The Physical and Mechanical Properties of Gellan Gum Films Incorporated Manuka Honey as Wound Dressing Materials

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Abstract. In this study, the physical and mechanical properties of gellan gum (GG) films incorporated manuka honey were investigated. The result shown that by increasing the honey content in GG film, the swelling, gel fraction and mechanical properties were decreased. Gellan gum films incorporated with highest concentration of honey at 10\% (GEL-H10) has lowest tensile strength and Yong’s modulus at 0.91 ± 0.2 MPa and 38.9 ± 1.7 kPa, respectively compared to GG films at lower concentration of honey. The water vapour transmission rate of GEL-H10 was recorded at 1145 ± 175 g m\(^{-2}\)d\(^{-1}\) which comparable with the commercial wound dressing product. This film has shown promising results to be used as wound dressing application.

1. Introduction

Biopolymers are receiving greater attention than synthetic petrochemical-based polymers due to the environmental concerns. A variety of renewable biopolymer such as a polysaccharide; i.e. gellan gum (GG) derived from \textit{Pseudomonas elodea}, has been studied in the development of wound dressings materials. GG is approved by the United States Food and Drug Administration (US FDA) and the European Union (EU) for use in the food industry and is an emerging scaffold material for tissue engineering application. For instance, GG has been studied for biomedical application as it has the potential to be used as matrices to repair and regenerate a wide variety of tissue and organ [1]. The development of GG hydrogels receives a lot of attentions due to the exceptional properties which meet the quintessential prerequisities of ideal wound dressings. A few studies have observed the good biocompatibility of GG against human skin fibroblasts cells (CRL-2522) [2], human fetal osteoblasts (hFOBs 1.19) [3] and rat bone marrow cells (rBMC) [4]. In wound dressing application, GG has shown promising results to proliferate the cell growth [5]. Although GG has been reported to biocompatible on live cells, researchers keep working to improve the proliferation rate of GG, for example by forming a complex gel with carboxymethyl chitosan [6] and incorporated with branched polyethyleneimine nanoparticles [7]. Honey is a well-known substance to threat the wounds and burns throughout the ages. Commonly, the healing or other properties of honey is studied in its pure state, i.e. in liquid form. Numerous studies have been reported the advantages of honey such as the anti-bacterial, anti-inflammatory, anti-viral and anti-oxidant effects [8-10]. Besides numerous studies of using pure honey has been used to treat wound and burns, limited studies have been reported to
incorporated honey as a composite with bio-polymer, i.e. transforming honey with biopolymers to a film or hydrogels. A few studies have been reported to used chitosan and gelatin incorporated with honey as a sheet [11], and some using polyvinylpyrrolidone (PVP) entrapped with honey to produce hydrogels [12]. Other study using alginate, chitosan and gelatin to produce hydrogel with an addition of honey [13]. Up to our knowledge, no single study has been conducted to produce gellan gum hydrogels incorporated with honey. This study fabricated and characterized the gellan gum hydrogels incorporating manuka honey. The mechanical characteristics, water vapour transmission rates, and swelling of the hydrogels were examined.

2. Materials and method

2.1. Materials

High-acyl gellan gum (KELCOGEL® Gellan Gum, lot number 5C1574A) was obtained from CP KELCO, USA. Glycerine (product number-G2289, lot number SHBC2650V), and anhydrous calcium chloride, CaCl$_2$ (product number-C5670, lot number SHBC2650V) were obtained from Sigma Aldrich, St Louis, MO, USA and Manuka honey (product number FL522990/1) was obtained from Nature’s Way, New Zealand. All materials were used as received without any further purification.

2.2. Preparation of gellan gum films incorporated Manuka honey (GEL-H)

The gellan gum (GG) films were prepared via casting method. The GG solution was prepared by dissolving 1% (w/v) of GG in 70 mL deionized water and with 55% (w/w) glycerine at continuous stirring for 1 hour and 45 minutes at 70°C. After GG was fully dissolved, 5 mM CaCl$_2$ was added into the mixture and stirred again. Lastly, Manuka honey was added and the solution was stirred for another 15 minutes. These gellan gum solutions containing 2%, 4%, 6%, 8% and 10% (w/v) Manuka honey were known as GEL-H2, GEL-H4, GEL-H6, GEL-H8 and GEL-H10 respectively. The solution was poured into petri dish (90 mm x 5 mm) and dried in the oven at 50°C for 24 hours.

2.3. Characterization of GEL-H films

2.3.1. FTIR Characterization. ATR-FTIR spectra were determined by using Perkin Elmer Spectrum 100 FTIR spectrophotometer with PIKE Miracle ATR accessory with single-bounce beam path, 45° incident angle, and 4 cm$^{-1}$ resolution. Then all spectra were corrected by using Perkin Elmer Spectrum 100 software. The wave number region studied was between 400-400 cm$^{-1}$. The resulting spectra and peak of functional groups were recorded.

2.3.2. Swelling Percentage. The swelling percentage test was carried out by immersing GEL-H (20 mm x 20 mm) in phosphate buffer solution at pH 7.2 in water bath (37 ± 0.5 °C). The samples were removed after 24 hours and lightly wipe with wet filter paper to expel surface solution. Finally, the swelling degree was determined from equilibrium swelling ratio as follow:

\[
\text{Swelling percentage} \, (\%) = \left( \frac{M_f - M_i}{M_i} \right) \times 100
\]

where, \(M_f\) = final weight of sample and \(M_i\) = initial weight of sample

2.3.3. Gel fraction. The film sample was cut into 20 mm x 20 mm and let dried for 24 hours. Then the film was weighed \((W_i)\) and the film was immersed in 10 mL deionised water at room temperature for 24 hours. After removing film from solution, the film was dried in oven at 50 °C for 24 hours and weighed again \((W_f)\) and gel content was calculated as below:

\[
\text{Gel fraction} \, (\%) = \left( \frac{W_i - W_f}{100} \right)
\]

where, \(W_i\) = initial weight, \(W_f\) = final weight.
2.3.4. Water Vapour Transmission Rates. The film was cut into 30 mm x 30 mm and put as a cap on a glass vial with diameter of 16 mm that contain 10 ml deionised water. Then it was weight (W_i) and left in the desiccator containing silica gel for 24 hours. After 24 hours, it was weighed again (W_f). The value of WVTR (g m^{-2} d^{-1}) was calculated as below:

\[ \text{WVTR} = \frac{(W_i - W_f)}{A} \]

where, \( W_i = \) initial weight, \( W_f = \) final weight and \( A = \) area of vial opening

2.3.5. Tensile Strength. The tensile strength was obtained by using Instron universal testing machine, model 3366 with cross-speed set at 10 mm/ min. All films were cut into 20 mm x 60 mm for tensile stress-strain measurement. The tensile stress and strain at break were calculated from the slope of linear part of stress-strain curve. The elastic modulus was also recorded. The tests were repeated triplicates per sample for defined ratio.

3. Results and discussion

3.1. FTIR Spectrum

Figure 1 shows the FTIR spectra of GEL film incorporated manuka honey. There are shifting and disappearance of few functional peaks in spectrum of GEL film compared to a representative of GEL-H10 film. Shifting or disappearance of the frequency in the functional groups of peaks indicates that there is an interaction between polymer and drug [14].

![Figure 1. ATR-FTIR spectra of (a) GEL-H10 film, (b) GEL film and (c) Manuka honey.](image)

GEL film shows a few main peaks at 3273 cm\(^{-1}\) due to the stretching vibration belong to O-H group. A peak was shown at wavenumber of 2935 cm\(^{-1}\) which due to sp\(^3\) hybridization of C-H stretching branched alkane, and a few other peaks at 1625 cm\(^{-1}\) and at 1427 cm\(^{-1}\) due to C=C bond and C-H bond saturated sp\(^3\) hybridization, respectively. There was also C=O stretching band form at1033 cm\(^{-1}\) and a peak 919 cm\(^{-1}\) due to alkane C-H bending. The incorporation of manuka honey in gellan
gum film shows a shifting of stretching vibration in gellan gum film, i.e. GEL film at 3273 cm\(^{-1}\) to 3290 cm\(^{-1}\) of GEL-H10 which indicate the hydroxyl O-H vibration of H-bonded between gellan gum and Manuka honey. The shifting of carbonyl group, C=O group was observed in GEL-H10 at 1035 cm\(^{-1}\) compared to GEL film at 1033 cm\(^{-1}\). The band of C-H stretching branched alkane of GEL film at 16255 cm\(^{-1}\) was shifted to 1638 cm\(^{-1}\) of GEL-H10 which suggested that the interaction between gellan gum and Manuka honey.

3.2. Swelling Percentage
Swelling behaviour is an important characteristic to indicate the ability of film to absorb any exudates or wound liquids from wound area. The ideal values for swelling behaviour depends on the wound condition, in which wound with huge amounts of exudates needed a dressing with high swelling behaviour and vice versa. The swelling percentages of all GEL-H films were summarized in Table 1. The swelling behaviour of GEL-H films was decreased upon addition of Manuka honey. For example, the GEL film absorbed 1321 ± 46% in PBS solution (pH 7.2) and decreased to 1174 ± 24% for GEL-H2 and further reduced to 302 ± 18% for GEL-H10 film. The decreased of swelling of GEL film with high content of honey (GEL-H10) could be due to lesser hydroxyl functional (OH) groups of GEL film available to bind with hydroxyl group from water, or in another word the addition of honey limits the hydrogen bond between GEL film with water [15]. The hydrogen bond is expected to occur between GEL and honey and thus decreased the swelling percentage of GEL-H films.

| SAMPLES   | SWELLING (%) | GEL FRACTION (%) | WVTR (g m\(^{-2}\)d\(^{-1}\)) |
|-----------|--------------|------------------|-----------------------------|
| GEL       | 1321 ± 46    | 29 ± 5           | 1841 ± 214                  |
| GEL-H2    | 1174 ± 24    | 23 ± 4           | 1734 ± 184                  |
| GEL-H4    | 786 ± 25     | 18 ± 3           | 1668 ± 146                  |
| GEL-H6    | 588 ± 14     | 15 ± 4           | 1442 ± 167                  |
| GEL-H8    | 437 ± 21     | 13 ± 4           | 1369 ± 153                  |
| GEL-H10   | 302 ± 18     | 11 ± 2           | 1145 ± 175                  |

3.3. Gel Fraction and Water Vapour Transmission Rates
Gel fraction is a parameter that was utilized to indicate the crosslinking behaviour of the samples. From the result obtained, GEL film has the highest gel fraction recorded at 29 ± 5% (Table 1). The inclusion of honey decreased the gel fraction values up to 11 ± 2% of GEL-H10. It can be deduced that the addition of honey disrupted the crosslinking properties of the film.

Water vapour transmission rates (WVTRs) is important to control the loss of body liquid due to the exudation and evaporation. The loss of body fluid may affect the decrease in body temperature thus accelerate metabolism rate. However, the WVTR value less than 76 g m\(^{-2}\) d\(^{-1}\) can cause accumulation of exudates and lead to growth of bacteria [16]. This problem can be improved by controlling the transmission of the body liquid in order to maintain high humidity area of wound itself. For that reason, WVTR test was necessary to determine the potential of film to allow the transmission of body fluid. Table 1 showed the result of WVTR values of gellan gum hydrogel with different concentration of manuka honey. WVTRs value of GEL-H10 film was significantly decreased to 1145 ± 175 g m\(^{-2}\) d\(^{-1}\) compared to GEL films at 1841 ± 214 g m\(^{-2}\) d\(^{-1}\). Lowest WVTR value for GEL-10 is expected due to huge amount of honey incorporated into the gellan gum film. The present of huge amount of honey (GEL-H10) disturbs the diffusion of water through the film and reflecting to the lowest WVTR obtained. In contrast, GEL-H2 film with lower amount of honey exhibited higher WVTR values.
Nevertheless, all the hydrogel samples show the WVTR values are suitable for wound dressing product because it is in the range of commercial wound dressing product (90-2893 g m⁻² d⁻¹) [16].

3.4. Tensile test

Tensile test was performed to determine the strength of the fabricated film. It is a versatile tool for the mechanical characterization of pharmaceutical film formulations including film dressings. Figure 2 shows the stress-strain curves of the GEL-H films and table 2 summarized the tensile strength, Young’s Modulus and tensile strain of the films. GEL-H2 film exhibited the highest tensile strength and tensile strain at 2.1 ± 0.9 MPa and 12 ± 5% respectively. The addition of higher amount of honey decreased the tensile strength and tensile strain of the film. The GEL-H10 shows the tensile strength at 0.9 ± 0.37 MPa and tensile strain at 3 ± 1%. This is a result of a decreased in hydrogen bonding interactions between gellan gum-gellan gum (GG-GG) due formation of hydrogen bonding between gellan gum-honey (GG-Honey) [17]. The GEL-H10 film physically soft which reflected to the low tensile performance compared to free-standing GEL films.

![Figure 2. Tensile stress-strain curve of GEL-H films.](image)

| SAMPLES  | σ (MPa) | YM (MPa) | ε (%) |
|----------|---------|----------|-------|
| GEL-H2   | 2.1 ± 0.96 | 32.2 ± 3 | 12 ± 5 |
| GEL-H4   | 1.2 ± 0.72 | 27.0 ± 3 | 8 ± 3  |
| GEL-H6   | 1.3 ± 0.53 | 31.5 ± 3 | 6 ± 3  |
| GEL-H8   | 1.2 ± 0.54 | 37.1 ± 2 | 4 ± 2  |
| GEL-H10  | 0.9 ± 0.37 | 38.9 ± 2 | 3 ± 1  |

4. Conclusion

Gellan gum films incorporated with Manuka honey (GEL-H) were successfully prepared. Inclusion of manuka honey into GEL films decreased the swelling and gel fraction of the films. Water vapour transmission rates of GEL-H films were in the range of commercial dressing products and the tensile performance shows all the films were flexible with higher strain values by lowering the concentration of manuka honey. The film has shown promising properties to be used in dressing application.
References

[1] Oliveira J T, Santos T C, Martins L, Picciochi R, Marques A P, Castro A G, Neves N M Tissue Engineering Part A 16 343-53
[2] Amin M, Anuar K, Gilmore K J, Matic J, Poon S, Walker M J and Wilson M R 2012 Macromolecular bioscience 12 374-82
[3] Wang C, Gong Y, Lin Y, Shen J and Wang D-A 2008 Acta Biomaterialia 4 1226-34
[4] Smith A M, Shelton R M, Perrie Y and Harris J J 2007 J Biomater Appl 22 241-54
[5] Mohd S S, Abdullah M A A and Mat Amin K A 2016 Journal of Bioactive and Compatible Polymers: Biomedical Applications 31 648-66
[6] Tang Y, Sun J, Fan H and Zhang X 2012 Carbohydrate Polymers 88 46-53
[7] Goyal R, Tripathi S K, Tyagi S, Ravi Ram K, Ansari K M, Shukla Y, Kar Chowdhuri D, Kumar P and Gupta K C 2011 European Journal of Pharmaceutics and Biopharmaceutics 79 3-14
[8] Lee H, Churey J J and Worobo R W 2008 Int. J. Food Microbiol. 126 240-4
[9] Tan H T, Rahman R A, Gan S H, Halim A S, Hassan S A, Sulaiman S A and Kirnpal-Kaur B 2009 BMC complementary and alternative medicine 9 34
[10] Yaghoobi R and Kazerouni A 2013 Jundishapur journal of natural pharmaceutical products 8 100-4
[11] Wang T, Zhu X-K, Xue X-T and Wu D-Y 2012 Carbohydrate polymers 88 75-83
[12] Yusof N, Hafiza A A, Zohdi R M and Bakar M Z A 2007 Radiation Physics and Chemistry 76 1767-70
[13] Kurhade S T, Momin M, Khanekar P and Mhatre S 2013 International Journal of Drug Delivery 5 353
[14] Prakash S J, Santhiagu A and Jasemine S 2014 Journal of Pharmaceutical and BioSciences 2 63-71
[15] Yang L and Paulson A 2000 Food Research International 33 563-70
[16] P. Wu, A. C. Fisher, P. P. Foo, D. Queen and Gaylor J D S 1995 Biomaterials 16 171
[17] Silva C L, Pereira J C, Ramalho A, Pais A A C C and Sousa J J S 2008 Journal of Membrane Science 320 268-79

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