Supporting Information

Gelatin/cellulose nanofiber-based functional nanocomposite film incorporated with zinc oxide nanoparticles

Swarup Roy[1,2]*, Deblina Biswas[2], Jong-Whan Rhim[1]*

[1]Department of Food and Nutrition, BioNanocomposite Research Institute, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
[2]School of Bioengineering and Food Technology, Shoolini University, Solan, Himachal Pradesh 173229, India. deblinabi@gmail.com (D.B)

*Correspondence: swaruproy2013@gmail.com (S.R); jwrhim@khu.ac.kr (J.-W.R)

Materials and methods

Film characterization methods

Characterization and properties

Surface color and light transmittance

The Hunter color (L, a, and b) and total color difference (ΔE) of the film sample were also measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan) using a white standard plate as a background. The total color difference (ΔE) and the whiteness index (WI) were calculated using the following equation.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad \text{(S1)}$$

$$WI = 100 - \sqrt{(100 - L) + a^2 + b^2} \quad \text{(S2)}$$

where ΔL, Δa, and Δb were differences between each color value of the control film and the test film specimen. The color properties of films were also calculated in the same way described above.

The UV-barrier and transparency properties of the films specimen were also assessed by determining the percent transmittance of the film sample at 280 nm (T_{280}) and 660 nm (T_{660}), respectively, in a UV-vis spectrophotometer [1] (Mecasys Optizen POP Series UV/Vis, Seoul, Korea).

Morphology, XRD, and FTIR

The films' surface and cross-section topology was inspected using the FESEM (FE-SEM, SU 8010, Hitachi Co., Ltd., Matsuda, Japan). All the film samples were sputter-coated with platinum for 2 min before the measurement. XRD pattern was noted in a multi-purpose X-ray Diffractometer (DMAX-2500, Rigaku, Tokyo, Japan). The FTIR spectra of all the films were noted in the ATR mode using an FTIR spectrometer (TENSOR 37 Spectrophotometer with OPUS 6.0 software, Billerica, MA, USA) wavenumber ranging from 4000-650 cm^{-1}.

WVP and WCA

The water vapor permeability of all the films was measured gravimetrically using a WVP cup following the ASTM E96-95 standard method. The WVP cup was first filled with a prescribed amount of water, then covered with films, sealed, and kept in the controlled environmental chamber (25 ^{o}C and 50% RH). After equilibration, the WVP cup's weight was checked at a pre-decided time interval, and the weight loss was determined. The WVTR ($g/m^2.s$) was determined from the slope (linear) of the steady-state portion of weight loss of the cup versus the time curve. Then, the WVP of the films was calculated in $g.m/m^2.Pa.s$ using the following equation:

$$WVP = (WVTR \times L)/\Delta p \quad \text{(S3)}$$

where L was the thickness of the film (m), and Δp was water vapor partial pressure difference (Pa) across the film [1].

The film's surface wettability was evaluated by measuring the water contact angle of the film surface using a WCA analyzer (Phoneix 150, Surface Electro Optics Co., Ltd., Kunpo, Korea). The film sample was fixed on the film holder, and a drop of water (~10 μL) was added to the film's surface and checked the WCA [1].

Moisture content (MC), swelling ratio (SR), and water solubility (WS)

The films' MC, SR, and WS were evaluated using previously reported methods [2]. The film's MC content was determined by determining the weight change of the film after drying at 105 °C for 24 h. The percentage of MC content of the films was calculated in triplicate using the following equation:

$$\text{MC}\ (\%) = \frac{W_i - W_f}{W_i} \times 100 \qquad \text{(S4)}$$

where W_i and W_f refer to the initial and final mass of the film samples correspondingly.

A pre-weighed film sample was put in a beaker containing 20 mL DI water, removed from the water after one h, removed from the surface water using blotting paper, and then weighed [3]. The SR of the films was calculated in triplicate using the following equation:

$$\text{SR}\ (\%) = \frac{W_f - W_i}{W_i} \times 100 \qquad \text{(S5)}$$

where W_i and W_f refer to the initial and final weight of the film samples correspondingly.

The pre-dried film sample was put in a beaker that contained 30 mL of DI water, and the beaker was covered and kept for 24 h at room temperature with gentle agitation [2]. The film specimen was removed, dried in a hot air oven at 105 °C for 24 h, and weighed. The WS of the film sample was calculated using the following equation:

$$\text{WS}\ (\%) = \frac{W_i - W_f}{W_i} \times 100 \qquad \text{(S6)}$$

Mechanical and thermal properties

Film thickness was measured using an electronic digital micrometer (Digimatic Micrometer, QuantuMike IP 65, Mitutoyo, Japan) with an accuracy of 1 μm. The film specimen's mechanical properties were tested following the standard method (ASTM D 882-88) using an Instron Universal Testing Machine (Model 5565, Instron Engineering Corporation, Canton, MA, USA). The Instron machine functioned with an initial grip separation of 50 mm and a 50 mm/min crosshead speed [4].

The film sample's thermal stability was evaluated using a thermogravimetric analyzer (Hi-Res TGA 2950, TA Instrument, New Castle, DE, USA). For this, ~10 mg of film sample was taken in a standard aluminum pan and scanned in a temperature range of 30-600 °C under nitrogen flow (50 mL/min).

Antibacterial activity

The films' antibacterial activity was calculated using a total viable colony count (TVCC) method [4]. The food-borne pathogenic bacteria, *L. monocytogenes* and *E. coli*, were used in this test. The test bacteria were inoculated in the BHI and TSB broth, respectively, cultured overnight at 37 °C with gentle agitation for 16 h. The inoculum was then suitably diluted, and ~100 μL of the diluted inoculum was aseptically transferred to the broth of TSB and BHI containing ~150 mg of the film samples incubated at 37 °C for with gentle agitation. The sample was taken out and plated on agar plates at regular intervals after suitable dilution to evaluate the TVCC. The antibacterial test was also carried out using a culture medium without film, and a control film served as negative control and positive control, respectively.

Statistical analysis

All the tests were carried out in triplicate, and the average value was reported. One-way analysis of variance (ANOVA) was done, and the significance of each mean property value was evaluated ($p < 0.05$) by Duncan's multiple range test using the SPSS statistical analysis computer program (SPSS Inc., Chicago, IL, USA).

Results and discussion

XRD analysis

The XRD pattern of the film is shown in Fig. S1. In the case of all films, the peaks appeared around 2θ = 15° and 22°, ascribed to the (110) and (002) planes of cellulose [5]. After blending of ZnONP, some prominent peaks of ZnONP (100, 101, 110, 103, etc.) can be seen in the nanocomposite films, which confirmed the presence of the nanoparticles in the films [6]. Similar results were reported in the case of ZnONP incorporated CNF-based films previously [5].

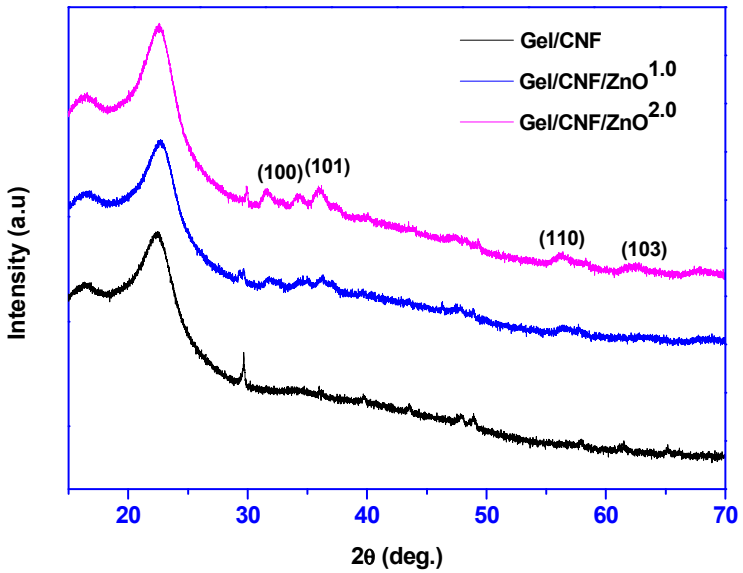

Figure S1. XRD analysis of the nanocomposite films.

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