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Silver nanoparticle-loaded chitosan–starch based films: Fabrication and evaluation of tensile, barrier and antimicrobial properties

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ABSTRACT

The fabrication of silver nanoparticles was accomplished by γ-ray irradiation reduction of silver nitrate in a chitosan solution. The obtained nanoparticles were stable in the solution for more than six months, and showed the characteristic surface plasmon band at 411 nm as well as a positively charged surface with 40.4 ± 2.0 mV. The silver nanoparticles presented a spherical shape with an average size of 20–25 nm, as observed by TEM. Minimum inhibitory concentration (MIC) against *E. coli*, *S. aureus* and *B. cereus* of the silver nanoparticles dispersed in the γ-ray irradiated chitosan solution was 5.64 μg/mL. The silver nanoparticle-loaded chitosan–starch based films were prepared by a solution casting method. The incorporation of silver nanoparticles led to a slight improvement of the tensile and oxygen gas barrier properties of the polysaccharide-based films, with diminished water vapor/moisture barrier properties. In addition, silver nanoparticle-loaded films exhibited enhanced antimicrobial activity against *E. coli*, *S. aureus* and *B. cereus*. The results suggest that silver nanoparticle-loaded chitosan–starch based films can be feasibly used as antimicrobial materials for food packaging and/or biomedical applications.

1. Introduction

For decades, silver nanoparticles have been widely used as antimicrobial agents in a number of areas, including dental [1,2], medical and pharmaceutical [3–9], textile and fiber [10–16], coating and paint [17,18], film [19], membrane [20] and food packaging purposes [21]. Conventionally, silver nanoparticles are produced by the reduction of silver salt precursors using chemical reducing agents in the presence of stabilizers. Although chemical reduction is an easy and convenient method, chemical reducing agents such as $NaBH_4$ [22], formamide [23], dimethylformamide [23,24], triethanolamine [23], hydrazine [25], etc., are involved in the production. The removal of these reducing agents is cost- and time-intensive and their residues are toxic. The reduction of silver salts by γ-ray irradiation [26–29], microwave irradiation [30–32], photochemical process [33,34] and sonochemical process [35] has been reported to produce metal nanoparticles without, or with fewer, chemical concerns.

Previously, we studied the effects of γ-ray doses (2.5–25.0 kGy) as well as concentrations of silver nitrate salt precursor (0.02–0.10 mmol) and chitosan solution (0.1 and 0.5% w/v) on the size and number of the formed silver nanoparticles (Ag^0) [36]. However, the antimicrobial activities of the obtained nanoparticles dispersed in γ-ray irradiated chitosan and their feasible application as antimicrobial agents for food packaging and biomedical devices have not been elaborated.

The application of silver nanoparticles in antimicrobial food packaging is possible, as revealed in previous reports [21,37,38]. Many efforts have been made to load and/or to incorporate silver nanoparticles into acceptable packaging materials such as filter paper [21], low density polyethylene (LDPE) [37], and poly(methyl methacrylate) (PMMA) [38]. Also, numerous biodegradable materials, e.g. poly(vinyl alcohol) [39] and polysaccharides such as starch [40], chitosan [19,41–43], alginate [43] and konjak glucomannan [33] have been used to fabricate silver nanoparticle-based composite films.

Starch and chitosan are naturally abundant polysaccharides which are generally non-toxic, available from renewable agricultural sources, and suitable for film formation. Although starch film is cheap and easily biodegradable, it is very sensitive to moisture and exhibits poor mechanical properties. Chitosan possesses many unique properties, including antimicrobial characteristics; hence it has been used in various applications, such as medical, pharmaceutical, textile, water treatment, food, cosmetics, packaging, etc. [44]. The blending of starch and chitosan is an alternative way to not only improve the mechanical and water vapor barrier properties, as well as the antimicrobial attributes, of starch film [45–48], but also to reduce the cost and enhance the biodegradability of chitosan film [48].

The aims of the present research are thus to determine the minimum inhibitory concentration (MIC) of silver nanoparticles dispersed in chitosan solution, and to investigate the effects of silver nanoparticle content on the tensile properties, the water vapor and oxygen

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gas barrier properties, and the antimicrobial qualities of silver nanoparticle-loaded chitosan–starch based films.

2. Experimental

2.1. Materials

Chitosan (deacetylation degree of 0.95 and molecular weight of ~700,000 Da) was purchased from Seafresh Chitosan (Lab) Co. Ltd., Thailand. Rice flour and waxy rice flour were acquired from Cho Heng Rice Vermicelli Factory Co. Ltd., Thailand. Silver nitrate and acetic acid were supplied by Merck, Germany. Magnesium nitrate was obtained from Ajax Finechem Pty. Ltd., Australia. Mueller-Hinton broth was purchased from Becton Dickinson, USA. Nutrient broth was supplied by HiMedia Laboratories Pvt. Ltd., India. The agar powder was a product of Purified Agar Co. Ltd., Thailand. The glycerol applied was of commercial grade. All chemicals were used as delivered and without any further purification.

2.2. Preparation of silver nanoparticles

Silver nanoparticles dispersed in chitosan solution were prepared according to a γ-ray irradiation reduction method described previously [36]. Briefly, chitosan solution (0.5% w/v) was prepared by stirring chitosan flakes in an aqueous acetic acid solution (1% v/v) at an ambient temperature overnight. Freshly prepared silver nitrate ($AgNO_3$) solution (50 mM, 0.04 mmol, 0.8 mL) and aqueous acetic acid solution (1% v/v, 1.2 mL) were then added to the chitosan solution (20 mL). The homogeneous mixture was irradiated by γ-rays with a dose of 25 kGy in a ^{60}Co Gammacell irradiator (Best Theratronics Ltd., Canada) at a dose rate of 12 kGy/h.

2.3. Characterization of silver nanoparticles

The UV–Vis absorption spectrum of the obtained particles dispersed in γ-ray irradiated chitosan solution was recorded over a wavelength range from 300 to 500 nm using a Helios Gamma spectrometer (Thermo Scientific, UK). Zeta potential and particle size were determined at 20 °C using a Zetasizer 3600 (Malvern Instruments Ltd., UK) equipped with a He–Ne laser operating at 4.0 mW and 633 nm with a fixed scattering angle of 90°. Transmission electron microscopy (TEM) analysis was performed using a Hitachi H-7650 (Hitachi High-Technologies Corp., Japan) at an accelerating voltage of 100 kV.

2.4. Study of antimicrobial activity of silver nanoparticles

Minimum inhibitory concentration (MIC) was determined using a tube dilution method. Three strains of bacteria — a Gram-negative bacterium, *Escherichia coli* (*E. coli*, ATCC35218), and two Gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*, ATCC6538) and *Bacillus cereus* (*B. cereus*, ATCC11778) — were applied as test organisms. Serial dilutions were made of the samples in Mueller-Hinton broth (MHB) which was used as a bacterial growth medium. The test organisms in MHB (10^6 CFU/mL, 1 mL) were then added to those sample dilutions (1 mL). The mixtures (2 mL) were homogeneously mixed using a vortex, subsequently incubated at an ambient temperature for 24 h, and then scored for growth. The MIC was defined as the lowest concentration resulting in the lack of visible growth of microorganisms.

2.5. Preparation of silver nanoparticle-loaded chitosan–starch based films

Silver nanoparticle-loaded chitosan–starch based films were prepared by a solution casting method. Chitosan flakes were dissolved in aqueous acetic acid solution (1% v/v) by stirring overnight to obtain a homogeneous chitosan solution (1.2% w/v). Two solutions of starches, i.e. rice starch (RS) and waxy rice starch (WRS) (2.0% w/v), were prepared by agitation of starches in water: at 100 °C for 60 min and 85 °C for 40 min for RS and WRS, respectively. Chitosan, RS and WRS solutions were mixed together at 50 °C to obtain a mixture with a weight ratio of chitosan to RS to WRS of 2:1:1. Glycerol (20% w/w), γ-ray irradiated chitosan solution, and γ-ray irradiated chitosan solution containing silver nanoparticles were then individually added to samples of the mixture. Different amounts of those components were used to prepare four types of film, as tabulated in Table 1. The homogeneous mixtures were poured onto acrylic plates (31×31 cm^2) and dried in a hot air oven at 50 °C overnight. The obtained films were peeled from the plates, neutralized in a closed chamber containing ammonia solution (25% v/v), thoroughly washed with water and dried at an ambient temperature (25 °C).

Neat chitosan and starch films were also prepared by a solution casting method as described above and used as controls for the antimicrobial activity test. Chitosan film was fabricated from 544 mL of chitosan solution (1.2% w/v), while starch film was produced from 163 mL of each of RS and WRS solutions (2.0% w/v).

2.6. Study of tensile properties of silver nanoparticle-loaded chitosan–starch based films

A film sample (2.5×15 cm^2) was preconditioned in a closed chamber containing saturated magnesium nitrate solution ($Mg(NO_3)_2$) at an ambient temperature (~50 ± 2% RH) for 2 days. Tensile testing was performed with a universal testing machine (model 1000, Instron, USA) according to the ASTM D882-91, with a crosshead speed of 50 mm/min and a grip separation of 100 mm. For each sample, 8–10 specimens were tested and the tensile properties (tensile strength, modulus of elasticity and elongation at break) were reported as mean ± SD. Tensile strength was calculated by dividing the maximum load by the initial cross-sectional area of the specimen. Modulus of elasticity was determined by extending the linear portion of the load–extension curve and dividing the difference in stress corresponding to any segment of section on this straight line by the corresponding difference in strain. The tensile strength and elastic modulus were expressed in MPa. Elongation at break was reported as a percentage and calculated by dividing the extension length (change in length) at the point of specimen rupture by the initial length of the specimen and multiplying by 100.

2.7. Study of barrier properties of silver nanoparticle-loaded chitosan–starch based films

Water vapor transmission rate (WVTR) was evaluated by a Gravimetric Modified Cup Method based on ASTM E96 [48,49]. A film sample with a diameter of 7.5 cm was covered and sealed to the open mouth of a test cup, with an inner diameter of 6.3 cm, which contained dried desiccants (25 mL). The assembly was weighed and

Table 1
Components and their contents used for preparation of chitosan–starch based film (A) and silver nanoparticle-loaded chitosan–starch based films (B–D).

Component	Film type			
	A	B	C	D
γ-Ray irradiated chitosan solution (0.5% w/v) containing silver nanoparticles (mL)[a,b]	0.0	26.6	53.2	106.4
γ-Ray irradiated chitosan solution (0.5% w/v) (mL)[a]	106.4	79.8	53.2	0.0
Chitosan solution (1.2% w/v) (mL)[a]	250	250	250	250
Rice starch solution (2.0% w/v) (mL)[c]	75	75	75	75
Waxy rice starch solution (2.0% w/v) (mL)[c]	75	75	75	75
Glycerol (g)	1.2	1.2	1.2	1.2

[a] 1% (v/v) aqueous acetic acid solution was used as a solvent.
[b] Concentration of silver nanoparticles was 0.1806 mg/mL.
[c] Water was used as a solvent.