Production and analysis of alginate oligosaccharides and their effect on the physical properties of gellan gum

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Abstract: Alginate oligosaccharides, in particular G-blocks, have recently been shown to have some interesting biomedical applications. In this work, G-blocks were extracted from sodium alginate salt and was characterised by ¹H-NMR, ¹³C-NMR. In addition, molecular weight and polymerization degree for these compoundswere measured by using size exclusion chromatography-multiangle laser light scattering (SEC-MALLS). The prepared G-blocks were added to several types of polysaccharides including gellan gum and two kinds of alginates containing different amount of G residues. It was found that G-block addition seemed to be more effective on gellan gum viscosity than on alginates; gellan gum viscosity increase with adding 2.0% w/v of G-block then G-block addition (3.3% w/v) lead to decrease the viscosity. Rheological measurements of viscoelastic properties of hydrogels containing increasing concentrations of G-blocks were also performed. The elastic modulus of gellan gum gel hugely decreased with adding 0.6% w/v of G-block then increase gradually with 2.0% w/v G-block. These results highlight the potential of G-block oligosaccharides in modifying and controlling the mechanical properties of other polysaccharides, which could have applications in developing drug delivery systems and biomaterials.

Keywords: Gellan, gellan gum, gellan gum viscosity, G-block, alginate

I. Introduction

Alginate is polysaccharide being in kelp including marine brown algae and soil bacteria. Although production by microbial fermentation is technically possible commercial, alginate is prepared primarily from algae including Laminariahyperborea, macrocystispyrifera, Laminariadigitata, Ecklonia maxima and Ascophylumnodosum.

The most important and significant feature regarding the physical properties of alginate is that it can form hydrogels on exposure to divalent cations. This selective binding occurs with the block of G to the formation of gel. The structural features in the G-blocks also lead to chelation of multivalent cations "egg-box" model Fig. 1.

There fore, when there is an increase in the content of L-guluronate residues in the chains, gel strength increase. In recent years, studies on alginate gels involving the use of small angle X-ray scattering report lateral association of G-blocks when cations (Ca²⁺) and G-content of alginateincreases (Draget and Taylor, 2011). Moreover, the strength selectivity of divalent cations differs from one to other which take this order $Pb^{2+}>Cu^{2+}>Ba^{2+}>Sr^{2+}>Ca^{2+}>Mn^{2+}>Mg^{2+}(Smith, Miri, 2011)$. Temperature has no particular influence on sol/gel transition of alginates.

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Figure 1: Egg-box model (Patel, 2006)

Alginates have wide range of applications in several areas including medicine, pharmacy and food industry. With regard to controlled release, alginates have long history of application in hydrophilic matrix system and it can be used alone or with other hydrophilic polymers to provide sustained drug release with acidic, basic and neutral grugs for more than 12 hs (FMC, 2008). Several researches in recent years have shown that oligoguluronates can act as a modifier modifying both the kinetics of the gel as well as the equilibrium properties of alginate. It has been reported recently that G-blocks have the ability to transiently alter the mucin network structures. This property of alginate oligoelectrolytes can be used for the treatment and curing of pathological respiratory conditions. It can also be used for altering the structure of mucosal surfaces, for instance, drug delivery systems. Moreover, alginates have immunogenic features and biological effect in body. The first time tried was done to control of diabetes in animals. In these animal transplantation trials, phagocytes and fibroblasts led to overgrowth of alginate capsules this research showed that amount of mannuronate in the sample of alginate was responsible for the inducibility. Capsule overgrowth that was reported in the animal transplantation trials was due to the content of mannurate-rich fragments in the sample. Some fragments that have no participation in the formation of gel network protrude out of the capsules and leads to the triggering of an immune response (Draget and Taylor, 2011). Alginate also has a thickening property, which is highly useful in food industry. Propylene glycol alginate (PGA) can be effectively used in such applications because it is static in moderate acid conditions. With the help of alginate, the texture, body and sheen of yoghurt can be effectively improved (Draget et al., 2005).

Gellan gum

Gellan gum is a linear, anionic hetero-polysaccharide created by a micro-organism, *Sphingomonaselodea* and has been utilized in the food production primarily as a gelling agent. The molecular structure of gellan gum is based on a tetra-saccharide replicating unit consisting of (1-3)- β -D-glucose, (1-4)- β -D-glucuronic acid, (1-4)- β -D-glucose, and (1-4)- α -L-rhamnose as the spine with acyl substituents of L-glycerate and acetate at the C-2 and C-6 (approximately 50%) positions of the (1-3)-linked D-glucose, respectively (Fig. 2). Gellan gum makes gels with different physical and textural features depending on the kind and concentration of cation added and on the acyl capacity. normally, the low-acyl type forms texturally hard and brittle gels in the presence of cations, in particular gel-promoting cations that include Ca²⁺ and K⁺, with greater thermo-irreversibility, while the high-acyl types form texturally soft and flexible gels even in the absence of cations (Funami et al., 2008).



Figure 2: Chemical structure of gellan gum (Chaplin, 2011)

Gellan gum is widely used in the food industry and biomedical fields. This material has a great ability of processing into transparent gels, which are usually unsusceptible to heat and acid stress. This is one of the biggest reasons behind the excess use of this material. There are two most popular and available forms of gellan gum; acetylated and deacetylated. Following steps are followed by gellan gum for gel formation at high temperature, gellan gum is found to be in the coil form and at decreased temperature, a transition between thermally reversible coils to double helix occurs, which is highly critical for the formation of gels.

After then, the formation of a structure, which is composed of anti-parallel double helices, is self -assembled in order to form oriented bundles known as junction zones. Untwined regions of polysaccharide chains are found to be in the form of extended helical chains. A linkage between these untwined regions and junction zones occurs, and it leads to the creation of a three-dimensional network, which forms the gel. The chemical nature and quantity of cations exist in the solution, which has a huge impact on the gelation of gellan gum solutions. The presence of cations is highly essential during preparation of functionally stable gel. On the other hand, the helix formation and its incomplete grouping often form an arranged structure, but it fails to lead to gel formation. The reason behind this is that the number of helical groups are not enough capable to give rise to a perpetual network in the entire volume. In such instances, carboxyl side groups are considered the biggest barriers, as they repel one another through electrostatic interaction. Hence, these groups obstruct the tight binding of helices and their adhesive grouping. Cations protect the electrostatic repulsion and permit the tight binding of helices and their adhesive grouping. (Lee et al., 2011).

The nature of cations has a huge impact on gelation properties of gellan gum. For example, divalent cations have much capability of promoting the gelation as compared to monovalent cations. In monovalent cations, gelation occurs mainly due to the screening of the electrostatic repulsion among the ionized carboxylate groups and the chains of gellan gum. In contrast, chemical bonding between divalent cations and two carboxylate groups, which are associated with guluronic acid molecules in the gellan chains in addition to the screening effect, is mainly responsible for the gelation and grouping of gellan. Gellan gum has a several good advantageous characteristics due to which, it is excessively used in pharmaceuticals industry and peculiarly environmental bioremediation. It is also considered an appealing biomaterial for fibrocartilage tissue engineering applications because it has great compatibility with cell and is able to inject into a defect and gels at body temperature(Oliveira et al., 2009).

It is widely used in medical industry, as it is quite useful for producing easy to swallow solid dosage forms like gels and coated tablets. At the same time, it is also used for doing modification in the releasing rate of active ingredients from capsules and tablets. It is of great use for controlling or sustaining the rate of release of several drugs and preparation of microencapsulation. There is an increase seen in the bioavailability of theophylline from the gellan gels (Tako et al., 2009). Gellan gum has a great use in the food industry and is considered to food additive as, it is used in the form of stabilizer, thickening agent, structuring

and versatile gelling agent in the variety of food products. It also has an ability of producing gel textures in various food products (Oliveira et al., 2009).

The purpose of this work is to extract G-block (guluronic acid) from alginate and identify its chemical composition by nuclear magnetic resonance (NMR) spectroscopy and to calculate its molecular weight and polymerization degree by (SEC-MALLS). Moreover, the effect of G-block on the viscosity of several types of alginates as aqueous solution and gel will be studied examine how G-block influence gelation point of gellan gum.

II Methods and materials

In this work, G-block was extracted by two different ways using different types of alginates according to (PCT, 1998).

In the first procedure, 10g of sodium alginate (protanalTM LFR 5/60 65% G units) (Schmid and Picker-Freyer, 2008) was weighed out and dissolved in 500mLHCl (0.3M) and mixed properly on magnetic stirrer for about three hours. The sample was heated to adequate boiling on water bath (6-7 hours at 100C°), and then the acid poured out carefully. The precipitate was washedtwice by 50ml of deionised water, and then50 mL of deionised water was add. The sample was leftovernight on stirrer after adjusting the pH to 3.3 with sodium hydroxide(0.1M).Centrifuge and about 10 ml of fresh deionised water was add to the precipitate and its pH was readjusted to 6.5-7.The sample was then frozen 20C° overnight then frozen dry.

In the second method, similar procedure was applied with one exception. Where, the type of sodium alginate that was protanal LF 200 M (35-45% G units) (Schmid and Picker-Freyer, 2008), homogeniser (Silverson L2R) was used for mixing the sample and rotary vacuum was used for drying.

The analysis of G-block was done bynuclear magnetic resonance (NMR) spectroscopy by using the freeze-dried G-block which was dissolved in deuterated D_2O and preformed to ¹H, and ¹³C NMR analysis using Bruker Advance 500MHz.

Molecular mass distribution by size exclusion chromatography-multiangle laser light scattering (SEC-MALLS)

Alginates are polydisperse in nature and their molecular structure resemble the molecular structure of synthetic polymers. Being polydisperse, alginate has an average molecular weight when looking over the whole distribution of molecular weights. The following formula defines the number and the weight average (Draget et al., 2005).

$$\overline{M_n} = \frac{\sum_i N_i M_i}{\sum_i N_i}$$
$$\overline{M_w} = \frac{\sum_i w_i M_i}{\sum_i w_i} = \frac{\sum_i N w_i M_i^2}{\sum_i N_i M_i}$$

Where, N_i is the number of molecules, M_n is the number of average molecular weight, M_w is the weight of average molecular weight, and w_i is the weight of molecules having a specific molecular weight M_i . For a polymer which is randomly degraded, the $Mw \approx 2 M_n$, and the polydispersity index = M_w/M_n .

A SEC-MALLS measurement was done on an HPLC system equipped with three straight connected columns. The column outlet was connected to a Dawn Heleos-II multiangle laser light scattering photometer (λ = 658 nm). The mobile phase was used 0.05M Na₂SO₄/ 0.01M EDTA (pH 6) and the flow rate was 0.7 mL/min. The injection volume was 100µL, and the concentration of sample (G-block) was 0.2% for suitable light scattering intensity. Moreover, the sample solution was filtered with a 0.22µm filter before injection (Suzuki et al., 2010). Under the same conditions, the SEC-MALLS measurement was done again except using one column instead three columns.

Solution alginates (4% w/v) and gellan gum preparation and measuring their viscosity with G-block

A 4.0g Protanal LF 200 M alginate was weighted out and dissolved in 100 mL DI water. The solution was mixed well by stirrer. As well as protanal LV low viscosity alginate was prepared. Gellan gum was prepared by dissolving 1.0g gum and in 100 mL deionised water (85 °C) and mixed completely. Preformed each sample to viscometer to analysisby using rheometer (bohlin rheometer CS-50)· 25°C and the gap on 150 μ m. 2mL of each sample was placed on plate (lower fixture) and covered and run the sample. The last step was repeated after adding 100mg of G-block to 15mL of each aqueous solution (0.6% w/v).

Measurement the viscosity of gellan gum with different amounts of G-block

A 0.1, 0.3, 0.5 and 0.5g G-block were added to 15mL of gellan gum solution in separated beakers and mixed very well. Viscosity was measured using similar technique described previously using viscometer at 25 °C.

Preparation gel of gellan gum, protanal LF 200 M alginate alone and protanal LV low viscosity alginate with G-block

A 0.1g of G-block was added to 15mL of each aqueous solution that prepared previously, and then 4 mL of each mixture were placed into well plate up of filter paper saturated with 10 mM calciumchloride. Soon after that, the surface of the sample was covered by other filter paper saturated with 10 mM calcium chloride. Then about 2mL of calcium chloride was poured on sample and left overnight. Viscous modulus and elastic modulus were measured using rheometer(2cm, 25°C).

Preparation gel of gellan gum with protanal LF 200 M alginate, protanal LV low viscosity alginate and G-block

A 0.1g of protanal LF 200 M alginate, protanal LV low viscosity alginate and G-block were weighted in separate beakers; then15 mL of gellan gum solution was added to each beaker and well mixed. Gel prepared in well plate as described previously and were left overnight. Viscous modulus and elastic modulus were then measured.

Determination of gelation point of gellan gum with different amounts of G-block

A 1% w/v of gellan gum was placed on water path (85° C).A 0.05g of CaCl₂ (powder) was added to the solution to make the concentration 2mM. The temperature of rheometer was set between 80-10°C. The up plate was made close to the lower plate (to ma the sample in hot condition). 15 ml of gellan gum solution was added to 0.1g of G-block and mix completely under hot condition (85° C). About 2 mL of the sample was placedonthe plate and then covered with upper plate. The sample was coveredby paraffin oil to avoid sample evaporation. The same step was done with 0.3, 0.5, 0.7 g and gellan gum alone.

III Results and discussion

The percentage yield of G-block in the first procedure was 55.3%, and was 52.02 % for the second procedure. It can be seen that there is no huge difference in yields between the two procedures. The slight variation might be attributed to some of precipitate was poured with the acid after hydrolysis step.

Chemical composition analysis by NMR

With regard to NMR result, it can be seen that chemical composition of G-block produced by the first procedure (Figs.3a &3b) is identical with that produced by the second procedure (Figs.4a &4b) in terms of ¹H and ¹³C NMR spectra. In terms of chemical shift, the highest chemical shifts in ¹H NMR spectra are H-1and H-5 (5.03, 4.4 ppm, respectively),whereas H-2, H-3 and H-4 are relatively close. In addition, it can be noticed that in ¹³C NMR the highest chemical shift are C-1 and C-6 (102,175.4ppm), respectively.This is due to the closeness of these atoms to the oxygen atom that are of high electronegativity,while the chemical shift of

C-2, C-3, C-4 and C-5 are 68, 72, 83 and 68.6 ppm respectively. Moreover, when compared with previous study (Fig.5) the spectra obtained were very similar.



Figure 3a: G-block structure of H¹ NMR (procedure I)



Figure 3b: G-block structure of ¹³C NMR (procedure I)



Figure 4a: G-block structure of H¹ NMR (procedure II)



Figure 4b: G-block structure of ¹³C NMR (procedure II)



Figure 5: ¹H and ¹³C NMR spectra for G-block collocated from previous study (Zhang, 2006)

Molecular mass distribution by SEC-MALLS



Figure 6a: SEC-MALLS chromatography of G-block with used 0.05M Na $_2$ SO $_4$ / 0.01M EDTA



Figure 6b: SEC-MALLS Chromatography of G-block with used 0.05M Na₂SO₄/ 0.01M EDTA and AUX, 90° Detector and one column

The degree of polymerization (DP) means the number of monomeric units in a macromolecule or polymer or oligomer molecule.

 $DP_n = \frac{\textit{Total Mw of the polymer}}{\textit{Mw of the monomer unit}} = \frac{\textit{Mn}}{\textit{M0}}$

: Mw of guluronic acid= 334-18=316From Fig.6a the total Mw of G-block (M_n) = 2.341×10^4

DP_n=

...... (1) From Fig.6b the total Mw of G-block $(M_n) = 2.146 \times 10^4$

With respect to SEC-MALLS result, when we look at degrees polymerization 1, 2 that means guluronic acid unit repeated 74.0 times and 67.9 times for DP_n one and two, respectively, and it has ability to make network by cross-link property. However, when the sample preformed for gel formation it does not made gel. Hence, this evidence indicates that this result is unlikely. This situation may be attributed to the concentration that used or instrumental error such as types of columns utilized.

In terms of Viscosity (Table 1), it seems to be that G-block addition led to increase in gellan gum viscosity more than protanal LF 200 M alginate and protanal LV low viscosity alginate. This may be due to the special characteristics of gellan gum that has different types of binds in aqueous solution. That might be stronger with G-block molecules. Hydrogen bonding might take place between OH-4 group of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the L-rhamnosyl residue, and between OH-3 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the D-glucuronosyl residue, making the gellan gum molecule rigid. Van der Waals forces, which is the intermolecular association alsomight take place between the methyl group and the hemiacetal oxygen atom of the L-rhamnosyl residues on different molecules (Tako et al., 2009).

It can be seen from Table 1, that, there is no difference between alginate 200 M viscosities and alginate 200M, with G-block viscosity 11.3166±0.77 and 11.3966±0.87 respectively. No effect of G-block on LV alginate viscosity was observed. In contrast, G-block addition led to

increase gellan gum viscosity (Gellan gum only= 0.51 ± 0.26 Pa &Gellan gum with G-block = 1.7 ± 0.15 Pa).

Table 1: Viscosity /Pa of protanal LF 200 M alginate, protanal LV low viscosity alginate and gellan gum and viscosity /Pa of these polysaccharides with G-block

Sample	Viscosity /Pa
Alginate 200M only	11.3166±0.77
Alginate 200M with G-block	11.3966±0.87
LV alginate only	0.0351±0.095
LV alginate with G-block	0.0364±0.1
Gellan gum only	0.51±0.26
Gellan gum with G-block	1.7±0.15

Measurement the viscosity of gellan gum with different amounts of G-block

Addition of G-block to gellan gum (Fig.7) had led to a decrease in viscosity until particular concentration (optimum concentration). Then increasing the concentration reduces the viscosity. This situation seems to be due to the ionic bonding (Van der Waals forces of attraction) will be stronger with particular amount of negative charge, hence the viscosity increases. However, too much negative charge decreases the force of these bonds and viscosity.



Figure 7: Viscosity (Pa) of gellan gum only, 0.6 % w/v G-block, 2.0 % w/v G-block, 3.3% w/v G-block and 5.0 % w/v G-block

In Fig. 7, we can find that although 5.0% w/v of G-block is the highest concentration, it is the low viscosity after gellan gum only. However, 2.0% w/v % G-block is the highest viscosity followed by 0.6% w/v G-block.

In Fig. 8, it can be seen that G-block addition also increases viscosity of gellan gum while no change in instantaneous viscosity of other types of alginates when G-block added. Figure 8a clearly shows that instantaneous viscosity of both LV alginate and LV alginate with Gblock are very close and are steady over shear stress while in alginate 200M alginate 200M with G-block (Fig. 8c) instantaneous viscosity is identical and decline with increasing shear stress. In gellangum case the situation is different (Fig. 8b); instantaneous viscosity of gellan gum with G-block is higher than gellan gum alone over shear stress. According to Fig.9, adding G-block did not affect elastic modulus of gellan gum but in alginate 200M and LV alginate elastic modulus increased.When adding alginate 200M to gellan gum increase elastic modulus while in cases LV alginate and G-block additions there are no effect; this my attributed to the difference in molecular weights for material used.





Figure 8: Instantaneous viscosity /Pa against shear stress /Pa. (a) comparison between LV alginate and LV alginate withe G-block. (b) comparison between gellan gum and gellan gun with G-block.(c) comparison between alginate 200M and alginate 200M with G-block





Gelation point

In Fig.10 it can be seen that there is no difference in gelation point temperatures betweengellan gum alone (Fig.10a) and 0.6% w/v G-block (Fig 10-b) 41.8 Pa, 42.8 Pa

respectively. While 3.3 % w/v G-block (Fig.10c) is the highest gelation point temperature followed by 5.0 % w/v G-block (Fig.10d) (56 Pa, 53 Pa, respectively). This might because G-block increase potential energy of gel which increase the temperature. With regard to viscous elastic properties, in gellan gum alone and 0.6% w/v G-block viscous modulus is distant from elastic modulus that means the gel seems to be more viscous. However, with 5.0 % w/v G-block viscous modulus and elastic modulus are convergent from 25 to 10° C, while in 3.3 % w/v G-block viscous modulus goes in parallel with elastic modulus. Although each Fig. 10b and Fig. 12b have the same percentage of G-block, the results are different this because difference in method preparation and concentration of CaCl₂ used.

We can notice (Fig. 11) that 0.6 is the lowest elastic modulus on the other hand gellan gum alone is the highest elastic modulus followed by 5.0 % w/v G-block and 3.3 % w/v G-block. That means presence amount of g-block in gellan gum gel led to increase hardness of the gel.This because G-block is an anionic oligosaccharide negative charge andalkaline earth metal ions (Ca) has negativecharge hence G-block addition increase force of attraction in the gel while too much of G-block increase spread of negative charge in gel media consequently repulsion force and decrease hardness as illustrated in Fig.12.





temperature/°C ;(b) viscous modulus /Pas (G[°]) and elastic modulus/Pa (G[°]) of gellan gum with G-block (0.6% w/v) against temperature /°C;(c) viscous modulus /Pas (G[°]) and elastic modulus/Pa (G[°]) of gellan gum with G-block (3.3% w/v) against temperature/°C;(d) viscous modulus /Pas (G[°]) and elastic modulus/Pa (G[°]) of gellan gum with G-block (3.3% w/v) against temperature/°C[2 mM CaCl2 used]







Figure 12: Domain model" of gellan gum gelation and effect of G-block

Figure 13 shows that tan delta of gellan gum alone, 0.6 % w/v G-block, 3.3 % w/v G-block and 5.0% w/v G-block. Tan delta defines as ratio of viscous modulus and elastic modulus.

Tan
$$(\delta) = \frac{G''}{G'}$$

Tan delta is greater when G'' is higher than G' (Kasapis et al., 2009). Hence, in this graph, tan delta increases with increasing the amount of G-block. The figure indicated that increasing concentrations of G-block causes the stiffness of the gel to decrease.





IV Conclusion

In this work, G-block was extracted from alginates salts and was analysed by ¹H-NMR, and ¹³C-NMR. In addition, SEC-MALLS was used to define molecular weight and polymerization degree for this compound. The prepared G blocks were mixed with several types of polysaccharides including gellan gum and two kinds of alginates containing different amount of G residues. It was found that adding G-block lead to increase gellan gum viscosity more than alginates; in which-block addition increases gellan gum viscosity with adding 2.0% w/v of G-block nevertheless adding (3.3% w/v) G-block lead to decrease the viscosity. Rheological measurements of viscoelastic properties of hydrogels containing increasing concentrations of G blocks were also achieved. The elastic modulus of gellan gum gel hugely decreased with adding 0.6% w/v of G-block while with 2.0% w/v G-block increase gradually. Moreover, it was found that the gelation point temperature of the gel became higher with G-block addition. These consequences emphasize the potential of G block oligosaccharides in improving and controlling the mechanical properties of other polysaccharides which could have applications in developing drug delivery systems and biomaterials.

المستخلص: تبين مؤخراً أن الجينات السكريات قليلة السكريدولا سيما G-block لما بعض التطبيقات الطبية الحيوية المثيرة للاهتمام. وفي هذا المبحث تم استخلاص G-block من ملح الجينات الصوديوم وتم التعرف على خواصها باستخدام الرنين المغناطيسي النووي-11 البحث تم استخلاص G-block من ملح الجينات الصوديوم وتم التعرف على خواصها باستخدام الرنين المغناطيسي النووي-11 البحث تم استخلاص SEC-NMR13 وG-block من ملح الجينات الصوديوم وتم التعرف على خواصها باستخدام الرنين المغناطيسي النووي-11 الزوايا (SEC-MAL13). ثم تم قياس الوزن الجزيئي ودرجة البلمرة لهذا المركب باستخدام كروماتوجرافيا استبعاد الحجم- تشتت ضوء الليزر متعدد الزوايا (SEC-MALLS). وتمت إضافة G-block المحضرة إلى عدة أنواع من السكريات بما في ذلك صمغ جيلان ونوعين من الألجينات التي تعتوي على كمية مختلفة من بقايالمادي المحامة وأثبت نتيجة البحث أن إضافة Joc ملح الجروات بعلى لزوجة صمغ الجيلان من الألجينات التي تعتوي على كمية معتلفة من بقايلة على لزوجة صمغ الجيلان من محافزي يلماني وتزداد لزوجة صمغ حيلان يوضافة G-block وزن/جم من G-block، ويتم تقليل اللزوجة بإضافة Joc من الألجينات التي كتوي على كمية من بقايلة من بقايلة على لزوجة صمغ الجيلان من الألجينات، وتزداد لزوجة صمغ جيلان بإضافة 2.0% وزن/جم من G-block، ويتم تقليل اللزوجة بإضافة Joc ما مرونة الألجينات، وتزداد لزوجة صمغ جيلان بإضافة 6.0% وزن/جم من G-block، ويتم تقليل اللزوجة بإضافة Joc ما مرونة ما ألر لينات، وتزداد لزوجة صمغ جيلان بإضافة 6.0% وزن/جم من G-block، ويتم تقليل اللزوجة بإضافة على لروجة ما مام مرونة ما ألر ما ألر ما إلحليات، وتزداد لزوجة مالماليوجة الميدوجيان وزن/جم من G-block، ويتم تعزيزات متزايدة من G-block، وزن/حجم من مامل مرونة حما إلى ما مرونة الجليدي بلدي بن بشكل كبير مع إضافة 0.0% وزن/جم من G-block، من ماحل ما منزيزات متزايدة من G-block، وزن/جم من G-block، من مامل مرونة ما إجراء قياسات ريولوجية الحمانة للهيدروجيلات التي تحتوي على زرديزيياً بنيبة 2.0% وزن/جم ما مرونة جل صمغ الجيلان بشكل كبير مع إضافة 0.0% وزن/جم من G-block، من ماحل من المنيبة ما الميكانيكية للسكريات الأخرى المنوي ما مامل مال مونا وزن مع ما مال مونا ما معاني ما ماليكانيكية السكريات المالي ما مال مول ما طبيقات في مولوا ألممة توصيل الأدوية والواد الحيوية.

References

Draget, K.I., Smidsrod, O. and Braek, S.G. (2005). Alginates from algae. *Polysaccharides and Polyamides in the Food Industry-Properties, Production and Patents*. [Online]. Available at: <u>http://www.media.wiley.com/product_data/excerpt/51/35273134/3527313451-</u> 1.pdf[Accessed 1 July 2021].

Draget, K.I. and Taylor, C. (2011). Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids*.October25(2),251-256.

FMC Biopolymer. (2008). Alginates for pharmaceutical and medical applications. FMC. [Fried J.R. (2003) *Polymer Science and Technology*. Pearson Prentice-Hall

Funami, T. Noda, S. Nakauma, M. Ishisara, S. Takahshi, R. Al-assaf, A. Ikeda, S.

Lee, H. Fisher, S. Kallos, M. and Hunter, C. (2011). Optimizing gelling parameters of gellan gum for fibrocartilage tissue engineering. *Journal of Biomedical Materials Research B: Applied Biomaterials*, February 98B (2), 238-245.

Kasapis, S. Norton, I. andUbbink, J (2009). *Modern biopolymer science*. London: academic press.

Oliveira, J.T., Martins, L. and Picciochi, R. (2009). "Gellan gum: A new biomaterial for cartilage tissue engineering applications. *Journal of Biomedical Materials* Research, 852-863.

Suzuki, S. Christensen, B. and Kitamura, S. (2010). Effect of mannuronate content and molecular weight of alginates on intestinal immunological activity through Peyer's patch cells of C3H/HeJ mice. *Carbohydrate Polymers*. August 83 (2011) 629–634.

Zhang, Zhao, X. Liu, H and Guan, H. (2006).Sequenceanalysis of alginate-derived oligosaccharides by negative-ion electrospray tandem mass spectrometry. *Institute of Marine Drug and Food*, 17, 621–630.

PCT. (1998) Procedure for producing uronic acid blocks from alginate. *Word intellectual property organization*. November 98, 1-25.

Patel, G. (2006). Algae's Functional ExcipentSide. *Pharmaceutical Formulation & Quality*. [Online]. Available at:

http://www.pharmaquality.com/ME2/Audiences/dirmod.asp?sid=325598564E8C4B3EB736C 7159241312D&nm=&type=Publishing&mod=Publications%3A%3AArticle&mid=D3E3C71 9D8D44216836DCA4F4144BEC4&tier=4&id=31AEAEED467E498A88520D670948F40A &AudID=5648A5C28C97462DBBDB309539B820EF# [Accessed 5 June 2021].

Chaplin, M. (2011).Gellan gum. *Water Structure and Science* [Online] Available at <u>http://www.btinternet.com/~martin.chaplin/hygellan.html#str</u> [Accessed 5 August 2011].

Tako, M. Teruya, T. Tamaki, Y and Konishi, T. (2009). Molecular origin for rheological characteristics of native gellan gum. *Original Contribution August* 287, 1445–1454.

Schmid, W and Picker-Freyer,K.(2008).Tableting and tablet properties of alginates: Characterisation and potential for soft tableting. *European Journal of Pharmaceutics and Biopharmaceutics*,October 72 (2009),165–172.