Sodium alginate film: the effect of crosslinker on physical and mechanical properties

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Abstract. In this work, the effect of calcium chloride (CaCl₂) at different concentrations and immersion duration on physical and mechanical properties of sodium alginate (SA) films was examined. The results show that the swelling properties of SA films were decreased upon immersed in longer duration of CaCl₂ due to improved crosslink between carboxyl group (-COOH) of SA to calcium ion (Ca²⁺) of the CaCl₂. In contrast, gel fractions of the films were increased at longer duration of immersions. The mechanical performances of the SA film immersed in 0.8 M of CaCl₂ for 8 minutes exhibited highest tensile stress, tensile strain and Young’s modulus at 3.92 ± 0.3 MPa, 21.08 ± 1.3% and 27.81 ± 7 MPa, respectively compared to SA films. The results show that the optimization of crosslinker could improve the physical and mechanical performances of the SA films.

1. Introduction
Sodium alginate is a biodegradable film which has been widely used in biomedical applications such as wound dressing, tissue engineering and drug delivery system due to its biocompatibility, biodegradability as well as its properties which similar to the human tissues [1]. Alginates are hydrophilic polysaccharide and can be found in brown seaweed, made up of 40% dry mater consisting another ionic polymer [2]. Alginates polymers primarily consist of blocks containing two uronic acids consist of two chain-forming heteropolysaccharides made up of blocks of β-(1,4)-linked D-mannuronic (M) and α-(1,4)-linked L-guluronic (G) acids. The latter can forms gels by complexing with divalent cations, and their structure varies depending to the monomer position in the chain, forming either homopolymeric (MM or GG) or heteropolymeric (MG or GM) blocks [3]. Physical and mechanical properties of alginate and the formation of gels depend on the relative proportion of these blocks, in which higher concentration of alginate promotes better strength. The ability of alginates to form gels in the presence of divalent calcium ions is one of their main bio-functional properties has a great industrial significance [4, 5]. This study fabricated and characterized the sodium alginate hydrogel films at different concentrations of crosslinker. The swelling, water vapour transmission rates, gel fraction and mechanical characteristics of the hydrogel films were investigated.
2. Materials and method

2.1. Materials
Sodium alginate (SA) powders were obtained from Merck Sdn. Bhd (Malaysia). Glycerol, calcium chloride was purchased from Sigma Aldrich, St Louis, MO, USA. All materials were used as received without any purification.

2.2. Preparation of sodium alginate film
A film-forming solution was prepared by dissolving 2% (w/v) of sodium alginate (SA) in 80 mL deionized water and 50% (w/w) glycerine at continuous stirring for 1 hour and 45 minutes at 60°C. The solution was poured into petri dishes (90 mm (x) 5 mm) and dried in oven at 60°C for 24 hr. The SA films were peeled off, and immersed in different concentrations of CaCl₂ solution, i.e. 0.2M, 0.4M, 0.6M and 0.8M with immersion durations at 2, 4, 6 and 8 minutes. The SA films were then dried again in oven at 60°C for 24 hr prior to any characterizations.

2.3. Swelling properties
The swelling behaviour was carried out by immersing SA film (20mm (x) 20mm) in phosphate buffer saline (pH 7.2). The samples were removed after 24 hours and carefully blotted with filter paper to remove the excess of phosphate buffer saline (PBS) from the surface and reweighed. The percentage of swelling was calculated using Eqn. 1:

\[
\text{Swelling (\%) = \left( \frac{M_w - M_o}{M_o} \right) \times 100}
\]  
(Eqn. 1)

where \( M_w \) is the final weight of samples and \( M_o \) is the initial weight of sample

2.4. Water vapour transmission rate
The WVTR of the films was measured by using a modification of the ASTM E96 (ASTM, 1995). The films were cut into 30 mm x 30 mm and fixed on a glass vial with diameter of 16 mm that contained 10 mL deionized water. The glass vials were placed inside a desiccator containing silica for 24 hours at 25°C. The analysis was carried out in triplicates to get the average weight. The WVTR (g m⁻² day⁻¹) was calculated using the following Eqn. 2:

\[
\text{WVTR} = \left( \frac{W_i - W_f}{A} \right)
\]  
(Eqn. 2)

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

2.5. Gel fraction
The films were cut into 20mm x 20mm dimension and dried at 60°C for 24 hours. Then the film was weighed (\( W_1 \)) and swelled in 20 mL of PBS solution at room temperature for 24 hr. Then, the wet samples were removed and the films were then dried again in an oven at 60°C for another 24 h and then reweighed (\( W_2 \)). The gel content of the film was calculated using the following equation Eqn. 3:

\[
\text{Gel content (%) = \left[ \frac{W_2}{W_1} \right] \times 100}
\]  
(Eqn. 3)

where, \( W_2 \) is the weight of sample after immersed in PBS and \( W_1 \) is the weight of sample after dried in the oven.

2.6. Mechanical properties
Mechanical testing was obtained by using an Instron Universal Testing Machine (Model 3366) with cross-speed set at 10 mm/min. The SA films were cut into 60mm (x) 20mm for tensile stress-strain measurement. Initial grip separation was set at 50 mm. The tensile stress and strain at break were calculated from the slope of linear part of stress-strain curve. The elastic modulus was also examined. The test was repeated triplicates per sample.
3. Result and discussion

3.1. Swelling properties

The swelling degree indicates the water retention ability of the films. The swelling percentages of sodium alginate (SA) films treated with different concentrations of CaCl \(_2\) and immersion period were recorded in Table 1. No result for free-standing SA film without treated with CaCl \(_2\) reported because it is not possible to produce the latter deprived of crosslinker. The results show that swelling degree (\%) of the SA films decreased upon immersed for longer duration (2 min – 8 min) in all concentration of CaCl \(_2\) (0.2M, 0.4M, 0.6M and 0.8M). For example, SA film immersed in 0.2M CaCl \(_2\) for 2 min recorded 54±1.8\% of swelling degree and further decreased to 26±0.9\% after immersed for 8 min. A slightly higher swelling degree for SA film immersed in 0.8M for 2 min at 69±1.4\% and decreased after immersed for 8 min to 51±1.2\%.

The difference in the swelling behaviour of the SA films can be explained as follows; when SA films are immersed in CaCl \(_2\) solution, Ca\(^{2+}\) ion present in the swelling medium penetrates into the SA film matrix and leads to the ion exchange with Na\(^+\) ions, which attached to -COO\(^-\) groups of SA blocks [6]. This process strengthens the crosslinking of SA film with crosslinker, and therefore further improved the structural integrity upon increased the molarity of CaCl \(_2\). That is the reason the swelling degree were higher after immersed in higher concentration of CaCl \(_2\) (0.8M). However, the decreased of swelling degree upon exposed to longer duration of immersion is due to degradation of SA cross-linked calcium in phosphate buffer saline (PBS) at a neutral pH. Since the affinity of PO\(_4\)^{3-} ions in the PBS was higher than Ca\(^{2+}\) ions of SA, it caused the breakage of the interaction between Ca\(^{2+}\) ions and the carboxylate groups of calcium-crosslink alginate [7], thus decreased the swelling degree at longer immersion period.

| Molarity CaCl \(_2\) (M) | Immersion time in CaCl \(_2\) (min) | Swelling degree (%) | WVTR (g m\(^{-2}\) d\(^{-1}\)) | Gel fraction (%) |
|--------------------------|-------------------------------------|---------------------|-----------------------------|-----------------|
| 0.2                      | 2                                   | 54 ± 1.8            | 2137                        | 20 ± 1.2        |
|                          | 4                                   | 22 ± 0.8            | 2582                        | 22 ± 1.8        |
|                          | 6                                   | 31 ± 1.7            | 3171                        | 19 ± 1.1        |
|                          | 8                                   | 26 ± 0.9            | 3115                        | 21 ± 0.9        |
| 0.4                      | 2                                   | 43 ± 1.4            | 1837                        | 24 ± 1.6        |
|                          | 4                                   | 42 ± 2.0            | 1966                        | 23 ± 1.2        |
|                          | 6                                   | 40 ± 1.8            | 2209                        | 26 ± 1.3        |
|                          | 8                                   | 31 ± 2.1            | 2412                        | 27 ± 0.6        |
| 0.6                      | 2                                   | 44 ± 1.3            | 1504                        | 23 ± 0.8        |
|                          | 4                                   | 43 ± 1.3            | 1835                        | 25 ± 1.5        |
|                          | 6                                   | 34 ± 1.2            | 2037                        | 26 ± 0.3        |
|                          | 8                                   | 27 ± 1.7            | 2214                        | 28 ± 0.7        |
| 0.8                      | 2                                   | 69 ± 1.4            | 1156                        | 26 ± 1.2        |
|                          | 4                                   | 64 ± 1.5            | 1535                        | 28 ± 1.2        |
|                          | 6                                   | 51 ± 1.3            | 1738                        | 28 ± 0.9        |
|                          | 8                                   | 51 ± 1.2            | 1870                        | 33 ± 1.6        |
3.2. Water vapour transmission rate

Water vapour transmission rates (WVTRs) is an important test to be determine in examine the loss of body fluid due to the evaporation process. The loss of huge quantity of exudates could causes the decrease in body temperature, and disrupted the process of evaporation, as well as could build up the pressure around wound and thus give pain to the patient [8]. Because of that, the WVTRs values are crucial to be examined and confirm the ability of the films to allow the transmission of body fluid. WVTRs of SA films at difference concentrations of CaCl$_2$ and immersion period were shown in Table 1. The results show that the WVTR values were decreased upon exposed to higher concentration of CaCl$_2$ at 0.8M due to stronger crosslinking between Ca$^{2+}$ with functional groups of SA. This strong crosslink acts as hindrance to diffuse molecule slow down the diffusion process. The WVTR values of SA immersed in 0.2M of CaCl$_2$ for 2 min at 2137 g m$^{-2}$ d$^{-1}$ compared to SA films immersed in 0.8M (2 min) at 1156 g m$^{-2}$ d$^{-1}$. The WVTRs values were further increased after immersed in longer period for all concentrations of CaCl$_2$. Nevertheless, the WVTR values of SA films are comparable to commercial wound dressing product such as OpSite at 792 g m$^{-2}$ d$^{-1}$ and Metalline at 1272 g m$^{-2}$ d$^{-1}$ that are suitable and safe as wound dressing materials [9].

3.3. Gel fraction

Gel fraction with various concentration of CaCl$_2$ solution was shown in Table 1. It was measured in order to examine the crosslinking degree of polymer chains within the films of sodium alginate. An increase in concentration of CaCl$_2$, increases in the percentage of gel content due to the formation of crosslink between Ca$^{2+}$ ions and the carboxylate group occurring in sodium alginate. Gel fraction of SA films immersed in 0.2M for 8 min was recorded at 21 ± 1.2% and further increased for SA films immersed in 0.8M (8 min) to 33± 1.6%.

3.4. Mechanical test

The tensile test is conducted to study the mechanical and to measure the strength and flexibility of the polymer’s films. The characterization of tensile is important for wound dressing materials to be examined and make sure it can be installed following the contour of our body and protect wounds. Fig. 1 shows the stress-strain curves of the SA films at different concentration of crosslinker and the tensile stress (σ), tensile strain (ε) and Young’s modulus (YM) of the films were summarized in Table 2. The results clearly show the significant effect of CaCl$_2$ to the mechanical properties of SA films. Higher concentration of CaCl$_2$ in SA films increased the σ, ε and YM of the films. The σ and YM values of SA films increased up to 4-fold after immersed in 0.8M compared to 0.2M of CaCl$_2$ for 8 minutes. The strain values of the SA films do not significantly change but increased from 15.19 ± 1.5% to 21.08 ± 1.3%.

The improved mechanical properties of the SA film in CaCl$_2$ could be due to the crosslinking reaction between Ca$^{2+}$ ions and carboxyl group of SA. The crosslink could form by a simple ionic bridging of two carboxyl groups on adjacent polymer chains with calcium ions. The results obtained is in agreement with the previous study [10].
Figure 1. Stress-strain curve of sodium alginate (SA) film after immersed in different concentrations of calcium chloride solution for (a) 2 min and (b) 8 min.

Table 2. The tensile stress (σ), tensile strain (ε) and Young’s modulus (YM) of sodium alginate film immersed in different concentration CaCl₂ for 2 and 8 min of contact time

| Molarity CaCl₂ (M) | Period (min) | σ (MPa)   | ε (%)    | YM (MPa) |
|-------------------|--------------|-----------|----------|----------|
| 0.2               | 2            | 1.00 ± 0.2| 15.21 ± 1.1 | 8.26 ± 2.3 |
|                   | 8            | 1.05 ± 0.2| 15.19 ± 1.5 | 9.23 ± 0.6  |
| 0.4               | 2            | 1.25 ± 0.2| 16.90 ± 2.5 | 9.64 ± 2.0  |
|                   | 8            | 2.43 ± 0.2| 16.25 ± 1.0 | 20.96 ± 10.6|
| 0.6               | 2            | 1.82 ± 0.2| 12.22 ± 3.2 | 17.88 ± 6.1 |
|                   | 8            | 2.90 ± 0.5| 18.63 ± 3.1 | 24.26 ± 12.1|
| 0.8               | 2            | 3.14 ± 1.2| 18.83 ± 3.0 | 24.97 ± 10.2|
|                   | 8            | 3.92 ± 0.3| 21.08 ± 1.3 | 27.81 ± 7.5 |

4. Conclusion

In this work, the sodium alginate (SA) film has been successfully prepared and characterized. The properties of the SA film were found to be affected by the concentration and contact time during the immersion in the calcium chloride (CaCl₂) solution. High concentration of CaCl₂ at 0.8M improved the swelling degree, water vapour transmission rates, gel fraction and mechanical properties of the films than 0.2M. These results show that the physical and mechanical properties of SA films can be modified by controlling the level of external crosslinking and acceptable to be applied as dressing materials.

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