The Effect of Acetaminophen on Physical, Compression Strength and Thermal Behaviours of Kelcogel Hydrogel Films

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Abstract. This study investigates the physical properties, compression strength and thermal behaviors of the Kelcogel hydrogel films. Kelcogel hydrogel film at 2 mm thickness (KEL-2) shows the optimum swelling ratio and compression strength at 99 ± 10% and 155 ± 33 kPa, respectively compared to other samples. The KEL-2 hydrogel films were further incorporated with acetaminophen and the properties were examined. The swelling ratio and compression strength of KEL-2 hydrogel films contained 30% (w/w) of acetaminophen (KEL-A30) show optimum values at 108 ± 10% and 423 ± 42 kPa, respectively. The WVTRs of the KEL hydrogel films incorporated acetaminophen were in the range of 2329 - 4699 g m⁻² d⁻¹. Thermal stability of KEL hydrogel films also increased depending to the amount of the acetaminophen added. This study shows that the addition of acetaminophen improved the physical properties, compressive strength and thermal behavior of the Kelcogel hydrogel film and promising to be applied as dressing materials.

1. Introduction

Hydrogel films are an appealing material due to its structure is similar to the extracellular matrix of many tissues, and often be processed under relatively mild conditions. Hydrogel films have been utilized as dressing materials, scaffold materials for drug and growth factor delivery, engineering tissue replacements, and a variety of other applications [1, 2]. Nowadays, biopolymers are the primary choice to be used in biomedical application than synthetic petrochemical based polymers. Several biopolymers are available such as chitosan, carrageenan, xanthan gum and gellan gum with different properties. In this study, gellan gum, i.e. a linear anionic polysaccharide produced by Pseudomonas elodea is chosen because it is approved by the United States Food and Drug Administration (US FDA) and the European Union (EU) to be used in the food industry [3]. Not limited to that, it is an emerging scaffold material for tissue engineering application [4, 5]. Various gellan gum brands are available in commercial market such as Kelcogel and Gelzan. Kelcogel is preferred for pharmaceutical, oral care and personal care, and Gelzan for microbiological media and plant tissue culture. Acetaminophen (AP) or also known as Paracetamol is mostly used as an analgesic and antipyretic [5]. It is considered as the safest analgesics available with 200 over products containing acetaminophen as an active ingredient. AP is also used as
a pain-killer to treat headache, back pain, acute pain resulting from soft tissue injury and post-operative pain [6]. Up to our knowledge, no study has been examined the effect of acetaminophen in kelnogel hydrogel films. This study examined the physical properties, compression strength and thermal behaviours of the hydrogel films specifically to be used as dressing materials.

2. Materials and Method

2.1 Materials
Low-acyl gellan gum (Kelcogel, batch number: 5C1574A) were obtained from CP Kelco, Chigaco, IL, USA. Glycerin (product number-G2289), anhydrous calcium chloride, CaCl₂ (product number-C5670), and acetaminophen (product number-A7085) were obtained from Sigma Aldrich, St Louis, MO, USA. All materials were used as received without any purification.

2.2 Preparation of hydrogel film at different thickness (KEL)
Kelcogel films were prepared using evaporation casting method. Hydrogels solution were prepared by dissolving 1% (w/v) of kelcogel in distilled water under continuous stirring with the temperature maintain at 80°C. Then, 50% (w/w) of glycerol was added into the hot solution, followed by addition of 0.1 M calcium chloride solution. Later, the solution was deposited onto petri (90 mm × 15 mm) up to 8 mm in thickness and dried in the oven at 50°C for 4 hours for 6 mm, 6 hours for 4 mm and 24 hours for 2 mm.

2.3 Preparation of hydrogel film incorporated acetaminophen (KEL-AP)
1% (w/v) of kelcogel was dissolve in distilled water with the temperature at 80°C under continuous stirring to produce hydrogel solution. After the kelcogel was completely dissolved, 50% (w/w) of glycerol was added into the solution. 10% (w/w) of acetaminophen by weight relative to hydrogel was prepared by dissolving using methanol to produce acetaminophen solution. Then the solution of acetaminophen was added drop wise into the hydrogel solution, and hereafter known as KEL-AP10. Lastly, 0.1 M CaCl₂ solution was added drop wise into the hydrogel solution under continuous stirring for 2 hours. After 2 hours of reaction, the hydrogel solution was pour into the petri dish at 8 mm thickness and dried in the oven at 50°C for 24 hours. The steps were repeated for 20% (w/w) and 30% (w/w) of acetaminophen, and the samples were known as KEL-AP20 and KEL-AP30 hydrogel films, respectively.

2.4 Characterization of hydrogel

2.4.1 Swelling Test.
Dried sample of 2 cm × 2 cm hydrogel were cut and immersed in 20 mL phosphate buffer solution (PBS) of pH 7.2 at 37 ± 0.5 °C for 24 hours. After 24 hours, the samples were removed and the excess water on the surface was wiped using tissue. The weight of hydrogel before (Wᵢ) and after (Wᵢ) immersed in PBS were recorded and the swelling percentage was calculated using the equation 2.1:

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

Eqn. 2.1

where Wᵢ and Wᵢ are initial and final weight, respectively.

2.4.2 Water Vapour Transmission Rate (WVTR).
The WVTR was carried out by cutting 3 cm × 3 cm hydrogel and placed them on the circular opening (test area) of cylindrical glass vial containing 10 mL water. Then the glass vials were left in the desiccator for 24 hours. The weight before and after the glass vials left in the desiccator were recorded and the WVTRs was calculated using the equation 2.2:

\[
\text{WVTR} = \frac{(Wᵢ - Wᵢ)}{A \times 24} \frac{g}{m²/\text{day}}
\]

Eqn. 2.2
where $W_i$ and $W_f$ are initial and final weight of glass vial and $A$ is the test area of the sample in m$^2$.

2.4.3 Compression Test.
The compression test was carried out using Instron Universal Mechanical machine (model 3366) at a cross speed set at 10 mm/min. All hydrogels were cut to 2 cm $\times$ 2 cm dimension. A minimum of three independent measurements will be obtained per sample of a defined ratio.

2.4.4 Thermogravimetric Analysis (TGA).
Thermogravimetric analyses were performed using Thermogravimetric Analyzer model Mettler Toledo. Films were cut into small pieces and about 10 mg were used. Then the films were heated from 30°C to 700°C at a heating rate of 10°C/min under nitrogen atmosphere with a nominal gas flow rate of 30 mL/min.

2.4.5 Differential Scanning Calorimetry (DSC).
Differential scanning calorimetry was performed using Pyris 6, Perkin-Elmer-TGA7. The samples were weighted about 4 mg. The heating rate was 10°C/min and the nitrogen gas was purged at a flow of 50 mL/min. The heating range of the sample was from room temperature to 200°C.

3. Result and Discussion
3.1 Swelling Test
Swelling test was conducted to measure the maximum amount of fluid absorbed and retained by the film. The swelling percentage of all hydrogel films were presented in Table 1. KEL-8 shows the lowest percentage of water uptake due to the thickest hydrogel (8 mm) at 2.2 ± 0.8% and has higher water content compare to other sample which resulting to the smallest absorption of water. Besides that, it is typical for a thick film (or known as hydrogel) of KEL-8 to absorb minimum water due to low water holding capacity. In contrast, KEL-2 film absorbs optimum water at 99 ± 10% due to its high holding capacity. This optimum swelling value is needed for wound dressing materials to absorb exudate from wound and promote moist environment to the wound bed. The swelling percentage of the KEL hydrogel films incorporated acetaminophen (KEL-AP) increased as the concentration of the latter increased. The enhancement of swelling values of KEL-AP film might be due to the present of higher number of the hydroxyl group (OH) in KEL-AP which able to bind with the water. The swelling percentage of KEL-AP30 film increased to 108 ± 10%. It is reported that an ideal wound dressing should be able to absorb water in the range of 100-900% [6]. Thus, the addition of acetaminophen enhanced the swelling properties of the hydrogel films.

Table 1. Swelling percentage and water vapour transmission rates (WVTRs) of the kelcogel film (KEL) and kelcogel film with acetaminophen (KEL-AP).

| Sample     | Swelling Percentage (%) | WVTR (g m$^{-2}$ d$^{-1}$) |
|------------|-------------------------|----------------------------|
| KEL-8      | 2 ± 0.2                 | N/A                        |
| KEL-6      | 18 ± 1                  | N/A                        |
| KEL-4      | 55 ± 18                 | 2690 ± 77                  |
| KEL-2      | 99 ± 10                 | 2385 ± 76                  |
| KEL-AP10   | 82 ± 7                  | 2329 ± 55                  |
| KEL-AP20   | 83 ± 8                  | 4361 ± 154                 |
| KEL-AP30   | 108 ± 10                | 4699 ± 596                 |

*N/A = not applicable*
3.2 Water Vapour Transmission Rate (WVTR)
Water vapour transmission rates (WVTRs) is used to measure the amount of water loss across the film over defined times. Film wound dressing should prevent or reduce the body liquid lost by controlling absorption and transmission as well as maintaining the humidity in the wound area, in order to accelerate the formation of granule and epithelisation process [7]. Therefore, WVTR is an important test to examine the potential of dressing materials in controlling the body liquid or wound exudate. The WVTRs values of all films were shown in Table 1. The WVTR values decreased as the thickness of the film decreased from 2690 ± 77 g m\(^{-2}\) d\(^{-1}\) of KEL-4 to 2385 ± 76 g m\(^{-2}\) g\(^{-1}\) of KEL-2. The WVTR values of KEL-8 and KEL-6 films were not carried out due to tendency of the samples to shrink within 24 hours of measurement and resulted in inaccurate results. The addition of acetaminophen in KEL films increased the WVTRs value to 4699 ± 596 g m\(^{-2}\) d\(^{-1}\). All the WVTRs values obtained were in the range of acceptable WVTRs values and comparable with commercial wound dressing products, i.e. in the range of 90-2893 g m\(^{-2}\) d\(^{-1}\) [8].

3.3 Compression Test
Compressive test was used to determine the mechanical strength of films and the effect of addition of acetaminophen into the films. The mechanical property of wound dressing materials is very important because certain strength was needed for handling and replacement of dressing materials at difference contours of human body [9]. The compressive stress (kPa), compressive strain (%) and Young’s Modulus (kPa) of the hydrogel films were summarized in Table 2. The compressive strength of the hydrogel films increased as the thickness of the hydrogel film decreased. KEL-2 yielded the highest compressive stress and Young’s Modulus at 155 ± 33 kPa and 2165 ± 470 kPa respectively. Inclusion of acetaminophen increased the compressive stress to 160 ± 10 for KEL-AP10 hydrogel film. The Young’s Modulus also increased to 2275 ± 284 kPa compare to free-standing KEL-2 film. The inclusion of higher concentration of acetaminophen further increased the stress to 423 ± 42 kPa for KEL-AP30, in which an increment of almost 3-fold compared to KEL-2 film. Meanwhile, the Young’s Modulus value also increased to 4-fold at 8773 ± 342 kPa compared to KEL-2. The increased of compressive strength and Young’s modulus of the KEL-AP films could be due to the improvement of cross-linking behaviour among the film network which creates the strong hydrogen bond interactions between kelcogel and acetaminophen. In contrast, the strain value for KEL-AP10 was decreased to almost 1-fold after the addition of acetaminophen due to limited stretching of the network in the film structure (hydrogen bond). KEL-AP30 recorded the lowest strain value at 9 ± 1 %. The addition of acetaminophen was successfully improved the strength of the hydrogel films and make it possible to be used on different part of our body as dressing materials.

| Sample   | Compressive Stress (kPa) | Compressive Strain (%) | Young’s Modulus (kPa) |
|----------|--------------------------|------------------------|----------------------|
| KEL-8    | 50 ±7                    | 20 ±4                  | 486±7                |
| KEL-6    | 55 ±3                    | 15 ±2                  | 590±26               |
| KEL-4    | 72 ±6                    | 19 ±1                  | 670±77               |
| KEL-2    | 155 ±33                  | 14±2                   | 2165±170             |
| KEL-AP10 | 160 ±10                  | 12±1                   | 2275±184             |
| KEL-AP20 | 360 ±75                  | 16±5                   | 5477±113             |
| KEL-AP30 | 423 ±42                  | 9±1                    | 8773±242             |
3.4 Thermogravimetric Analysis (TGA)
The thermogram and derivative thermogram of pure hydrogel film (KEL-2) and KEL-AP films were presented in Figure 1 and summarized in Table 3. The TGA/DTG result shows two main region observed, at (i) 30 °C to 140 °C, which resulting from the removal of free water from the polysaccharide [10], and (ii) 180 °C to 280 °C, which related to the decomposition of the aromatic bond of acetaminophen (Fig 1).

![Figure 1. (a) Thermogravimetric thermograms (TGA) and (b) derivative thermograms (DTG) of the kelcogel film (KEL) and kelcogel film with acetaminophen (KEL-AP).](image)

Table 3. Temperature onset ($T_o$), temperature completion ($T_c$) and weight loss (%) of KEL-2 and kelcogel film with acetaminophen (KEL-AP).

| Sample     | Temperature onset, $T_o$ (°C) | Temperature Completion, $T_c$ (°C) | Weight loss (%) |
|------------|-------------------------------|-----------------------------------|-----------------|
| KEL-2      | 38                            | 600                               | 93              |
| KEL-AP10   | 38                            | 69                                | 69              |
| KEL-AP20   | 39                            | 80                                | 80              |
| KEL-AP30   | 39                            | 92                                | 92              |

The degradation of KEL-2 film started approximately at temperature onset, $T_o=38^\circ C$ and the temperature completion, $T_c$ at 600°C (Table 3). Inclusion of acetaminophen increased the temperature onset $(T_o = 39^\circ C)$, and further increased the temperature completion, $T_c$ ($T_c = 580^\circ C$- $670^\circ C$) depending to the concentration of acetaminophen. KEL-AP20 films yield the highest temperature completion at $T_c=670^\circ C$.

3.5 Differential Scanning Calorimetry (DSC) analysis
DSC result shows that the inclusion of acetaminophen affect the thermal stability of the hydrogel film (Figure 2 and Table 4). The melting point, $T_m$ is increased depending to the concentration of acetaminophen, in the range of 106 °C to 147 °C. KEL-AP30 yields the highest melting point ($T_m=147^\circ C$) than KEL-2 hydrogels ($T_m= 104^\circ C$). Broad exothermic peaks were detected for KEL-A hydrogel
films. Higher content of acetaminophen increased the $T_c$ to 278 (KEL-AP30) and confirming the improved thermal stability of the films with addition of acetaminophen.

![Figure 2. Differential scanning calorimetry thermograms of kelcogel film and kelcogel film with acetaminophen (KEL-AP) at different concentrations.](image)

| Sample     | Temperature (°C) | Range (°C) |
|------------|------------------|------------|
|            | Glass Transition, $T_g$ | Temperature Onset, $T_o$ | Temperature Melting, $T_m$ | Temperature Completion, $T_c$ | $T_c - T_o$ |
| KEL-2      | 29               | 104        | 123        | 275             | 171     |
| KEL-AP10   | 28               | 63         | 106        | 278             | 215     |
| KEL-AP20   | 28               | 108        | 134        | 285             | 177     |
| KEL-AP30   | 29               | 128        | 147        | 278             | 150     |

4. Conclusion
Kelcogel film incorporated with acetaminophen (KEL-AP) was successfully examined for their physical properties, compression and thermal behaviours. It shows that the inclusion of acetaminophen improved all the properties particularly the swelling and compression strength of the films. The water vapour transmission rates of the KEL-AP film were comparable to the commercial dressings. In conclusion, the KEL-AP films shows promising properties to be used as wound dressing materials.

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