A Thesis

entitled

Rheological Properties and Decomposition Rates of Gellan Gum

by

Sulaiman Ali A. Dhameri

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Chemical and Environmental Engineering

Dr. Dong-Shik Kim, Committee Chair

Dr. Joshua Park, Committee Member

Dr. Maria R. Coleman, Committee Member

Dr. Cyndee Gruden, Dean
College of Graduate Studies

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An Abstract of

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Low acyl gellan gum (LA-GAGR) is a microbial polysaccharide that forms a viscoelastic gel when mixed with hyaluronic acid and β-tri- calcium phosphate (TCP). The effects of concentrations of the cross-linker (TCP) and LA-GAGR on the rheological properties and decomposition of the gels were studied. The degree of cross-linking of LA-GAGR was controlled with TCP content to determine its effects on the viscoelastic properties and release rate of LA-GAGR from the gel. The gels appeared thicker and opaquer with increasing TCP concentration. Three concentrations, 0.0 wt%, 0.5 wt%, and 1.0 wt% of TCP were examined with 0.15 wt% and 0.30 wt% of LA-GAGR. 5 wt% TCP is a maximum weight percentage that can be added to LA-GAGR, above which the TCP begins to precipitate out. Flow sweep tests showed that all tested gels are shear thinning and that the gel made with 1.0 wt% TCP and 0.30 wt% LA-GAGR displayed the highest viscosity with the experimental value of 675 Pa·s at 0.1 s^{-1}. However, gels made with 0.0 wt% TCP and 0.15 wt% LA-GAGR showed the lowest viscosity, 5.8 Pa·s at 0.1 s^{-1}. The gel made with 0.0 wt% TCP and 0.15 wt% LA-GAGR exhibited the lowest loss and storage moduli of 0.5 Pa and 0.056 Pa, respectively. The gel made with 1.0 wt% TCP and 0.30 wt% LA-GAGR exhibited the highest loss and storage moduli of 116 Pa and 437.8

Pa, respectively. The gels made with 0.30 wt% LA-GAGR and TCP concentrations of 0.5 wt% and 1.0 wt% showed typical viscoelastic behaviors. For the release rates of LA-GAGR the gels, the gel made with 1.0 wt% TCP and 0.30 wt% LA-GAGR exhibited the lowest average release rate of 0.10 mg h^{-1}, releasing all of LA-GAGR within 13 days. The low degree of crosslinking, due to the decrease of TCP, decreased the decomposition time of the gel to 8 days for the gel containing 0.30% LA-GAGR, 0.5% TCP. The properties of the gels varied depending on the method of mixing. When components were added gradually during mixing, the gel resulted in thicker and opaquer gels with slower decomposition time of 240 h compared to the gel formed via one time-addition of components decomposed in 216 h. It is concluded that, with a given LA-GAGR concentration, high TCP concentrations increase the degree of cross-linking of the polysaccharide, and therefore, the gel behaves more elastic and less fluidic, and decomposes slower.

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I would like to express my sincere gratitude to my advisor and committee chair Dr. Dong-Shik Kim for the continuous support, patience, guidance, motivation, and immense knowledge. I would like to thank my committee member Dr. Joshua Park for the useful comments, remarks and engagement through my studies. I am also very grateful for Dr. Maria Coleman for her efforts as a great professor and valuable advice. Furthermore, I wish to express my deep gratitude for the faculty and staff of the Department of Chimerical Engineering at the University of Toledo for providing excellent working conditions. Special thanks to Abdullah Baawad, Surachet (Ray) Duanghathaipornsuk, Abdelbaset Ali, Ehsan Fakhrabadi, and Haidar Alolaywi for their valuable insight and support. I wish to also express my deep appreciation for Drs. Ana Alba-Rubio, Matthew Liberatore, and Yakov Lapitsky for valuable suggestions which have contributed greatly to the improvement of this thesis. Finally, I would like to express my appreciation for friends and family who supported. I am very thankful to my wife Anastasiia and my daughter Thuraya for their patience, continuous encouragement, and valuable insight.

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List of Abbreviations

GAGR	Glucose, Glucuronic Acid, Glucose, Rhamnose
HA	Hyaluronic Acid sodium salt
HPSEC	High Pressure Size Exclusion Chromatography
LA	Low Acyl
TCP	β-Tri-Calcium Phosphate
TMACl	Tetra Methyl Ammonium Chloride

Chapter 1

Introduction

1.1 Motivation

Millions of people worldwide suffer from osteoporosis. High treatment cost and multiple side effects that of the available treatment encouraged the development of more effective and affordable treatment option. Low acyl gellan gum appears to be a safe, effective in enhancing the bone growth without significant side effects, strong osteogenic and antigenic properties. The aim of the current study is to investigate the rheological properties and decomposition rates of gels in terms of the concentrations of cross-linker (TCP) and LA-GAGR.

1.2 Orthopedic Background

Approximately 200 million people worldwide are diagnosed with osteoporosis. Annually, there are about 8.9 million osteoporotic fractures, with a bone fracture occurring every three seconds [1]. Osteoporosis comes from Greek "*osteo*" means bone and "*por*" referring to passage. Structural framework of a healthy bone is made up of calcium, magnesium, phosphate, various minerals, and collagen [2]. Healthy bone tissue constantly breaks and regenerates new cells. However, when the cells are destroyed faster than they are rebuilt, bone becomes thinner and less dense. Loss of minerals and calcium from the bone decreases bone density and results in thinner more fragile bone tissue. As shown in Figure 1-1, healthy femur on the left appears to have denser and less porous framework compared to the osteoporotic femur on the right. Contrary to the healthy bone which has sponge like structure as shown, osteoporotic bone has wide pores and thinner connecting bone, thus making fragile and brittle osteoporotic bone more susceptible to fractures.

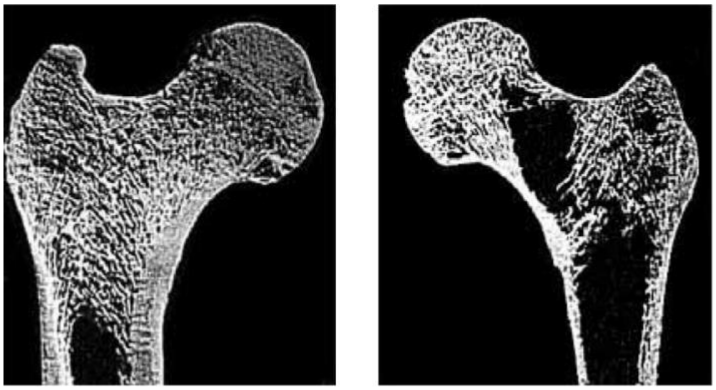


Figure 1-1: Healthy femur (left) vs osteoporotic femur (right) [3].

Elderly population is at higher risk of having osteoporosis. As we age, more bone tissue is lost due to the cells being rebuilt at a slower rate than they are removed [4]. This is due to the fact that older people are more likely to suffer from vitamin D deficiency due to multiple risk factors such as reduced skin thickness, limited sunlight exposure, lower dietary intake, damaged intestinal absorption, and hydroxylation in kidneys and liver [5]. Calcium and vitamin D are indispensable to bone health. Vitamin D helps bone to absorb calcium, which in turn is used to build strong bone framework. Therefore, vitamin D deficiency results in lower bone density and osteoporosis. Women over fifty are four times as likely to have osteoporosis as their male peers [6]. By the age of thirty both men and women build more bone cells than he or she loses, and once they turn thirty-five, bone cell growth gradually declines, and more cells are destroyed than regenerated.

Healing of osteoporotic bone fractures remains a major challenge in medical community. While there have been many major therapeutic improvements in the past decades, osteoporotic fractures remain significant clinical obstacle. Growing number of patients undergo surgery in order to repair fractured bones, and bone drafts are often use to enhance and stabilize the orthopedic implant. Consequently, various studies have been conducted in order to find a more suitable and cost-effective osteogenic agent with fewer side effects and longer plasma half-life. Strong osteogenic and angiogenic properties of low acyl gellan gum (referred to as LA-GAGR throughout this thesis) were discovered in the collaborative research between Drs. Dong-Shik Kim (Chemical Engineering) and Joshua Park (Neurosciences). Moreover, absence of harmful side effects, low production cost, and long plasma half-life of LA-GAGR make it suitable for pharmaceutical use.

1.3 Low acyl gellan gum background

1.3.1 Structure of Low acyl gellan gum

Gellan gum was first discovered and isolated by Kelco Division of Merck & Company, Inc. in 1978 from the Lily plant in Pennsylvania [7]. It is a naturally occurring polymer, produced as a linear extracellular polysaccharide by *Sphingomonas elodea*. Gellan gum is a linear, anionic heteropolysaccharide, consisting of a tetrasaccharide containing the repeating units of D-glucose, glucuronic acid, and L-rhamnose :[(1,3)-β-

D-Glc*p*-(1,4)-*β*-D-Glc*p*A-(1,4)-*β*-D-Glc-(1,4)-α-L- Rha-(1→)], As shown in figure 1-2, containing two acyl substituents. Depending on the degree of acyl substitution, gellan gums can be categorized as high acyl (HA) gellan gum and low acyl (LA) gellan gum. The presence of counter ions and degree of acylation are determining factors for the gellan properties, since the substituents in gellan interfere with aggregation process. The native form of gellan gum also known as high acyl (HA) contains two acyl substituents, acetate and glycerate, that are located on the glucose residues [8]. Acetyl and glyceryl are bulky substituents, thus the gellan polymer chains are unable to form close double-helix formation between them. As a result, HA gellan gels are weak, soft, and elastic [9]. On the other hand, LA gellan gums that are firm, non-elastic, and brittle [10]. Their ability to form three-dimensional networks, in the presence of alkali metals or alkali earth metal cations, which are capable of imbibing water or other biological fluids, made them particularly useful in pharmaceuticals [10, 12]. Furthermore, when cooled, LA gellan gum disordered coil rearranges to form double helix formation [9]. Consequently, controlled drug release can be achieved by controlling the crosslinking degree. Controlled release of drugs has been used for oral drug delivery, ophthalmic and nasal formulations without significant side effects [13,14].

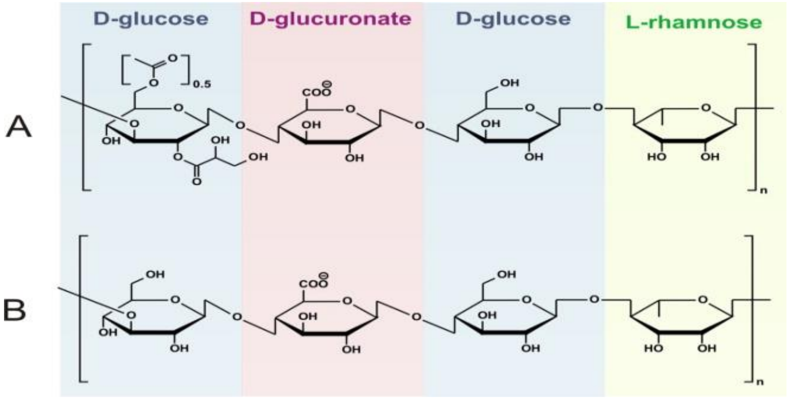


Figure 1-2: The structure of native (a) and low acyl (b) gellan gum [11].

Properties of LA-GAGR need to be controlled for easier injection and local stability. Rheological properties and decomposition rates are controlled with the contents of LA-GAGR and cross-linker, tricalcium phosphate (TCP). TCP was used to crosslink LA-GAGR as shown in Figure 1-3.

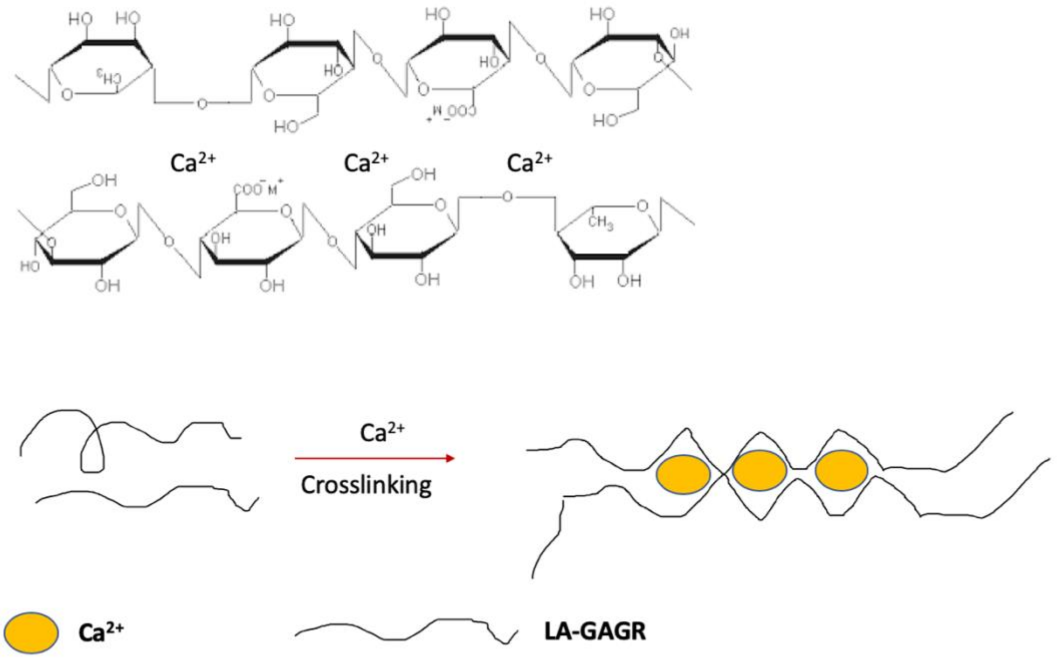


Figure 1-3: Schematic of the formation of TCP crosslinking and LA-GAGR [17][18].

1.3.2 Rheological Properties of Low Acyl Gellan Gum Gels

LA-GAGR was mixed with hyaluronic acid (HA) and β-tricalcium phosphate (TCP) to form a gel. The rheological properties of the gel were measured, and the results of oscillation frequency tests were analyzed. G′ and G″ moduli were determined by starting with the following equation:

$$\frac{\tau}{\gamma} = \frac{(\tau_o cos\delta)sin\omega}{\gamma} + \frac{(\tau_o sin\delta)cos\omega}{\gamma} \tag{1}$$

And substituting $\frac{\tau}{\gamma} = G$, $\frac{(\tau_o cos\delta)}{\gamma} = G'$, and $\frac{(\tau_o sin\delta)}{\gamma} = G''$ which yields,

$$G = G'sin\omega + G'' cos\omega \tag{2}$$

, where ω is the frequency; δ is the phase lag between stress (τ) and strain (γ); G″ represents the loss modulus, which measures energy loss, and it is the out-of-phase component; and G′ represents the storage modulus, which stores elastic energy, and it is the in-phase component of the modulus. G″ and G′ moduli are described as the ratio of the stress and the strain amplitudes [15]. The flow sweeps measured the dynamic viscosities of LA-GAGR gels as a function of frequency. G″ and G′ were tested in order to determine how elastic and viscous the gels.

1.3.3 Decomposition rate of LA-GAGR gel

Depending on the TCP concentration, the LA-GAGR gel decomposed at different rates. It is hypothesized that the cross-linker, TCP, slowly leaches out to DI water, and as a result, the gel is decomposed as the cross-linking of gel is loosened.

Chapter 2

Materials and Methods

2.1 Preparation of LA-GAGR gel

Low acyl gellan gum (LA-GAGR) was purchased from CP Kelco (USA). β-tricalcium phosphate (TCP), hyaluronic acid sodium salt (HA) and tetramethylammonium chloride (TMACl) were purchased from Sigma-Aldrich (USA). As shown in Figure 2-2 a 0.5 wt% HA solution was prepared by gradually adding 200 mg of HA in 40 ml water and heated to 85 °C while stirring for 20 min. Upon complete dissolution, 120 mg of LA-GAGR was added gradually to make a 0.30 wt% solution and stirred until a homogeneous solution was obtained. Then, 400 mg of TCP was added gradually to make a 1.0 wt% mixture and stirred until a white gel was obtained. The gel was left to cool down to room temperature while stirring. Additionally, five more gels were made. Two more gels were prepared with 0.0 and 0.5 wt% TCP while other components remained the same. The other three gels were made with 0.0, 0.5, and 1.0 wt% TCP and LA- GAGR of 0.15 wt%. The HA amount was constant for all gels. Two different methods of mixing were investigated to better understand the effects of mixing methods on the gel properties. The first gel was prepared by gradually adding 200 mg of HA in 40 ml water and heated to 85 °C while stirring for 20 min. Upon complete dissolution, 120 mg of LA-GAGR was added gradually to make a 0.30 wt% solution and stirred until a homogeneous solution was obtained. Then gradually adding TCP into the solution of 0.30 wt% LA-GAGR and 0.5 wt% HA until the TCP concentration reached 5 wt%. The second gel was prepared by added at once of 200 mg of HA in 40 ml water and heated to 85°C while stirring for 20 min. Upon complete dissolution, 120 mg of LA-GAGR was added at once to make a 0.30 wt% solution and stirred until a homogeneous solution was obtained. Then, TCP was added at once to make 5 wt%. All weight percentage based on the initial water volume. Above 5 wt% TCP, the TCP begins to precipitate and therefore 5 wt% TCP was chosen as the upper limit of this study and the lower limit is without TCP. Two different weight percentages of LA-GAGR, 0.15 wt% and 0.30 wt%, were added in order to study the gel behavior. Increasing LA-GAGR weight percentage more than 0.30 wt% makes the gel too thick to be injected. On the other hand, gels prepared with less than 0.15 wt% LA-GAGR are easy to flow and would not fit the end goal (i.e., localized gel injection for bone fracture incidents) of this research project, which this thesis is only a part of. Three different weight percentages of TCP were added in order to see the gels behavior with increasing TCP weight percentages.

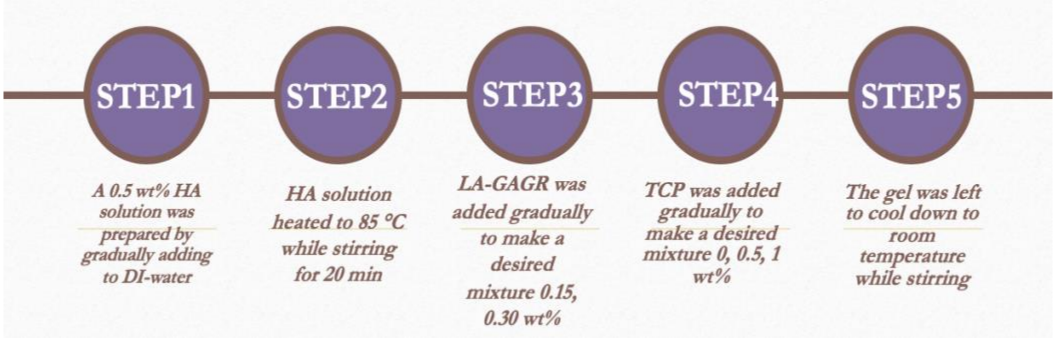


Figure 2-1: Preparation of LA-GAGR gel.

2.2 Oscillation Frequency and Flow Sweep

The prepared gels were tested using a Discovery Hybrid Rheometer (TA Instruments, USA). The gel was placed between parallel plates with 40 mm diameter. All tests were run in the linear viscoelastic region at 25 °C. Oscillation Frequency and Flow Sweep were used in order to determine rheological properties of the gels.

2.3 Decomposition of the gels

As shown in Figure 2-2 the gels were weighed and then added to flasks containing 80 ml DI water, incubated at 37 °C and shaken at 60 rpm. After 240 min shaking, the gels were taken out of the flasks, gently tapped to remove the extra liquid on the surface of the gel and weighed. Afterward, the gels were placed in flasks with fresh DI water, and the procedure was repeated every 24 h until all the gels completely decomposed. The used DI water from which the gel was removed was collected in a beaker. The collected DI water was vacuum dried at 37 °C until it reduced to 40 ml in order to verify that the decomposition occurred due to leaching of TCP into DI water.

High-pressure size exclusion chromatography (HPSEC) was used to measure the amount of LA-GAGR released from the gel. An Agilent PL aqua gel column (7.5×300 mm) with mixed pore sizes and an average particle size of 8 μm was used. The mobile phase was TMACl at a concentration of 0.075 M and at 40 °C. The sample of 50 μL was injected at a flow rate of 1.0 ml/min. A standard curve of LA-GAGR was constructed by serial dilutions of 1.5 mg/ml stock solution.

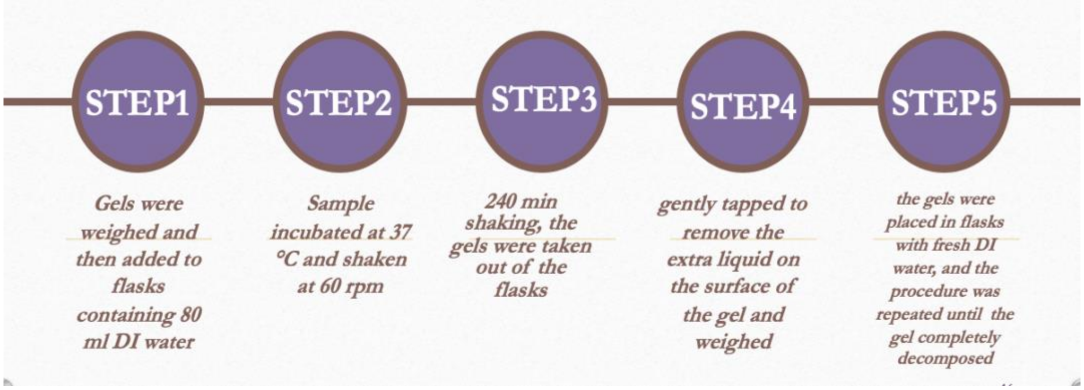


Figure 2-2: Decomposition of LA-GAGR gel.

2.4 Decomposition Rates

The gels were placed in a 250 ml flask filled with 80 ml of DI water, used as a release medium. The flask was kept at 37 °C while shaking at 60 rpm. For the initial 24 h, samples of 1 ml were withdrawn every 4 h of shaking, using a pipette, and replaced with an equal volume of DI water. After that, samples were taken every 24 h. The samples were analyzed for the amount of LA-GAGR in the samples using HPLC. The cumulative LA-GAGR released into the bulk water was calculated using Equation 3 and plotted in terms of time:

$$\text{Cumulative LA-GAGR released (\%)} = \frac{m_r}{m_t} \times 100 \qquad (3)$$

, where m_r is the mass of LA-GAGR released and m_t is the initial mass of LA- GAGR gel.

Chapter 3

Results and Discussion

3.1 Gel Appearance

The gels shown in Figure 3-1 (a) were made with gradual addition of TCP to 0.15 wt% LA-GAGR, 0.5 wt% HA. The gel on the left was prepared without TCP. The absence of TCP made the gel have liquid consistency and, it was the most fluidic among the prepared gels. The gel prepared with 0.5 wt% TCP appeared to have a thicker consistency and was less fluidic than the 0 wt% TCP gel. The gel made with 1.0 wt% TCP was the thickest, had the most gel-like appearance, and was the least fluidic of all. Therefore, the addition of TCP enhanced the gel-like appearance of the compound.

The compounds demonstrated in Figure 3-1 (b) were made with gradual addition of TCP to 0.30 wt% LA-GAGR and 0.5 wt% HA. The compound on the left, made in the absence of TCP had a water-like consistency. The compound prepared with 0.5 wt% TCP and 1.0 wt% TCP were thicker and more fluidic. Thus, like 0.15 wt% LA-GAGR shown in Figure 3-1 (a), the addition of TCP to 0.30 wt% LA-GAGR enhanced the gel-like appearance of the compound.

Figure 3-1 (c) shows two gels prepared with 0.30 wt% LA-GAGR, 0.5 wt% HA, and 5.0 wt% TCP. The gel on the left was formed by gradually adding all the components, whereas the gel on the right was formed by adding individually each compound all at once to DI water. Both gels appeared thick and opaque, but the one made by the gradual addition of components (left) seems to have more solid-like consistency than the one on the right. All the experiments below were performed using the gels prepared by gradual addition of components.

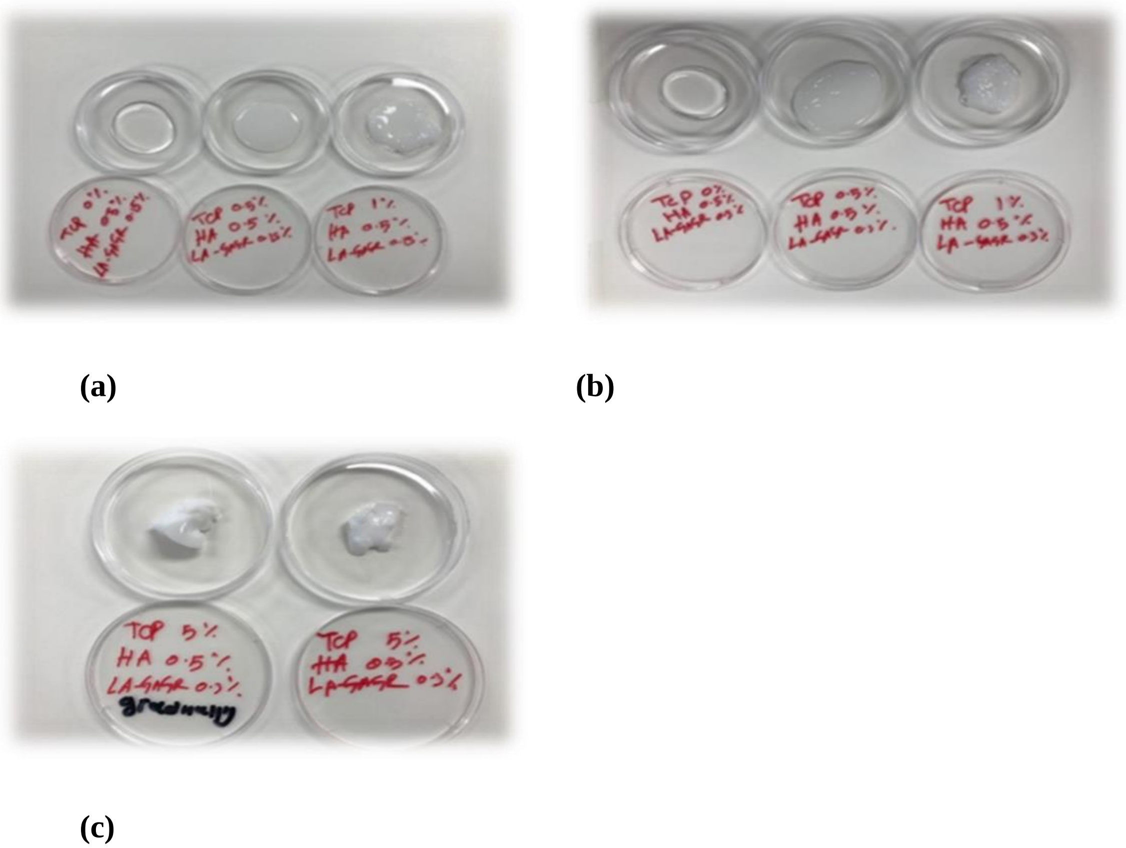


Figure 3-1: Appearance of gels made with (a) 0.15 wt% LA-GAGR, (b) 0.30 wt% LA-GAGR, and (c) 0.30 wt% LA-GAGR made with gradual addition (left) and one-time addition (right) of 5.0 wt% TCP.

3.2 Flow Sweep

Figure 3-2 shows the results of the dynamic viscosity measurements. The gels prepared with gradual addition of components to make 0.0, 0.5, and 1.0 wt% TCP and 0.15% LA-GAGR were tested to determine the effects of the cross-linker on the viscosity of the gels. All gels exhibited shear thinning behavior. At a shear rate of 0.1 s^{-1}, the viscosity of the gel with 0.0 wt% TCP is 15.8 Pa·s, while the viscosity of the gels containing 0.5 wt% and 1.0 wt% TCP are 22.7 Pa·s and 34.6 Pa·s, respectively. The viscosities of these gels decrease at the same rates with the shear rate. Overall, it was determined that the viscosity of the gel increases with the TCP concentration.

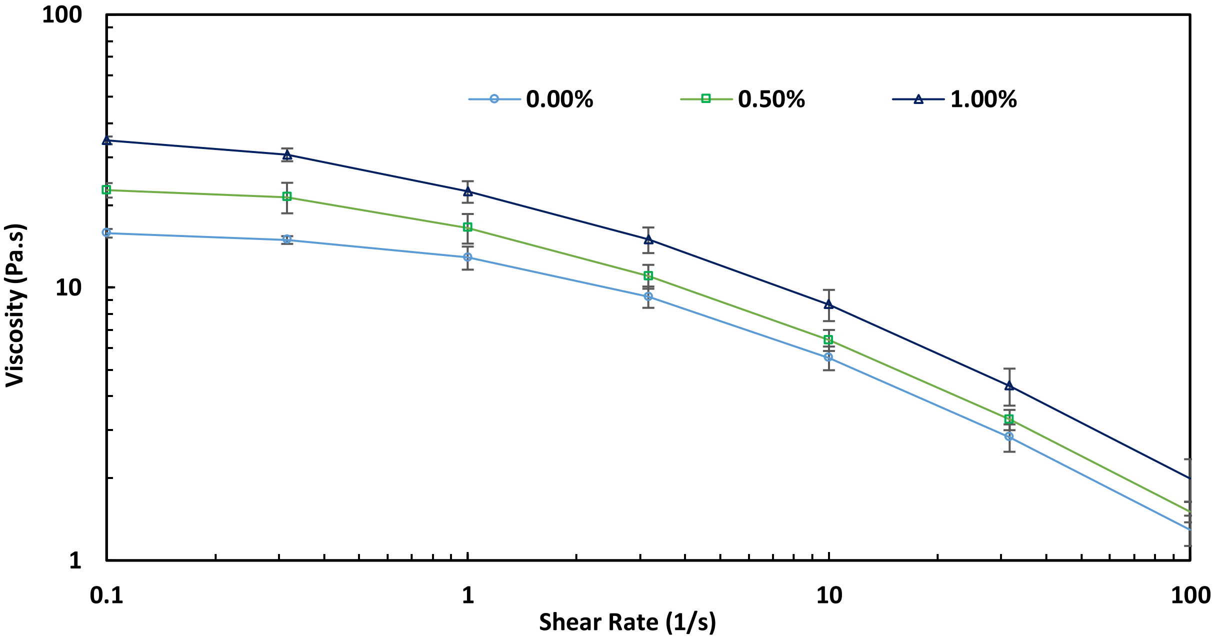


Figure 3-2: Flow sweep of the gradually added 0.15% LA-GAGR.

Figure 3-3 shows the results for 0.30 wt% LA-GAGR. The viscosities of gels with gradual addition of 0.0, 0.5, and 1.0 wt% TCP were measured. All tested gels are shear thinning. At a shear rate of 0.1 s^{-1}, the viscosity of the gel containing 0.0 wt% TCP is 24

Pa·s, while the viscosities of the gels with 0.5 and 1.0 wt% TCP are 32 Pa·s and 675 Pa·s, respectively. The viscosity of the gel with 1.0 wt% TCP is much higher than the other two because more crosslinking was formed. As the shear rate increases to 100 s^{-1}, the viscosity of the gel with 1.0 wt% TCP decreased more rapidly than those of the gels with 0.0 and 0.5 wt% TCP whose viscosities decreased at the same rates. The rapid decrease in the viscosity of the 1.0 wt% TCP gel at low shear rates is thought to be due to the greater degree of cross-linked LA-GAGR chains that are aligned more easily than the other gels with lower degrees of cross-linking [16]. Additionally, it is noted that the gel with 0.30 wt% LA-GAGR resulted in almost a factor of 20 increase in viscosity (675 Pa·s) compared to the gel with 0.15 wt% LA-GAGR (34.6 Pa·s). At shear rate of 1 s^{-1}, the viscosities of 0.15 and 0.30 wt% LA-GAGR with 1.0 wt% TCP were 22.5 Pa·s and 202.2 Pa·s, respectively, which indicates a greater number of polymeric chains were crossed-linked.

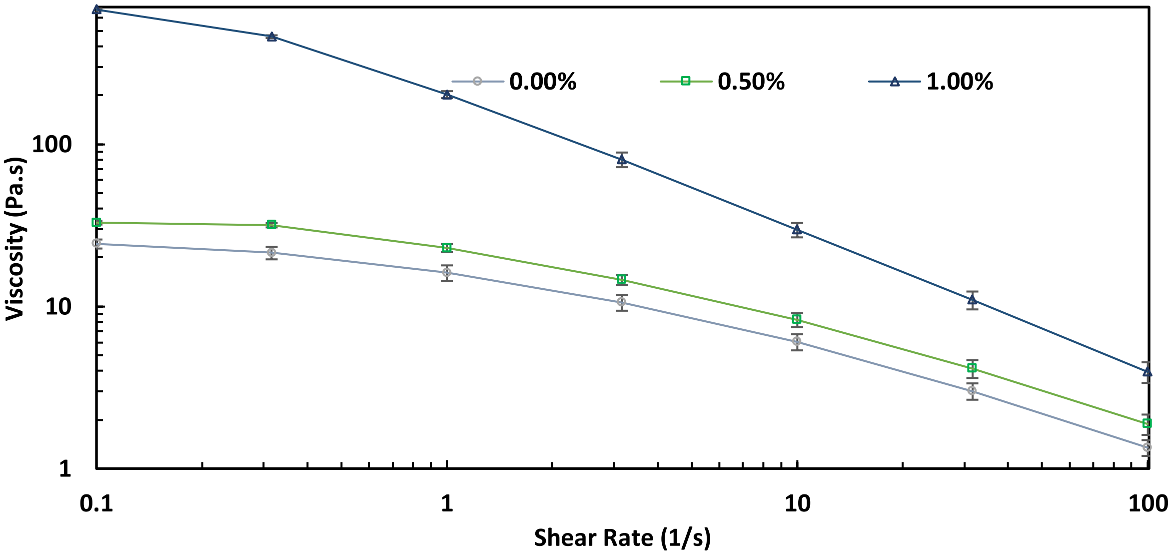
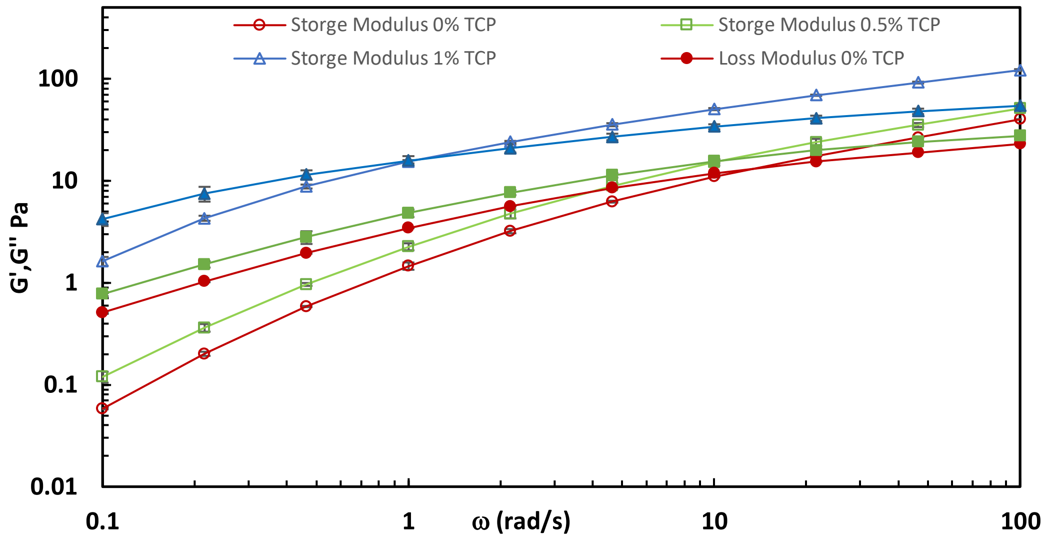


Figure 3-3: Flow sweep of gradually added 0.30% LA-GAGR.

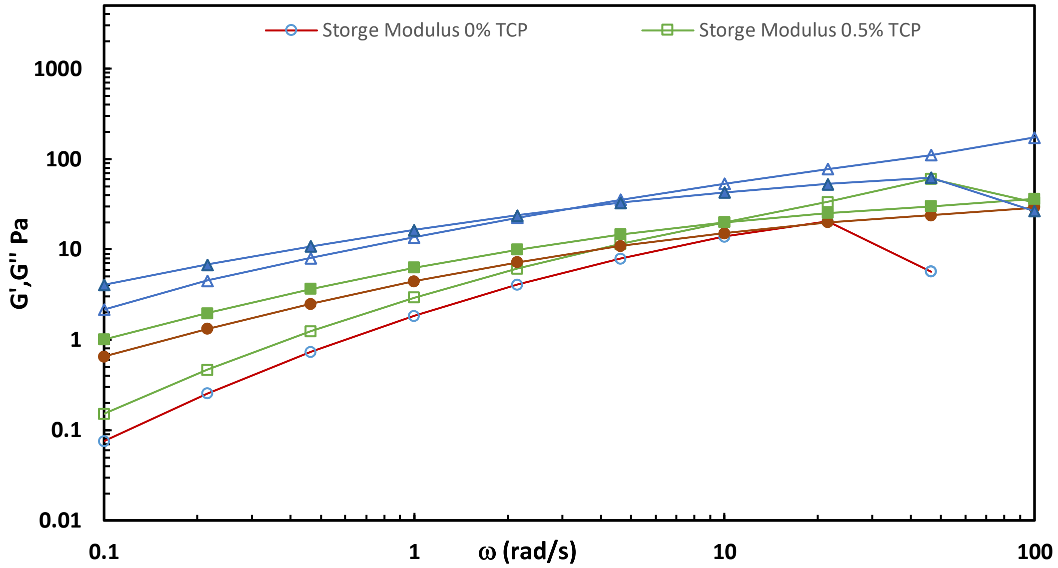
3.3 Oscillation Frequency

The oscillation frequency test was done using two geometries, 40 mm (Figure 3-4 (a)) and 20 mm (Figure 3-4 (b)). As shown in Figure 3-4, at 0.1 rad s^{-1}, the G' values are 0.056, 0.12, and 1.6 Pa for 0.0, 0.5, and 1.0 wt% TCP, respectively. The G' values of the gels increase in parallel, and at 100 rad s^{-1}, the G' values are 40, 51, and 121.5 Pa for the 0.0, 0.5, and 1.0 wt% TCP gels, respectively.

At 0.1 rad s^{-1}, G" values are 0.5, 0.8, and 4.2 Pa for the 0.0, 0.5, and 1.0 wt% TCP gels, respectively. For the gel with 0.0 wt% TCP, the G" curve is higher than G' until angular frequency of 15 rad s^{-1} indicating that it behaves more like a viscous liquid than an elastic gel until 15 rad s^{-1}. When the oscillation frequency increases above 15 rad s^{-1}, the curves switch, and it shows more elasticity. The same trend is observed at 10 rad s^{-1} and 1.3 rad s^{-1} for the 0.5 and 1.0 wt% TCP gels. The switch between the G' and G" curves occurs at lower frequencies as the TCP concentration increases. At high TCP concentrations, LA-GAGR is cross-linked more, and as a result, the gel becomes more elastic at low frequencies. Moreover, when performing the test using a 20 mm plate, which could explain the deformation observed only when using the 20 mm plate and not the 40 mm plate, at the same frequency of 21.5 0.0 wt% and 46 rad s^{-1} of 0.5, and 1.0 wt% TCP. When compared to 40 mm plate, the values of G' and G" increased moderately for all three compounds. However, a similar trend was observed in the crossing points between G' and G". The 0.0 % TCP at the crossing point between G' and G" at 21.5 rad s^{-1} where G' value drops. A smaller amount of the compound is needed, and thus less polymeric chains, when performing the test using a 20 mm plate, which could explain the deformation observed only when using the 20 mm plate and not the 40 mm plate, at the same frequency of 21.5 rad s^{-1}.



(a)



(b)

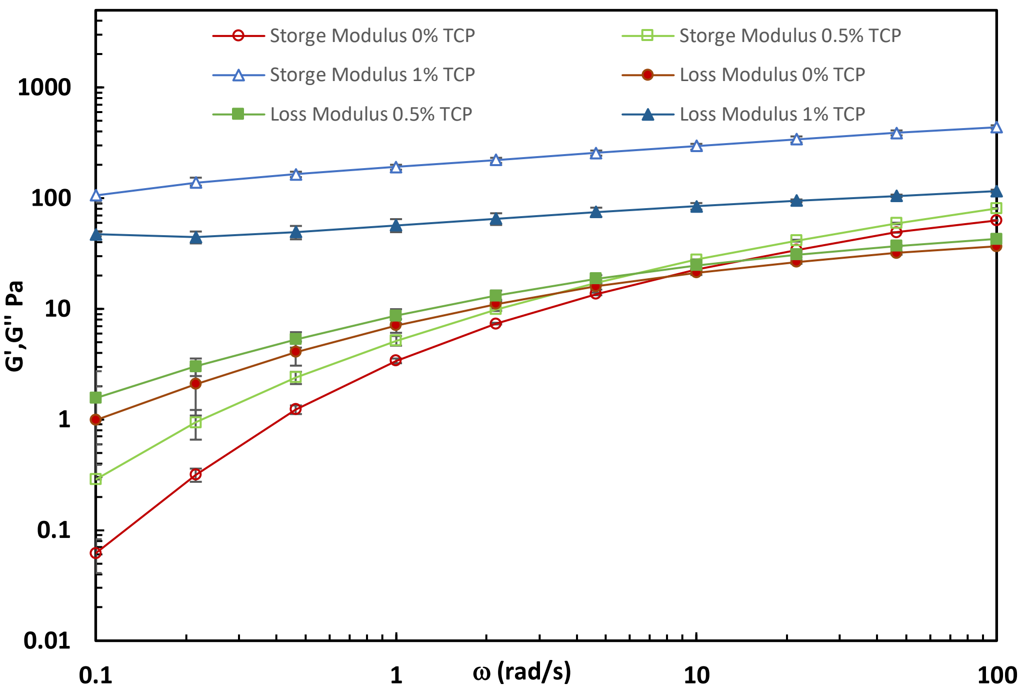
Figure 3-4: G' and G" from oscillation frequency tests for gradually added 0.15% LA-GAGR a) 40 mm plate b) 20 mm plate

Figure 3-5 (a) shows the results of using 40 mm plate geometry. It shows the G' and G" curves of the gels containing 0.30 wt% LA-GAGR, 0.5 wt% HA and a varied amount of TCP. Compared to the 0.15 wt% LA-GAGR shown in Figure 3-4, 0.30 wt% LA-GAGR behaves much different from the 0.15 wt% LA-GAGR gel. First of all, at 0.1 rad s^{-1} , the G' values are 0.06, 0.29, and 106 Pa for the 0.0, 0.5, and 1.0 wt% TCP gels, respectively, as compared to 0.056, 0.12, and 1.6 Pa for the 0.15 wt% gel in Fig. 5. The G' of the 1.0 wt% TCP gel seems almost independent on the frequency, which represents typical characteristics of a viscoelastic gel, compared to the significant increases observed in the 0.0, and 0.5 wt% TCP gels as frequency increases. At 100 rad s^{-1}, the G' values are 62.9, 80.8, and 437.8 Pa for the 0.0, 0.5, and 1.0 wt% TCP gels, respectively, where they are 40, 51, and 121.5 Pa for the 0.15 wt% LA-GAGR gels.

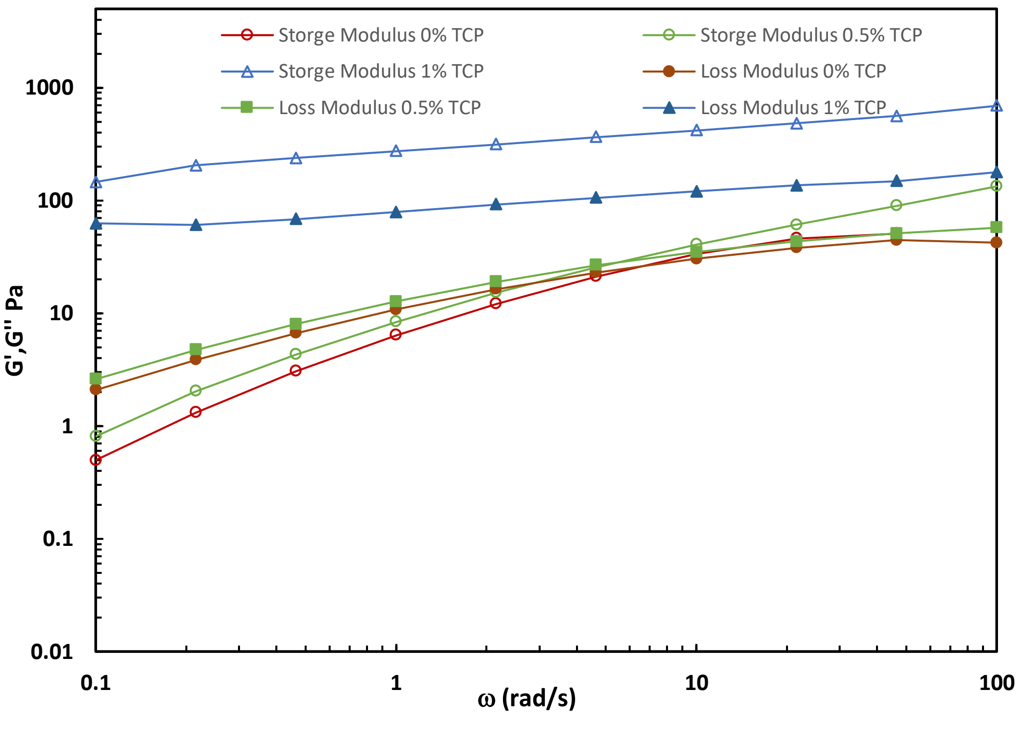
G" values for the 0.30 wt% gels are also greater than those of 0.15 wt% gels. At 0.1 rad s^{-1}, the G" values are 1, 1.6, and 47.3 Pa for the 0.0, 0.5, and 1.0 wt% TCP gels, respectively. Like G', the G" of the 1.0 wt% TCP gel is also almost independent of frequency, compared to the 0.0 and 0.5 wt% TCP gels. The G" values increase to 36.8, 42.9, and 101.06 Pa for 0.0, 0.5, and 1.0 wt%, respectively. The 1.0 wt% TCP gel is more elastic than viscous in the entire frequencies. The 0.0 and 0.5 wt% TCP gels are more like a viscous liquid than elastic gel until about 8.5 and 6.5 rad s^{-1}, respectively, above which they become more elastic gels. The cross-over between the G' and G" curves for the 0.0 and 0.5 wt% TCP gels with 0.30 wt% LA-GAGR occur at 8.5 and 6.5 rad s^{-1}, which occur earlier than those of the 0.15 wt% LA-GAGR gels (15, 10, 1.3 rad s^{-1} for 0.0, 0.5 and 1.0 wt% TCP, respectively). The more LA-GAGR and TCP are included in the gel, the more it behaves like an elastic gel due to high degrees of cross-linking. In viscoelastic polymers, the work of deformation is partially stored as potential energy, and the rest is transformed to heat and appears as mechanical damping [15]. Also, dynamic viscosity can be found by dividing G″ by the frequency [15]. When G′ < G″, the gel acts more viscous than elastic, and if G′ > G″, the gel elastic character becomes dominant [15]. The ratio of G″ to G′ in a viscoelastic material is represented as tan (δ), which offers a measure of damping in the materials. As tan (δ) increases, the damping value also increases. This happens when the loss modulus of the material is greater than the storage modulus [15]. A small tan (δ) value is an indication of a solid behavior, while a large value indicates a liquid behavior.

Figure 3-5 (b) shows the results of using 20 mm plate geometry. When compared to 40 mm plate, the values of G′ and G″ moderately increased for all three compounds. However, the 1% TCP still exhibited the same elastic behavior beyond 100 rad s^{-1}. Compounds containing 0.5% and 0% TCP also exhibited similar transition from viscous behavior to an elastic behavior at almost the same frequency when the 20 mm plate was used, i.e., 4.6 rad s^{-1} and 10 rad s^{-1} for 0.5% and 0.0% TCP, respectively.

Increasing LA-GAGR from 0.15% to 0.30% resulted in a significant increase of G’ and G” values for all three TCP concentrations. For 1% TCP, at 0.1 rad s^{-1}, G′ and G″ values are 1.6 Pa and 4 Pa, respectively, for 0.15% LA-GAGR, while G′ and G″ values are 106 Pa and 47.3 Pa, respectively, for 0.30% LA-GAGR. A greater dependence on frequency is exhibited by the 0.15% than 0.30% LA-GAGR, which scarcely depended on frequency. The greater dependence on frequency indicates a smaller number of cross-linked polymeric chains.



(a)



(b)

Figure 3-5: Oscillation Frequency of 0.30% LA-GAGR (a) 40 mm plate (b) 20 mm plate

Two different methods of preparing the compounds were investigated using a 20 mm diameter plate, added individually each component all at once of, and gradual addition Figure 3-6. At 0.1 rad s^{-1}, G′ values are 1192.4 Pa and 412.4 Pa for gradual and one-time addition, respectively. While G″ values are 412.4 Pa and 152.5 Pa for gradual and once addition, respectively. Both gels exhibited moderate dependence on frequency. At 100 rad s^{-1}, G′ values are 2499.3 Pa and 1130.8 Pa for gradual and added all at once, respectively. While G″ values are 414.2 Pa and 184.4 Pa for gradual and added all at once, respectively, the gradual addition method shows that the G′ and G″ values can be increased, without changing the composition, which is attributed to a greater cross-linking of the polymeric chains due to an enhanced mass transport. In the frequency range of 0.1-1 rad s^{-1}, G” values are decreasing, which means that the dissipated energy is decreasing, as the frequency increases. The low frequency gives more time of relaxation and thus more energy can be stored rather than dissipated. The decrease in the dissipated energy (G”) is accompanied with an increase in the storage modulus (G’), which means that the stored energy is increasing in the same frequency range. The frequency of 1 rad s^{-1} appears to be the limit after which the stored (G’) and the dissipated (G”) energies are increased with increasing frequency.

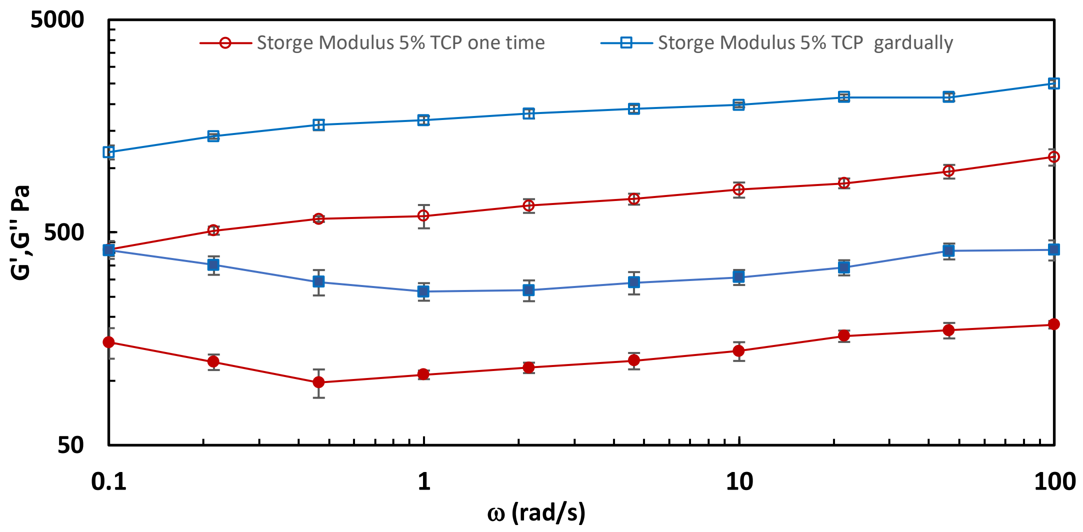


Figure 3-6: Oscillation Frequency Test of two different methods

3.4 Decomposition of the gel

Figure 3-7 shows the decomposition of the gel made with gradually added 0.15 wt% LA-GAGR, 0.50 wt% HA, and a varied amount of TCP. The gel containing 1.0 wt% TCP swells up and increases by 123.3% in weight in the initial 4 h, and then the weight of gel decreases until it decomposes completely in 144 h. The gel containing 0.5 wt% TCP increases by 23.5% in weight in the initial 4 h, and then decomposes in 48 h. The 0.0 wt% TCP decomposes within 4 h without swelling. Because the gel containing 1.0 wt% TCP was highly cross-linked, it imbibed water of 123% with its own weight. The absorbed water expanded the gel, weakened the cross-linking, and dissolved the cross-linker (i.e., TCP), which resulted in the decomposition of gel and the decrease of its weight. For the gel containing 0.5 wt% TCP, i.e., the less cross-linked than the 1.0 wt% TCP gel, the initial imbibition made the gel weight increase by 23.5%, and then, the weight decreased due to dissolution of TCP.

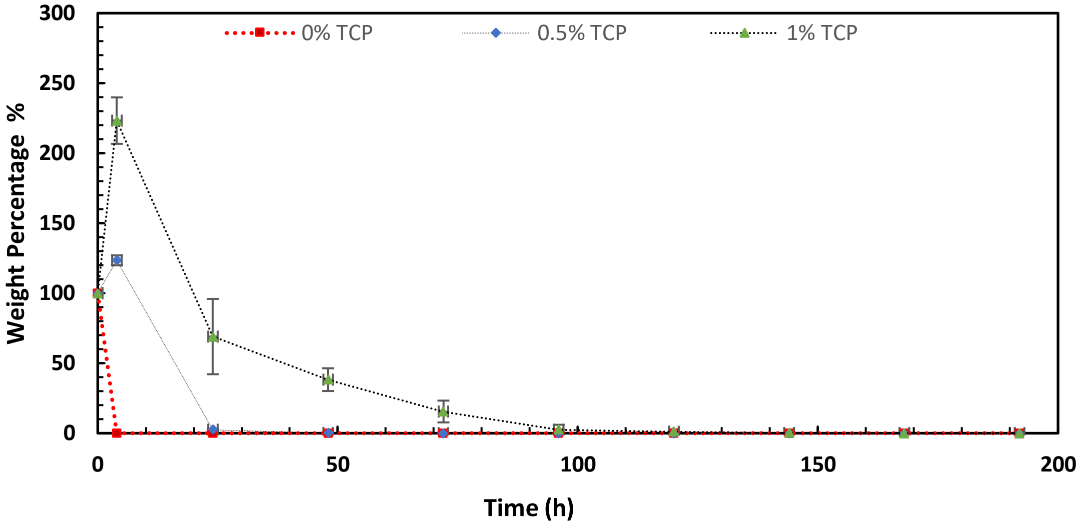


Figure 3-7: Swelling and decomposition of gradually added 0.15 wt% LA-GAGR gel

Figure 3-8 shows the swelling and decomposition of the gel made with gradually added 0.30 wt% LA-GAGR, 0.50 wt% HA, and a varied amount of TCP. The gel containing 1.0 wt% TCP swells up and increases by 145% of its initial weight in 4 h. Then, the weight of gel begins to decrease as it continues to decompose. The gel decomposes completely within 192 h. For the gel containing 0.5 wt% TCP, the weight of gel initially increases by 85% in 4 h, and then completely decomposes in 120 h. The 0.0 wt% TCP gel swells, and its weight increases by 37.5%, and then decomposes within 24 h. Compared with the gels containing 0.15 wt% LA-GAGR, the gels with 0.30 wt% LA-GAGR imbibes water more and take longer decomposition times. With the same TCP concentration of 1.0 wt%, for example, the 0.30 wt% LA-GAGR gel imbibes about 22% more water than 0.15 wt% LA-GAGR gel. Furthermore, it takes 48 hours longer for 0.30 wt% LA-GAGR to decompose than 0.15 wt% gel. The high content of LA-GAGR appears to result in more swelling and longer decompose rate due to its contribution to formation of stronger stretching network and more crosslinking. That led to more time to break the crosslinking.

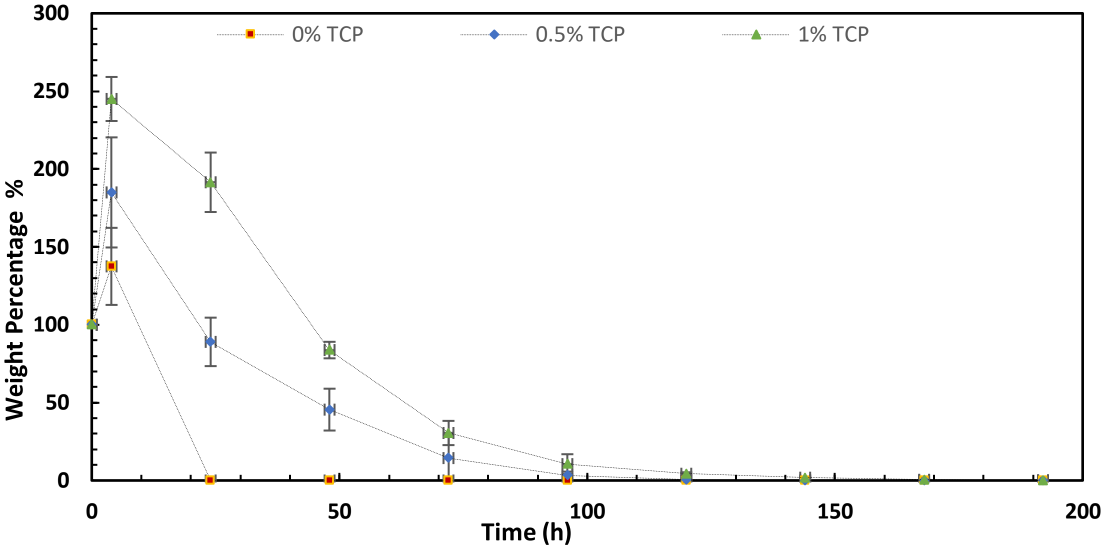


Figure 3-8: Swelling and decomposition of gradually added 0.30 wt% LA-GAGR gel

As shown in Figure 3-9, the method of mixing appears to affect the structure and possibly the properties of gels. Figure 3-9, shows the swelling and decomposition of two gels prepared with 0.30 wt% LA-GAGR, 0.5 wt% HA, and 5.0 wt% TCP using two different methods, a gradual addition of components and one-time addition of the components. The gradual addition of components resulted in a gel that swells and becomes heavier by 192.5% in weight. It takes 240 hours to decompose. For the gel prepared by one-time mixing shows less swelling (150% weight increase) and a shorter decomposition period (216 h). It is thought that gradual mixing resulted in higher degrees of cross-linking of LA-GAGR due to an enhanced mass transport of TCP and HA, which results in a longer decomposition time.

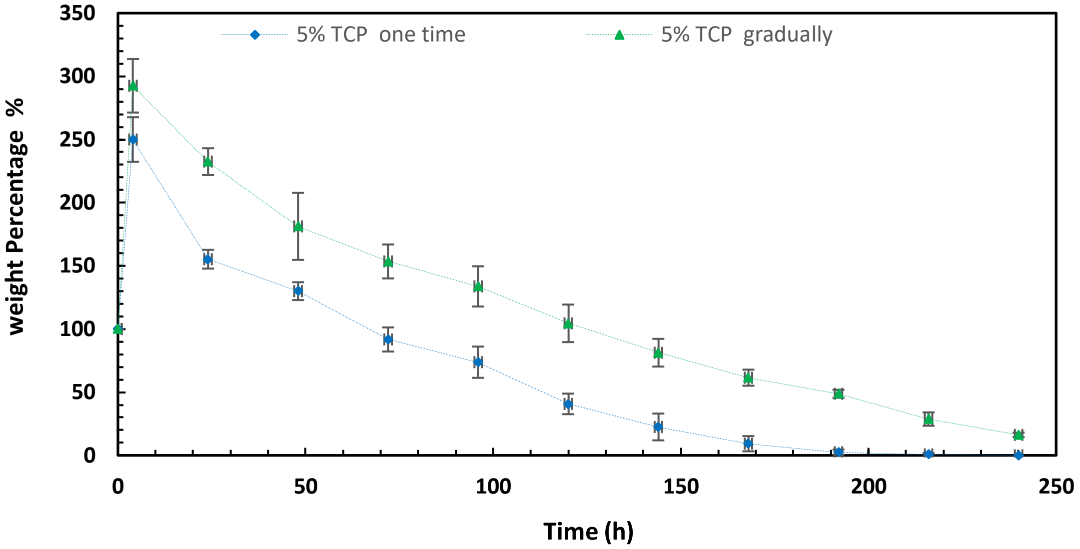


Figure 3-9: Swelling and decomposition of the gels of 0.30 wt% GAGR and 5 wt% TCP prepared by two different methods

3.5 Release rates of LA-GAGR

Figure 3-10 shows the results obtained from the release rate tests for the gels made with 0.15 wt% LA-GAGR, 0.5 wt% HA, and gradually added TCP. The thick, opaque 40 ml layer of the LA-GAGR gel was immersed into the release medium (DI water) over the recorded time span. It was placed in 80 ml DI water, and the cumulative concentration of LA-GAGR that decomposed in the DI water was measured in terms of time. The gel layer appeared to become thinner on the bottom of the flask due to the slow decomposition into the medium. As shown in Figure 3-10, the LA-GAGR with 0.0 wt% TCP decomposed rapidly within 8 h, whereas the LA-GAGR with 0.5 and 1.0 wt% TCP released LA-GAGR within 7 and 8 days, respectively. In order to calculate the average release rate, the actual release weight of each point was divided by the correlated time. The average release time for every run was the average release rate of the gel. The 0.0 wt% TCP gel has an average release rate of 4.30 mg h^{-1} whereas the average release rates of gels made with 0.5 and 1.0 wt% TCP are 1.94 mg h^{-1} and 0.19 mg h^{-1}. The significant reduction of the release rates (or, increase of decomposition period) of LA-GAGR from the gels containing high concentrations of TCP indicates that TCP controls the degree of cross-linking of LA-GAGR polymers.

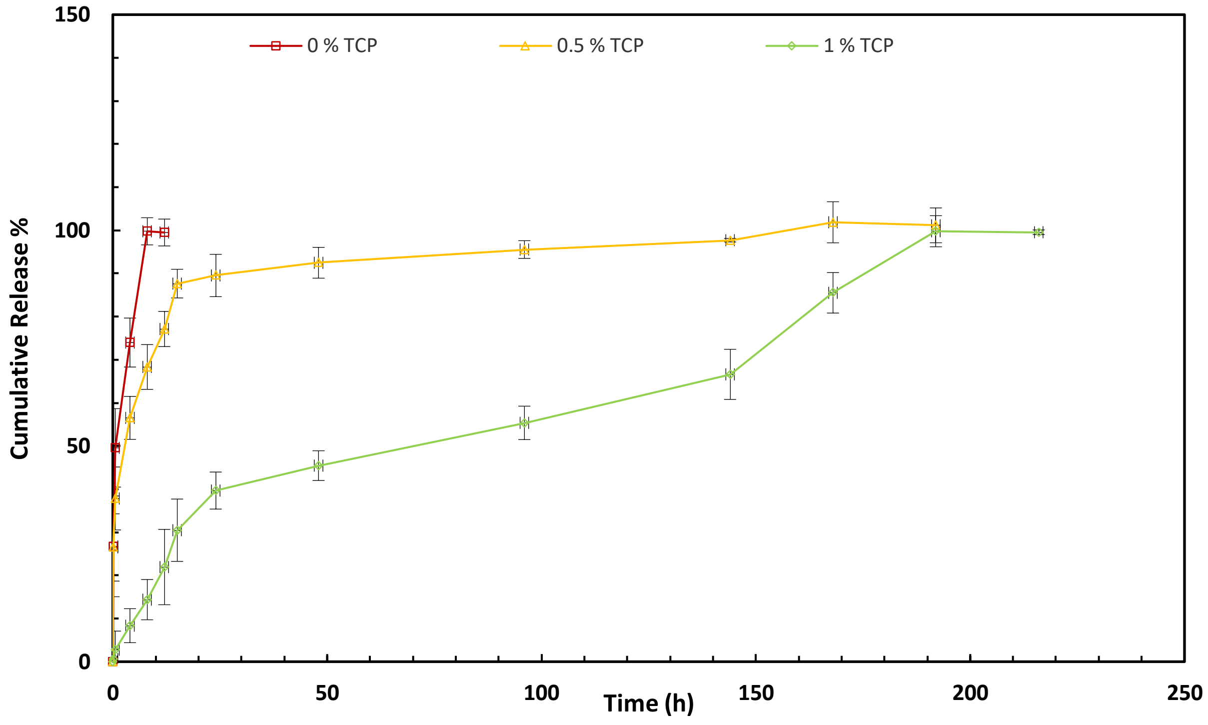


Figure 3-10: Cumulative release of gradually added 0.15% LA-GAGR in terms of time.

Figure 3-11 shows the release results of the gels made with 0.30 wt% LA-GAGR. Compared to the 0.15 wt% LA-GAGR gel in Figure 3-10, the gels made with 0.30 wt% LA-GAGR exhibit longer times to release LA-GAGR for the same TCP concentrations

(48 h, 9 days, and 13 days, for 0.0, 0.5 and 1.0% TCP). The gels with 0.0, 0.5 and 1.0 wt% TCP have average release rates of 1.10, 0.43, and 0.10 mg h^{-1}. For the 1.0 wt% TCP gels, it took 8 and, 13 days for 0.15 and 0.30 wt% LA-GAGR to be completely released, respectively. The more LA-GAGR is present in the gel, the longer it takes for LA-GAGR to be released. Just like the effects of TCP concentration on the LA-GAGR release rates shown in Figure 3-11, LA-GAGR contents in the gel plays an important role in holding the gel structure tight. Furthermore, the high LA-GAGR content shows not only the extended time period for release, but it also slows down the release rate. Therefore, it is verified that the concentrations of both TCP and LA-GAGR control the degree of cross-linking and release rate of LA-GAGR.

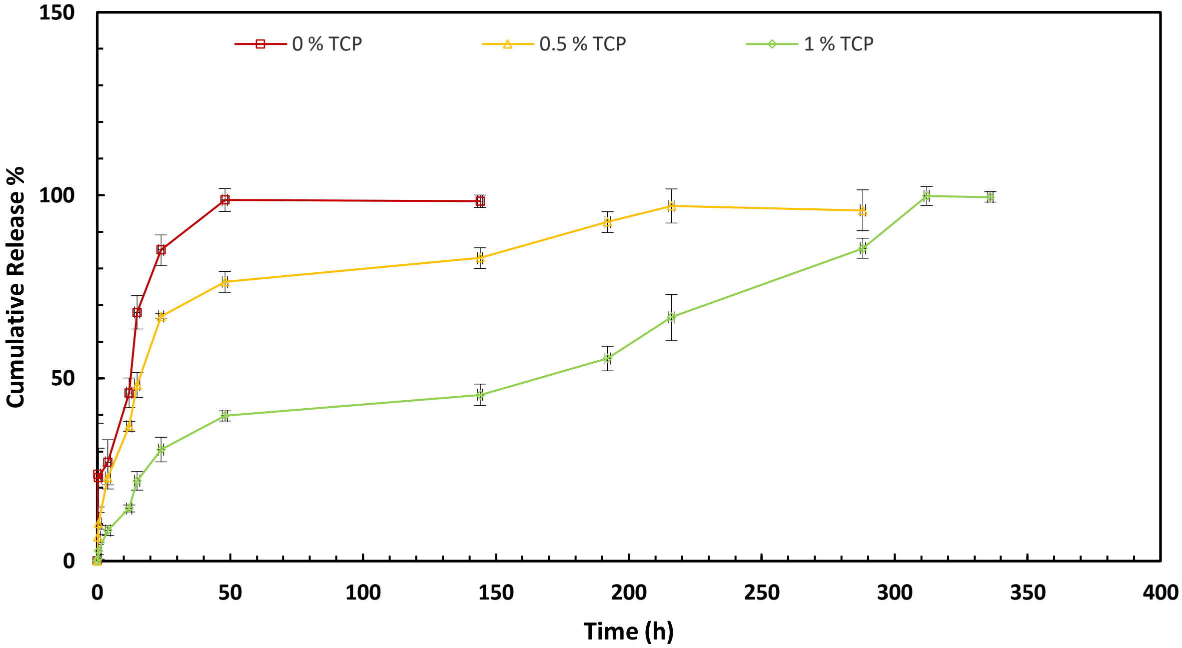


Figure 3-11: Cumulative release of gradually added 0.30 wt% LA-GAGR in terms of time

Table 2-1 shows the comparison between the release rates and full release periods of the tested gels. The gel made with 0.0 wt% TCP and 0.15 wt% LA-GAGR decomposed within 8 h with an average release rate of 4.30 mg h^{-1}, whereas the gel made with 0.5 wt % and 1.0 wt% TCP released LA-GAGR within 7 and 8 days, with the average release rates of 1.94 and 0.19 mg/h, respectively. Also, it shows that the gels made with 0.0, 0.5, and 1.0 wt% decompose within 48 h, 9 days, and 13 days with the average release rates of 1.10, 0.43, and 0.10 mg/h. When the amount of LA-GAGR was doubled from 0.15 wt % to 0.30 wt%, the average release rates decreased and the full release periods increased. These results occurred due to the increase of the degrees of cross-linking within the gels.

Table 2-1: Comparison of the release rates and full release periods between the tested gels.

TCP WT%	RELEASE RATE OF 0.15 WT% LA-GAGR [MG/H]	RELEASE RATE OF 0.30 WT% LA-GAGR [MG/H]	TIME FOR FULL RELEASE OF 0.15 WT% LA-GAGR [H]	TIME FOR FULL RELEASE OF 0.30 WT% LA-GAGR [H]
0.0	**4.30**	**1.10**	**8**	**48**
0.5	**1.94**	**0.43**	**168**	**216**
1.0	**0.19**	**0.10**	**192**	**312**

3.6 Color change of the gel

Figure 3-12 shows samples of the gel some of which were exposed to the open air while others were sealed; both were kept for 2 months. The gels in Fig. Figure 3-12 (a) were in the medium for two months and were kept in the sealed flask. The samples contained varying amount of TCP. The samples displayed had the composition of TCP varied from 0.0 wt% to 1.0 wt%. After two months the gels turned orange, where the gels containing more TCP displaying deeper orange color. The samples turned orange due to a possible oxidation reaction. Oxidation could have taken place because there were oxygen molecules in the medium.

The gels in Figure 3-12 (b) were made with 0.30% LA-GAGR, 0.5 % TCP (left) and 1.0 % (right) were exposed to the open air for two months. The gel contained calcium and phosphate ions. Since the samples were exposed to the air, the oxygen present in the air reacted the oxidation reaction took place. Electrons were transferring from the calcium and phosphate to the oxygen molecules through this process, and oxide was formed.

a) **b)**

Figure 3-12: Color change of the gel (a) in water (b) in air.

Chapter 4

Conclusion

The LA-GAGR gels were prepared with 0.5 wt% HA, 0.15 or 0.30 wt% LA-GAGR, and 0.0, 0.5, and 1.0 wt% TCP to investigate the effects of the concentrations of the cross-linker, TCP, and LA-GAGR on the rheological properties and decomposition rates of the gels. It was observed that the mixing methods affect the properties of the gels. The conclusion of the study is summarized as follows:

1) Differences in the gels' appearances were observed for the gels prepared by gradual addition of TCP, compared to the gels made by adding all the components at the same time. The gels from the gradually-added method showed more rigid appearance, more swelling and longer decomposition time than the other ones made by one-time addition.

2) Flow sweep tests show that an increase in the TCP concentration made the gels more viscous.

3) The flow sweep test results showed that all the gels exhibit shear thinning behavior. The gels exhibited much greater viscosity values when the LA-GAGR content was high; the highest viscosity value of 675 Pa·s was observed at 0.1 s^{-1} for the gel

containing 0.30 wt% LA-GAGR, 1.0 wt% TCP, and 0.5 wt% HA, whereas 0.15 wt% LA-GAGR with 0.0 wt% TCP showed the lowest viscosity of 15.8 Pa·s.

4) The oscillation frequency test results showed that at low frequencies, the gels behave more viscous, whereas the elastic behavior dominates at higher frequencies. The gel containing 0.0 wt% TCP and 0.30 LA-GAGR remained more viscous than elastic until 8.5 rad s^{-1}. The gel containing 0.5 wt% TCP and 0.30 LA-GAGR remained more viscous than elastic until 6.5 rad s^{-1}. However, the gel containing 1.0 wt% TCP and 0.30 LA-GAGR remained more elastic than viscous beyond 100 rad s^{-1}.

5) The decomposition time of the gels were 13 days for the gel containing 0.30 wt% LA-GAGR, 1.0 wt% TCP, and 0.5 wt% HA. The increase in the content of TCP resulted in an increase in the decomposition period due to the higher degree of crosslinking. The gels made of 0.30 wt% LA-GAGR with 0.0, 0.5 and 1.0 wt% showed typical viscoelastic properties.

6) The release rates of LA-GAGR from the gels were also much lower for the gels with 1.0 wt% TCP and 0.30 wt% LA-GAGR (13 days) than the gels with lower concentrations of TCP and LA-GAGR due to the high degree of cross-linking. The degree of cross-linking increases with the contents of TCP and LA-GAGR, which resulted in longer gel decomposition periods, slower release rates of LA-GAGR, and more elastic gels.

Chapter 5

Recommendation and Future Work

The present study explored rheological properties of low acyl gellan gum. The amounts of TCP and LA-GAGR were varied in order to see their impacts on the gel's properties and appearances. During the preliminary studies, low acyl gellan gum was enzymatically separated into two lower molecular weight products, named midi-GAGR and mini-GAGR. The gel prepared with midi-GAGR and mini-GAGR and the same amounts of cross-linking (TCP) and HA might be more effective than LA-GAGR due to a larger surface area of midi-GAGR and mini-GAGR. Breaking down LA-GAGR into small oligomers would increase its surface area and it would make more interaction with bones. Consequently, future studies would explore a way to control mini- and midi-LA-GAGR gels' rheological properties and release rates.

The LA-GAGR, midi-GAGR, and mini-GAGR gels would need to be tested in human bone cells to see their impacts on the blood vessel formation and bone growth. Polycardanol can also be added to enhance the LA-GAGR gel's antioxidant and anti-bacterial effects.

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