Physiochemical and Mechanical Properties Study of Gellan Gum Incorporating Norfloxacin Biofilm

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Abstract. The aim of this work was to investigate the physiochemical and mechanical properties of gellan gum incorporating norfloxacin (GG-NOR) biofilm at different concentration of NOR. Four different concentration of NOR (0.01%, 0.1%, 0.5%, and 1% (w/w)) were used to produce GG-NOR biofilms. The biofilms was successfully produced with good shape and were transparent at ~95%. The addition of norfloxacin was found to increase the toughness and elongation-at-break values of biofilm to 0.52 J g⁻¹ and 3.8 % at higher concentration of NOR. On top of that, the thermal stability of biofilm also was increased when the concentration of norfloxacin was increased attributed to the interaction between gellan gum and norfloxacin as proved by FTIR analysis.

1. Introduction

The ideal biodegradable materials are obtained from renewable biobased resources, usually called biopolymers, with excellent mechanical and barrier properties and biodegradable at the end of their life. Biopolymers have been considered as a potential environmentally-friendly substitute for the use of non-biodegradable and non-renewable plastic packaging materials. Biofilm materials may also serve as gas and solute barriers and complement other types of packaging by improving the quality and extending the shelf life of foods. Furthermore, biodegradable film packaging materials are excellent vehicles for incorporating a wide variety of additives such as antioxidants, antifungal agents, antimicrobials, colors, and other nutrients [1,2].

However, several concerns must be addressed before the commercial use of biobased primary film materials. These concerns include degradation rates under various conditions, changes in mechanical properties during storage, the potential for microbial growth, and release of harmful compounds into packaged foods. In reality, relatively poor mechanical and high hydrophilic properties coupled with the poor process ability of biopolymer-based packaging materials are a major limitation on their industrial use [3]. Thus, in this study norfloxacin has been incorporated into gellan gum (GG) biopolymer in order enhanced the mechanical properties of the film. Previously GG biopolymer
widely used in biomedical applications due to its biocompatibility and biodegradability properties [4-6]. The selection of norfloxacin because it offer an antibacterial activity due to the presence of fluoroquinolone group in the structure [7, 8]. Many studies have been reported the effectiveness of the norfloxacin in combating the Gram-positive and Gram-negative bacteria [9-16]. At the same time norfloxacin is more active and less cytotoxicity compared to other fluoroquinolone groups such as levofloxacin and ofloxacin [17-18].

2. Experiments

2.1. Materials
Low-acyl gellan gum (Gelzan™ CM, Mw ≈ 2-3 x 10^5 Da, product number G1910, lot number SLBB0374V) was obtained from Sigma-Aldrich, Malaysia. The commercial antibiotic, Norfloxacin (product number N9890) was obtained from Fluka, USA. All materials were used as initially received.

2.2 Preparation of Biofilm
A stock solution of gellan gum (GG) was made by dissolving 1% (w/v) of GG in 100 mL deionized water (18.2 MΩ) with continuous stirring for 2 h at 70 °C. GG-NOR solutions were prepared by adding 0.01%, 0.1%, 0.5% and 1% (w/w) norfloxacin (NOR) into the stock solution of GG. The GG and GG-NOR solutions were next deposited onto petri dishes (90 mm x 15 mm) and placed inside Venticell oven at 30 °C for at least 24 h. All biofilms were pre-conditioned in a desiccator (24 °C, 50 % relative humidity (RH)) for at least 2 days prior to testing [19].

2.3 Characterization of Biofilm
Ultraviolet (UV-vis) transmission spectra of biofilms were performed using a spectrophotometer (Varian, Cary 50) with data interval = 5 nm, scan rate = 24 000 nm/min and wavelength range 200-800 nm. Quartz cells with path length 10 mm were used. The UV-vis transmittance was conducted by measured the attached biofilms on the cuvette’s surface. ATR-FTIR spectra were collected using a Perkin Elmer Spectrum 100 FT-IR spectrophotometer with PIKE Miracle ATR accessory (single-bounce beam path, 45 ° incident angle, 16 scans, 4 cm⁻¹ resolution) and all spectra were corrected by the Perkin Elmer spectrum 100 software. Stress-strain measurements were obtained using an Instron Universal Testing machine (model 3366) with ± 10 kN grips and the cross-speed set at 20 mm/min. Biofilm thickness (2.0 cm x 6.0 cm) was measured by a hand-held micrometer (Mitutoyo). Young’s modulus (E), tensile strength (TS), and toughness (T) were calculated from the slope of the linear part of the stress-strain curve, maximum stress, and through integration of the area under the curve, respectively. Elongation-at-break (EAB) was also recorded. Thermogravimetric analyses were performed on a Pyris 6, Perkin-Elmer-TGA6. Biofilm samples were analysed in platinum pans at a heating rate of 10 °C/min to 900 °C in an atmosphere of N2 flow at 50 ml/min. Sample used was approximately 10 mg.

3. Results and Discussion
The addition of norfloxacin in gellan gum biofilm does not change the physical appearance of the biofilm compared to pure GG biofilm (Figure 1 (a)-(e)). All the biofilms, namely GG-NOR001, GG-NOR01, GG-NOR05, and GG-NOR1 which containing 0.01 %, 0.1 %, 0.5 %, and 1 % (w/w) of norfloxacin, respectively, were transparent with the transmittance at ≈95 % at 700 nm (Figure 1(f)). The transparent property of this biofilm can be an advantage for the packaging materials i.e., to increase the initial perception of food freshness, colors and texture of the products as well as to highlight the quality of food ingredient.

The chemical interaction of gellan gum and norfloxacin was further investigated by using ATR-FTIR spectroscopy as shown in Figure 2. Norfloxacin shows eight prominent peaks, i.e., at 3393 cm⁻¹ corresponding to the v(N-H) stretching vibration of the imino-moiety of piperazinyl groups [20], 2987 cm⁻¹ referred to the presence of ethyl group and 1716 cm⁻¹ represented the carbonyl v(C=O) stretching
of carboxylic acid. Apart from that, the peaks at 1619 cm\(^{-1}\) represented \(\nu\)(N-H) bending vibration of quinolones group, 1489 cm\(^{-1}\) due to \(\nu\)(O-C-O) of acids while 1029 cm\(^{-1}\) represented the \(\nu\)(C-F) group. Besides that, the peak at 921 cm\(^{-1}\) suggested the \(\nu\)(N-H) bending vibration of amines. Last but not least, for pure norfloxacin absorption peak was observed at 704 cm\(^{-1}\) which was assigned due to the meta distribution of the aromatic protons [21-24].

![Figure 1](image_url)

**Figure 1.** The appearance of biofilms (a) GG (b) GG-NOR001, (c) GG-NOR01, (d) GG-NOR05, (e) GG-NOR1 biofilms, and (f) transmittance of pure GG and GG-NOR biofilms. Scale bar represents 2 cm
The band at 3463 cm\(^{-1}\) and 1748 cm\(^{-1}\) in gellan gum spectrum was detected due to the O-H and C=O group stretching [25]. Further, the peaks at 1736-1748 cm\(^{-1}\) were detected in GG-NOR composite biofilms. All of these peaks are detected with strong intensity compared in pure norfloxacin as mentioned earlier. The intra-molecular hydrogen formation might be occurred between the atoms inside the gellan gum molecule due to the sharpened of absorption peak were detected [20]. Furthermore, the inclusion of norfloxacin into gellan gum produced the signal present at range 1622-1675 cm\(^{-1}\). All of these peaks were approved that those two materials have chemical interaction due to the \(\nu\)(N-H) bending, which is absent in free standing gellan gum structure but appeared in blends. The conformation interactions between gellan gum and norfloxacin also was proved by the presence of the fluoroquinolone group in the blends at range 1108-1122 cm\(^{-1}\) which is prominent in norfloxacin for antibacterial properties [26]. Further, the fourth distinctive peaks that appeared in the blends are the region 950-900 cm\(^{-1}\) which is due to the \(\nu\)(N-H) bending vibration of amines [20]. The \(\nu\)(N-H) bending vibrations were observed broadly appeared in GG-NOR05 and GG-NOR1 at 912 cm\(^{-1}\) and 906 cm\(^{-1}\), respectively, while almost absent in GG-NOR001 and GG-NOR01 due to the lesser interaction of norfloxacin added. Apart from that, the aromatic protons were detected at 720 cm\(^{-1}\), 751 cm\(^{-1}\), 706 cm\(^{-1}\), and 735 cm\(^{-1}\); for GG-NOR001, GG-NOR01, GG-NOR05, and GG-NOR1, respectively. All of these peaks were shifted to the higher wavelength, which is proved the interaction between the blends.

**Figure 2.** FTIR spectra of pure GG, NOR and GG-NOR biofilm

Figure 3 shows the TGA thermogram and derivative thermogram of GG, GG-NOR001, GG-NOR01, GG-NOR05, and GG-NOR1 composite biofilms. The initial and final degradation of the samples were observed from the onset and offset temperature. From the Figure 3, it can be summarized that GG and GG-NOR001 biofilms was the most stable in thermal based on its highest onset temperature at 246 °C (Table 1). However, GG-NOR05 biofilms shows the longest time to complete the degradation process due to the offset temperature recorded at 747 °C followed by GG-NOR1, GG-NOR001, and GG-NOR01 biofilms at 698 °C, 580 °C, and 579 °C, respectively. A consequent mass loss occurred due to the degradation of polymer matrix of all GG-NOR biofilms [27-28]. Addition of norfloxacin exhibits better
outcome in increasing thermal stability due to the certain degree of interaction between gellan gum and norfloxacin as demonstrated by FTIR spectroscopy.

**Figure 3.** (a) Thermogravimetric thermograms of GG, GG-NOR001, GG-NOR01, GG-NOR05 and GG-NOR1 biofilms

**Table 1.** Thermal gravimetric properties of GG and GG-NOR001, GG-NOR01, GG-NOR05, and GG-NOR1 biofilms

| Sample         | Onset temperature (˚C) | Offset temperature (˚C) | Weight loss (%) |
|----------------|------------------------|-------------------------|-----------------|
| GG             | 246                    | 570                     | 71              |
| GG-NOR001      | 246                    | 580                     | 71              |
| GG-NOR01       | 245                    | 579                     | 71              |
| GG-NOR05       | 244                    | 747                     | 92              |
| GG-NOR1        | 245                    | 698                     | 77              |

The strain curve of GG-NOR composite biofilms are shown in Figure 4. It can be seen that the stress and strain curve of GG-NOR composite biofilms increased with increased of norfloxacin concentration up to 1 % (w/w); i.e., GG-NOR01. The addition of norfloxacin in gellan gum biofilms increased the toughness (T) and elongation-at-break (EAB) values proportionally with addition of more norfloxacin. However, it decreased the tensile strength (TS) and Young’s modulus (E) of biofilms as summarized in Table 2.

The inclusion of norfloxacin at 0.01 % (w/w) in gellan gum biofilms insignificant increase the toughness to 0.28 J g⁻¹ compared to gellan gum biofilm at 0.26 J g⁻¹ which equivalent to 1-fold increase. However, the TS and E values of the GG-NOR001 biofilms were decreased to 59±11 MPa and 3671±639 MPa, respectively, compared to gellan gum biofilm, but it values are highest compared to the other GG-NOR blends. This is due to the modification imposed on the composite biofilms lead to weakening the TS of the composite biofilms. This is may be due to the contribution of norfloxacin into the GG biofilm which is changed the chemical structure in the blends. Our result show that the reason as to the increase of toughness and strain-at-break of GG-NOR biofilm was due to the formation of
hydrogen bonds by reaction of carbonyl group of norfloxacin with gellan gum as proved by ATR spectroscopy.

**Figure 4.** Typical stress-strain curves of pure GG and GG-NOR biofilms

**Table 2.** The mechanical properties through GG, GG-NOR001, GG-NOR01, GG-NOR05, and GG-NOR1 biofilms (mean±SD) (n=3)

|        | Thick (µm) | TS (MPa) | E (MPa) | T (J g⁻¹) | EAB (%) |
|--------|------------|----------|---------|-----------|---------|
| GG     | 30±0.010   | 80±12    | 4600±330| 0.26±0.09 | 2.1±0.2 |
| GG-NOR001 | 27±0.010   | 59±11    | 3671±639| 0.28±0.06 | 2.1±0.5 |
| GG-NOR01 | 27±0.004   | 55±10    | 3375±935| 0.32±0.03 | 2.2±0.5 |
| GG-NOR05 | 34±0.001   | 53±6     | 3292±380| 0.39±0.09 | 2.4±0.4 |
| GG-NOR1  | 36±0.001   | 47±9     | 2349±617| 0.52±0.18 | 3.8±0.4 |

**4. Conclusion**

The chemical, physical and thermal properties of GG-NOR biofilms were investigated. Inclusion of different concentrations of norfloxacin to the gellan gum caused the toughness property increased with increasing the amount of norfloxacin added. Spectroscopy analysis proved that the hydrogen bonding involved in the bonds formation. The GG-NOR biofilms were thermally stable.

**5. References**

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