a constant stress of 1 Pa and a cooling rate of 1 ^{o}C/min

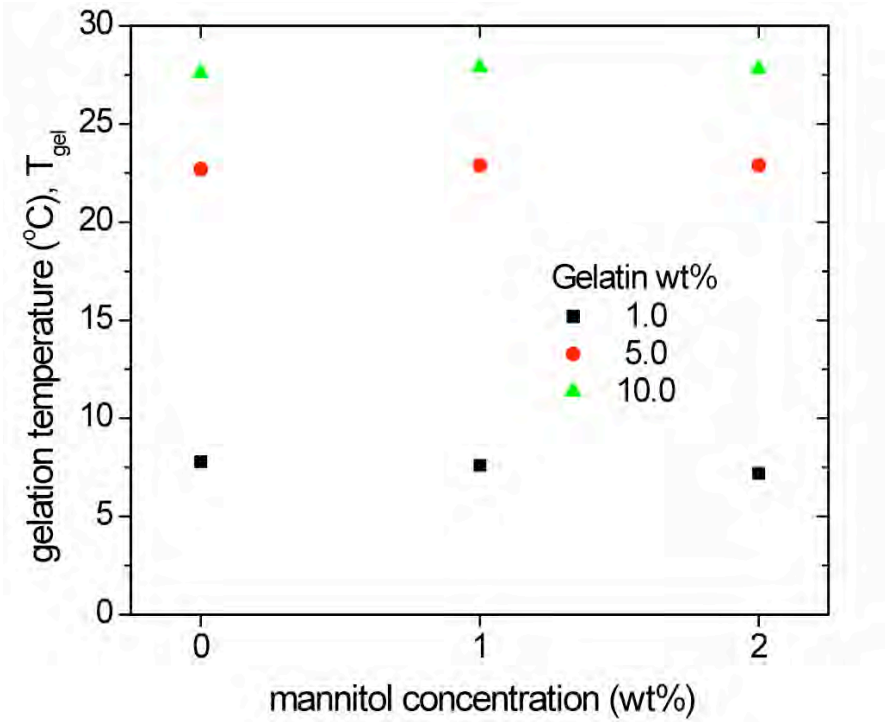

Figure 6.6 The effect of mannitol addition on the gelation temperature of aqueous gelatin solutions

6.3 Effect of salt type on transition temperature of gelatin-xanthan mixture

As part of the experiments of gelatin-xanthan mixture interactions with additives, the addition effect of sodium chloride (NaCl), sodium acetate ($C_2H_3NaO_2$), and sodium citrate ($Na_3C_6H_5O_7$) was studied. The pharmaceutical active ingredient (API) of the formulation was not provided by the local pharmaceutical industry for safety issues. These experiments were performed in order to choose a salt as a drug model to study its interactions with gelatin-xanthan mixtures and to determine the effect of additive size. The salts were used in a 0.17 M (equal to 1 wt%) concentration because it is near to the API concentration range. In Figure 6.7, it was observed that the size of the additives used did not have a significant effect on the transition temperatures at each xanthan concentration. In addition, Figure 6.7 illustrates the effects of salt addition to gelatin (1 wt%) - xanthan (0.1 and 0.5 wt%) mixtures. At low concentration of

xanthan, the addition effect of salts on the rheology was similar. At high concentrations of xanthan, sodium chloride and sodium acetate had similar effect but sodium citrate had a higher transition temperature. A summary of the transition temperatures is presented in Figures 6.8 and 6.9. The viscosity before the transition of the mixtures containing sodium citrate was lower than that of the mixtures containing the other salts. This can be attributed to a reduction of the hydrodynamic volume of the xanthan molecule because sodium citrate provides three times more sodium ions to screen xanthan carboxylic group charges compared to the other salts. This was observed in a higher extent at high concentration of xanthan. Viturawong et al.[97] related the increasing ionic strength to a viscosity decrease of xanthan aqueous solutions due to the sidechains of xanthan collapsing to the backbone of its structure, thus, giving a more compact structure and reducing its hydrodynamic volume. Sodium chloride was chosen as the drug model in the next sections to determine its effect on interactions and rheological properties of gelatin-xanthan mixtures.

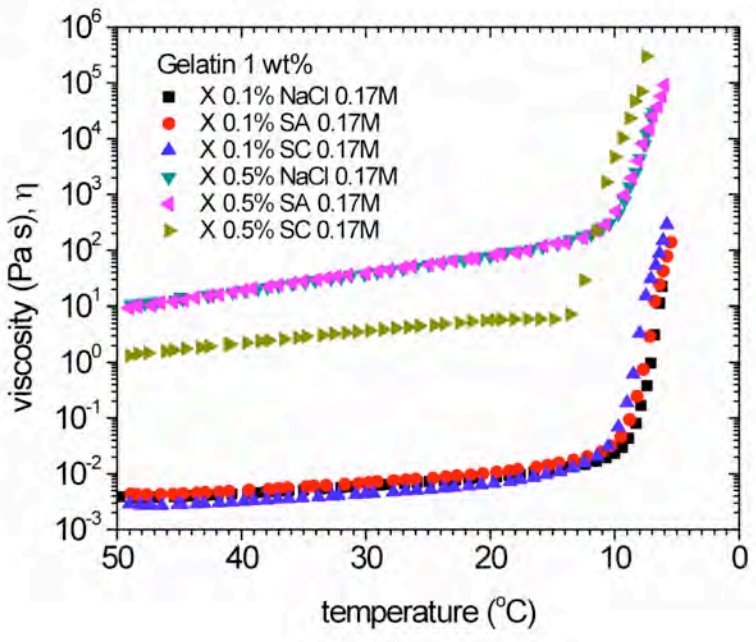

Figure 6.7 Sodium chloride (NaCl), sodium acetate (SA), and sodium citrate (SC) effect on the viscosity as a function of temperature of gelatin-xanthan mixtures

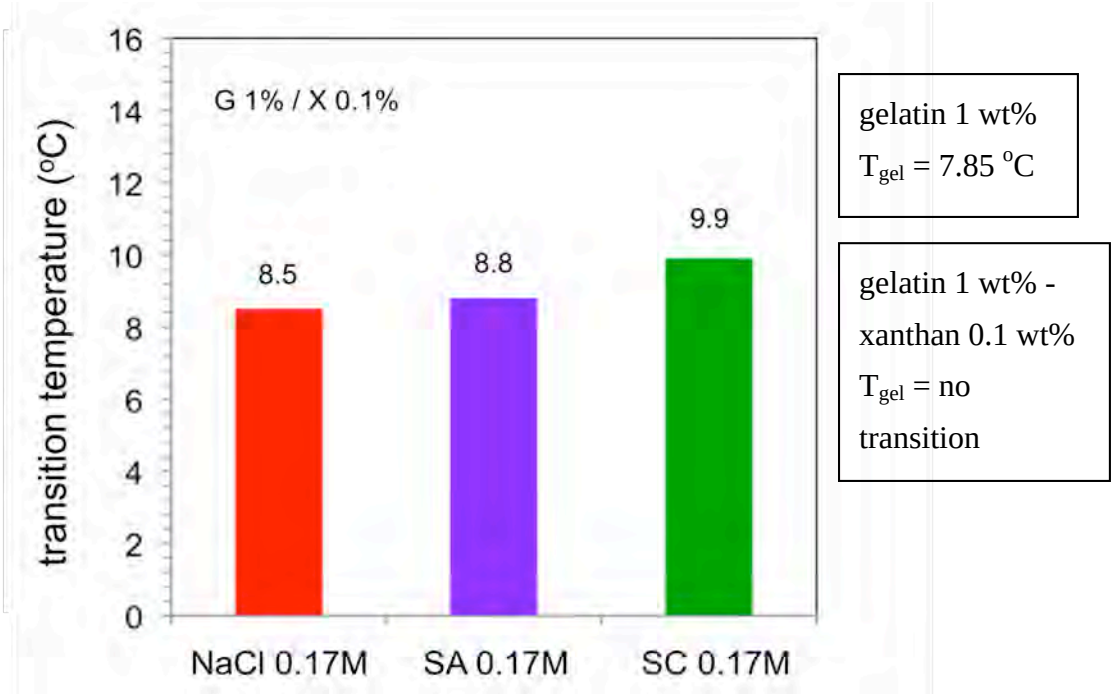

Figure 6.8 Transition temperatures comparison for gelatin (1 wt%) - xanthan (0.1 wt%) mixture containing sodium chloride, sodium acetate or sodium citrate

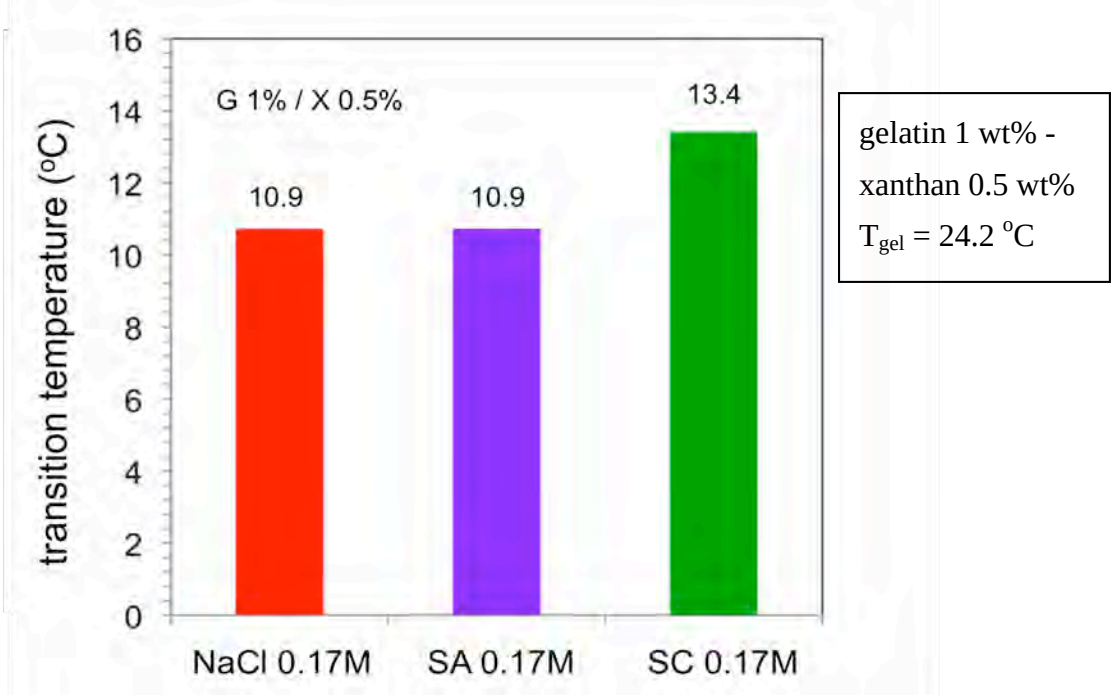

Figure 6.9 Transition temperatures comparison for gelatin (1 wt%) - xanthan (0.5 wt%) mixture containing sodium chloride, sodium acetate or sodium citrate

6.4 NaCl effect on transition temperature of gelatin-xanthan mixtures

All temperature transitions for gelatin-xanthan mixtures with and without NaCl are illustrated in Figures 6.11 to 6.14. As previously discussed, no transitions were observed for mixtures of gelatin (1 wt%) - xanthan (0.05-0.1 wt%), gelatin (1.3 wt%) - xanthan (0.05-0.1 wt%), and gelatin (2.5 wt%) - xanthan (0.2-0.3 wt%). These results suggest the formation of electrostatic complexes between gelatin and xanthan molecules. The gelatin (1.3 wt%) - xanthan (0.1 wt%) mixture had no transition under CSTR test conditions, for this reason it was used as an example to illustrate better the effect of NaCl addition. In Figure 6.10 it is observed that as the amount of salt added increases, the mixture was nearer to reach the gel state until it reaches it. Refer to Figures A.4 to A.6 in the Appendix for additional information. Also, the resulting transition temperature was similar to the transition temperature of gelatin alone. As salt was added, electrostatic interactions decreased between xanthan and gelatin due to screening of xanthan charges by sodium cations. This inference is supported by the fact that the mixture reaches the gel state and the gelation temperature was similar to that of gelatin alone. Moreover, Higiro et al. [116] studied the effect of salt on xanthan-locust bean gum mixtures and determined that the electrostatic interactions between these biopolymers were reduced or vanished when adding and increasing salt concentration.

Figures 6.11 and 6.12 show that for gelatin 1 and 1.3 wt% mixtures with NaCl 0.25 wt% there are transitions observed at very low xanthan concentrations or no xanthan at all. The other mixtures that had no transitions at NaCl 0.25 wt% may be due to having not sufficient salt to stabilize xanthan molecules. This inference is supported by the fact that passed certain concentration of xanthan there are xanthan molecules with unscreened charges that interact with gelatin molecules causing a partial or total reduction of the gelatin available for gel formation. A

partial reduction of the gelatin available for gel formation can cause a decrease of the transition temperature value, making it not appreciable on the experimental window. Moreover, a partial or total reduction of the gelatin available for gel formation can impede the mixture from reaching the transition temperature. Temperature transitions were observed for gelatin 1 and 1.3 wt% mixtures with NaCl 0.5 wt%. For gelatin 2.5 wt%, experiments were only done with NaCl 1 wt% and without NaCl. Also, temperature transitions were observed for gelatin 1, 1.3 and 2.5 wt% mixtures with NaCl 1 wt%. As an observation, an increase in the transition temperature is observed as xanthan concentration increases in a higher extent for gelatin 1 and 1.3 wt% mixtures with NaCl addition. In contrast, all gelatin 5 wt% mixtures with and without salt addition showed transitions since the gelatin to xanthan concentration ratio is higher than for the other mixtures. All the results discussed above described the effect of salt addition over gelatin interactions with xanthan.

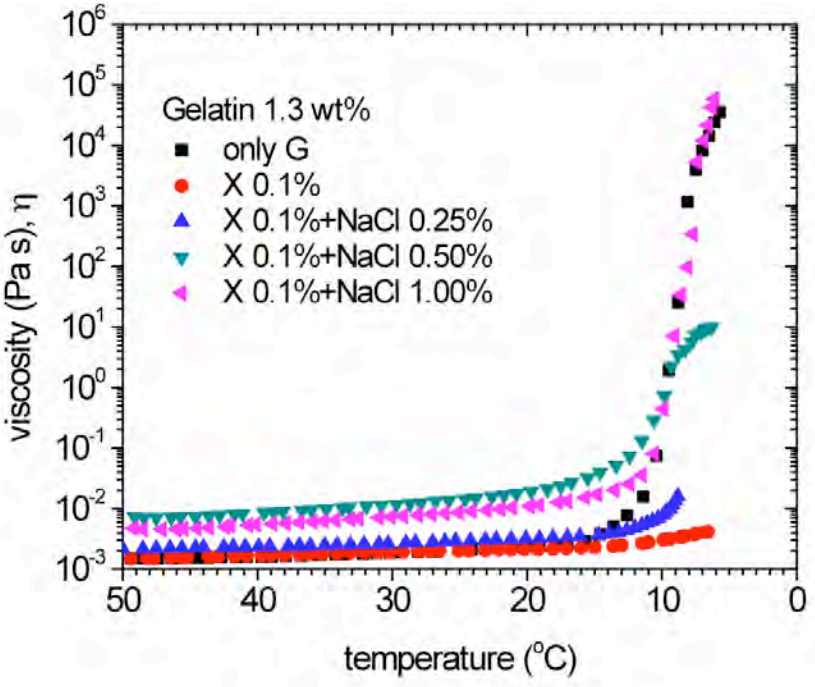

Figure 6.10 Sodium chloride (NaCl) effect on the sol-gel transition of a gelatin (1.3 wt%) - xanthan (0.1 wt%) mixture

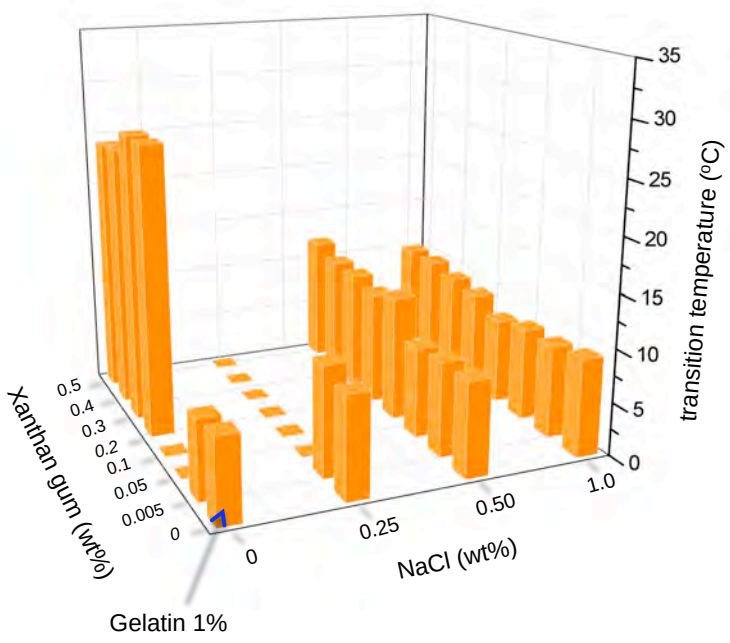

Figure 6.11 Transition temperatures for 1 wt% gelatin solution as a function of xanthan and sodium chloride addition

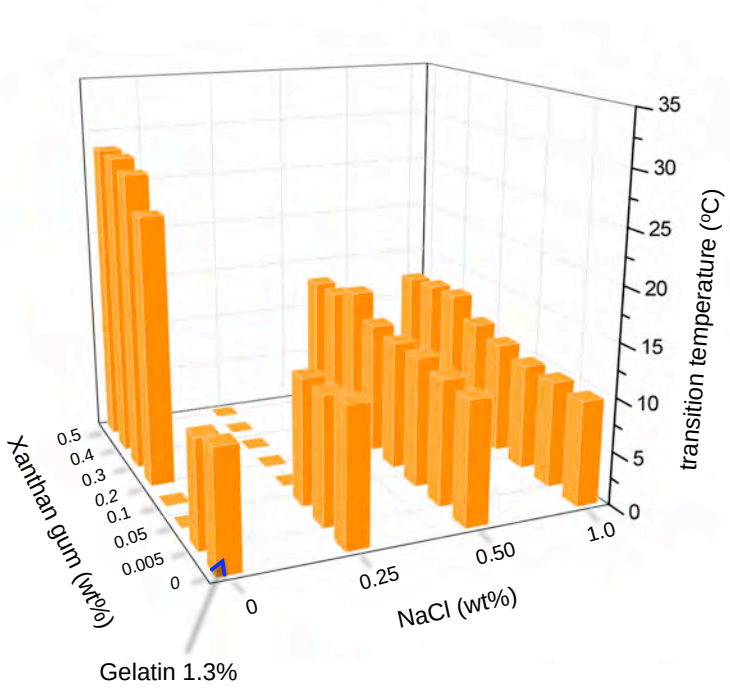

Figure 6.12 Transition temperatures for 1.3 wt% gelatin solution as a function of xanthan and sodium chloride addition

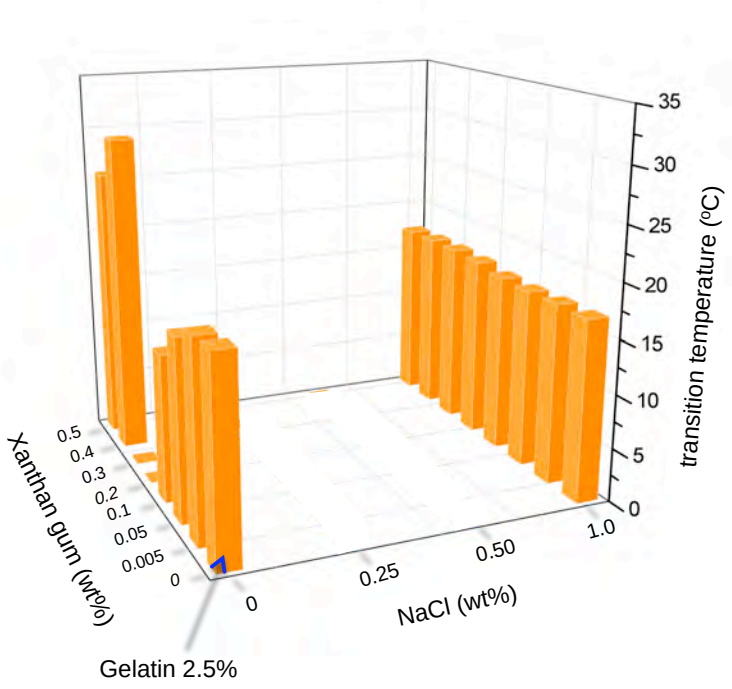

Figure 6.13 Transition temperatures for 2.5 wt% gelatin solution as a function of xanthan and sodium chloride addition

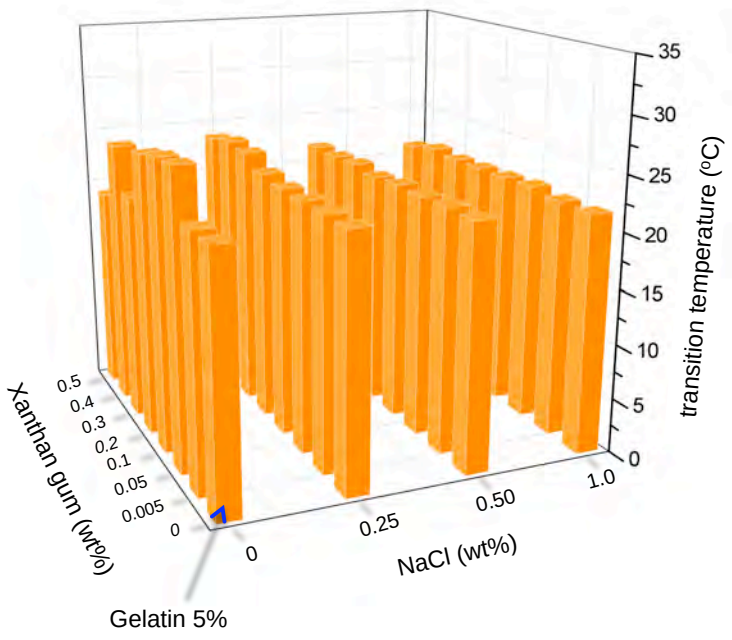

Figure 6.14 Transition temperatures for 5 wt% gelatin solution as a function of xanthan and sodium chloride addition

6.5 Dynamic rheology of gelatin-xanthan mixtures

The following tests were performed after a CSTR test (50-10 ^{o}C, 1 ^{o}C/min, 1 Pa) and a 15 minutes rest in order to determine the strength of the gelled gelatin-xanthan mixtures with and without NaCl. Figure 6.15 illustrates the storage modulus of gelatin (1 wt%) -xanthan mixtures without NaCl. An abrupt increase (14x to 25x) of the storage modulus is observed for xanthan concentrations over 0.2 wt%. The storage modulus was analyzed at different aging times to determine if it followed similar trends, as depicted in Figure 6.16. An aging time of 60 minutes was chosen to present a summary of the storage modulus for each gelatin-xanthan mixture, as depicted in Figure 6.17. Refer to Figures A.7 to A.11 in the Appendix for additional details. It was observed a decrease of the storage modulus when adding small amounts of xanthan. The lowest storage modulus of each gelatin concentration corresponds to the same gelatin-xanthan mixtures that did not showed transitions, thus it further suggest that the system is not gelling. These results complement the hypothesis on the formation of electrostatic complexes between gelatin and xanthan molecules.

Figure 6.18 illustrates the storage modulus of gelatin-xanthan mixtures with NaCl 1 wt%. It is observed that the storage modulus increases with xanthan concentration increase. As explained before, NaCl hinders the formation of electrostatic complexes allowing all mixtures to gel at the NaCl concentration used. Figure 6.19 presents a summary of the storage modulus for each gelatin-xanthan mixture with NaCl at an aging time of 60 minutes. In addition, a minimum on the storage modulus curves was not observed in Figure 6.19, therefore, confirming that there is no complex formation occurring between gelatin and xanthan. Moreover, the change of storage modulus magnitude as xanthan concentrations increases was larger for small gelatin concentrations. It is clearly observed that for xanthan concentrations equal and over 0.2 wt% the

magnitude of the storage modulus is highly reduced with NaCl addition but still it is higher than that of gelatin alone.

The mixtures comprised of gelatin 1.3, 2.5, and 5 wt% follow similar trends as the presented for mixtures of gelatin 1 wt%, as depicted in Figure 6.19.

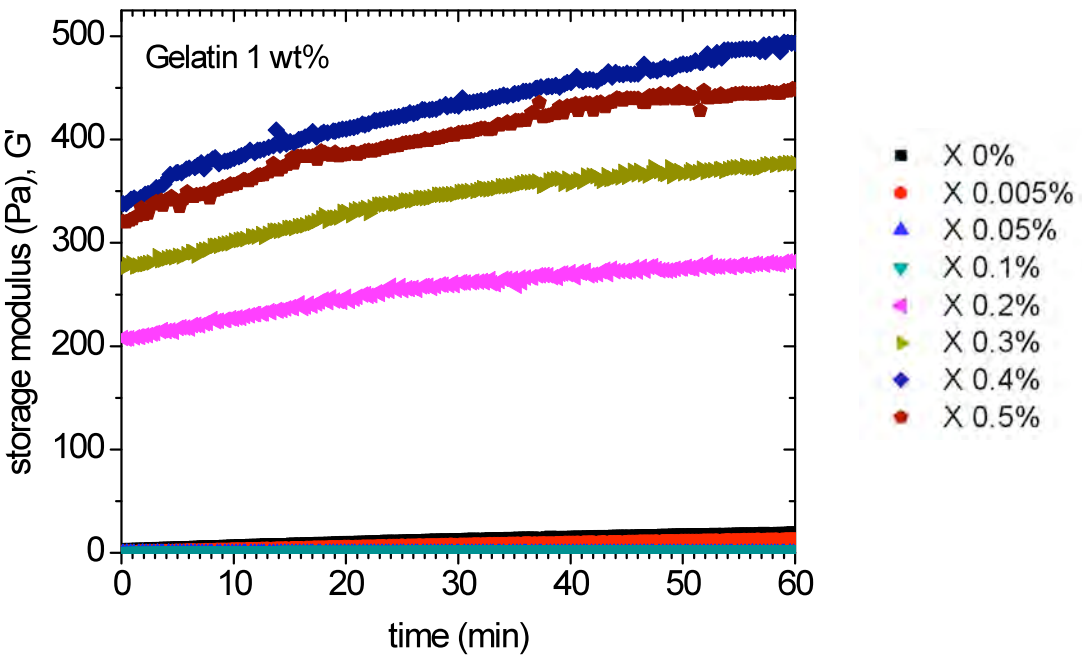

Figure 6.15 Storage modulus (G') of gelatin (1 wt%)-xanthan as a function of xanthan concentration and time. Test conditions: frequency of 1 Hz, strain of 1%, and temperature of 10 ^{o}C

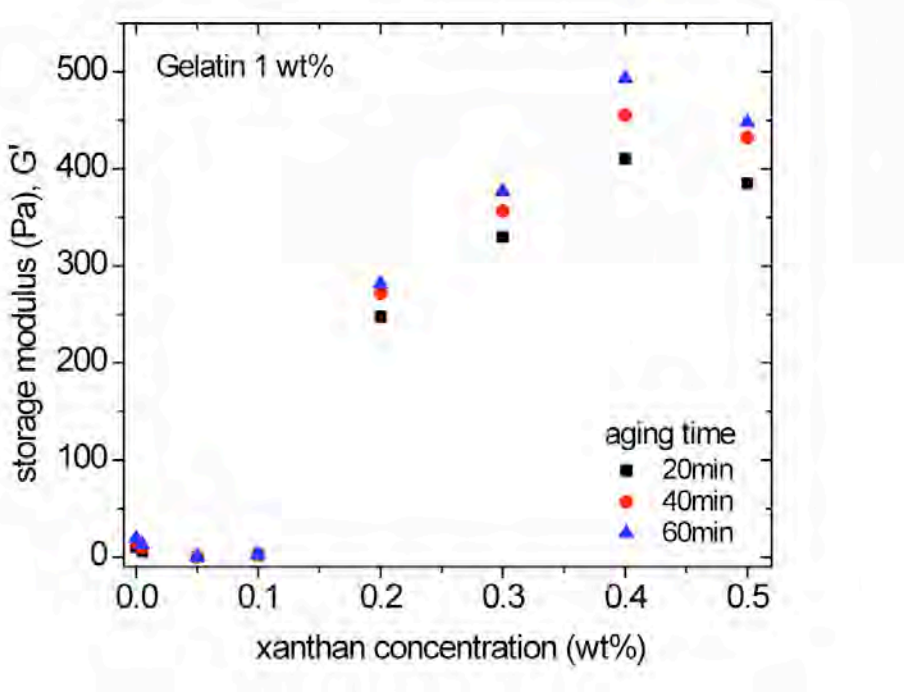

Figure 6.16 Storage modulus (G') of gelatin 1 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration

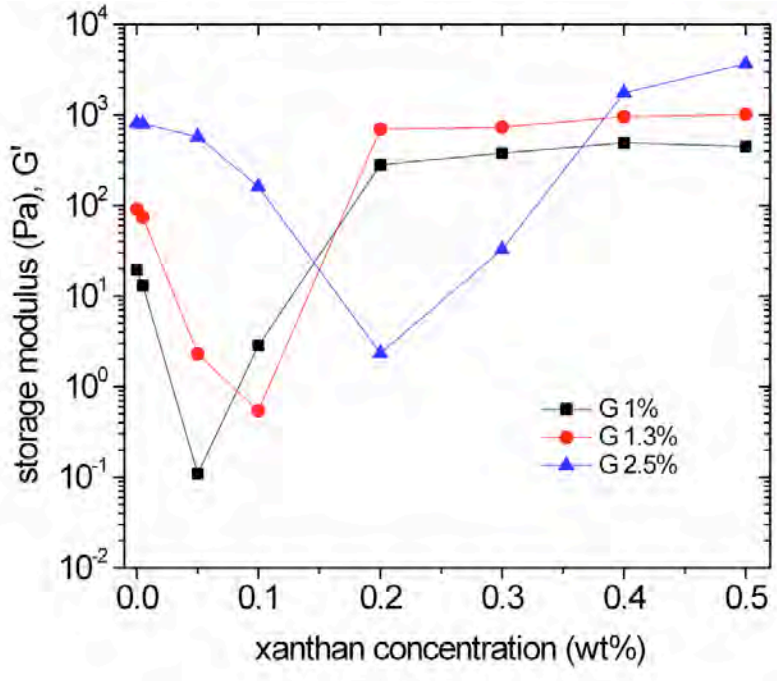

Figure 6.17 Storage modulus (G') at an aging time of 60 min after CSTR and 15 min rest for gelatin-xanthan mixtures without additives

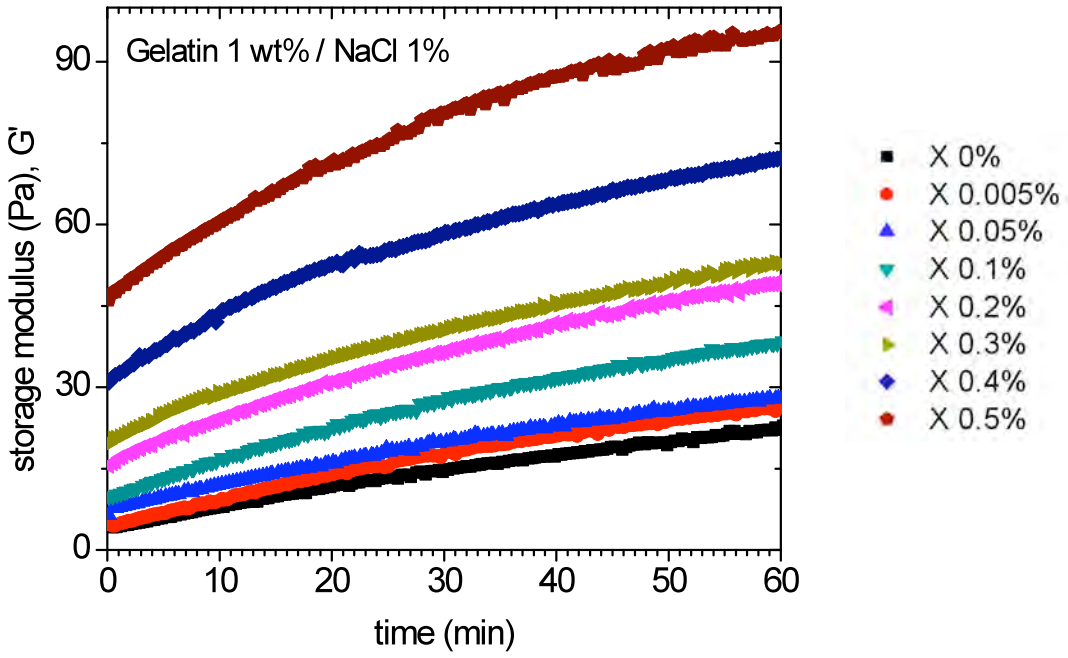

Figure 6.18 Storage modulus of gelatin (1 wt%)-xanthan-NaCl (1 wt%) as a function of xanthan concentration and time. Test conditions: frequency of 1 Hz, strain of 1%, and temperature of 10 ^{o}C

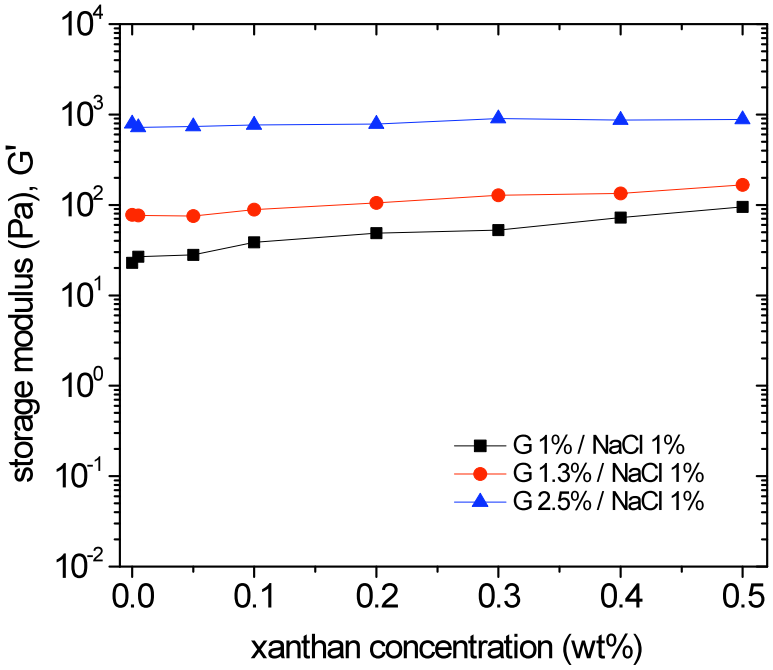

Figure 6.19 Storage modulus (G') at an aging time of 60 min after CSTR and 15 min rest for gelatin-xanthan mixtures with sodium chloride (1 wt%) addition

6.6 Zeta potential of gelatin-xanthan mixtures

Zeta potential was used to determine the molecules charge interactions in gelatin-xanthan mixtures. The zeta potential of gelatin solutions was measured as a function of xanthan concentration at 40^{o}C. The pH was not adjusted. The zeta potential value near 0 at a gelatin to xanthan ratio of 12.4 and 14.2 for 1.3 and 2.5 wt% gelatin mixtures, respectively, indicates that attraction between opposed charges dominates, which confirms the formation of complexes hypothesis from previous sections. Meanwhile, a zeta potential value near zero was observed at a gelatin xanthan ratio of 110 for 1 wt% gelatin mixture. This can be attributed to an equipment limitation at low biopolymer concentrations, which makes measurements non-reproducible without the addition of salts.

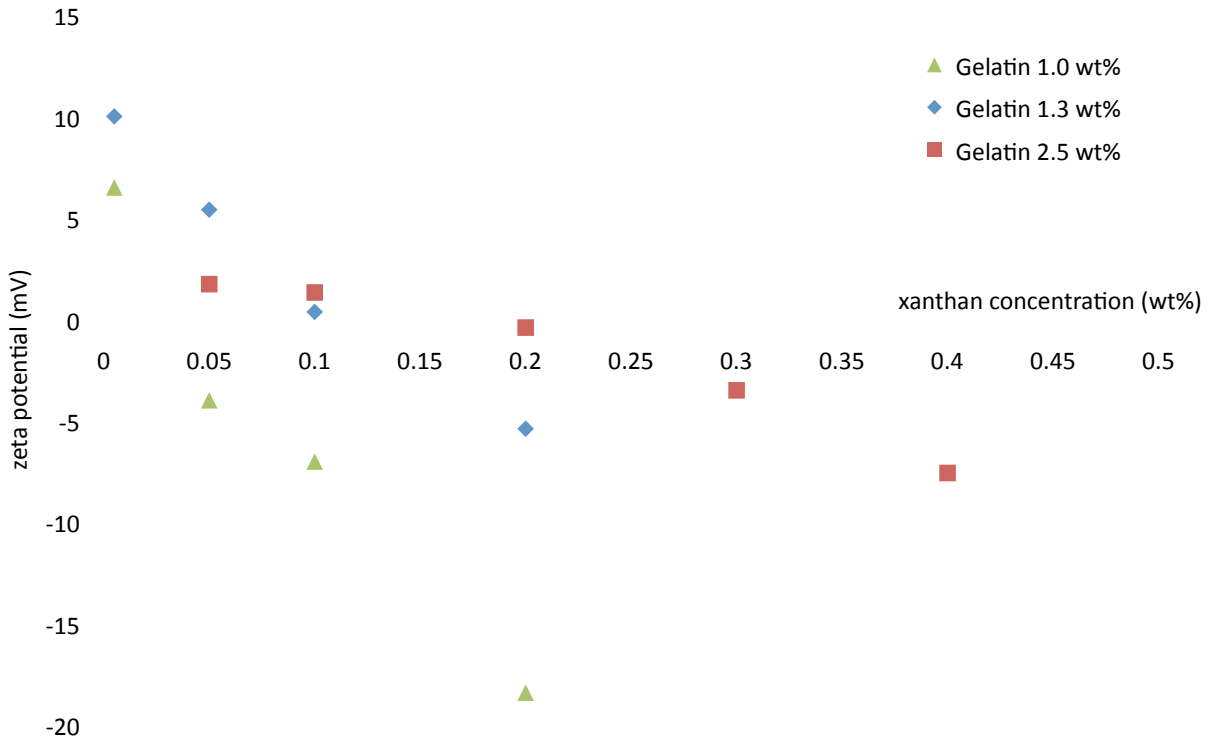

Figure 6.20 Zeta potential of gelatin solutions as a function of xanthan concentration

6.7 Conclusions

The gelation temperature of gelatin solutions was studied as a function of xanthan concentration. Also, mannitol addition to gelatin and gelatin-xanthan solutions did not have a significant effect on the sol-gel transitions, even though it is classified as a structural agent. CSTR experiments of gelatin-xanthan mixtures showed no gelation for some mixtures attributed to complexes formation due to electrostatic interactions between these polyelectrolytes. Storage modulus results evidenced the same observation of complexes formation. The results indicated that the ratio of gelatin to xanthan for maximum formation of complexes was approximately 20, 13, and 12.5 for gelatin 1, 1.3, 2.5 wt% mixtures, respectively. Similarly, zeta potential measurements showed an isoelectric point at a ratio of approximately 13.3. NaCl addition to gelatin-xanthan mixtures reduced or vanished the interactions between gelatin and xanthan by stabilizing carboxilic group charges on xanthan side chains. These results suggest that if xanthan is not well stabilized it can have a detrimental effect on the viscosity.

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APPENDIX A

Table A.1 Properties of common biopolymers

Agents	Gel formation	Thickening effect	Transparency of the gel	Cold water solubility	pH-stability
Gelatin	+ + + Thermo-reversible	+ + +	+ + +	0 (With some exception)	+ +
Agar-agar (polysaccharide)	+ + + Thermo-reversible	+ + +	+	0	+ +
Alginates	+ + + (with calcium)	+ + +	+ + +	+ + +	+
Carboxymethyl cellulose (CMC)	0	+ + +	-	+ + +	+ + pH (3-11)
Hydroxypropyl-methyl cellulose (HPMC)	+ + + Gel formation on heating	+ + +	+	+ + +	+ + + pH (1-10)
Modified starches (polysaccharide)	+ + +	+ + +	+	0 (With exception of physically modified starches)	+ +
Pectin (polysaccharide)	+ + +	+ +	+ + +	+ +	+

+++ excellent

+ poor

0 none

Table A.2 Placebo formulation properties at 50^{o}C

Placebo Formulation	pH	Ω (µS) S=siemens
1	6.25	439.7
2	5.13	180
3	5.56	168.5
4	5.3	390
5	5.74	167
6	5.22	423
7	4.87	364
8	6.67	260
9	5.39	265
10	5.12	370.3
11	6.02	504.3
12	4.93	119
13	4.73	363
14	6.28	312.7
15	5.51	161
16	5.21	415.1
17	5.37	301
18	5.37	318
19	5.36	309.7

Table A.3 Gelatin (1 wt%)-xanthan mixtures properties at 40^{o}C

gelatin (wt%)	xanthan (wt%)	NaCl (wt%)	pH	Ω (μS)	ρ (g/mL)
1	0	0	4.64	75.4	0.9954
1	0.005	0	4.7	107.8	0.9956
1	0.05	0	5.83	139.4	0.9955
1	0.1	0	5.16	176.5	0.9958
1	0.2	0	5.66	226	0.9963
1	0.3	0	5.3	283	0.9964
1	0.4	0	5.54	342	0.9966
1	0.5	0	5.59	415	0.9967
1	0	0.25	5.19	$4.98 * 10^3$	0.9969
1	0	0.5	5.17	$9.36 * 10^3$	0.9986
1	0	1	5.14	$17.35 * 10^3$	1.002
1	0.005	0.25	5.2	$4.984 * 10^3$	0.9969
1	0.005	0.5	5.23	$9.69 * 10^3$	0.9989
1	0.005	1	5.58	$17.46 * 10^3$	1.002
1	0.05	0.25	5.24	$4.978 * 10^3$	0.997
1	0.05	0.5	5.2	$9.32 * 10^3$	0.9988
1	0.05	1	5.56	$17.41 * 10^3$	1.0021
1	0.1	0.25	5.24	$5.066 * 10^3$	0.9973
1	0.1	0.5	5.2	$9.205 * 10^3$	0.999
1	0.1	1	5.54	$17.57 * 10^3$	1.0022
1	0.2	0.25	5.24	$5.124 * 10^3$	0.9976
1	0.2	0.5	5.15	$9.177 * 10^3$	0.9993
1	0.2	1	5.52	$17.66 * 10^3$	1.0026
1	0.3	0.25	5.3	$5.185 * 10^3$	0.9979
1	0.3	0.5	5.18	$9.575 * 10^3$	0.9997
1	0.3	1	5.51	$17.77 * 10^3$	1.0018
1	0.4	0.25	5.22	$5.272 * 10^3$	0.9981
1	0.4	0.5	5.17	$9.439 * 10^3$	0.9979
1	0.4	1	5.5	$17.80 * 10^3$	0.9966
1	0.5	0.25	5.26	$5.036 * 10^3$	0.9984
1	0.5	0.5	5.19	$9.49 * 10^3$	1.0005
1	0.5	1	5.02	$17.94 * 10^3$	1.002

Table A.4 Gelatin (1.3 wt%)-xanthan mixtures properties at 40°C

gelatin (wt%)	xanthan (wt%)	NaCl (wt%)	pH	Ω (μS)	ρ (g/mL)
1.3	0	0	5.15	283	0.9958
1.3	0.005	0	5.18	309	0.9961
1.3	0.05	0	5.19	334	0.9962
1.3	0.1	0	5.17	371	0.9964
1.3	0.2	0	5.23	405	0.9966
1.3	0.3	0	5.3	438	9.9968
1.3	0.4	0	5.32	461	0.9969
1.3	0.5	0	6.06	488	0.9971
1.3	0	0.25	5.16	$4.952 * 10^3$	0.9977
1.3	0	0.5	5.17	$9.441 * 10^3$	0.9994
1.3	0	1	5.16	$17.23 * 10^3$	1.0001
1.3	0.005	0.25	5.17	$4.98 * 10^3$	0.9977
1.3	0.005	0.5	5.14	$9.48 * 10^3$	0.9994
1.3	0.005	1	5.11	$17.30 * 10^3$	1.0027
1.3	0.05	0.25	5.18	$4.985 * 10^3$	0.9978
1.3	0.05	0.5	5.17	$9.38 * 10^3$	0.9996
1.3	0.05	1	5.13	$17.40 * 10^3$	1.0024
1.3	0.1	0.25	5.2	$5.08 * 10^3$	0.998
1.3	0.1	0.5	5.11	$9.54 * 10^3$	0.9997
1.3	0.1	1	5.08	$17.45 * 10^3$	1.0028
1.3	0.2	0.25	5.17	$5.07 * 10^3$	0.9983
1.3	0.2	0.5	5.13	$9.77 * 10^3$	1.0001
1.3	0.2	1	5.1	$17.50 * 10^3$	1.0032
1.3	0.3	0.25	5.15	$5.191 * 10^3$	0.9986
1.3	0.3	0.5	5.13	$9.15 * 10^3$	1.0004
1.3	0.3	1	5.11	$17.55 * 10^3$	1.0036
1.3	0.4	0.25	5.16	$5.015 * 10^3$	0.999
1.3	0.4	0.5	5.15	$9.15 * 10^3$	1.0008
1.3	0.4	1	5.12	$17.63 * 10^3$	1.0037
1.3	0.5	0.25	5.17	$5.103 * 10^3$	0.9994
1.3	0.5	0.5	5.1	$9.185 * 10^3$	1.0011
1.3	0.5	1	5.05	$17.75 * 10^3$	1.0044

Table A.5 Gelatin (2.5 wt%)-xanthan mixtures properties at 40°C

gelatin (wt%)	xanthan (wt%)	NaCl (wt%)	pH	Ω (μS)	ρ (g/mL)
2.5	0	0	-	-	-
2.5	0.005	0	-	-	-
2.5	0.05	0	-	-	-
2.5	0.1	0	-	-	-
2.5	0.2	0	-	-	-
2.5	0.3	0	-	-	-
2.5	0.4	0	-	-	-
2.5	0.5	0	-	-	-
2.5	0	0.25	-	-	-
2.5	0	0.5	-	-	-
2.5	0	1	-	-	-
2.5	0.005	0.25	-	-	-
2.5	0.005	0.5	-	-	-
2.5	0.005	1	-	-	-
2.5	0.05	0.25	-	-	-
2.5	0.05	0.5	-	-	-
2.5	0.05	1	-	-	-
2.5	0.1	0.25	-	-	-
2.5	0.1	0.5	-	-	-
2.5	0.1	1	-	-	-
2.5	0.2	0.25	-	-	-
2.5	0.2	0.5	-	-	-
2.5	0.2	1	-	-	-
2.5	0.3	0.25	-	-	-
2.5	0.3	0.5	-	-	-
2.5	0.3	1	-	-	-
2.5	0.4	0.25	-	-	-
2.5	0.4	0.5	-	-	-
2.5	0.4	1	-	-	-
2.5	0.5	0.25	-	-	-
2.5	0.5	0.5	-	-	-
2.5	0.5	1	-	-	-

- not determined

Table A.6 Gelatin (5 wt%)-xanthan mixtures properties at 40°C

gelatin (wt%)	xanthan (wt%)	NaCl (wt%)	pH	Ω (μS)	ρ (g/mL)
5	0	0	5.17	357	0.9987
5	0.005	0	5.21	383	0.9991
5	0.05	0	4.98	398	1.006
5	0.1	0	5.01	441	1.0032
5	0.2	0	4.97	492.6	1.0018
5	0.3	0	5.03	536	1.0064
5	0.4	0	3.23	618.4	1.007
5	0.5	0	5.05	588.9	1.0075
5	0	0.25	5.17	$4.841 * 10^3$	1.0076
5	0	0.5	5.18	$8.95 * 10^3$	1.0093
5	0	1	5.19	$16.65 * 10^3$	1.0129
5	0.005	0.25	5.2	$4.9 * 10^3$	1.0078
5	0.005	0.5	5.15	$9.3 * 10^3$	1.009
5	0.005	1	5.45	$16.47 * 10^3$	1.0129
5	0.05	0.25	5.19	$4.92 * 10^3$	1.0078
5	0.05	0.5	5.2	$9.147 * 10^3$	1.0095
5	0.05	1	5.4	$16.53 * 10^3$	1.013
5	0.1	0.25	5.19	$4.733 * 10^3$	1.0079
5	0.1	0.5	5.18	$8.78 * 10^3$	1.0097
5	0.1	1	5.12	$16.6 * 10^3$	1.0131
5	0.2	0.25	5.2	$4.71 * 10^3$	1.0082
5	0.2	0.5	5.19	$8.742 * 10^3$	1.01
5	0.2	1	5.42	$16.71 * 10^3$	1.0133
5	0.3	0.25	5.19	$4.86 * 10^3$	1.0087
5	0.3	0.5	5.2	$8.74 * 10^3$	1.0102
5	0.3	1	5.41	$16.91 * 10^3$	1.0136
5	0.4	0.25	5.24	$4.79 * 10^3$	1.009
5	0.4	0.5	5.21	$8.83 * 10^3$	1.0107
5	0.4	1	5.41	$16.94 * 10^3$	1.0143
5	0.5	0.25	5.22	$4.9 * 10^3$	1.0093
5	0.5	0.5	5.21	$8.75 * 10^3$	1.0112
5	0.5	1	5.13	$16.42 * 10^3$	1.014

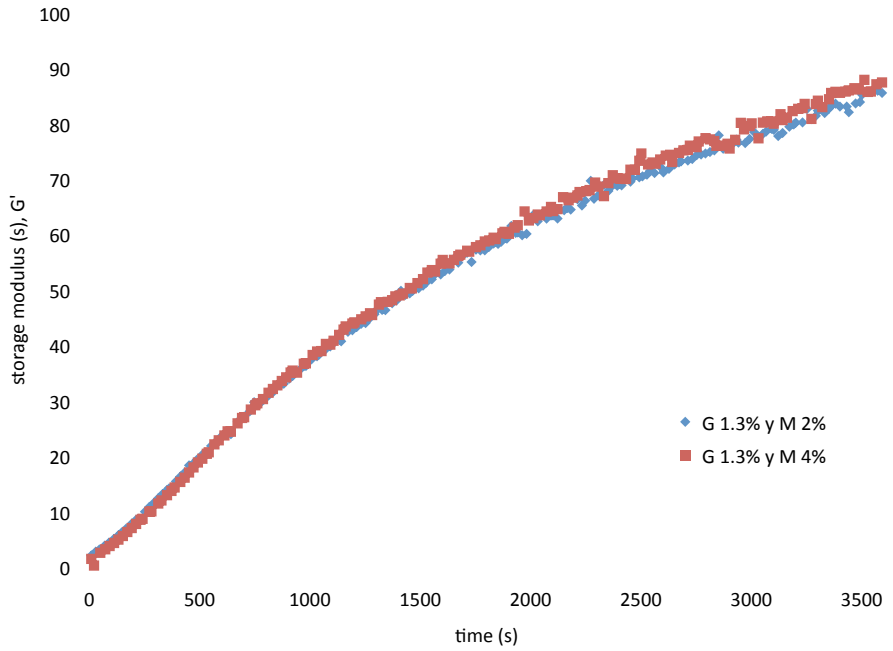

Figure A.1 Gel strength of gelatin 1.3 wt% containing 2 and 4 wt% of mannitol

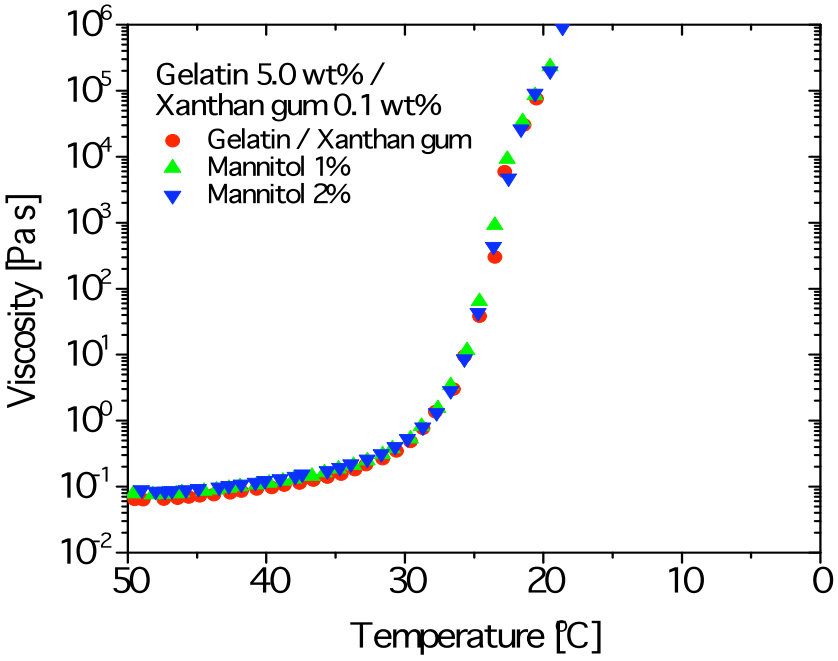

Figure A.2 Mannitol addition effect on gelatin (5 wt%)-xanthan (0.1 wt%)

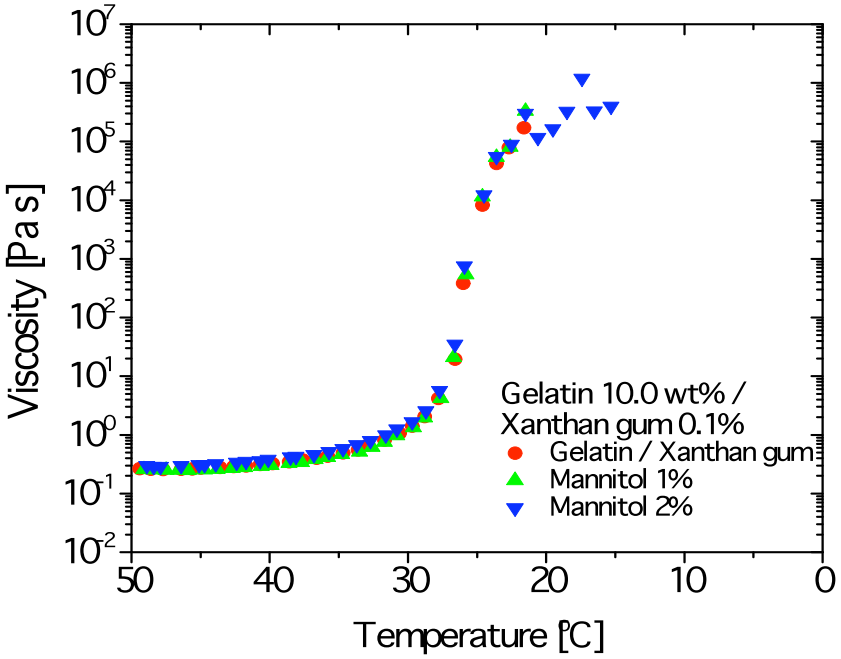

Figure A.3 Mannitol addition effect on gelatin (10 wt%)-xanthan (0.1 wt%)

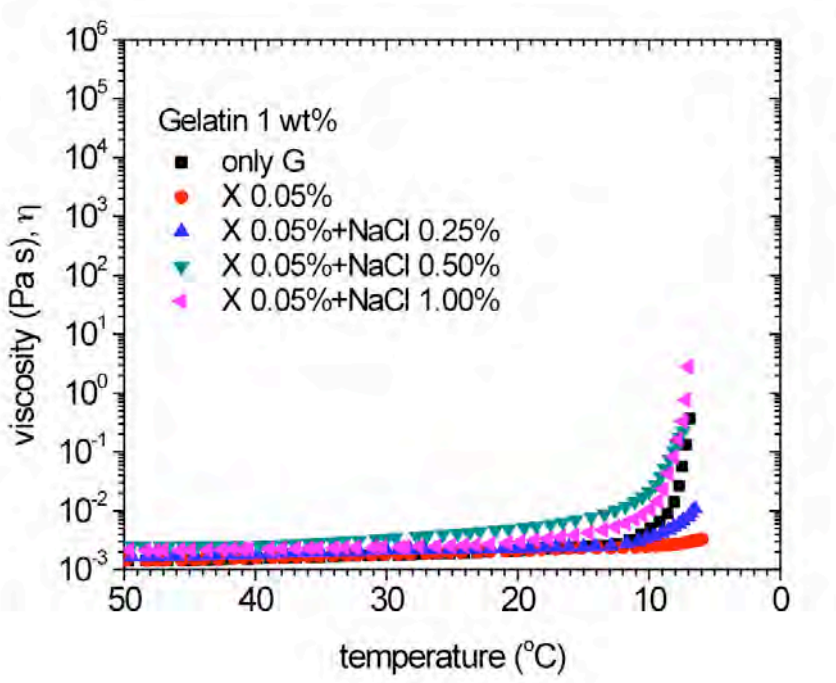

Figure A.4 Sodium chloride (NaCl) effect on the sol-gel transition of gelatin (1 wt%) - xanthan (0.05 wt%) mixture

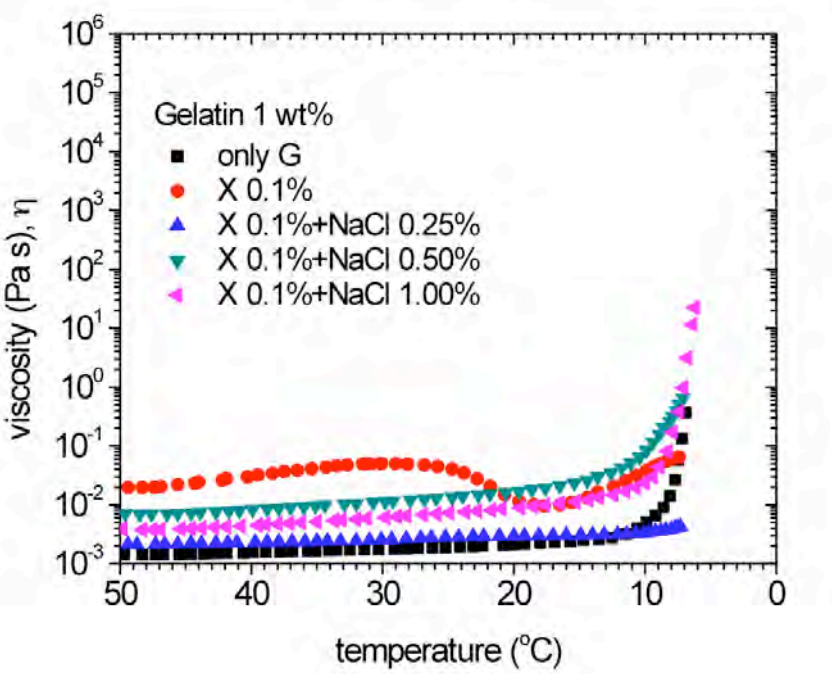

Figure A.5 Sodium chloride (NaCl) effect on the sol-gel transition of gelatin (1 wt%) - xanthan (0.1 wt%) mixture

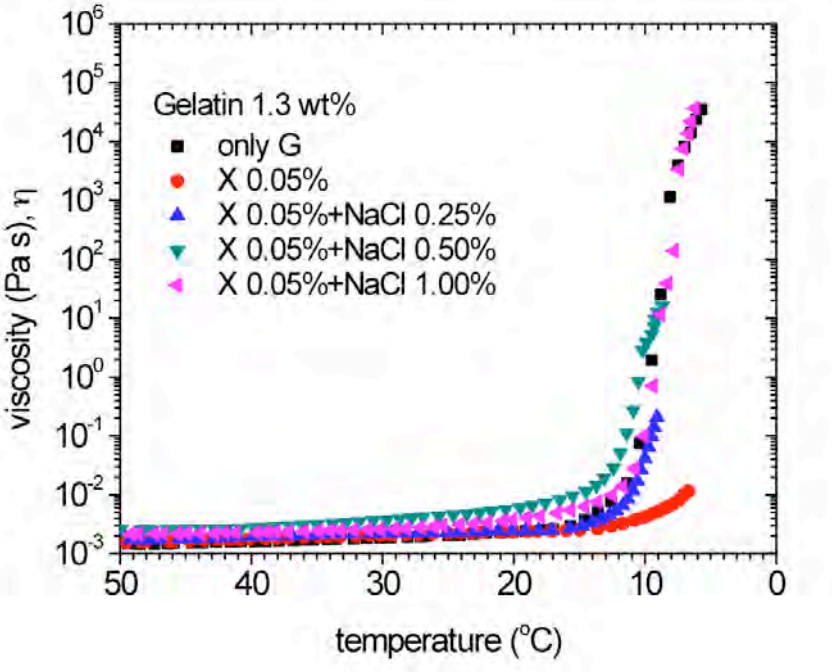

Figure A.6 Sodium chloride (NaCl) effect on the sol-gel transition of gelatin (1.3 wt%) - xanthan (0.05 wt%) mixture

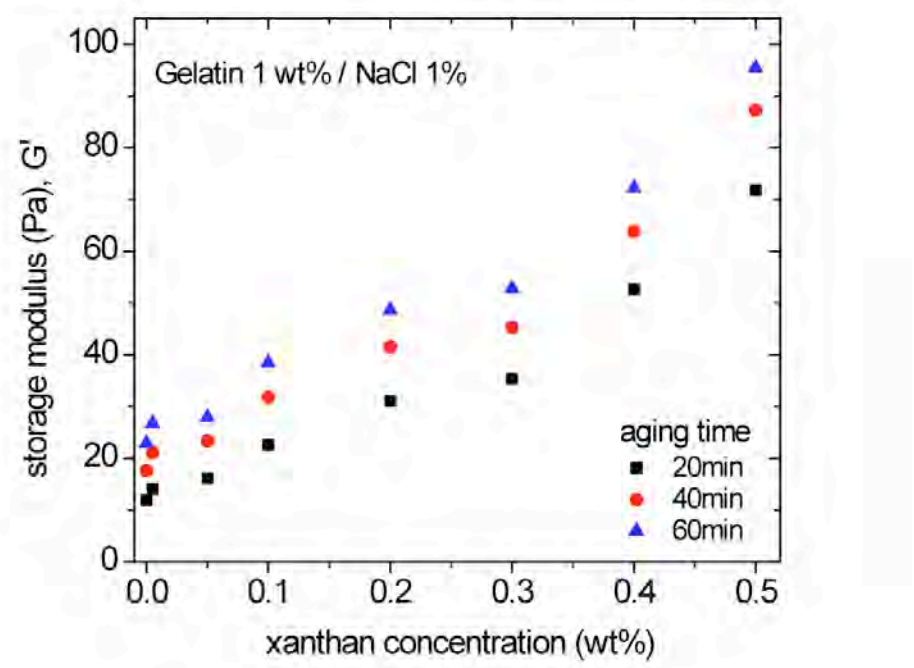

Figure A.7 Storage modulus (G') of gelatin 1 wt% with NaCl 1 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration

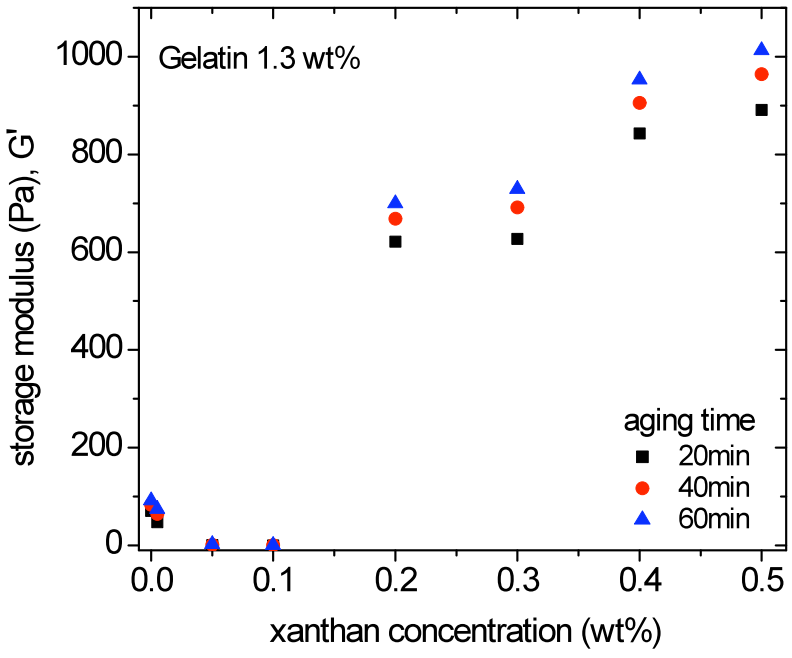

Figure A.8 Storage modulus (G') of gelatin 1.3 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration

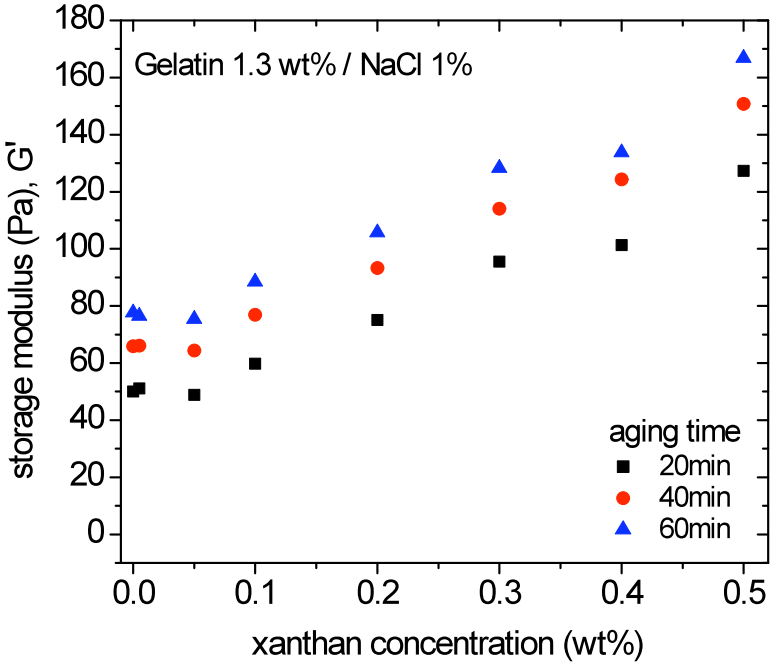

Figure A.9 Storage modulus (G') of gelatin 1.3 wt% with NaCl 1 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration

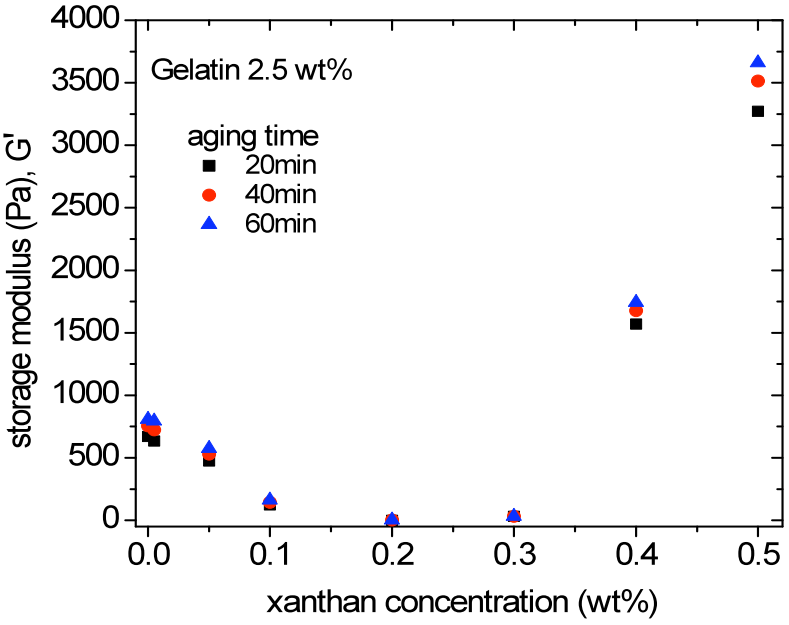

Figure A.10 Storage modulus (G') of gelatin 2.5 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration

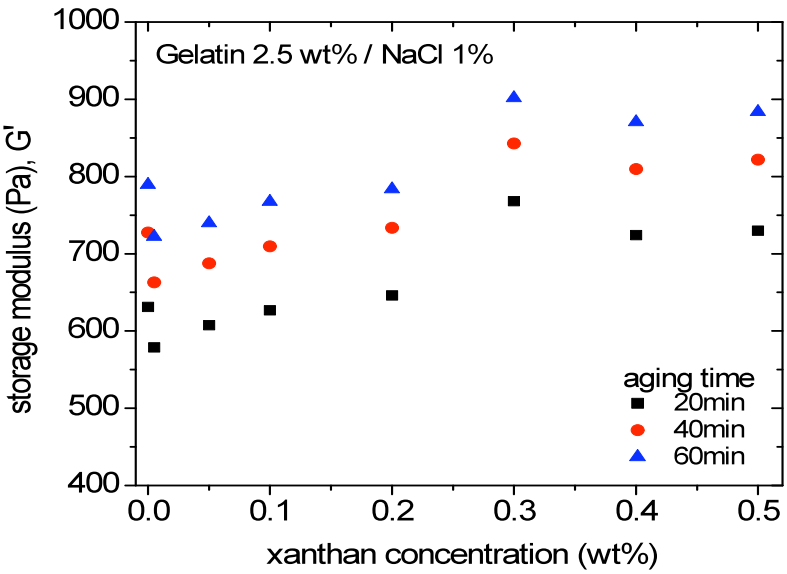

Figure A.11 Storage modulus (G') of gelatin 2.5 wt% with NaCl 1 wt% at aging times of 20, 40, and 60 minutes as a function of xanthan concentration