Characterization and ornidazole release *in vitro* of a novel composite film prepared with chitosan/poly(vinyl alcohol)/alginate

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Abstract: To create a moist environment for rapid wound healing, a new C-P-A film with sustained antibacterial capacity had been developed by the casting/solvent evaporation method. This new type of C-P-A film consists of a chitosan top layer and sodium alginate sublayer separated by an ornidazole-incorporated poly(vinyl alcohol) layer, exhibited perfect binding characteristics among the three layers. Physical characterization of the C-P-A film showed that the triple-layer film had excellent light transmittance, control of water vapor transmission rate, and fluid drainage ability promotion, compared with the single-layer film. From the *in vitro* release studies, about 90% of OD was released from the composite films within 60 min, and no significant difference was observed in cumulative release percentage with increases in the drug content. The composite film at low concentration of OD (1.0 mg/cm2) showed effective antimicrobial activity in the cultures of *Staphylococcus aureus* and *Escherichia coli* in agar plates. The results obtained in this work indicated that the new type of C-P-A composite film incorporated with ornidazole has the potential for wound dressing application. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 85A: 566–572, 2008

Key words: chitosan; sodium alginate; ornidazole; composite film; drug delivery

INTRODUCTION

Wound healing is a dynamic process in which a variety of cellular and matrix components act in concern to reestablish the integrity of injured tissue, and the requirements for the dressing could change as the healing progresses.[1] With substantial advancements in wound dressing, it was widely accepted that a moist wound free of infection provides an optimum microenvironment rich in white blood cells, enzymes, cytokines, and growth factors beneficial to continuous tissue repair processes. The ideal wound dressing, therefore, should protect the wound from bacterial infection, prevent excessive fluid loss, maintain a moist healing environment, and be biocompatible.[2,3] Besides, dressings should have proper adherence to the wound surface and must be easy to apply and remove to improve patient compliance and comfort. Based on these ideas, the concept of the triple-layer wound dressing was put forward,[4,5] which assumed that the sublayer would contact the wound surface, absorb wound exudates, and promote tissue regeneration; the midlayer, with antimicrobial agents, could release drugs in a controlled manner; and the top layer would prevent bacterial invasion and control water vapor evaporation.

In the past two decades, numerous natural (e.g. gelatin, chitosan, alginate, and collagen) and synthetic polymers (e.g. polyurethane, polyethylene, poly(vinyl alcohol) (PVA)) had been investigated for use in wound dressings.[6–8] The structures of the dressings comprised films, hydrogels, sponges, bilayered systems with homogeneous structures, or composite laminates of two or more materials.[9–11] However, the triple-layer wound dressing had not been found until now. Hence, an attempt was made to design a three-layered composite film with an antibacterial agent as shown in Figure 1 to meet the divergent demands of the healing process.

The strategy for designing the new composite film was principally based on the utilization of polymers with suitable physicochemical properties. Chitosan (CS) was selected as the top-layer polymer because of its extremely interesting properties. CS is a unique polysaccharide obtained by the partial deacetilation

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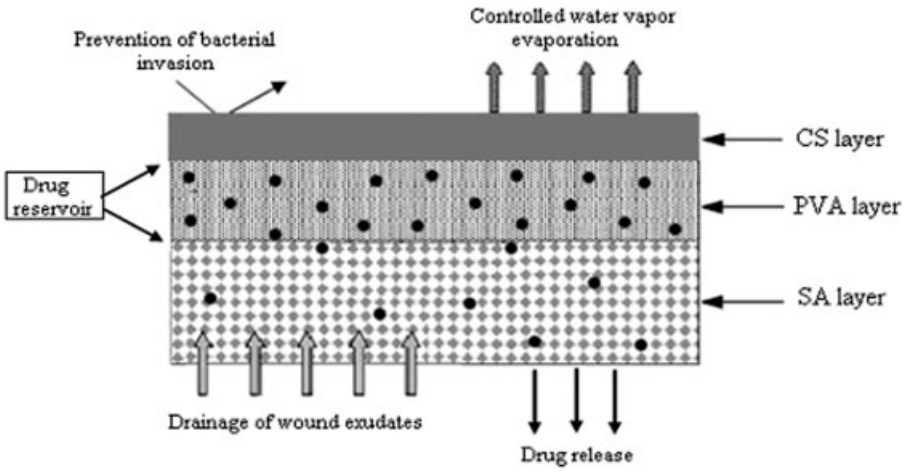

Figure 1. Schematic explanation of the structure of the C-P-A composite film.

of chitin, which had been used in a wide variety of biomedical applications such as drug delivery carriers, surgical thread, and wound dressings owing to its good biocompatibility, biodegradability, nontoxicity, antibacterial activity, and film, fiber, and gel forming capacities.[12] The midlayer contained PVA and ornidazole (OD). PVA is a hydrophilic biodegradable polymer mainly composed of C—C bonds.[13] PVA-based membranes show promising performance for functional materials because of their good hydrophilicity, film-forming property, excellent mechanical strength, and swelling behavior.[14] They have been used extensively for pharmaceutical applications containing bioactive drugs in controlled release systems, such as wound dressings, artificial skin, and dialysis membranes.[15,16] The sublayer, which adheres to the wound surface, should possess good bioadhesive strength, accelerate wound healing, and also control the release of drug. Sodium alginate, which is included in the sublayer, is a sodium salt of alginic acid, a naturally occurring nontoxic polysaccharide found in brown algae. It has many attractive physical and biological properties, such as solubility in water, moisture retention, gel-forming capability, and good biocompatibility, all of which make it a promising biomaterial in a number of biomedical applications such as wound dressings, tissue engineering, and drug delivery. There are reports suggesting that certain alginate-based dressings have hemostatic properties and could enhance wound healing by the stimulation of human monocytes.[17–19]

In addition, both single and composite films were developed by the casting/solvent evaporation method and tested *in vitro* for required properties of wound dressing applications, including film morphology, water vapor transmission rate, and water uptake capacity. With OD as the antimicrobial agent, the *in vitro* drug release behavior and antimicrobial activities of the composite films were investigated.

MATERIALS AND METHODS

Materials

Chitosan (CS) from crab shell was obtained from Biochemical Medical Plant of Qingdao (Qingdao, China). Poly (vinyl alcohol) (PVA) was purchased from Sigma (USA). Sodium alginate (SA) ($M_w = 1.2 \times 10^5$, $\mu = 280$ mPa s) was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Ornidazole (OD) powder was from Yangzhou Tengda Chemical Factory Co. (Jiangsu, China).

Preparation of films

The films were prepared using the casting/solvent evaporation technique as follows.

Preparation of single films

CS was dissolved by stirring in distilled water containing 1.0 wt % acetic acid. Insoluble substances and air bubbles were removed by filtration through a medium-pore-size glass funnel to yield a CS solution (2%, w/v). Then the solution (40 mL) was spread on a glass plate (12 cm × 12 cm) and dried at room temperature. The dried film was immersed in 10% NaOH solution to neutralize the acetic acid and washed with distilled water until a neutral pH was obtained. PVA and SA were dissolved in distilled water for the preparation of PVA (3%, w/v) and SA (2%, w/v) solution, respectively. Then the solution was cast on the glass plate and dried.

Preparation of three-layered composite films

A certain amount of the CS solution was poured onto a glass plate (12 cm × 12 cm) and allowed to dry under air at room temperature. Then the PVA (3%, w/v) solution with various loading concentrations of OD was cast above

the CS layer. The resulting two-layered films were slowly dried before spreading a third layer. Subsequently, the SA solution was cast on top of the previous one. The final three-layered films were dried until constant weight was reached and removed from the glass plate. The composite films with varied drug content were designated as C-P-A-1, C-P-A-2, and C-P-A-3. The blank matrix film without the drug was marked C-P-A-0.

Thickness of films

The thickness of each film was measured at five different places using a micrometer, and a mean value of five measurements was used as the film thickness.

Morphology study

The transparency of film samples was measured by UV-spectrophotometer (Hewlett Packard 8453, Palo Alto, CA). The films were cut into quadrate pieces, and attached to the euphotic surface of the colorimetric utensil. The percentage transmittance of calibrated white standard and film samples was measured in the range of 400–800 nm.

The surface morphology and cross sections of the C-P-A films were examined by scanning electron microscope (KYKY2800B, KYKY Technology Development Ltd., China). The dried films were coated under an argon atmosphere with gold–palladium (Sputter coater, Balzers SCD 004, Liechtenstein) and then observed under a scanning electron microscope.

Fourier transform infrared spectroscopy

FTIR spectra of samples were recorded with an Avater-360 Fourier Transform Infrared Spectroscope (Nicolet) in the wave number range of 400–4000 cm^{-1}. Each sample was pulverized, gently triturated with KBr powder, and compressed into a sheet.

Water uptake capacities

The water uptake capacity of the films was determined by swelling the films in phosphate buffer solution (PBS; pH 7.4) at room temperature. A known weight of the film was placed in the medium for the required period of time. The swollen films were removed, carefully blotted with filter paper to remove excess surface water, and immediately weighed. The water uptake was calculated from the following formula:

$$\text{Water Uptake } (\%) = \frac{W_2 - W_1}{W_1} \times 100$$

where W_2 is the weight of swollen film and W_1 is the initial weight of the film. Results are reported as the mean of five determinations.

Water vapor transmission rate

Water vapor transmission rate (WVTR) across the films was determined according to the ASTM method E96-90, Procedure D.[20] The tested film was positioned across the opening glass vial containing distilled water, and then the glass vial was placed upright in a desiccator at 35°C and with relative humidity maintained at 45%. Evaporation of water through the test film was determined by weighing the glass vial. An open vial was used as the control. The WVTR of the material was determined in units of grams per square meter per day.

In vitro drug release studies

The *in vitro* drug release studies of C-P-A film were carried out using a modified dissolution apparatus, which consisted of a 250-mL beaker as a receptor compartment and a glass rod with an attached plastic disk as a donor tube. The CS layer of the film was attached to the plastic disk with double side adhesive. The donor tube was then dipped into the receptor compartment containing dissolution medium. By this means, only the SA layer was exposed to the dissolution medium. The rotation rate was 100 rpm and 200 mL of PBS (pH 7.4) maintained at 37 ± 0.5°C was used as the dissolution medium. At predetermined time intervals, 5 mL samples were collected and replaced with an equal volume of fresh medium. The OD content of the samples was determined spectrophotometrically at 320 nm and reported as an average of three determinations. All calculations of the amount of drug released were made by taking into account the relative content of the drug in the film.

In vitro antibacterial studies

The antibacterial properties of the OD-incorporated C-P-A film (8 mm diameter discs) were tested on agar plates inoculated with *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) using the inhibition zone test.[21] OD was released from films into inoculated medium to inhibit the growth of the two bacterial strains. After 48 h of incubation at 37°C, inhibition zones (diameter of inhibitory circle) around the OD-incorporated film were measured and compared with that of blank film without OD formulation.

RESULTS AND DISCUSSION

Characteristics of films

Table I summarizes the physical characteristics of the formulated films. The thickness of single films ranged from 59 to 70 μm, and the C-P-A film was 150 μm thick on average. The differences in thickness among drug-containing films were within the acceptance range. Regarding peelability, the films of CS and PVA were easily taken off the glass plate,

TABLE I
Physical Properties of the Films

Film Formulation	OD Content (mg/cm^2)	Thickness[a] (μm)	WVTR (gm^{-2} day $^{-1}$)[b]	Physical Characteristics of Films
CS	0	59 ± 9	2093	Soft, homogeneous surface; easy to peel
PVA	0	60 ± 12	1846	Soft, colorless, homogeneous surface; easy to peel
SA	0	65 ± 10	2925	Little brittle, homogeneous surface; hard to peel
C-P-A-0	0	147 ± 11	2084	Soft, homogeneous surface; easy to peel
C-P-A-1	0.5	152 ± 17	2211	Soft, homogeneous surface; easy to peel
C-P-A-2	1.0	156 ± 14	2270	Soft, homogeneous surface; easy to peel
C-P-A-3	1.5	155 ± 12	2295	Soft, smooth surface; easy to peel

[a]Data are means ± SD, n = 5.
[b]Data are means, n = 3.

while the SA film attached more strongly to the glass plate after drying. The C-P-A films, both the drug-free and the drug-containing, were flexible, homogenous, and smooth surfaced.

Morphology of the films

It can be clearly seen in Figure 2 that both CS and PVA films possessed high diaphaneity, and the percentage transmittance was between 82 and 91%. However, the SA film, whose transmittance value was in the range of 79–85%, exhibited translucence and had a whitish appearance. So the C-P-A films showed lower transmittance in comparison to single films, and the presence of OD caused a slight decrease in transmittance.

The SEM of the surface and cross section for the C-P-A-2 prepared by the casting/solvent evaporation technique are presented in Figure 3. It is important to mention that mixing of the different layers could occur upon evaporation of the solvent, causing changes in the polymeric matrix, and that a perfect binding among the three layers was achieved, as was clearly demonstrated by the photomicrographs of the cross section, Figure 3(a). Therefore, the three-layered structure of C-P-A film was not found in the picture. There were only a dense top layer and a loose sublayer. The dense CS layer [Fig. 3(b)] displayed a smooth nonporous structure and was clear. However, the homogenous crystals of OD were visible in scanning electron micrographs of the SA layer [Fig. 3(c)], which apparently indicated that precipitation of OD, previously dissolved and finely dispersed in the PVA solution, occurred during film formation.

FTIR study

FTIR spectroscopy was used to examine the interactions of the materials. The infrared spectra of CS, PVA, SA, OD, and the C-P-A-2 film are presented in Figure 4. The bands of SA [Fig. 4(b)] appeared at 3566 cm^{-1} for the hydroxyl groups, and the ones at 1615 cm^{-1} and 1426 cm^{-1} were attributed to the asymmetric stretching vibration and symmetric stretching of —COO group, respectively.[22] The CS spectrum [Fig. 4(c)] was similar to those in previous reports.[23,24] The broad band at 3413 cm^{-1} was the O—H stretching, and the band at 1654 cm^{-1} was the N—H bending. The FTIR spectrum of pure PVA [Fig. 4(d)] showed absorption peaks at about 3613 cm^{-1} (O—H) and at about 1140 and 1458 cm^{-1} for the —C—O group.[25] From the FTIR spectrum of the C-P-A-2 film, in Figure 4(a), it could be seen that the characteristic absorption band of the O—H stretching shifted to lower wave number, suggesting an increase in the hydrogen bonding. The change indicated that the model drug used in this study had strong intermolecular interactions with the matrixes of the composite film. At the same time, there were no new characteristic absorption bands of C-P-A-2 film, which meant that there was no obvious chemical reaction between the drug and the matrix.

Water uptake capacity study

The equilibrium water sorption behavior of the films is shown in Figure 5. The films rapidly swelled and reached equilibrium within 30–45 min (data not

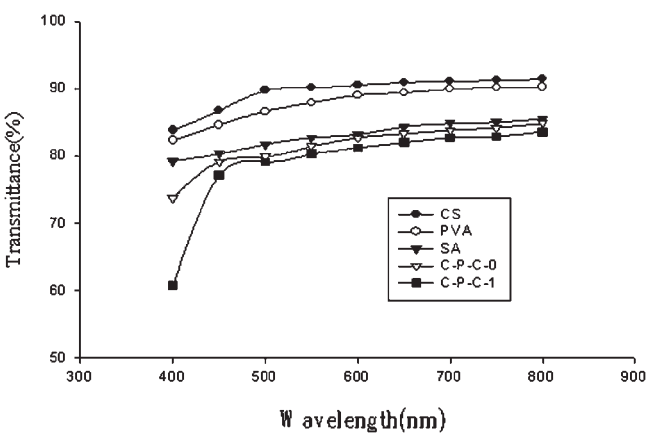

Figure 2. Transmittance of the films.

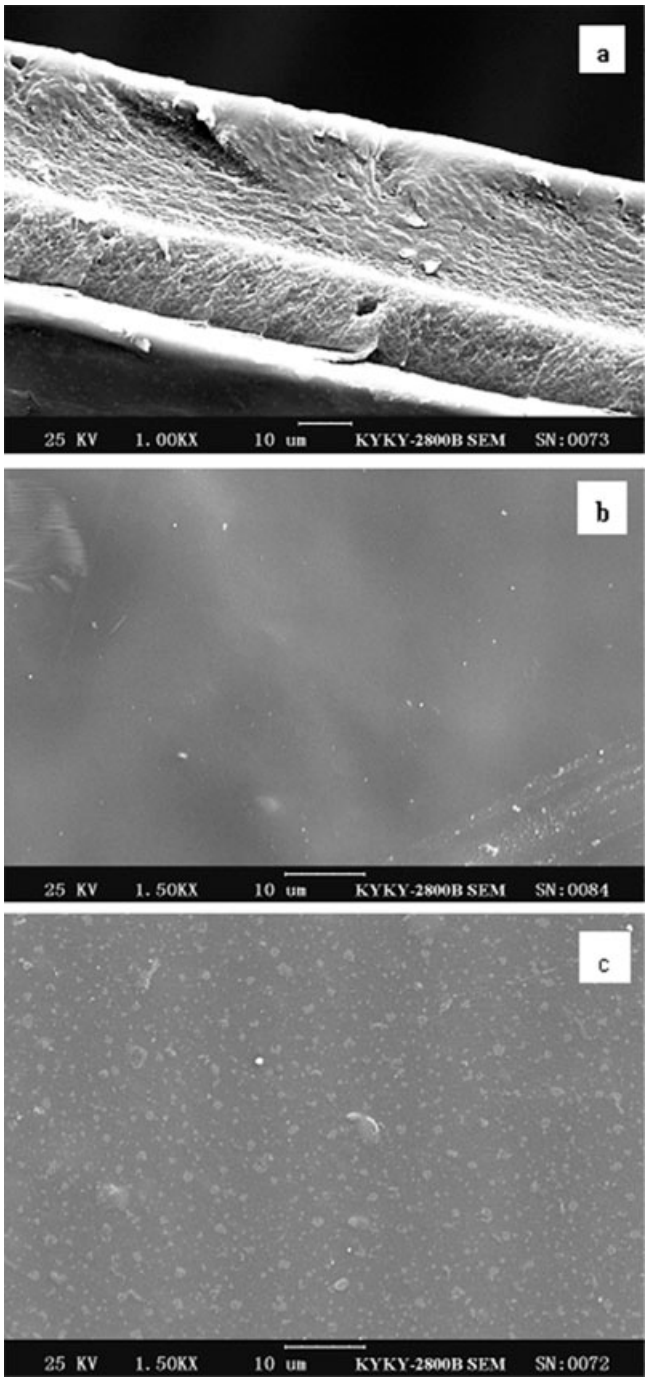

Figure 3. Scanning electron micrographs of cross section and surfaces of drug-containing C-P-A-2. (a) Cross section, (b) the chitosan layer, and (c) the sodium alginate layer.

shown). The water uptake values of CS, PVA, and SA single films are about 256, 162, and 1084%, respectively. The C-P-A films were found to have high water absorption capacity, showing 720% uptake, and the presence of OD did not cause any changes. The acquired results were found to be in good agreement with those of a previous study:[26] SA uptook more water than CS and PVA, and

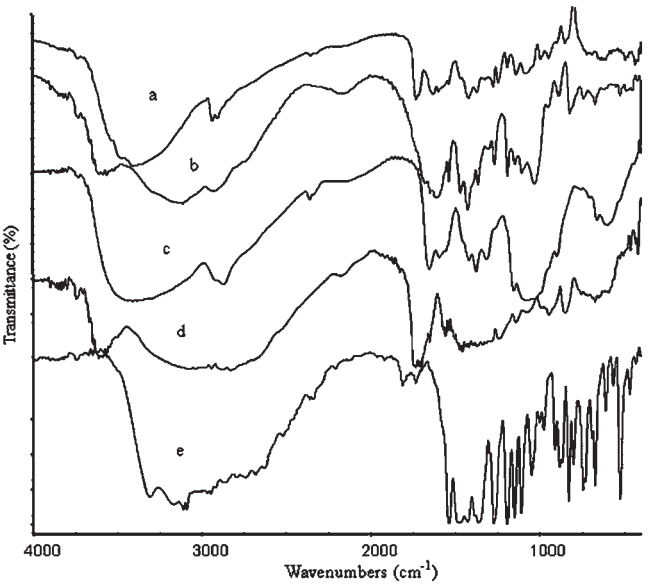

Figure 4. FTIR spectra of (a) C-P-A-2 film, (b) sodium alginate, (c) chitosan, (d) polyvinyl alcohol, and (e) ornidazole.

the addition of SA enhanced the swelling rate of the composite films. This evidence suggested that the composite film could have the potential to prevent wounds from accumulating fluid by the adsorption of exudates and create a moist wound healing environment for wound healing.

Water vapor transmission rate

An ideal dressing would control the evaporative water loss at an optimal rate to prevent the accumulation of fluid in heavily exuding burn wounds while ensuring that wound dehydration does not occur. By keeping the wound area moist but not macerated, wound healing beneath the dressing

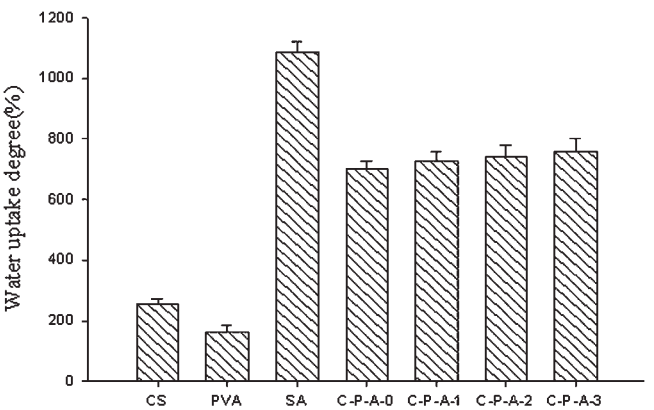

Figure 5. Water sorption behavior of the films in phosphate buffer solution (pH 7.4).

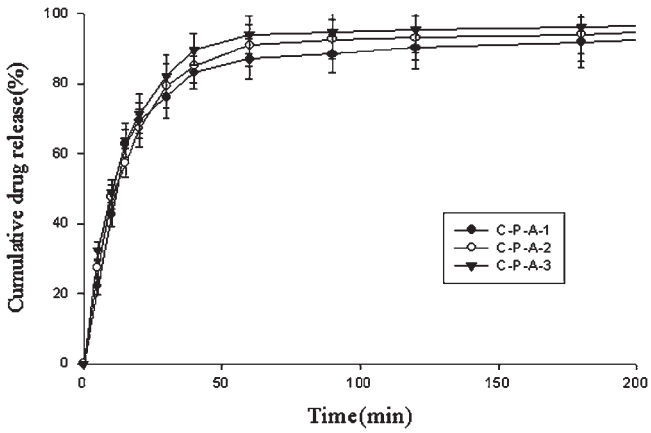

Figure 6. The release profile of OD from the composite film incorporated with various concentrations of drug.

should be accelerated. The evaporative water loss for normal skin is 204 ± 12 g m^{-2} day^{-1}, while that for injured skin could range from 279 ± 26 g m^{-2} day^{-1} for a first-degree burn to 5138 ± 202 g m^{-2} day^{-1} for a granulating wound.[27] It had been recommended that a rate of 2000–2500 g m^{-2} day^{-1}, which is in the midrange of loss rates from injured skin, would provide an adequate level of moisture without risking wound dehydration. As shown in Table I, the WVTR values of single films were about 2039, 1846, and 2925 g m^{-2} day^{-1}, respectively, and the WVTR values of the C-P-A films were about 2084 to 2295 g m^{-2} day^{-1}. As can be seen, the WVTR tended to increase with increasing content of OD in the composite films. Therefore, the drug-containing composite films gave an ideal WVTR compared with that of the single films. This finding suggested that the change in internal matrix structure of the C-P-A films affected the permeation of water vapor, which is a small molecule.

In vitro drug release studies

The *in vitro* release profile of OD from the C-P-A films in PBS (pH 7.4) is shown in Figure 6. In all drug-containing composite films, about 90% of OD was released within 60 min, and the percentage then gradually increased. No significant difference was observed in cumulative released percentage when concentration of OD was increased from 0.5 to 1.5 mg/cm^2, indicating that composite film formulations would enable application of OD at lower concentrations. It was observed that the exposure of the films to the aqueous medium caused neither rupture nor rapid erosion. The SA layer rapidly hydrated and swelled to form a gel layer over the film surface, whereas the attached CS layer and drug-containing PVA layer, which slowly swelled, helped to maintain the original shape of composite films and controlled

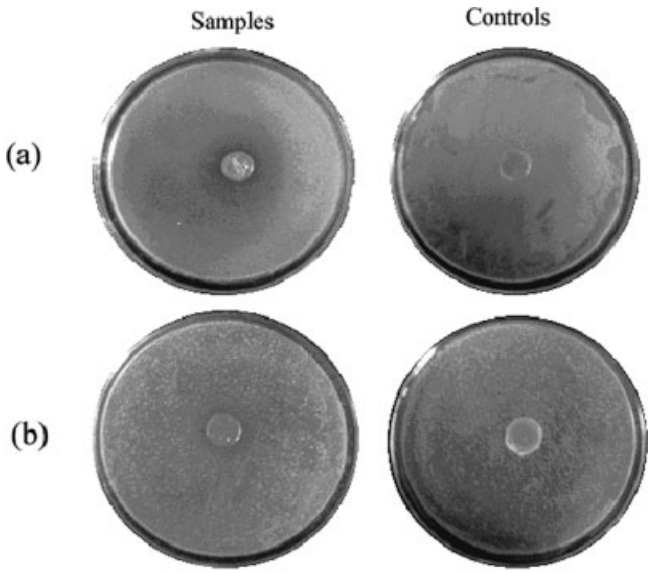

Figure 7. Inhibitory zones of the C-P-A-2 composite film, compared with controls (C-P-A-0) against (a) *Staphylococcus aureus* and (b) *Escherichia coli.*

the drug release. Therefore, it can be assumed that the drug release from the composite films was probably governed first by drug diffusion through the swollen/gelled polymer and then by the erosion of the composite film.

In vitro antibacterial studies

The antibacterial ability of the composite films loaded with OD was examined using the Bauer-Kirby Disk Diffusion Test. Zones of no growth (inhibition zones) were clearly seen around the discs of OD-incorporated films in culture plates inoculated with either Gram-negative (*E. coli*) or Gram-positive bacteria (*S. aureus*) [Fig. 7]. Similar inhibition zones were not seen with discs of drug-free composite films, thus indicating that the active antibacterial agent of OD could be immobilized in the composite film and subsequently released, thereby effectively inhibiting target microorganisms. It can be clearly observed in Table II that the antimicrobial activity of OD increased with increasing content of drug from 0.5 to 1.0 mg/cm^2, but the antimicrobial effects did

TABLE II
Inhibitory Zones of Composite Film Incorporated with Various Concentrations of Drug

Film Formulation	*Staphylococcus aureus*	*Escherichia coli*
C-P-A-1	17.4 ± 0.66	14.6 ± 0.54
C-P-A-2	21.0 ± 0.75	19.5 ± 0.65
C-P-A-3	22.3 ± 0.67	20.5 ± 0.57

Data are means ± SD, n = 5.

not show significant differences when the drug content ranged from 1.0 to 1.5 mg/cm^2. Between these two types of bacteria, the antimicrobial capability of the drug-containing composite films was more effective against the Gram-positive bacteria, as indicated by the bigger inhibition zone.

CONCLUSION

The films were formulated by the casting/solvent evaporation method. With OD as a model drug, we studied the structure and character of the films, especially their potential capacity for use in wound dressings. It was demonstrated that the composite films showed excellent light transmittance, water vapor evaporation control, and fluid drainage promoting ability compared with the single-layer films. From SEM images and FTIR, it was found that the C-P-A film exhibited perfect binding among the three layers, and the polymers had no obvious chemical reaction with the drug. The results of the *in vitro* drug release and antibacterial studies indicated a promising application of OD at a low concentration (1.0 mg/cm^2) in the C-P-A film wound dressing.

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