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Preparation and Characterization of Composite Gels and Films Containing Gelatin and Hydroxypropyl Methylcellulose Phthalate

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ABSTRACT: Composite gels and films were prepared by the blending of hydrated gelatin as a base material and hydroxypropyl methylcellulose phthalate (HPMCP) at various mass ratios. A composite technology was applied to obtain improved mechanical, physicochemical, and antimicrobial properties of the gelatin used as a base material. We investigated the effects of different experimental conditions on the rheological and mechanical properties and antimicrobial activities of the composite gels, films, and solutions. The rheological values (storage modulus, loss modulus, and complex viscosity) of the composite solutions and gels increased with added HPMCP. Aerobic microorganisms, yeasts, and molds were not detected throughout the testing period in the gelatin–HPMCP composite solution. In contrast, many microorganisms were detected in the gelatin-only samples beginning with day 3 of storage. The composite films exhibited relatively good mechanical and physical properties compared with the gelatin-only film. The composite film containing HPMCP at a mass ratio greater than 1:4 did not dissolve in gastric juice (pH 1.2) for at least 2 h, but all other samples, including the gelatin-only film dissolved in enteric juice (pH 6.8). © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39597.

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INTRODUCTION

Food and pharmaceutical manufacturers worldwide are facing an escalating demand for collagen and gelatin. Mammalian gelatins (porcine and bovine) are currently the most popular and widely used gelatin materials. Global gelatin production is currently 326,000 tons, and 75% of this is derived from pig skin and bovine hides (46 and 29.4%, respectively).[1] Gelatin has attracted attention for diverse applications because of its abundance,[2] and it exhibits excellent film-forming properties.[3]

However, gelatin possesses poor mechanical characteristics and is very susceptible to microbial spoilage. Many alternatives to improve these drawbacks have been investigated, and the composite application of chemical agents or biomaterials has been actively studied with different materials to provide additional functionality.[4] For example, the addition of chemical crosslinking agents, such as glutaraldehyde, formaldehyde, and glyoxal, or enzymes, such as microbial transglutaminase, improves the properties of gelatin.[5]

Many researchers have also investigated various approaches with biopolymers to modify gelatin to improve its functionality. The blending of gelatin with other biopolymers (e.g., k-carrageenan, chitosan, and pectin) has been proposed as a potential approach for improving various properties.[6–9]

One of the available biopolymer derivatives is hydroxypropyl methylcellulose phthalate (HPMCP), a cellulose derivative. HPMCP is manufactured from plant resources and retains hydroxyl propyl, methyl, and phthalyl groups on cellulose chains and exhibits an excellent film-forming ability.[10] The interactions between gelatin and HPMCP result in the enhancement of the gel and film properties, and the antimicrobial activities of composite solutions are derived from HPMCP. The cellulose derivative is also widely used in the pharmaceutical industry because it is less susceptible to hydrolysis than cellulose acetate phthalate. In particular, HPMCP is widely used as an enteric coating agent by the pharmaceutical industry, as it dissolves at pH 5–5.5, and dissolution can be controlled by the variation of the phthalyl content.[11] Two types of different pH-soluble HPMCPs are available, HP-55 and HP-50. A suitable grade of HPMCP should be selected in accordance with the properties and formulation required for the application.

Hydrogen-bonding interactions are observed between the carbonyl groups of HPMCP and the amine groups of gelatin.[12] This interaction can be optimized by the addition of the proper concentration of HPMCP and improves the mechanical properties of gelatin.

In this study, composite technology was applied to obtain improved physicochemical and mechanical properties of gelatin. We investigated the effects of different experimental conditions on the rheological and mechanical properties of the composite system, and the antimicrobial activities derived from the phthalic acid half-ester moieties of HPMCP were also studied. The main aim of this study was to establish a composite technique platform with HPMCP to improve the physicochemical, mechanical, and microbiological properties of gelatin to extend its application range.

EXPERIMENTAL

Materials

Gelatin was purchased from Geltech Co., Ltd. (Busan, Korea). HPMCP (HP-50, generally used for enteric coating in pharmaceutical industry) was obtained from Samsung Fine Chemical Co., Ltd. (Ulsan-Si, Korea). It was substituted with methoxyl, hydroxypropoxyl, and phthalyl at 20–24, 6–10, and 21–27% (220824 type), respectively. All other chemicals were analytical grade, were purchased from Duksan (Seoul, Korea) and Samchun Chemical (Seoul, Korea), and were used as received.

Composite Solution and Gel Preparation

HPMCP powder was weighed and hydrated with distilled water containing NaOH (6% w/w of HPMCP) for 1 h at 400 rpm. At first, gelatin was weighed, hydrated by mixing with a prepared HPMCP solution, stirred for 10 min, incubated at room temperature for 30 min (for swelling), and then stored in a 60°C water bath for 1 h. The composite solutions were prepared at various mass ratios of HPMCP to gelatin (mass ratio = 1:32 to 1:2). After 1 h of storage at room temperature, the gelatin and composite solutions were matured at 4 and 10°C for 17 h until gelation was achieved.[13]

Gel Strength

The gel strength was measured in 10 replicates following the Bloom test.[14] A 12.7-mm diameter flat plunger was used to press 4 mm into one end of 105 mL of mature gelatin (150 Bloom) or composite gels at a speed of 30 mm/min with a texture analyzer equipped with a 4.5-kg load cell (CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA).

Combination Gelation Analysis

A combination gelation analysis was performed according to the method of Gilsenan et al.,[15] and the absorbance values of HPMCP, gelatin, and the composite samples were measured at 400 nm after maturation at 10°C for 1 h. The rise in the absorbance value of the composite sample reflected an increase in the complex formed by the composite gelation.

Rheological Properties

The rheological properties of the gelatin alone and the composite samples were determined before maturation with a dynamic rheometer (Physica MCR 301, Anton-Paar, Ashland, VA). The dynamic rheometer was equipped with plate geometry [distance between plates (d) = 40 mm]. Before the temperature ramp test, frequency and strain sweeps were conducted to obtain the linear viscoelastic region, after which the frequency and strain were fixed at 1 Hz and 1%, respectively. Temperature sweeps were done from 0 to 60°C. Grease was used to prevent the dehydration of the exposed sample surface.[16] Three replicate scans were made on the storage modulus (G'), loss modulus (G''), and complex viscosity (η). The composite solutions were prepared at various mass ratios of HPMCP (0, 1, 2, and 3% w/w) to gelatin (6.67% w/w) fixed. A representative curve was selected and used to analyze the gel rheological properties from at least three replicate scans.

Physical Stability Test on Composite Solution

The physical stability of the gelatin alone and the composite solutions prepared under specific conditions, as described in the Experimental section, was determined by the measurement of changes in the viscosity at 37°C over a 9-day storage period. In this experiment, the instrument of choice for measuring viscosity was the Brookfield viscometer (LV type, Brookfield Engineering Laboratories, Inc., Middleboro, MA). We measured the viscosity by checking the force required to spin a plate through the fluid. This stability analysis method was described in the previous study.[17] In this experiment, different mass ratios of HPMCP to gelatin (HPMCP/gelatin = 0, 1:10, 1:5, 1:3, and 1:2) were used in the preparation of the solutions.

Film Casting

Approximately 50 mL of the prepared film solution was cast onto a 25 × 35 cm^2 glass plate, which was formed with a custom-designed film applicator. After drying at room temperature, the films were peeled off from the glass plates and cut into test specimens. The test specimens were immediately placed back into a constant temperature and humidity chamber (model TR-001-1, Jeio Tech Co., Ltd., Seoul, Korea, 25°C, 50% relative humidity) and held for 48 h before testing.[18] The gelatin concentration was fixed [20% w/w] during this process, and different weight ratios between gelatin and HPMCP (HPMCP/gelatin = 0, 1:10, 1:5, 1:3, and 1:2) were applied for the mechanical analysis and disintegration test.

Mechanical Properties of the Film

The tensile strength (TS) and elongation at break (E) of each film were measured with a Lloyd Instrument testing machine (LRX plus, Lloyd Instrument, West Sussex, United Kingdom). Fifteen specimen samples were cut from the film samples prepared on glass plates (P25 × 35 cm^2) and conditioned for 48 h at 25°C and 50% relative humidity in a constant temperature and humidity chamber before measurements. The initial grip separation and crosshead speed were set at 5 cm and 500 mm/min, respectively. TS was calculated by the division of the maximum load by the cross-sectional area of the film, and E was calculated and expressed as the percentage change in the original length of a specimen between grips (5 cm) according to ASTM D 882-88. Both TS and E values of the test samples were measured as quickly as possible upon removal from the environmental chamber.[19] The film hardness (g) was measured in 15 replicates with a texture analyzer.

Disintegration Test on the Films

This test was conducted with a disintegration apparatus (PTZ-E, Pharma Test, Frankfurt, Germany). The prepared gelatin–HPMCP composite films and the gelatin-only film were placed in a basket rack (Figure 1). This test was also carried out with the disk-type samples of the prepared films. The frequency of

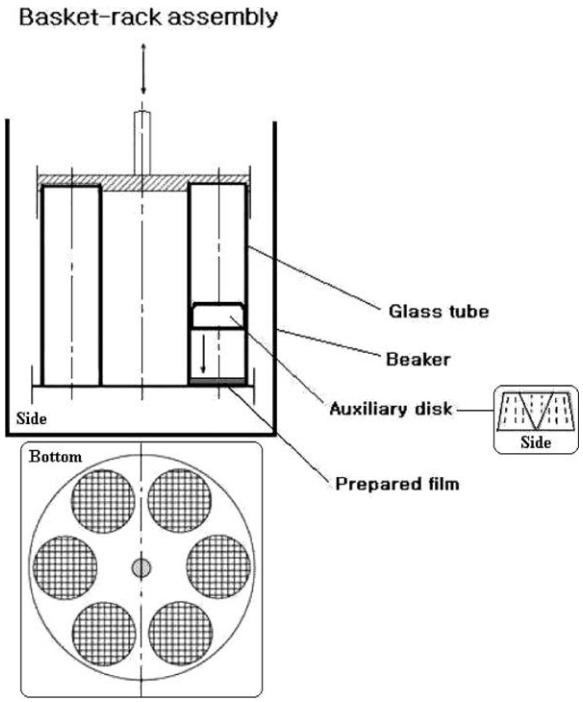

Figure 1. Schematic of the disintegration apparatus.

the basket-rack movement was set at 30 cycles/min.[20] The prepared films were exposed to gastric juice for 2 h at 37.5°C (pH 1.2), rinsed with water, and immediately immersed in enteric juice (pH 6.8). Through this experiment, we could identify the acid-resistance properties of the composite films, including the appropriate mass ratios of HPMCP.

Microbial Assay on Solutions

The antimicrobial effects of HPMCP against aerobic microorganisms, yeasts, and molds was evaluated with the solid-media culture method. The gelatin (16% w/w, fixed) and gelatin–HPMCP composite (weight ratios of gelatin to HPMCP = 32:1, 16:1, 10:1, 4:1, and 2:1) solutions were incubated at 37°C for 120 h.[21]

We applied HPMCP as an antimicrobial agent, and the composite solutions that could be prepared by changing the HPMCP concentration were analyzed for antimicrobial effectiveness for 5 days. The microorganisms generated in the incubated samples were grown in agar-based culture media (Wako Chemicals, Inc., Richmond, VA) and incubated at 37°C for 48 h, and viable cell numbers were determined as colony forming units (log cfu/g).[22]

Statistical Analysis

The values are expressed as mean plus or minus standard deviation from at least three independent experiments calculated with Microsoft Excel software.[23] The data were analyzed with the Duncan's multiple comparison method of SAS version 9.1 software (SAS Institute, Cary, NC). A value of $p < 0.01$ was considered significant.

Table I. Gel Strength Values of Composite Gel Samples Prepared with 6.67% w/w Gelatin and Diverse Concentrations of HPMCP

Added HPMCP (% w/w)	0	0.5	1.0	1.5	2.0
Gel strength (g)	150.8	155.7	168.5	184.4	195.3

RESULTS AND DISCUSSION

Interaction Between Gelatin and HPMCP

The gel strength of gelatin (150 Bloom, 6.67% w/w) was 150 g measured with a generally established method and increased to 195 g when 2% w/w HPMCP was added to form a composite gel (Table I). The noticeable improvement in the gel strength was obtained in the composite samples with 1% w/w or more HPMCP added. No significant difference was observed among the gel strength values of the composite gel samples containing 2% w/w or more HPMCP. Thus, the addition of 2% w/w HPMCP was considered sufficient to enhance the gelatin gel strength.

The absorbance value of distilled water at 400 nm was used as a control for the gelatin and gelatin–HPMCP composite samples. As the proportion of HPMCP increased, the absorbance of the composite gel also increased (Table II). Gilsenan et al.[15] reported that the insoluble complex induced from the combination of gelatin and polysaccharides resulted in higher absorbance values than those of the raw materials. It was clear that HPMCP and the gelatin molecules interacted to form a composite gel.

Composite Gel Properties

The strength of the composite gel was 1.3–1.5 times higher than that of gelatin and increased continuously with maturation, showing its highest values after 17 h of maturation (Figure 2).

Furthermore, the increase in the gel strength of the gelatin and the composite gel during maturation at both 4 and 10°C was noticeable, and the gel strength at 10°C maturation was lower than that at 4°C. In this study, the maturation test was conducted after the prepared gelatin and composite solutions were stored at room temperature for 1 h. At least 8 h of maturation was proposed as the minimum maturation duration at 10 or 4°C for effective gelation of the gelatin and composite gel.

Many researchers have reported that different gelatin gel strengths have been achieved with different maturation temperatures.[24] Large crystalline regions were formed with a higher gel

Table II. Effect of the HPMCP Content on the Absorbance (A_{400}) of Gelatin Only and the Composite Gel

				Gelatin–HPMCP composite gel (HPMCP/gelatin)			
	6.67% gelatin	2% HPMCP	4% HPMCP	1/6.67	2/6.67	3/6.67	4/6.67
A_{400} (OD)	0.081	0.006	0.011	0.125	0.151	0.173	0.210

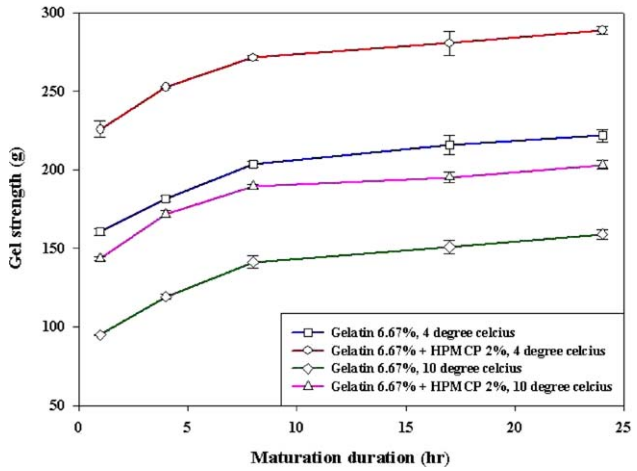

Figure 2. Effect of the maturation temperature and duration on the gel strength of the gelatin and composite gels with 2% HPMCP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

strength after longer maturation at lower temperatures than after shorter maturation at higher temperatures.

Although HPMCP could not form a solid gel, the strength of the composite gel was slightly higher than that of gelatin. The absorbance values of the composite samples were also higher than the sum of the gelatin and HPMCP values. The results show that HPMCP played a synergistic role in the composite gelation.

Rheological Properties of the Composite Gel

G', G'', and η of gelatin decreased as the temperature increased. A drastic decrease in these values occurred at 8–14°C, and the values were approximately 0 Pa (G' and G'') and 0 Pa s (η) when the temperature was >22°C. This indicated that the melting point of gelatin gel was around 20°C.

With the addition of HPMCP, G', G'', and η of gelatin increased across the board. G' reached 5.9 kPa with the addition of 3% w/w HPMCP, and this value was approximately 55% higher than that of the gelatin without HPMCP. The temperature-dependent changes in G'' (Figure 3) and η (Figure 4) of the composite gel were similar to that of G'.

Generally speaking, the hardness of the molecular chain, bond quantities, and junction zone strength contributed to G' and G'' and were closely associated with the mobility, movement, friction of small molecules, and vibration and rotation of the functional groups.[25]

In this study, these moduli and η were used to analyze the gelation behavior, gel-network-forming behavior, and structural characteristics of the gelatin and composite gels. These characteristic parameters also mirrored diverse inner structural molecular interactions during the formation of the gel network.[26]

The addition of HPMCP boosted G', G'', and η of the gelatin gels. A firm composite gel was obtained after sufficient maturation.

Far-reaching hydrogen bonds encouraged interactions among the gelatin molecules and between the gelatin and HPMCP molecules. The combination of the gelatin and cellulose derivative increased the junction zone strength in the composite gel and enhanced the mechanical properties.

Physical Stability Analysis of the Composite Solution

The physical stability of the gelatin and composite solutions was determined by the examination of the changes in the viscosity over time.

The results of the stability test are shown in Figure 5. No significant reduction in the viscosity was observed in the HPMCP-added gelatin solutions until 9 days of storage, whereas a marked reduction in the viscosity occurred in the gelatin solution. This suggests that HPMCP had a preserving effect on the physical properties of the gelatin solution by protecting it from microbial spoilage. The changes in the physical characteristics

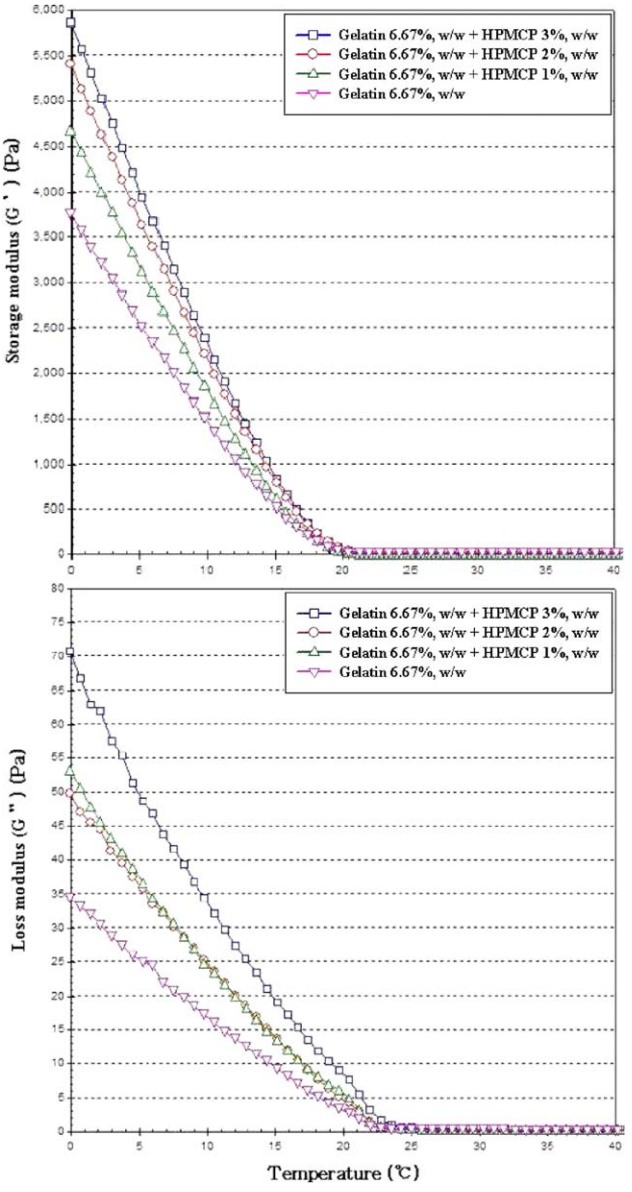

Figure 3. Effect of the HPMCP content on G' and G'' of the gel samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

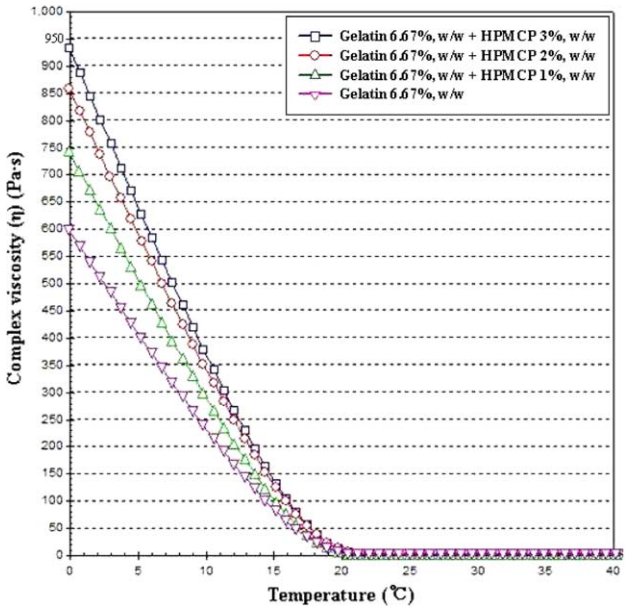

Figure 4. Effect of the HPMCP content on η of the gel samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

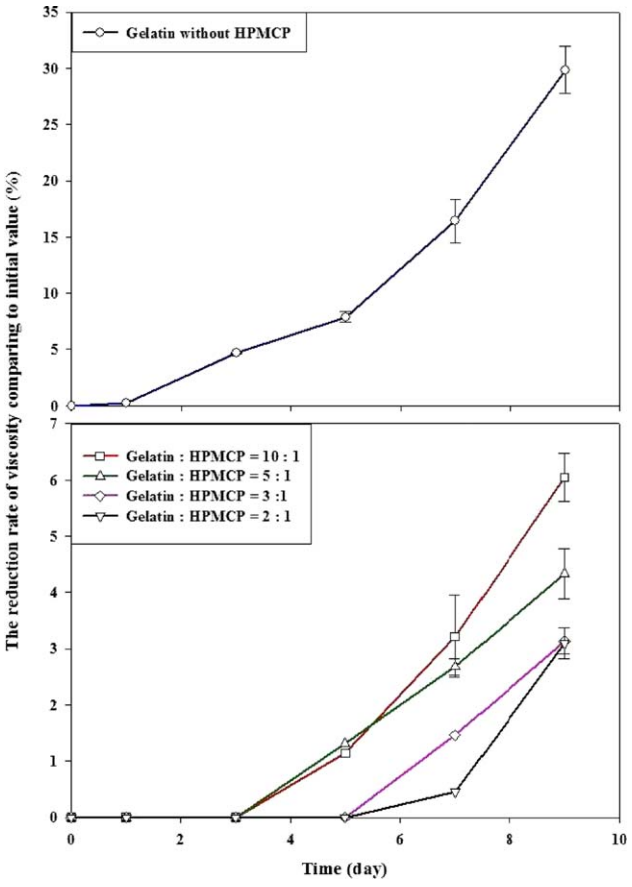

Figure 5. Effect of the HPMCP content on the reduction rate of the viscosity of the gelatin and gelatin–HPMCP solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

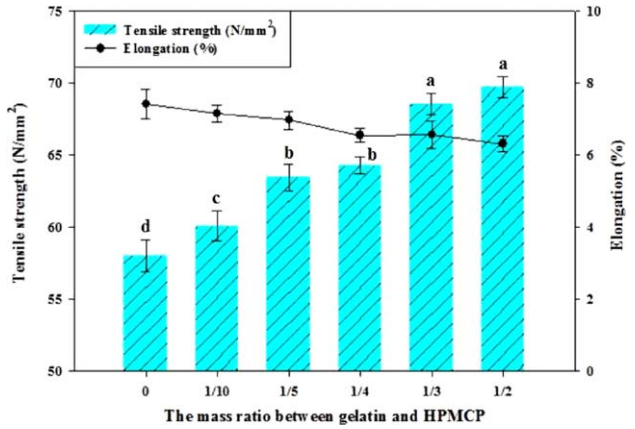

Figure 6. Effect of the mass ratio of HPMCP to gelatin on the TS and E of the film. Different letters (a–d) within a column indicate significant differences ($p < 0.01$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

are usually accompanied with the spoilage. The preserving effect of HPMCP is believed to be due to the phthalic acid half-ester moieties.[27]

Mechanical Properties of the Composite Film

The TS and E values were measured and applied to describe how the mechanical properties of the composite films were related to their chemical composition.[28]

The mechanical properties of the gelatin and composite films are presented in Figures 6 and 7. The results indicate that the TS values of the composite films were significantly different from that of the gelatin alone film. Among the composite film samples, the composite film with 10% w/w HPMCP (mass ratio of HPMCP to gelatin = 1/2) added had the highest TS. As the HPMCP concentration increased, the TS of the composite film increased proportionally. The TS values of the gelatin-only and 2, 4, 5, 6.67, and 10% w/w HPMCP-added composite films

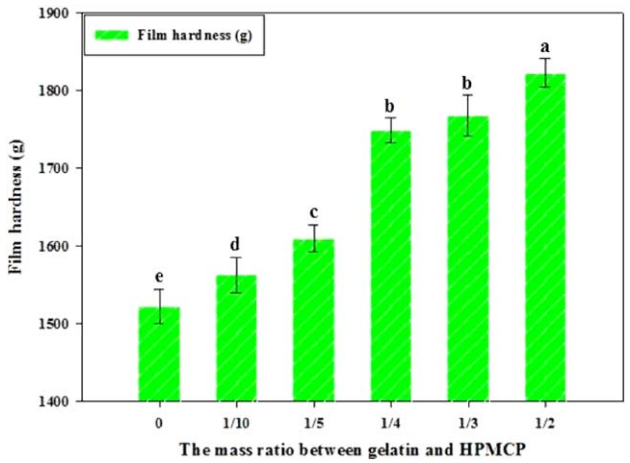

Figure 7. Effect of the mass ratio of HPMCP to gelatin on the hardness of the film. Different letters (a–e) within a column indicate significant differences ($p < 0.01$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Sizes and Shapes of the Films Remaining After the Disintegration Test Conducted in Gastric Juice (pH 1.2) and Then Enteric Juice (pH 6.8)

	Sizes and Shapes of Remaining Film Samples				
In pH 1.2 after 2 h				Completely dissolved within 1 h	Completely dissolved within 1 h
In pH 6.8	Completely dissolved within 10 min	Completely dissolved within 10 min	Completely dissolved within 10 min	–	–
HPMCP/gelatin mass ratio	1:2	1:4	1:6	1:8	Gelatin-only film

(20% w/w, fixed gelatin concentration, weight ratio of HPMCP to gelatin = 1:10, 1:5, 1:4, 1:3, and 1/2, respectively) were 58.05, 60.11, 63.46, 64.55, 68.57, and 69.77 N/mm^2, respectively.

The gelatin film had the highest *E* property value of 7.42%. However, no significant differences were observed among the values of the prepared samples. The *E* value of the composite film containing a relatively high mass ratio of HPMCP was lower than that of gelatin. The lowest *E* was 6.32% for the composite film at a mass ratio of 2:1 (gelatin/HPMCP).

The film hardness also had a similar tendency as the TS. As the added amount of HPMCP increased, the composite film hardness increased.

In summary, the addition of HPMCP increased the TS, and the film hardness values increased but slightly decreased the *E* value.

Disintegration Test of Composite Film

This experiment was conducted to investigate the influence of the HPMCP content in the composite film on the dissolution behavior in gastric juice (pH 1.2).

Table III shows the size, shape, and clarity of the remaining films after exposure to gastric juice for 2 h and then to enteric juice (pH 6.8). The gelatin film without HPMCP readily dissolved in gastric juice within 10 min. However, the composite film with a mass ratio of 1:4 or greater of HPMCP retained their initial appearance in gastric juice for 2 h or longer.

In case of the sample containing a 1:6 mass ratio of HPMCP had a relatively lower acid resistance than the composite film containing HPMCP at a mass ratio of greater than 1:4 (HPMCP/gelatin). Therefore, the sample showed partial shape decomposition (partial dissolution) during the disintegration test in gastric juice for 2 h.

The results demonstrate that the composite film containing a mass ratio of HPMCP greater than approximately 1:4 (HPMCP/gelatin) had enteric properties and could be used as a resource for intestinal applications. Both the composite and gelatin films dissolved in enteric juice within 10 min.

Microbial Assay of the Composite Solution

Different proportions of HPMCP and storage terms (0–5 days) were used as variables. The results on the growth of aerobic microorganisms, yeasts, and molds in gelatin-based solutions at a constant 37°C as affected by HPMCP are given in Table IV.

No aerobic microorganisms, yeasts, or molds were detected in any of the gelatin–HPMCP composite solutions throughout the storage period. In contrast, many microorganisms were detected in the gelatin-only samples from day 3 of storage.

HPMCP derived from plant sources is a promising preservative with no side effects in the human body.

The antimicrobial activity of the HPMCP appeared to be due to the phthalic acid half-ester moieties.[27]

Table IV. Antimicrobial Effect of HPMCP Against Aerobic Microorganisms, Yeasts, and Molds

	Total aerobic microbial count (log cfu/g)					Total yeasts and molds count (log cfu/g)				
[G:P][a]	1 day	2 day	3 day	4 day	5 day	1 day	2 day	3 day	4 day	5 day
Gelatin	N/D[b]	N/D	2.41	2.67	3.02	N/D	N/D	1.69	1.83	1.95
1/32	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1/16	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1/10	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1/4	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1/2	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

[a] Weight ratio of gelatin to HPMCP [HPMCP/gelatin].
[b] N/D, not detectable.

CONCLUSIONS

The addition of HPMCP generated a composite gel with enhanced the rheological and mechanical properties, and the maturation process reinforced the composite gel properties.

The viscosity values of the composite solutions were retained during the stability test, whereas a significant reduction was indicated in the gelatin-only solution.

Aerobic microorganisms, yeasts, and molds were not detected in any of the gelatin–HPMCP composite solutions, whereas many microorganisms were detected in the gelatin-only samples.

The films and gels exhibited relatively good mechanical and physical properties compared to the gelatin-only film as a result of HPMCP–gelatin molecular interaction, which was described identically in previous studies on the effect of the interaction between the gelatin and a polysaccharide derived from cellulose.

Because of its enhanced mechanical properties and resistance to gastric juice, the gelatin–HPMCP composite system offers potential applications in the pharmaceutical, food packaging, and other industries.

In the future, we plan to use this composite system to conduct application studies, including acid-resistant formulations for pharmaceutical and functional food fields. We also hope to more fully integrate the influence of various additives on the overall properties of the composite system.

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