

Preparation of Gellan Gum (GG) Film: The effect of GG, Calcium Chloride (CaCl_2), Glycerol Concentration and Heat Treatment

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Abstract. The aim of this work was to investigate the effect of gellan gum (GG), calcium chloride (CaCl_2) and glycerol concentration on physical appearance of the film. It was found that, after heated at 50 °C for 24, hours the films were successfully produced using low amount of GG (0.50, 0.75, and 1.00 g) with effective concentration of CaCl_2 (5 mM) and glycerol (50 % (w/w)). The film was not well dried at 40 °C while at 70 and 100 °C, the film formed was easily to rupture due to the overheated. At 1.5 g GG the film cannot be produced due to the formation of highly viscos and sticky formulated solution. The effective glycerol concentration was within the range of 30-50 % (w/w, based on GG weight). At 60 and 70 % (w/w) of glycerol, the films started to distort at the edge due to high concentration of the plasticizer.

1. Introduction

Gellan gum is a type of microbial polysaccharide that is produced from fermentation of the *Pseudomonas elodea*. It is composed of a tetrasaccharide repeating unit of two β -D-glucose, one β -D-glucuronic acid and one α -L-rhamnose [1, 2]. Native gellan contains a substitution of an ι -glycerate group at C₂ and a partial substitution of acetate group at C₆ of 1,3- β -D-glucose. However, the commercial product based on gellan gum has much lower sol-gel transition temperature and higher in gel strength compared to native product. The commercial gellan gum product has been completely deacetylated and de-esterified by alkali treatment [3-6]. The remarkable properties which has been displayed by the gellan gum gel compared to the other polysaccharide gels are that they are heatresistant and less pH-sensitive [4]. Therefore, gellan gum has been widely used in food industry as a thickening agent [7, 8]. Moreover, gellan gum is one of the most promising in situ gelling polymers in the human body and applicable for biomedicine technology, such as drug delivery vehicles, protein immobilization media and wound dressing materials [9-14]. However, the applications of GG gel have their own limitation due to the poor stability in physiological conditions and low in mechanical strength. Thus, researchers focused on synthesizing GG film with good mechanical properties to extend their applications on food packaging, edible film and coating. It can be obtained by incorporating the crosslinker and plasticizer into the GG during the formulation. It is important to note that GG carries a net negative charge from the carboxylic groups present in the glucuronic acid residues which is the determining factor to the ability of GG hydrogel to transform into film after sufficient heat treatment.



Thus the presence of cation as cross-linker to the negative charge of GG is important for gelation process and film forming [15, 16]. The GG chain will be rapidly cross-linked to form the three-dimensional network with addition of cations. K^+ , Na^+ , and Ca^{2+} are among the commonly used cations to form strong and durable GG film. Somehow, divalent Ca^{2+} is widely used as can form stronger gels than monovalent cations [17]. Besides the cross-linker, plasticizer is another important additive in the film formulation especially for biodegradable films [18-20]. The addition of plasticizers can improve the mechanical properties of edible films and various plasticizers, such as polyols, have been employed to increase the flexibility and workability of these films. Among the plasticizers, glycerol is one of the most broadly used in film-making techniques, and it has been successfully employed in the production of polysaccharide-based edible films [21-24]. It is a water soluble, polar, and low molecular weight non-volatile substance, which makes glycerol a suitable plasticizer to be used with a compatible water-soluble polymer [22]. However, to our best of knowledge there are no specific investigations about the effect of plasticizer concentration on the properties of GG film so far. Therefore, the purposes of this work were to: (1) develop a novel edible film based on GG; and (2) determine the effect of heat treatment, GG content, and concentration of cross-linker ($CaCl_2$) and plasticizer (glycerol) on the physical appearance of the resulting films.

2. Experiments

2.1 Materials

Low-acyl gellan gum (Kelcogel®, CP Kelco, lot number 5C1574A), Glycerol (lot number STBC1888V) were purchased from Sigma-Aldrich, Malaysia; as a plasticizer to reduce the brittleness of film. Calcium Chloride ($CaCl_2$, lot number K47117278604, Mw 110.98 g/mol) was obtained from Merck, Malaysia. All materials were used as received.

2.2 Preparation of GG Film

Gellan gum (GG) solution was prepared by dissolving GG (0.00, 0.50, 0.75, 1.00 and 1.50 g) into 100 mL distilled water under continuous stirring for 2 h at 70°C. (30, 40, 50, 60, and 70 % (w/w), relative to GG) of glycerol and (2, 5 and 10 mM) calcium chloride ($CaCl_2$) was added into the gellan gum solution. Then, the mixture was stirred for 15 minutes and the resulting suspension was casted onto an acrylic plate. After removing bubbles, the samples were dried for 24 h (at room temperature, 40, 50, 70 and 100°C) in an oven. All films were preconditioned in a desiccator (27 °C, 50% relative humidity (RH)) for at least 2 days prior to testing.

Table 1. Testing parameter of the experimental design

Type	Testing Parameter
Heat temperature, °C	Room temperature, 40, 50, 70, and 100
Concentration of gellan gum, g	0.00, 0.50, 0.75, 1.00, and 1.50
Concentration of $CaCl_2$, mM	2, 5, 10
Concentration of glycerol, % (w/w)	30, 40, 50, 60, and 70

*percentage weight relative to gellan gum

3. Results and Discussion

Figure 1 shows the produced samples when gellan gum suspension was dried at different temperature for 24 h. After drying at room temperature (RT), the samples were presented in hydrogel form (figure 1(a)). Hydrogel can be described as a solution that shows a behaviour similar to gels at low shear stresses however act as fluids at higher shear stresses [27]. Hydrogel resulted from gellan gum is not a real gel

but a fluid or paste-like material which shows soft texture with a yield stress. It provides an appropriate environment for a proper texture formation and inhibition of sedimentation or suspension of particles in liquid gel structures [28]. This hydrogel is classified as physical gels category because in these gels joining points are formed through physical reactions such as hydrogen bonds, hydrophobic bonds and cross-sectional joints by the aid of cations [29]. The random coils would form double helices structure with neighbouring chains through hydrogen bonds and van der Waals forces [29]. Subsequently, the aggregation would occur to generate a continuous three dimensional (3D) network giving rise to a gel.

Heat treatment was carried out at higher temperature because the existence of -OH groups is important for changes occurred in solution during heating, as it is involved in reactions with other sugars [31]. At 40 °C, the GG film was formed however it is not well dried thus the film was stacked on the casting plate and cannot be peel off (figure 1(b)). At this temperature, the gellan gum which was initially in the membrane suspension liquid, is fully stretched and displaced, thus the integration among them is promoted to produce the film. The formation of film is an initial stage of gellan gum gelatinization accompanied by formation of double helical structures and during heating process strands are formed from the structures which aggregate in the presence of cation to form a three-dimensional hard and brittle gelatin network [32]. After being heated at 50 °C for 24 hours, free standing GG film was formed (figure 1(c)). Meanwhile, at 70 and 100 °C, the produced films were torn apart and ruptured attributed to overheat as the degeneration of film-forming materials may occur, resulting in larger molecules break within the polymer chain (figure 1(d), 1(e)).

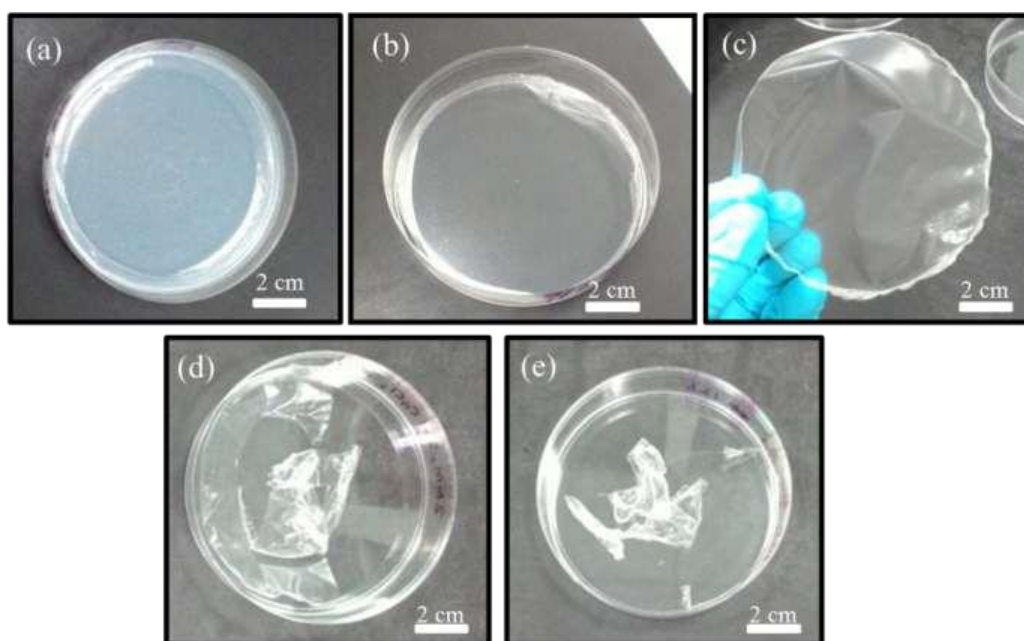


Figure 1. Samples after drying at (a) room temperature, (b) 40 °C, (c) 50 °C, (d) 70 °C, and (e) 100 °C for 24 hours.

The effect of GG mass which were 0.00, 0.50, 0.75, 1.00 and 1.50 g on the physical appearance of films was examined. Films are not able to be formed when the formulation without the gellan gum, presumably due to the lack of intermolecular interactions upon film dehydration. When 0.50, 0.75 and 1.00 g of gellan gum was used, a films was successfully produced as shown in figure 2(a), 2(b) and 2(c), respectively. Among the produced films, 1.00 g GG is the best film as nice spherical shape was observed without shrinkage and distortion as compared to 0.5 and 0.75 g GG films. At the highest amount of GG studied (1.50 g), the film cannot be produced due to the formation of highly viscous and sticky suspension

as observed visually (figure 3(a)) and determined by mildly rubbing between two fingers (figure 3(b)). In general, the stickiness of gel was increased with the increasing of polymer concentration as it leads to more intense aggregation of the polymer chains and stronger physical and chemical crosslinking [33]. Thus, at higher polymer concentrations more inter-chain cross linking occurs leading to a significant increase in viscosity of gel.



Figure 2. The produced film using (a) 0.50 g, (b) 0.75 g, and (c) 1.00 g of GG.



Figure 3. (a) Highly viscos, and (b) sticky GG suspension produced using 1.50 g GG.

The gelation mechanism of GG suspension depends on the nature of cations present as cross-linker. The divalent cation cross-linker promotes the gelation much more effectively than the monovalent cation as they can form ionic bonds directly with the carboxylic acid groups at different GG chains, resulting in the aggregation of the double helices by the inter-chain bridging [34, 35]. One of the capabilities of GG is forming a physical gelation by addition of physical cross-linker (Ca^{2+}). The physical gelation mechanism actually occurred at two stages which is when the temperature of hot GG solution is decreased, the molecules of GG undergo a conformational thermo reversible change from random coils structure to double helices structure [36, 37]. This occurs in GG either with or without additions of ions. However, with addition of cations, the gelation was follow up with the aggregation of the double helical elements with water through hydrogen formation to form three-dimensional (3D) network [38]. In this research the cross-linker (Ca^{2+}) function was observed at 2, 5 and 10 mM. As shown in figure 4(a)-4(c), the films were successfully produced at all studied concentration. It can be observed that increasing the concentration of cross-linker resulted in a significant decrease of the solubility of the biofilms. This indicates that the Ca^{2+} actually promoted to the crosslinking formation between the polymer chains, thus increasing the concentration of Ca^{2+} makes the intermolecular bonds more cohesive and arranged, thereby reduce the solubility of the films.

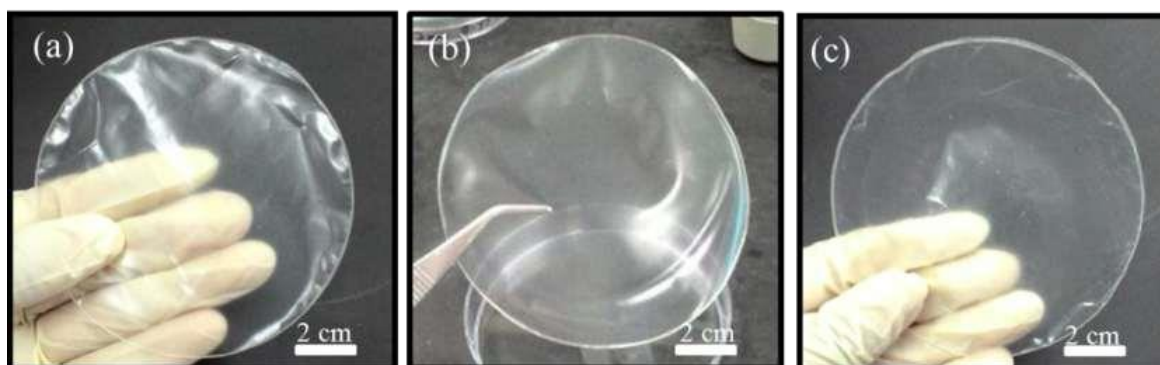


Figure 4. The film produced using different concentration of CaCl_2 cross-linker (a) 2 mM (b) 5 mM, and (c) 10 mM.

In film formulation, the plasticizer was used to enhance the film flexibility and decreased brittleness. There are varieties of plasticizers available commercially in the market such as polyethylene glycol 400 (PEG), sorbitol, propylene glycol (PG), ethylene glycol (EG), and glycerol. However in this study glycerol was selected due as it is the most effective plasticizers for GG films fabrication as recorded in other previous study [39]. Figure 5 shows the produced film using different concentration of glycerol. Good films were produced at low concentration of glycerol used in film formulation especially at 50 w/w %. They are in good shape as well. However, the shape of the films can be altered depending on the casting plate used in the experiment. At higher concentration of glycerol (60 and 70 w/w %), a defect can be observed on the produced film as they were distorted attributed to the limited dispersion capability of the GG solution at higher glycerol concentration. When the plasticizer concentration exceeds polymer compatibility limits, it will cause the phase separation as well as physical exclusion of the plasticizer [40]. The other problems that often encountered in the formation of the films at higher glycerol concentration are the films tend to be sticky and they will be difficult to handle either for characterization or application.

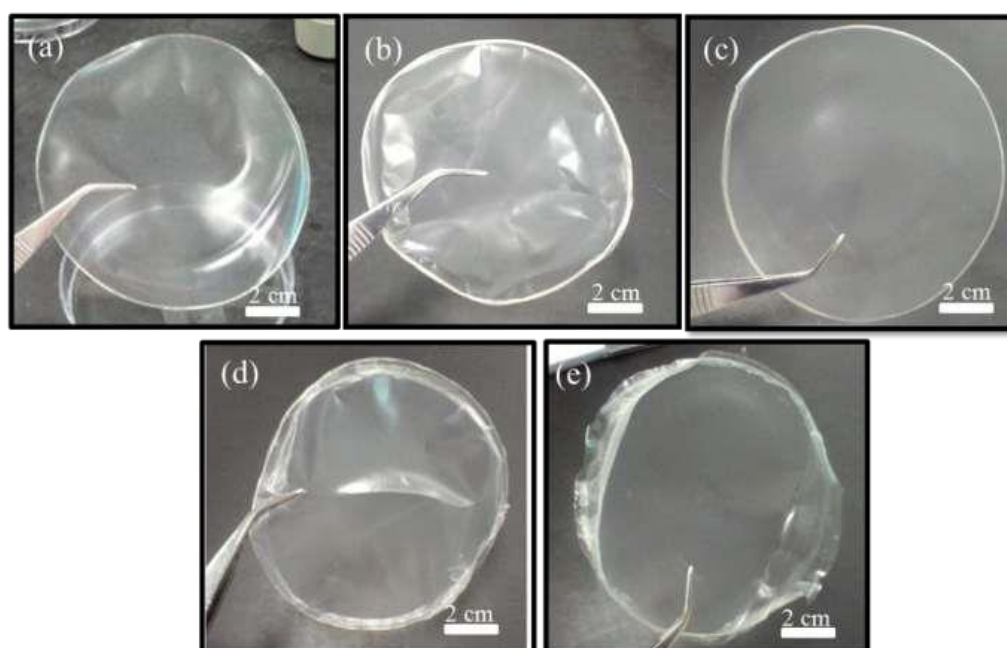


Figure 5. The produced films at different concentration of glycerol (a) 30 % (w/w), (b) 40 % (w/w), (c) 50 % (w/w), (d) 60 % (w/w), and (e) 70 % (w/w).

4. Conclusion

This study demonstrated that GG could be used as a film-forming material. Good film was produced at 1.00 g GG with 5 mM CaCl_2 cross-linker and 50 w/w % glycerol as plasticizer after dried at 50 °C for 24 hours. Based on the visual and preliminary characterisations, it can be concluded that glycerol is an appropriate plasticizer for GG films. Overall, the results of the present study indicates that GG with the effective CaCl_2 and glycerol could be regarded as an appropriate formulation for films synthesis.

5. References

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Acknowledgments

The authors are grateful to Universiti Malaysia Terengganu (UMT) for providing the facilities to carry out this project and Malaysia Ministry of Higher Education for the financial support vote FRGS 59358 and MyBrain15 (MyPhD).