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Development and In Vitro Evaluation of Chitosan–Eudragit RS 30D Composite Wound Dressings

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ABSTRACT

The purpose of this research was to design and evaluate chitosan-based films intended for wound dressing application. Required properties for successful wound dressing, such as liquid uptake, vapor and oxygen penetration, bioadhesiveness, and film elasticity, were examined. Water uptake and vapor penetration of the films were determined gravimetrically, while oxygen penetration was determined by Winkler's method. The bioadhesive properties were determined with an in-house pulley system instrument using a pig gut model. Film elasticity was determined with a stretch test using an Instron apparatus. The results showed that pure chitosan films exhibited relatively high liquid uptake and the adsorption tended to decrease with the addition of Eudragit RS 30D. Moisture vapor and oxygen were found to be able to penetrate through all film formulations in comparable amounts. The bioadhesiveness test tended to show lower bioadhesive properties with the addition of Eudragit RS 30D. The formulation containing only chitosan exhibited low elongation of the film at 2 N, but the film elasticity increased with the addition of Eudragit RS 30D. In conclusion, the addition of Eudragit RS 30D could improve a film's mechanical properties but lower its bioadhesiveness.

KEYWORDS: wound dressing, chitosan hydrogels, Eudragit RS 30D.

INTRODUCTION

It has been reported that conventional wound dressing passively provides wound protection.[1,2] Today, effective wound dressing requires not only protecting the wound from its surroundings but also promoting healing by providing an optimum microenvironment for healing, removing any excess wound exudate, and allowing continuous tissue reconstruction.[3-6] The ideal wound dressing, therefore, should (1) be able to protect the wound from secondary infection, (2) provide a moisturized wound-healing environment, (3) provide thermal insulation, (4) be able to be removed without causing trauma to the wound, (5) remove drainage and debris, (6) be free from particulate and toxic products, and (7) promote tissue reconstruction.

Chitosan has received great attention from those developing medical and pharmaceutical applications because of its beneficial intrinsic properties. It is one of the natural polymers that has a high potential for helping with wound healing. This polycationic polymer is generally obtained by alkaline deacetylation of chitin, which is an extracted component of the crustacean exoskeleton. Both chitin and chitosan possess many properties that are advantageous for wound dressing, namely biocompatibility, biodegradability,[7] hemostatic activity,[8] healing acceleration, and antiinfection properties.[9,10] However, pure chitosan films have a poor tensile strength and elasticity due to their brittleness. Hence, addition of other polymers is necessary to achieve films with improved strength and elasticity.

Recently, solvent-based systems have begun to be replaced by aqueous polymeric dispersions, which are more environmentally friendly.[11] Tons of Eudragit RS 30D is used each day as a film former in pharmaceutical applications, especially as a membrane for controlled drug release. Eudragit RS 30D is a polymer containing acrylic and methacrylic acids esters with some hydrophilic properties because of the presence of quaternary ammonium groups. This polymer is insoluble in water but swells in physiological fluid independent of the pH and becomes water permeable. It is mainly used in film coating of tablets, granules, and other small particles and can also be used in matrix formation.[12] However, Eudragit RS 30D is also known to form highly flexible film because of its low glass transition temperature.[13] The tackiness of the films creates tremendous handling problems during the tablet-coating process, as the coated substrates stick to each other as well as to the wall of the coating chamber.[14,15] Because of this stickiness and flexibility and these matrix-forming properties, Eudragit RS 30D can be used in wound-dressing applications, especially in chitosan-based composite films.

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In this study, composite wound dressings were developed based on 2 main requirements: that they help with wound healing and that they be simple to produce. Chitosan-based composite films were prepared by adding Eudragit RS 30D as a composite polymer and glutaraldehyde as a crosslinker. The films were tested in vitro for properties required for wound-dressing applications, including water vapor penetration, water uptake, oxygen penetration, bioadhesive properties, film elasticity, bacterial penetration, and residual glutaraldehyde content.

Materials and Methods

Materials

Chemicals were obtained from commercial suppliers and were used as received. High–molecular weight (MW) chitosan (MW 474 kDa) with a deacetylation degree of 96% was purchased from Aqua Premier Co, Ltd (Chonburi, Thailand). Eudragit RS 30D was kindly donated by Rohm GmbH (Darmstadt, Germany). Analytical-grade lactic acid and glutaraldehyde (25% vol/vol) were purchased from Riedel deHaen (Darmstadt, Germany).

Preparation of the Composite Films

The films were prepared using casting and solvent evaporation. To prepare chitosan films, a specified amount of chitosan was dispersed in deionized water. Lactic acid (3% vol/vol) was then added to dissolve the chitosan dispersion and agitated for 1 hour, followed by the addition of glutaraldehyde (3%-30% wt/vol) as a crosslinker under gentle agitation. The mixed solution was left to stand until air bubbles had disappeared. The solution was then poured onto a dry glass Petri dish in a dust-free environment and allowed to air-dry at 40°C for 24 hours. The obtained films were tested for their properties.

To prepare chitosan–Eudragit RS 30D films, the colloidal dispersion of Eudragit RS 30D (0.5%, 1.0%, 1.5% wt/vol) was dispersed in deionized water, then added to the chitosan solution (2.5% and 3.0% wt/vol) and agitated for 1 hour. Glutaraldehyde (3% wt/vol) was added to the mixed solution under gentle agitation. The resultant mixture was left to stand until the air bubbles disappeared and was then poured onto a dry glass Petri dish in a dust-free environment and allowed to air-dry at 40°C for 24 hours. The obtained films were tested for their properties.

Characterization of the Composite Films

Water Vapor Penetration

To measure the water vapor penetration, the films were cut and placed on top of open 2.5-cm bottles containing 5 g of silica gel and held in place with a screw lid (test area: 4.9 cm^2). The bottles were conditioned in a desiccator containing silica gel for 12 hours. The bottles were then placed in a desiccator containing a saturated solution of NaCl at 30°C (75% relative humidity). The equilibrium vapor penetration was determined by weighing the bottles at 0, 12, 24, and 48 hours, respectively.

Water Uptake

The water uptake was assessed gravimetrically. The weights of the completely dried films were determined with an analytical balance. Strips of chitosan-based films (1 × 2 cm^2) were immersed in deionized water at 37°C in an incubator for 24 hours. The resultant swollen film was gently blotted with filter paper to remove excess surface water and weighed again. The water uptake of the film is the increase in weight, expressed as a percentage.

Mechanical Properties

To determine the mechanical properties of the films, a stretch test was performed on an Instron apparatus (Model 3342, Instron Corp, Canton, MA). In the stretch test, the test films (0.5 × 3 cm^2 test sections) were held in place by clippers, which were attached to the cell of an Instron device. The upper clipper was driven upward, stretching the films at various forces. The mechanical properties of the films were the elongation of the films under the stress of 2 N.

Oxygen Penetration

Oxygen penetration through films was studied by placing the films on top of open 250-mL flasks containing 200 mL of deionized water and held in place with a screw lid (test area: 4.9 cm^2). The negative control was the closed flask with an airtight cap (preventing oxygen from entering the flask), while the positive control was the open flask (allowing oxygen to enter the flask and dissolve in the water as recipient). The test flasks were placed in an open environment under constant agitation for 24 hours. The collected water samples were then analyzed for dissolved oxygen according to Winkler's method.[16] Following Winkler's method, a divalent manganese solution was added to the test solution, followed by a strong alkali (NaOH). Under such conditions, any dissolved oxygen in the test solution rapidly oxidizes an equivalent amount of divalent manganese (Mn^{+2}) to manganese dioxide (MnO_2(s)) of a higher valence state (Mn^{+4}). The MnO_2(s) exists as a precipitate in the solution. When the solution is acidified in the presence of iodide (KI), free iodine (I_2) is produced in a concentration that is equivalent to the original concentration of dissolved oxygen in the test solution. The sample was titrated

with 0.25N sodium thiosulfate solution with the addition of starch as an indicator until a blue color was reached. The results were expressed as the amount of dissolved oxygen in milligrams per milliliter.

Bioadhesive Properties

The in vitro evaluation of the bioadhesive properties of the films was performed using an in-house pulley system instrument (Figure 1). The proximal portion of a pig's large intestine was used to represent the mucous-like texture of a fresh wound. The freshly slaughtered pig's large intestine was washed with physiological saline at 4°C and attached to a platform (test area: 4.9 cm^2). A prewetted film was placed atop the intestine and held under 100 g weight for 2 minutes, with the other side of the weight connected to a pulley system. The bioadhesiveness of the film was measured by adding water to a water container connected to the pulley system until the film was detached from the intestine. The weight of water needed to detach the film from the intestine was recorded.

Microbial Penetration

The films' ability to prevent microbial penetration was tested by placing the films on open 10-mL vials containing 5 mL of nutrient broth (Merck, Darmstadt, Germany) and held in place with a screw lid (test area: 0.8 cm^2). The negative control was a vial closed with a tightly packed cotton ball, while the positive control was an open vial. The tested vials were placed in an open environment for 1 week. The cloudiness of the nutrient broth in any vial was recorded as microbial contamination.

Residual Glutaraldehyde Measurement

The 0.5 × 0.5 cm^2 film was dissolved in 1.0% (wt/vol) acetic acid, and the sample solution was assessed for the amount of residual glutaraldehyde using das chromatography equipped with mass spectroscopy (GC-MS) (GP 2010, Shimadzu, Kyoto, Japan) with an injection temperature of 200°C, a column temperature of 150 to 250°C, split injection mode, and 100 kPa pressure.

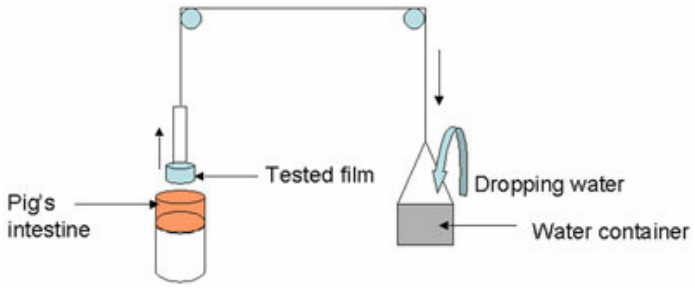

Figure 1. Diagram of the instrument for in vitro evaluation of bioadhesive properties of the films.

Statistical Analysis

All of the characterizations of the composite film experiments were done in triplicate. One-way analysis of variance was performed to determine significant differences for each property among the formulated films. The differences were considered to be significant at a level of $P < .05$.

Results and Discussion

In preliminary tests, noncrosslinked chitosan films were found to be transparent and water-soluble. However, the films broke apart shortly after rehydration. Glutaraldehyde (3% wt/vol) was added to weakly crosslink the films. During crosslinking, glutaraldehyde diffused into the polymeric solution, forming either intermolecular or intramolecular linkages.[17,18] The crosslinked films were not soluble in water and showed substantially stronger than non-crosslinked films membrane properties both before and after the rehydration process. Increasing the concentration of glutaraldehyde from 3% to 30% wt/vol while keeping the chitosan concentration constant at 3% wt/vol changed the color of the films from pale to dark brown. Moreover, the water absorption of the films decreased because of the lower water solubility of chitosan after crosslinking. Increasing the chitosan concentrations resulted in less flexibility and poor elasticity of the films. The formulation of 3% wt/vol total polymer concentration of either chitosan alone or chitosan in combination with Eudragit RS 30D and 3% wt/vol glutaraldehyde was found to be optimal, producing films with good flexibility. This formulation was used for the experiments that followed. The composite transparent films without Eudragit RS 30D were not flexible but became flexible after the addition of Eudragit RS 30D. However, at the Eudragit RS 30D concentrations of 1.0% wt/vol and above, the films were very flexible and very sticky, so they were difficult to handle.

Water Vapor Penetration

The water vapor penetration across the films at 6, 12, 24, and 48 hours was measured and expressed as a percentage of weight increase of the dried silica gel. Figure 2 shows that chitosan and chitosan–Eudragit RS 30D increased as a function of time with similar profiles. The vapor transmission was measured under steady-state conditions. Therefore, the contribution of the moisture absorbed by the film can be considered negligible. The vapor penetration through the films at 48 hours showed that the films containing chitosan 3% wt/vol (CS3%), chitosan 2.5% wt/vol + Eudragit RS 30D 0.5% wt/vol (CS2.5% + EU0.5%), chitosan 3% wt/vol + Eudragit RS 30D 0.5% wt/vol (CS3% + EU0.5%), chitosan 3% wt/vol + Eudragit RS 30D 1.0% wt/vol (CS3% + EU1.0%), and chitosan 3% wt/vol + Eudragit RS 30D

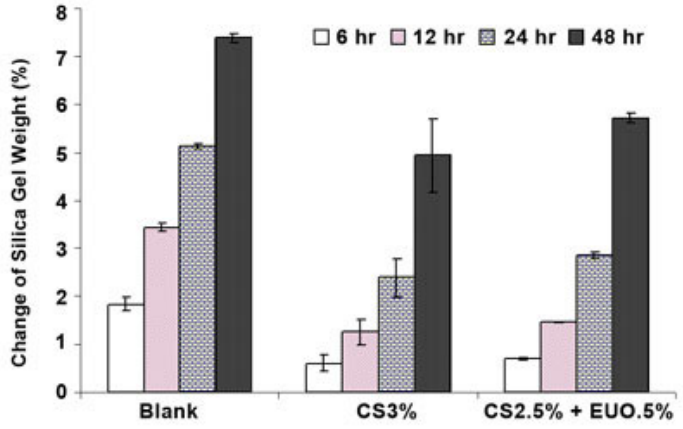

Figure 2. The vapor penetration through the films at 6, 12, 24, and 48 hours. CS indicates chitosan; EU, Eudragit RS 30D.

1.5% wt/vol (CS3% + EU1.5%) did not have statistically different vapor penetrations, with values of 4.93 ± 0.75, 5.72 ± 0.10, 5.11 ± 0.67, 4.19 ± 0.23, and 4.05 ± 0.55% weight increases of the dried silica gel, respectively. At 3% wt/vol of chitosan, increasing Eudragit RS 30D from 0% to 0.5% to 1.0% to 1.5% wt/vol only minimally reduced vapor penetration through the films. It has been reported that the desired permeability to water can be improved by changing the film density.[19] However, our results showed slightly decreased vapor penetration at 48 hours, and the concentration range from 3% to 4.5% wt/vol of total solid content was too narrow to obtain statistically different values for the formulations.

Water Uptake

Figure 3 shows the equilibrium water uptake of chitosan and chitosan–Eudragit RS 30D films with different concentrations of Eudragit RS 30D. Chitosan films showed the highest increase in equilibrium water uptake, while keeping the concentration of chitosan constant at 3% wt/vol and increasing the concentration of Eudragit RS 30D from 0.5% to 1.0% to 1.5% wt/vol resulted in lower water uptake. However, the difference was not statistically significant. The films containing chitosan 2.5% wt/vol + Eudragit RS 30D 0.5 wt/vol (CS2.5% + EU0.5%) exhibited the lowest water uptake because of the lower concentration of chitosan. These results are expected since chitosan is a more hydrophilic polymer than Eudragit RS 30D. Therefore, having a higher amount of chitosan in the films improved their ability to adsorb water.

Mechanical Properties

As shown in Figure 4, the films containing only chitosan 3% wt/vol showed the lowest elongation at force 2 N, while the composite films containing chitosan 2.5% wt/vol + Eudragit RS 30D 0.5% wt/vol (CS2.5% + EU0.5%) showed the highest elongation at force 2 N, followed by the composite films containing chitosan 3% wt/vol + Eudragit RS 30D 0.5% wt/vol (CS3% + EU0.5%). Addition of Eudragit RS 30D to chitosan films increased elongation at the same level of solid content (3% wt/vol). This result indicated the flexibility improvement of the film with the inclusion of Eudragit RS 30D. Increasing the total solid concentration from 3% wt/vol to 3.5% wt/vol (CS3% + EU0.5%) to 4.0% wt/vol (CS3% + EU1.0%) to 4.5% wt/vol (CS3% + EU1.5%) resulted in decreasing elongation at force 2 N. These results imply that increasing the films' density by increasing the total solid content decreased the films' flexibility.

Oxygen Penetration

Under normal circumstances, purified water has a dissolved oxygen value in the range of 7.0 to 14.6 mg/mL at 0 to 35°C. The tested solutions from the airtight flask (negative control) and the opened flask (positive control) had dissolved oxygen

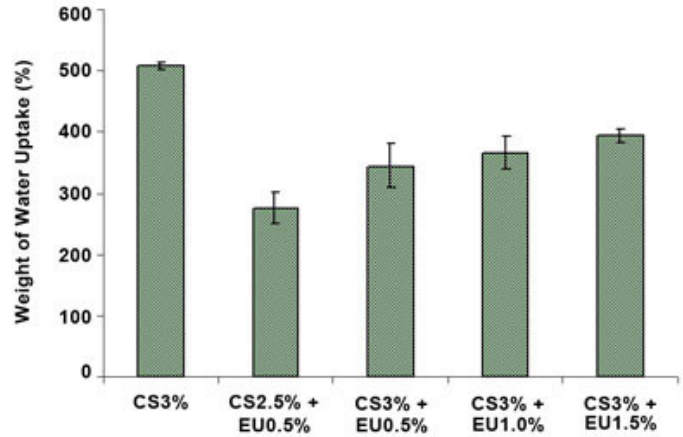

Figure 3. The equilibrium water uptake of the films. CS indicates chitosan; EU, Eudragit RS 30D.

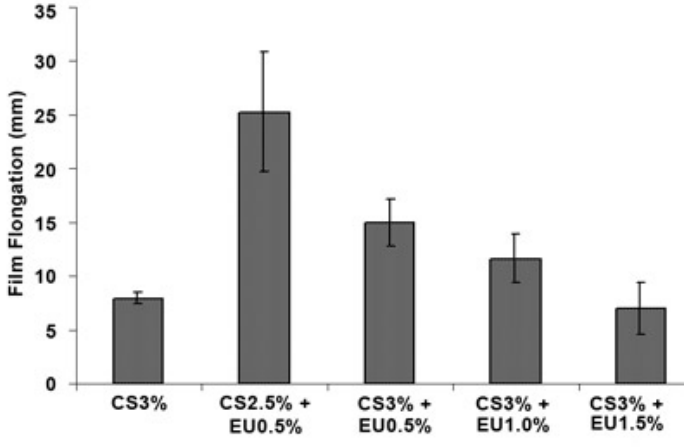

Figure 4. The elongation of the films under the stretch test at force 2N. CS indicates chitosan; EU, Eudragit RS 30D.

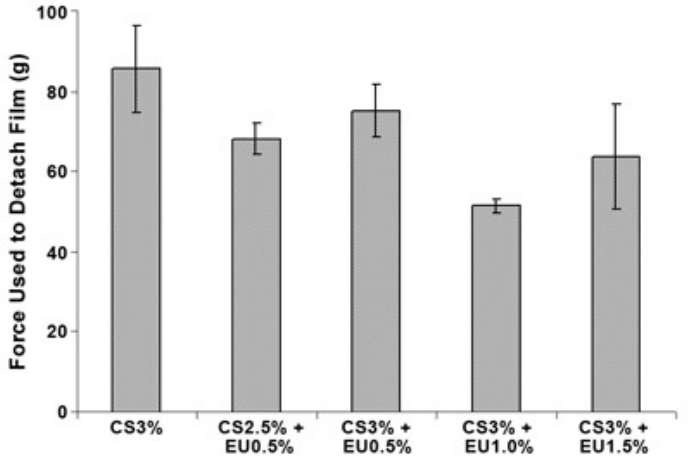

Figure 5. The weight required to detach the attached films from the pig's intestine. CS indicates chitosan; EU, Eudragit RS 30D.

7.70 ± 0.20 mg/mL and 8.85 ± 0.48 mg/mL, respectively, whereas those from flasks covered with chitosan (CS3%) and chitosan–Eudragit RS 30D (CS3% + EU1.5%) had dissolved oxygen 8.10 ± 0.20 and 8.21 ± 0.24 mg/mL, respectively. The oxygen penetrations were not significantly different among the composite films. From the results, it can be concluded that oxygen was able to penetrate through all the film formulations.

Bioadhesive Properties

The weight of water required to detach the attached film from the pig's intestine was used to represent the relative magnitude of bioadhesive force of the tested film. The films composed of only chitosan showed the highest bioadhesiveness, while the films containing Eudragit RS 30D (CS3% + EU1.0%) showed the lowest and most homogeneous detachment forces. However, the results do not show a real trend, and the differences are not statistically significant (Figure 5).

Microbial Penetration

In the microbial penetration tests, the positive control tubes were tested to ensure that the nutrient broth was suitable for bacterial growth, while the negative control tubes were tested because they represented a condition free from intrinsic bacterial contamination. The results showed that microbial contamination was not observed in the chitosan–Eudragit RS 30D tubes and the negative control tubes. Only the positive control tubes had bacterial contamination. This indicates that the developed composite films have good potential for use as wound dressing because of their ability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer by reducing the primary wound contamination[20] and because of their ability to protect the wound from secondary bacterial infection.

Residual Glutaraldehyde

As mentioned earlier, glutaraldehyde was used as a crosslinker to form either intermolecular or intramolecular linkages. An excess of unreacted glutaraldehyde from the crosslinking process can be toxic for the wound. Weakly crosslinked composite films without any residual glutaraldehyde can be safely used without compromising their integrity. Since the optimal concentration of crosslinking agent (glutaraldehyde) was 3% wt/vol, the residual glutaraldehyde in the film of this concentration was analyzed by GC-MS. There was no residual glutaraldehyde left in the films.

CONCLUSION

The in vitro evaluation revealed that Eudragit RS 30D can be incorporated into chitosan film to improve its mechanical properties while substantially maintaining vapor penetration, water uptake, and oxygen penetration properties. The films showed good protection against microbial penetration, indicating a strong potential for wound dressing application. The crosslinked films with 3% wt/vol glutaraldehyde developed in this study did not show any residual glutaraldehyde. Hence, the films can be safely used as good wound-dressing systems.

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