Electrospun Allantoin-loaded Cellulose Acetate Fiber Mats for Wound Dressing Application

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Abstract

Encapsulation of allantoin (AL) in cellulose acetate (CA) were achieved by means of electrospinning. The obtained electrospun fiber mats containing 5-20% w/w AL (based on the weight of CA) exhibited smooth fiber surface without aggregation of AL. The average diameters of the obtained nanofibers were 256-353 nm. The release behaviors of AL from the nanofibers was compared with those from the cast films. The degree of water retention, weight loss, and release profiles of AL from nanofiber mats and films were studied in acetate buffer (ACB; pH 5.5) at 32ºC and phosphate buffer saline (PBS; pH 7.4) at 37ºC. The degree of water retention of the AL-loaded nanofiber mats was about 206-226% which were much higher than those of the films (i.e. 19-26%). Two types of release methods including the total immersion and the transdermal diffusion through pig skin were performed in both buffers for 48 h. For both fiber mats and films, the burst release followed by a sustained release of AL was observed. However, the fiber mats allowed more AL released comparing to the films. For the total immersion method, the highest percentages of AL released from the fiber mats in ACB and PBS were in a range of 51-96% and 36-94%, respectively. Whereas, these values from the films were in a range of 10-41% and 7-22%, respectively. For the transdermal diffusion through pig skin method, the highest percentages of AL released from the fiber mats in ACB and PBS were in a range of 60-96% and 43-89%, respectively. Moreover, the amounts of AL loading in the fiber mats affected to the percentages of AL released. The fiber mat containing 5% AL exhibited the higher percentage of AL released than those containing 10 and 20% AL which could be a result of the smaller size of fibers.

Keywords: Allantoin; Cellulose acetate; Electrospinning; Wound dressing; Drug release

1. Introduction

Recently, electrospinning as the interesting method for fabrication of nanofibers, has drawn highly attention in many areas of research. The principle of the fiber formation is the application of electric forces to polymer liquid [1]. By applying the electrostatic field to the polymer melts or solutions, the charged species are accumulated and the stream of polymer liquid is subsequently ejected. The ultrafine fibers are deposited on a collecting device as a non-woven fibrous membrane. Because of the very small size of electrospun fibers, they have high surface area to volume or mass ratio. Also, the electrospun fibers have a porous structure in a form of non-woven mats. Therefore, they have been investigated as the ideal materials in many applications. Some of the proposed applications are as filtrations [2], electrical and optical devices [3], scaffolding in tissue engineering [4], and wound dressings [5]. For the drug delivery applications, the electrospun fibersexhibited a sustained release of drug and allowed more drug molecules to be released more than the cast films that have no pores [5, 6]. The controlled and sustained release of drugs or other functional substances can be achieved when the electrospun fiber mats were used as carriers [5-7].

Allantoin (AL), also called 5-ureidohydantoin with a chemical formula C4H6N4O3, is a heterocyclic compound produced by animals, bacteria and plants. AL has been widely used in cosmetics, pharmaceutical, and oral hygiene products [8] according to its good properties such as hydrating, repairing, healing and soothing for skin. AL is one of the interesting functional compounds for use in wound healing. It has been reported to regulate an inflammatory response [9, 10] and stimulate fibroblastic proliferation and extracellular matrix synthesis [11].

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In this work, the polymer used as a matrix for fiber formation was cellulose acetate (CA), one of the common biopolymers. The optimum conditions for preparation of the electrospun CA

fibers were reported by Liu and Hsieh [12]. The concentrations of CA in a range of 12.5-20% w/w and a mixed solvent of 2:1 v/v acetone/N,N-dimethylacetamide (DMAc) were recommended. According to the good properties of CA which are the ease of fiber formation and tissue compatibility, the electrospun CA fibers are widely used in the drug delivery system. The encapsulation of many types of drugs or substances such as gallic acid [5], asiaticoside [6], curcumin [13], amoxicillin [14], and gabapeptin [15] into the electrospun CA fibers and the release profiles of these chemical agents were reported.

In the present contribution, the release behaviors of AL from the electrospun CA fiber mats was investigated. The encapsulation of AL in CA nanofibers was achieved by electrospinning technique. Two types of release assays, i.e. the total immersion and the transdermal diffusion through a pig skin methods were carried out. Two types of medium, i.e. an acetate buffer (ACB; pH 5.5) and a phosphate buffer saline (PBS; pH 7.4) were used in the release study to simulate the pH of human skin and physiological fluids, respectively. Moreover, water retention and weight loss behaviors of the AL-loaded CA fiber mats were also studied.

2. Methods

2.1. Materials

CA with molecular weight about 30,000 g/mol (39.7% acetyl content) and AL were purchased from Sigma Aldrich (Switzerland). DMAc and acetone were purchased from Labscan, Asia (Thailand). Acetic acid and sodium acetate were purchased from Carlo Erba (Italy). Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were purchased from Acros Organics (Belgium). All solvents were analytical reagent grade.

2.2. Electrospinning

CA solution was prepared in a mixed solvent of 2:1 v/v acetone/DMAc at 17% w/v. AL was mixed into the CA solution at different concentrations including 5, 10, and 20% w/w based on the dry weight of CA. The AL-loaded CA solutions were fabricated into ultrafine fibers Cannon Fenske Routine viscometer (size 450). The AL-loaded films were also fabricated from solvent casting method at the same concentration of AL loading with those of the electrospun fibers for use in comparative studies. The thickness of fiber mats and films samples were controlled to about 50-60 μm.

2.3. Characterizations of the AL-loaded CA fiber mats and films

A JEOL JSM-LV5410 scanning electron microscope (SEM) was used to observe the surface morphology of the AL-loaded fibers. The diameters of fibers were measured by using the SemAfore 4.0 software. Water retention and weight loss behaviors of both fiber mats and cast films after submersion in ACB and PBS for 48 h were investigated and calculated according to the following equations:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100 \quad (1)$$

$$\text{Weight loss (\%)} = \frac{M_i - M_d - M_r}{M_i - M_r} \times 100 \quad (2)$$

where M and M_d are the weight of each sample after submersion in a medium for 48 h in wet state and dry state, respectively. M_i is the initial weight of the sample in dry state. M_r is the weight of AL that was released from the sample at 48 h.

For preparation of ACB (pH 5.5), 15 g of sodium acetate was dissolved in about 50 ml of distilled water. 1.5 ml of concentrated acetic acid was then added. Finally, distilled water was added to make up 100 ml solution. For preparation of PBS (pH 7.4), 0.101 g of sodium dihydrogen orthophosphate, 0.618 g of disodium hydrogen orthophosphate, and 0.87 g of sodium chloride were dissolved in about 50 ml of distilled water. Lastly, distilled water was added to make up 100 ml solution.

2.4. Release of AL

The release behaviors of AL from the AL-loaded CA fiber mats and films were studied by two methods, i.e. the total immersion and the transdermal diffusion through pig skin method. Two types of the releasing medium including ACB (pH 5.5) and PBS (pH 7.4) were used. For the total immersion method, the samples (the size of 2 x 2 cm) were placed into 40 ml of either ACB at 32ºC or PBS at 37ºC. The solution was withdrawn at a given time points and quantified for the amount of AL released by measuring its absorbance at 325 nm using a UV-visible spectrophotometer. For the transdermal diffusion method, an abdomen pig skin was treated by removal of hair, subcutaneous fat, and underlying tissues. The thickness of treated pig skin was about 1-1.2 mm. Each fibers and films specimens were placed on top of pig skin. They were then placed on a modified Franz diffusion cell. The released amount of AL were also measured from its absorbance at 325 nm. The triplicate of experiments were performed.

3. Results and Discussion

3.1. Electrospinning

The surface morphology of the AL-loaded electrospun CA fibers were smooth with the round cross-sectional shape (see Fig. 1). The average diameter of the 5% AL-loaded CA fibers was 256 ± 49 nm which was slightly smaller than that of the neat CA fibers (353 ± 56 nm) (see Table 1). However, upon increasing more AL to 10 and 20%, the average diameters of fibers were increased. The initial decreasing in the size of fibers upon addition of AL (i.e. 5%) could be due to the lowering of the viscosity of solution as shown in Table 1, which could be resulted from disturbing of the entanglement of CA molecules by AL molecules.

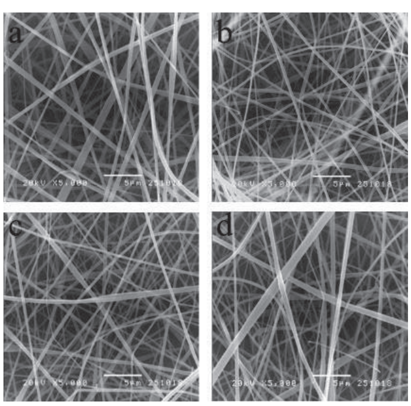

Fig. 1. SEM images of (a) neat electrospun CA fibers (b) 5% AL (c) 10% AL and (d) 20% AL-loaded electrospun CA fibers

The viscosity is one of the most important parameters indicating the size of the electrospun fibers. Generally, the lower viscosity of solution will be resulted in the smaller size of electrospun fibers. The polymer solution with lower viscosity has smaller viscoelastic force that has less ability to resist the elongation of the polymer jets according to the electrostatic force and Coulombic repulsion force [16]. Upon addition of AL to 10 and 20%, the resulting fibers were larger as commonly known that the presence of rigid particles in fluid can increase the viscosity. The more particles of AL in the CA solutions could be a reason of higher viscosities (see Table 1) and subsequently yielded the larger fibers.

Table 1. Viscosity of the solutions and average diameters of the obtained fibers

Solutions	Viscosity (*cP*)	Average diameter of fibers (*nm*)
Neat CA solution 5%	484 ± 11	353 ± 56
AL-loaded CA 10% AL-	444 ± 14	256 ± 49
loaded CA 20% AL-	474 ± 3	287 ± 59
loaded CA	500 ± 14	296 ± 67

3.2. Water retention and weight loss

The degree of water retention and weight loss of the specimens were determined in either ACB and PBS after 48 h of immersion. The amounts of water retention of the AL-loaded CA fiber mats were in a range of 223-226% in ACB and 206-221% in PBS which were much higher than those of the cast film specimens (i.e. 19-23% in ACB and 21-26% in PBS) (Fig. 2a).

The results of weight loss were found in a same trend as the results of the water retention. The percentages of weight loss of the AL-loaded CA fiber mats were in a range of 21-22% in ACB and 20-22% in PBS. These values for the films were in a range of 3.8-4.2% in ACB and 3.7-4.0% in PBS (Fig. 2b). However, the difference in concentrations of AL did

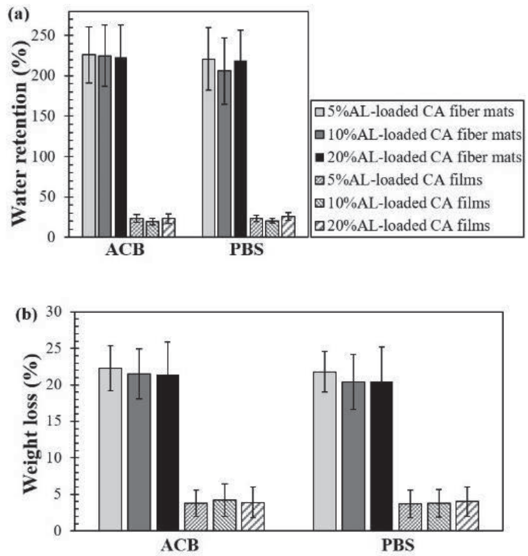

Fig. 2. The percentages of (a) water retention and (b) weight loss of the AL-loaded CA fiber mats and films

not give the significant difference in the amounts of water retention and weight loss. The higher amount of these values of the electrospun fiber mats comparing to the films could be caused by its porous structure that can hold more water inside and also the high surface area of the ultrafine fibers [5, 6, 13].

3.3. Release of allantoin

The release behaviors of AL from the AL-loaded electrospun CA fiber mats and cast films were investigated under two simulated conditions with different pH and temperature. The experiments were carried out by the total immersion and the transdermal through a pig skin method using ACB (32°C, pH 5.5) and PBS (37°C, pH 7.4) as similar to the human skin and physiological conditions, respectively. Fig. 3a and 3b present the cumulative percentage of AL release from the fiber mats and films from the total immersion method in ACB and PBS, respectively. At initial time period from 0-180 min, both the AL-loaded fiber mats and films showed a burst release of AL. Later, the sustained release of AL was observed until reaching an equilibrium that had no change of the cumulative release amounts. Interestingly, the greater values of AL released were observed from the fiber mat specimens comparing to the films. For the 5, 10, and 20% AL-loaded CA fiber mats, the highest cumulative percentages of AL released in ACB were about 96, 75, and 51%, respectively. Whereas, these values from the films with similar AL loading contents were about 41, 33, and 10%, respectively. The similar trend of results was observed from the release study in PBS. For the 5, 10, and 20% AL-loaded CA fiber mats, the highest cumulative percentages of AL released in PBS were about 94, 67, and 36%, respectively. The lower values were observed in case of the films which were about 22, 14, and 7%, respectively. Many factors can affect to the rate and efficiency or the highest amounts of drug that release into media. Amounts of water retention or swelling ability was reported as one of the factors that contribute to the ability of carrier to release drug molecules [5, 6, 13]. The higher amounts of weight loss and water retention of fiber specimens according to its porous structure comparing to the films as discussed in the previous section, could contribute to the greater ability for media to penetrate into the membrane and therefore allowing more AL to diffuse or release. A number of reports revealed the similar results that the electrospun fiber mats can allow the substances to release more than from the cast films that has no pores [5, 6, 13].

Comparing the maximum percentages of released substances with the related research work, the amounts of AL released in this work were lower than those from Suwantong et. al. [6] that reported about 75-95% of asiaticoside released from electrospun CA fibers in ACB (32°C, pH 5.5) and about 90-99% in PBS (37°C, pH 7.4). The lower amounts of released substances compared with another work could be caused by the different compositionsin media in which the media containing 10% v/v methanol was used [6].

Comparing among the fiber specimens with different amounts of AL loading for both types of releasing media, the amount of AL released from 5 %AL-loaded fiber mat was higher than those of 10, and 20%, respectively. This could be resulted from the smaller diameter of 5% AL-loaded CA fibers (i.e. 256 nm) comparing to those of 10 and 20% AL-loaded CA fibers (i.e. 287 and 296 nm) that provided higher porosity and higher surface area to volume ratio and subsequently contributed to the higher amount of AL released.

In case of the cast film specimens, the percentage of AL released from 20% AL-loaded CA film was lower than those of 10, and 5%, respectively. Because of the dense structure of the cast film, the higher amount of substance loaded could lead to the higher amount of substance trapped inside the film and provided smaller amounts of drug released.

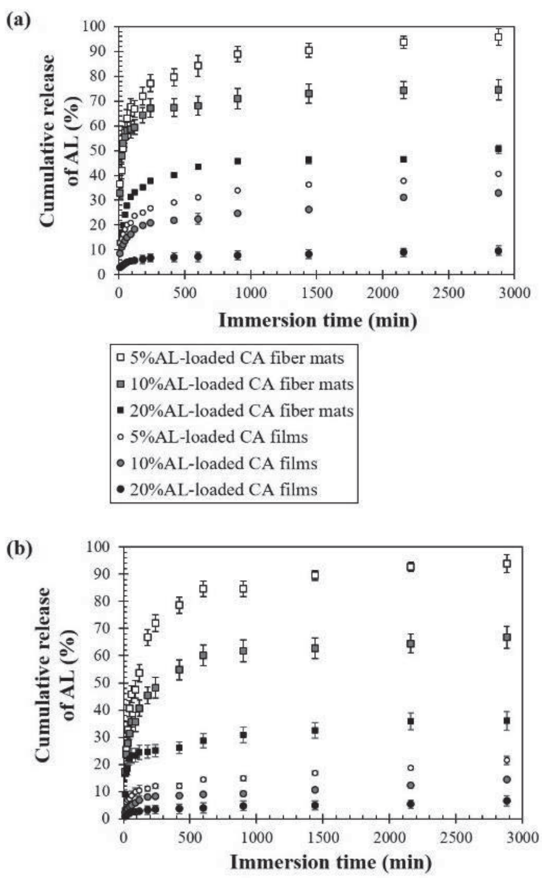

Fig. 3. Cumulative release profiles of AL from the electrospun CA fiber mats and films from total immersion method in (a) ACB and (b) PBS

In the transdermal diffusion through a pig skin method, the cumulative release amounts of AL from the fiber mats in ACB and PBS are shown in Fig. 4a and 4b, respectively. The initial burst released followed the sustained released was also observed. For the 5, 10, and 20% AL-loaded CA fiber mats, the highest cumulative percentages of AL released in ACB were about 96, 81, and 60%, respectively, while those in PBS were about 89, 66, and 43%, respectively. Moreover, the highest cumulative percentages of AL released from 5% AL-loaded CA fiber mats in both ACB and PBS were greater than those from 10 and 20% AL-loaded CA fiber mats, respectively. The reason could be the smaller size of fibers of 5% AL-loaded fibers comparing to those of 10 and 20% AL-loaded fibers as already discussed earlier. Evidently, the AL-loaded CA fiber mats have revealed the high potential for use in wound dressing applications as observed that the sustained release or controlled release of AL was achieved.

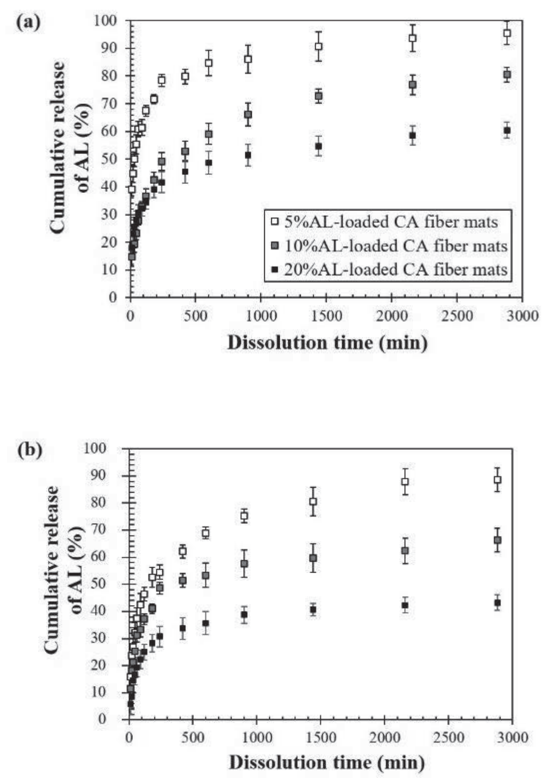

Fig. 4. Cumulative release profiles of AL from the electrospun CA fiber mats and films from transdermal diffusion method in (a) ACB and (b) PBS

4. Conclusions

The nanofibers of CA containing AL at concentrations of 5, 10, and 20% w/w were fabricated by electrospinning. There were no beads or agglomerates of AL on the surface of the electrospun fibers as investigated by SEM. The average diameter of fibers was in a range of 256-353 nm. The release characteristics of the electrospun fibers were compared with those of the cast films. The release experiments were studied under the simulated conditions of human skin (32 ºC, pH 5.5) using acetate buffer and physiological fluids (37ºC, pH 7.4) using phosphate buffer saline. Two types of release methods were carried out including the total immersion and the transdermal diffusion through pig skin methods. For both fibers and films specimens, the initial burst release of AL followed by a sustained released was observed. However, the highest percentages of AL released from the fiber mats were greater than those of the films. The greater amounts of weight loss and water retention of the fiber mats could be reasons that the fiber samples can allow more AL molecules to be released. For the total immersion method, the highest cumulative percentages of AL released from the fiber mats in ACB and PBS were in a range of 51-96% and 36-94%, respectively. Whereas, these values from the films were in a range of 10-41% and 7-22%, respectively. For the transdermal diffusion through pig skin method, the highest cumulative percentages of AL released from the fiber mats in ACB and PBS were in a range of 60-96% and 43-89%, respectively. Moreover, the percentages of AL released were decreased as the amounts of AL loaded increased. This could be caused by the difference of fiber sizes. The fiber mat containing 5% AL that has a smaller average fiber diameter exhibited the higher percentage of AL released than those containing 10 and 20% AL. Lastly, the AL encapsulated in the electrospun CA fibers have revealed the high potential for use in wound dressing applications as the sustained release or controlled release of AL was achieved.

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