



CHAPTER V

ELECTROSPUN CELLULOSE ACETATE FIBER MATS CONTAINING ASIATICOSIDE OR CENTELLA ASIATICA CRUDE EXTRACT AND THE RELEASE CHARACTERISTICS OF ASIATICOSIDE

5.1 Abstract

Ultra-fine cellulose acetate (CA; $M_w \approx 30,000$ Da; degree of acetyl substitution ≈ 2.4) fiber mats containing asiaticoside (AC) from the plant *Centella asiatica* L. either in the form of pure substance (PAC) or a crude extract (CACE) were fabricated by electrospinning. Incorporation of either PAC or CACE (40 wt.% based on the weight of CA) in the neat CA solution (17% w/v in 2:1 v/v acetone/dimethylacetamide) did not affect the morphology of the obtained fibers, as both the neat and the herb-loaded CA fibers were smooth. The average diameters of these fibers ranged between 301 and 545 nm. Determination of the release characteristics of AC from the herb-loaded CA fiber mats was carried out by the total immersion and the transdermal diffusion through a pig skin methods in acetate or phosphate buffer solution that contained methanol (hereafter, A/B/M or P/B/M medium) at either 32 or 37 °C, respectively. In the total immersion method, the maximum amounts of the AC released from the PAC- and the CACE-loaded CA fiber mats into the A/B/M medium were ~ 24 and $\sim 10\%$ (based on the weight of the specimens), while those of the AC released into the P/B/M medium were ~ 26 and $\sim 12\%$, respectively. Considerably lower values were, however, obtained when the materials were placed on top of a piece of pig skin. Lastly, the herb-loaded CA fiber mats released no substance that was harmful to normal human dermal fibroblasts, rendering their potential for use as topical/transdermal or wound dressing patches.

(Key-words): Topical/transdermal drug delivery; Wound dressing; Electrospinning; Cellulose acetate

5.2 Introduction

Electrospinning (e-spinning) is a process by which ultra-fine fibers with diameters in the micrometer down to nanometer range can be fabricated. This process involves the application of a strong electric field across a conductive capillary attaching to a polymer liquid-containing reservoir and a collector (Reneker, 1996; Doshi, 1995). When the electric field exceeds a critical value where the Coulombic repulsion of the accumulated charges overcomes the surface tension of the polymer droplet at the tip of the capillary, a charged jet is ejected. During its flight to the collector, the charged jet thins down and, simultaneously, dries out or solidifies, leaving ultra-fine fibers on the collector (Deitzel, 2001). Ultra-fine fibers obtained from this process exhibit various interesting characteristics (e.g., high surface area to mass or volume ratio, high density of micro- or nanometer-sized pores of the non-woven mat, and vast possibilities for surface functionalization). These unique properties render electrospun (e-spun) fibers as excellent candidates for various biomedical applications, e.g., scaffolding materials for cell/tissue culture (Yoshimoto, 2003; Wutticharoenmongkol, 2006; Suwantong, 2007), wound-dressing materials (Min, 2004; Noh, 2006), and carriers for topical/transdermal delivery of drugs (Kenawy, 2002; Taepaiboon, 2006; Taepaiboon, 2007; Tungprapa, 2007; Suwantong, 2007).

Cellulose acetate (CA) is the acetate ester of cellulose, the primary structural component of cell walls of green plants and is one of the most common biopolymers on earth (Anonymous, 2006). Ultra-fine CA fiber mats and regenerated cellulose membrane have been prepared by e-spinning. The most suitable solvent system for producing e-spun CA fibers was 2:1 v/v acetone/dimethylacetamide (DMAc) mixture, which allowed the resulting CA solutions (i.e., 12.5-20 wt.%) to be e-spun into fibers with average diameters ranging between ~ 100 nm and ~ 1 μ m (Liu, 2002). In another report (Son, 2004), a mixed solvent of acetone/water with the water content in the range of 10 to 15 wt.% was used to produce the e-spun CA fibers with average diameters being ~ 2 μ m. In yet another report (Ma, 2005), 3:1:1 v/v/v acetone/dimethylformamide (DMF)/trifluoroethylene (TFE) was used to prepare a

CA solution that resulted in the e-spun fibers with diameters ranging from ~200 nm to ~1 μm .

Among various applications, e-spun CA fiber mats have been developed as carriers for topical/transdermal delivery of drugs (Taepaiboon, 2007; Tungprapa, 2007; Suwantong, 2007). Taepaiboon *et al.* (2007) developed e-spun CA fiber mats as carriers for topical/transdermal delivery of all-trans retinoic acid or vitamin A acid (Retin-A) and α -tocopherol or vitamin E (Vit-E) from CA solutions in 2:1 v/v acetone/DMAc containing Retin-A and Vit-E in the amount of 0.5 and 5 wt.% (based on the weight of CA), respectively. E-spun CA fiber mats as carriers for topical/transdermal delivery of four different non-steroidal anti-inflammatory drugs (NSAIDs), i.e., naproxen (NAP), indomethacin (IND), ibuprofen (IBU), and sulindac (SUL), were reported by Tungprapa *et al.* (2007) who used CA solutions in 2:1 v/v acetone/DMAc as the base spinning solutions into which NAP, IND, IBU and SUL in the amount of 20 wt.% (base on the weight of CA) were added. Very recently, Suwantong *et al.* (2007) reported the use of e-spun CA fiber mats as carriers for topical/transdermal delivery of curcumin, a herbal compound found in the plant *Curcuma longa* L. from CA solutions in 2:1 v/v acetone/DMAc that contained curcumin in various amounts (i.e., 5-20 wt.% based on the weight of CA).

Among various other herbs, extracts from *Centella asiatica* (L.) Urban, also known as Asiatic Pennywort or Buabok (in Thai), have been reported to heal wounds, burns, and ulcerous abnormalities of the skin, cure stomach, and duodenal ulcers, and are effective in the treatment of leprosy, lupus, scleroderma, and diseases of the veins (Kartnig, 1988). Among the four major triterpenoid components of *Centella asiatica* (i.e., asiatic acid, asiaticoside, madecassic acid and madecassoside), asiaticoside (see Figure 5.1), a trisaccharide triterpene, is supposedly the most active compound associated with the healing of wounds, as evidenced by the observed increase in antioxidant levels at an initial stage of healing of excision-type cutaneous wounds in rats (Shukla, 1999), the observed increase in the proliferation and the production of types I and III pro-collagen mRNA and protein levels of human dermal fibroblasts (Maquart, 1990; Shim, 1996), and the stimulation of extracellular matrix accumulation in rat experimental wounds (Suguna, 1996; Maquart, 1999) in response to the presence of this substance.

The aim of the present contribution was to investigate the potential for use of the e-spun CA fiber mats as carriers for topical/transdermal delivery of asiaticoside (AC) from the fiber mats that contained either pure asiaticoside (PAC) or *Centella asiatica* crude extract (CACE). Various properties (i.e., morphological, water retention, weight loss, and cytotoxicity) of both the neat and the herb-loaded e-spun CA fiber mats and release characteristics of AC from the herb-loaded CA fiber mats in two types of media (i.e., acetate and phosphate buffer solutions that contained methanol) were investigated. Comparisons were made against the corresponding solvent-cast CA films.

5.3 Experimental

5.3.1 Materials

Cellulose acetate (CA; white powder; $M_w \approx 30,000$ Da; acetyl content = 39.7 wt.%; degree of acetyl substitution ≈ 2.4) was purchased from Sigma-Aldrich (Switzerland). Pure asiaticoside (PAC; 90% purity) and *Centella asiatica* crude extract (CACE; triterpene content = 95%; asiaticoside = 37.5% (HPLC) and madecassic and asiatic acids = 56.2% (HPLC)) were purchased from Shanghai Angoal Chemical Co., Ltd. (China). Acetone (Carlo Erba, Italy), *N,N*-dimethylacetamide [DMAc, Labscan (Asia), Thailand], sodium acetate, sodium chloride, anhydrous disodium hydrogen orthophosphate, and sodium dihydrogen orthophosphate (Ajax Chemicals, Australia), and glacial acetic acid (Carlo Erba, Italy) were of analytical reagent grade and used without further purification.

5.3.2 Preparation of Neat and Herb-Loaded CA Fiber Mats and Films

A weighed amount of CA powder was dissolved in 2:1 v/v acetone/dimethylacetamide (DMAc) to prepare the base CA solution at a fixed concentration of 17% w/v. Herb-containing CA solutions were prepared by dissolving the same amount of CA powder and either PAC or CACE in the amount of 40 wt.% based on the weight of CA powder in the acetone/DMAc mixture. Prior to e-spinning, the as-prepared solutions were characterized for their viscosity and conductivity using a Brookfield DV-III programmable viscometer and a SUNTEX conductivity meter, respectively. The measurements were carried out at 25 ± 1 °C.

The as-prepared solutions were then e-spun under a fixed electric field of 17.5 kV/15 cm at a controlled feeding rate of $\sim 1 \text{ mL}\cdot\text{h}^{-1}$ (by means of a Kd Scientific syringe pump). Unless otherwise noted, the collection time was $\sim 18 \text{ h}$ (resulting in the fiber mats of $90 \pm 10 \text{ }\mu\text{m}$ in thickness). For comparison purposes, both the neat and the herb-loaded CA films were also prepared by solvent casting technique from 4% w/v CA solution in 2:1 v/v acetone/DMAc and the same solution that contained either 40 wt% of PAC or CACE. Unless otherwise noted, the thickness of the as-cast films was $80 \pm 10 \text{ }\mu\text{m}$.

5.3.3 Characterization of Neat and Herb-Loaded CA Fiber Mats and Films

Morphological appearance of both the neat and the herb-loaded e-spun CA fiber mats and the corresponding as-cast films was observed by a JEOL JSM-6400 scanning electron microscope (SEM). Each specimen was coated with a thin layer of gold using a JEOL JFC-1100E sputtering device prior to SEM observation. Diameters of the e-spun fibers were measured directly from SEM images using a SemAphore 4.0 software.

Water retention and weight loss behavior of both the neat and the herb-loaded e-spun CA fiber mats and the corresponding as-cast films were measured in an acetate or a phosphate buffer solution containing 10% v/v methanol (hereafter, the A/B/M and the P/B/M medium, respectively; see below for the preparation of the media) at the skin and the physiological temperatures of 32 and 37 °C, respectively, for 24 h according to the following equations:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100, \quad (5.1)$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d - M_r}{M_i - M_r} \times 100, \quad (5.2)$$

where M is the weight of each specimen after submersion in the buffer solution for 24 h, M_d is the weight of the specimen after submersion in the buffer solution for 24 h in its dry state, M_i is the initial weight of the specimen in its dry state, and M_r is the weight of AC that was released from the specimen.

5.3.4 Release of Asiaticoside from Herb-Loaded CA Fiber Mats and Films

5.3.4.1 *Preparation of Releasing Medium*

To prepare 1,000 mL of the acetate buffer solution, 150 g of sodium acetate was dissolved in 250 mL of distilled water. Exactly 15 mL of glacial acetic acid was then added very slowly into the aqueous sodium acetate solution. Finally, distilled water was added to the solution to fill the required volume. The pH of the as-prepared acetate buffer solution was 5.5. On the other hand, to prepare 1,000 mL of the phosphate buffer solution, 6.177 g of anhydrous disodium hydrogen orthophosphate and 1.014 g of sodium dihydrogen orthophosphate were dissolved in 100 mL of distilled water. Exactly 8.7 g of sodium chloride was then added into 20 mL of the solution. Finally, distilled water was added to the solution to fill the required volume. The pH of the as-prepared phosphate buffer solution was 7.4.

For intended use of the herb-loaded e-spun CA fiber mats and the corresponding as-cast films as tropical/transdermal patches, the acetate buffer solution (pH = 5.5) containing 10% v/v of methanol (hereafter, the A/B/M medium) was used as the releasing medium. On the other hand, for intended use of the herb-loaded materials as wound dressings, the phosphate buffer solution (pH = 7.4) containing 10% v/v methanol (hereafter, the P/B/M medium) was instead used. The addition of methanol was to facilitate the dissolution of the AC in the media. The use of methanol as the dissolution aid for investigating the release of a substance that is not readily soluble in water in an aqueous medium is not uncommon (Shukla, 1991; Birnbaum, 2000; Maeda, 2004).

5.3.4.2 *Actual Asiaticoside Content*

To determine the actual amount of AC in the herb-loaded e-spun CA fiber mats and the corresponding as-cast films, each specimen (circular disc; ~2.8 cm in diameter) was first dissolved in 4 mL of 2:1 v/v acetone/DMAc. After that, 0.5 mL of the solution was added into 8 mL of either A/B/M or P/B/M medium and the actual amount of AC was measured by a high-performance liquid chromatography (HPLC) (see later). The actual amount of AC in the herb-loaded e-spun CA fiber mats and the corresponding as-cast films was then back-calculated from the obtained data against a predetermined calibration curve for AC.

5.3.4.3 *Asiaticoside-Release Assay*

The release characteristics of AC from the herb-loaded e-spun CA fiber mats and the corresponding as-cast films was investigated by two types of the release assay, i.e., total immersion and transdermal diffusion through a pig skin methods. Again, two types of the releasing medium, i.e., A/B/M (pH 5.5) or P/B/M (pH 7.4), were used. Each specimen (circular disc; ~2.8 cm in diameter) was immersed in 20 mL of a medium at the skin and the physiological temperatures of 32 °C (for A/B/M medium) or 37 °C (for P/B/M medium). At a specific immersion or diffusion time point ranging between 0 and 24 h (1440 min), either 0.5 mL (for the total immersion method) or 0.3 mL (for the transdermal diffusion through a pig skin method) of a sample solution was withdrawn and an equal amount of the fresh medium was refilled. For the transdermal diffusion through a pig skin method, each specimen was placed on a fresh piece of pig skin (abdomen; epidermal hair, subcutaneous fat, and underlying tissues removed; final thickness = 1-1.5 mm), which, in turn, was placed on top of the medium on a modified Franz diffusion cell. The amount of AC in the sample solutions was determined by HPLC (see later). The obtained data were carefully calculated to determine the cumulative amount of AC released from the specimens at each immersion or diffusion time point. The experiments were carried out in triplicate.

5.3.4.4 *HPLC Analysis*

A Shimadzu LC-10 AD HPLC was used to quantify the amount of AC in the sample solutions. Chromatographic separation of the herbal substances was achieved by the use of an Inertsil ODS-3 C18 column (particle size = 5 μm ; column dimension = 4.6 x 250 mm) with an Inertsil ODS-3 guard column (particle size = 5 μm ; column dimension = 4.0 x 10 mm) operating at 1 $\text{mL}\cdot\text{min}^{-1}$. The mobile phase for AC separation was 26:24:50 v/v/v acetonitrile/methanol/distilled water. The injection volume was 50 μL . A UV detector for AC was set at (λ_{max}) 204 nm. All of the sample solutions were filtered through a nylon filter (average pore size = 0.45 μm) prior to injection. AC was separated out over a range of elution periods of 7.5-7.7 min. Due to the difference in the AC content in PAC (i.e., 90%) and CACE (i.e., 37%), the calibration curves for

AC were carried out over the concentration ranges of PAC of 0.14-2.95 mg·mL⁻¹ and CACE of 1.84-4.60 mg·mL⁻¹, respectively.

5.3.5 Indirect Cytotoxicity Evaluation

The indirect cytotoxicity evaluation of both the neat and the herb-loaded e-spun CA fiber mats and the corresponding as-cast films was conducted in adaptation from the ISO 10993-5 standard test method in a 96-well tissue-culture polystyrene plate (TCPS; Nunclon™, Denmark) using normal human dermal fibroblasts (NHDF; sixth passage) as reference. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Corp., USA), supplemented by 10% fetal bovine serum (FBS; Invitrogen Corp., USA), 1% L-glutamine (Invitrogen Corp., USA) and 1% antibiotic and antimycotic formulation [containing penicillin G sodium, streptomycin sulfate, and amphotericin B (Invitrogen Corp., USA)]. The specimens (circular discs of ~7 mm in diameter) were sterilized by UV radiation for ~1 h and were then immersed in serum-free medium (SFM; containing DMEM, 1% L glutamine, 1% lactalbumin, and 1% antibiotic and antimycotic formulation) for 24 h in incubation to produce extraction media of varying concentration (i.e., 10, 5, and 0.5 mg·mL⁻¹). NHDF were separately cultured in wells of TCPS at 8,000 cells/well in serum-containing DMEM for 24 h to allow cell attachment. The cells were then starved with SFM for 24 h. After that, the medium was replaced with an extraction medium and cells were re-incubated for 24 h. Finally, the viability of the cells cultured by each of the extraction media was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, with the viability of the cells cultured by fresh SFM being used as control.

The MTT assay is based on the reduction of the yellow tetrazolium salt to purple formazan crystals by dehydrogenase enzymes secreted from the mitochondria of metabolically active cells. The amount of purple formazan crystals formed is proportional to the number of viable cells. First, each culture medium was aspirated and replaced with 100 ml/well of MTT solution at 5 mg ml⁻¹ for a 96-well TCPS. Secondly, the plate was incubated for 1 h at 37 °C. The solution was then aspirated and 100 ml/well of DMSO was added to dissolve the formazan crystals. Finally, after 3 min of rotary agitation, the absorbance at the wavelength of 570 nm

representing the viability of the cells was measured using a SpectraMax M2 Microplate Reader.

5.3.6 Statistical Analysis

Data were presented as means \pm standard error of mean. A one-way ANOVA was used to compare the means of different data sets, and statistical significance was accepted at a 0.05 confidence level.

5.4 Results and discussion

5.4.1 E-spinning of Neat and Herb-Containing CA Solutions

Prior to e-spinning, both the neat and the herb-containing CA solutions were measured for their shear viscosity and electrical conductivity, in which the results are summarized in Table 5.1. The presence of both PAC and CACE in the base CA solution increased the shear viscosity of the resulting herb-containing CA solutions, while it decreased the electrical conductivity of the resulting solutions. E-spinning of these solutions was carried out at a fixed electric field of 17.5 kV/15 cm. Selected SEM images of the e-spun fibers from these solutions are shown in Table 5.2. Evidently, cross-sectionally round fibers with smooth surface were obtained and no presence of any kind of aggregation was observed on the surface of the fibers, indicating that the as-loaded PAC and CACE were incorporated well within the fibers. According to Table 5.1, the diameters of the neat CA fibers were 301 ± 64 nm, while those of the PAC- and the CACE-loaded CA fibers were 485 ± 91 and 545 ± 96 nm, respectively. The observed increase in the diameters of the PAC- and the CACE-loaded CA fibers in comparison with those of the neat ones should be a result of the greater shear viscosity of the herb-containing CA solutions in comparison with that of the neat one. Comparatively, Taepaiboon *et al.* (2007) reported that the diameters of the neat and the vitamin-loaded CA fibers ranged between 247 and 265 nm; Tungprapa *et al.* (2007) reported that the diameters of the neat and the drug-loaded CA fibers ranged between 231 and 297 nm; and, lastly, Suwantong *et al.* (2007) reported that the diameters of the neat and the curcumin-loaded CA fibers ranged between 301 and 340 nm. They also found that all of the

medicated CA fibers were smooth, with no evidence of any kind of aggregation being observed on the surface of the fibers (Taepaiboon, 2007; Tungprapa, 2007; Suwantong, 2007). Though not relevant to the purpose of the present report, some mechanical properties of both the neat and the herb-loaded e-spun CA fiber mats were also investigated (see additional experiment in Supplementary data).

In comparison, both the neat and the herb-containing CA solutions were also fabricated into films by solvent-casting technique. The surface morphology of the as-cast films is also shown in Table 5.2. Evidently, the surface of the as-cast films was relatively smooth without any evidence of any kind of aggregation on the surface of the films, indicating that the as-loaded PAC and CACE were incorporated well within the films, as in the case of the PAC- and the CACE-loaded CA fibers.

5.4.2 Water Retention and Weight Loss Behavior of Neat and Herb-Loaded CA Fiber Mats and Films

The water retention and the weight loss behavior of both the neat and the herb-loaded e-spun CA fiber mats and the corresponding as-cast films after submersion in either A/B/M (at 32 °C) or P/B/M (at 37 °C) medium for 24 h were characterized and the results are shown in Figure 5.2. The water retention of the neat CA fiber mats was ~319 and ~384% in the A/B/M and the P/B/M medium, respectively. Tungprapa *et al.* (2007) reported that such the property value of the neat CA fiber mats (20-35 µm in thickness with the average diameter of the individual fibers being ~230 nm) after 24 h of submersion in the acetate buffer solution at 37 °C was ~715%, while Suwantong *et al.* (2007) showed that such the property value of the neat CA fiber mats with a similar physical characteristic to the ones used in this work after 48 h of submersion in the acetate buffer solution containing 0.5% v/v polysorbate 80 and 3% v/v methanol (hereafter, B/T/M medium) at 37 °C was ~370%. In comparison with those of the neat materials, such the water retention values of the PAC- and the CACE-loaded e-spun CA fiber mats were ~391 and ~460% in the A/B/M and the P/B/M medium and ~369 and ~394% in the A/B/M and the P/B/M medium, respectively. On the other hand, such the property values of the as-cast film counterparts were much lower, with the values being ~10-40% in the A/B/M medium and ~14-52% in the P/B/M medium, respectively.

According to Figure 5.2, the loss in the weight of the neat CA fiber mats was ~6.5 and ~12.0% in the A/B/M and the P/B/M medium, respectively. Tungprapa *et al.* (2007) showed that such the property value of the neat CA fiber mats after 24 h of submersion in the acetate buffer solution at 37 °C was much lower at ~1.1%, while Suwantong *et al.* (2007) showed that such the property value of the neat CA fiber mats after 48 h of submersion in the B/T/M medium at 37 °C was much greater at ~13.5%. The greater weight loss of the neat CA fiber mats in the acetate buffer solution that contained methanol than that of the materials in the neat acetate buffer solution was due possibly to the presence of methanol that facilitates the dissolution of CA in the testing medium. In comparison with those of the neat materials, such the weight loss values of the PAC- and the CACE-loaded e-spun CA fiber mats were ~8.8 and ~18.9% in the A/B/M and the P/B/M medium and ~8.0 and ~16.6% in the A/B/M and the P/B/M medium, respectively. On the other hand, such the property values of the corresponding as-cast films were much lower, with the values being ~0.9-5.4% in the A/B/M medium and ~2.3-6.7% in the P/B/M medium, respectively.

The observed greater values of both the water retention and the weight loss of the neat and the herb-loaded e-spun CA fiber mats than those of the corresponding as-cast CA films should be due to the highly porous nature, hence the greater surface area, of the fiber mats in comparison with the dense structure of the films. The high porosity of the fiber mats allows for greater accessibility of the medium, which, in the case of them being used as drug carriers, should provide greater possibility for the drug to be leached out from the fibrous matrix, when compared with the film counterparts.

5.4.3 Release of Asiaticoside from Herb-Loaded CA Fiber Mats and Films

Prior to investigating the release characteristics of AC from the herb-loaded e-spun CA fiber mats and corresponding as-cast CA films, the actual amount of AC within these samples needs to be measured. It should be noted that the chemical integrity of AC in both the PAC- and the CACE-loaded e-spun CA fiber mats was proven to be intact after the e-spinning process (see additional experiment in Supplementary data) Table 5.3 summarizes the actual amount of AC in these samples (reported as the percentage of the initial content of the AC contained in both

the PAC- and the CACE-containing spinning and the casting solutions) in the A/B/M or the P/B/M medium. In the A/B/M medium, the actual amounts of AC in the PAC- and the CACE-loaded e-spun CA fiber mats were ~84 and ~80%, respectively, while those in the film counterparts were ~70 and ~72%, respectively. In the P/B/M medium, different values were obtained. They were ~92 and ~75% for the PAC- and the CACE-loaded e-spun CA fiber mats, respectively, while they were ~79 and ~74% for the films. These values were used as the basis to arrive at the cumulative release of AC from these herb-loaded materials.

The release of AC from the herb-loaded e-spun CA fiber mats and the corresponding as-cast CA films was carried out by the total immersion and the transdermal diffusion through a pig skin methods in two types of the releasing medium, i.e., the A/B/M (at 32 °C) or the P/B/M (at 37 °C) medium. Previously, Taepaiboon *et al.* (2007) and Suwantong *et al.* (2007) used an acetate buffer solution that contained methanol as the releasing medium in their studies on the release characteristics of vitamins (i.e., Retin-A and Vit-E) and curcumin from the vitamin- and the curcumin-loaded e-spun CA fiber mats and the corresponding as-cast films, respectively. Here, the cumulative profiles of AC released from these herb-loaded materials were to be reported in two different manners, i.e., as the percentage of the weight of AC released divided by the actual weight of AC being present in the specimens and as the percentage of the weight of AC released divided by the actual weight of the specimens.

In the total immersion method, about half of the AC originally loaded in both the PAC- and the CACE-loaded e-spun CA fiber mats was released into the A/B/M medium within the first 180 and 60 min after immersion, with the amounts of the AC released from these materials increasing further to reach a plateau value at ~720 and ~480 min after immersion, respectively (see Figure 5.3). These corresponded to the maximum amounts of AC released from these materials of ~24 and ~10% (based on the weight of the specimens; i.e., mg of AC released/mg of specimens \times 100), which were ~98 and ~77% of all of the AC that was originally present in these specimens, respectively. On the other hand, about half of the AC originally loaded in both the PAC- and the CACE-loaded e-spun CA fiber mats was released into the P/B/M medium within the first 30 and 20 min after immersion, with

the amounts of the AC released from these materials increasing further to reach a plateau value at ~1200 and ~720 min after immersion, respectively (see Figure 5.4). These corresponded to the maximum amounts of AC released from these materials of ~26 and ~12% (based on the weight of the specimens), which were ~99 and ~92% of all of the AC that was originally present in these specimens.

According to Figures 5.3 and 5.4, it is apparent that the amounts of AC released from both the PAC- and the CACE-loaded as-cast CA films were significantly lower than those released from the corresponding e-spun fiber mats. Specifically, in the A/B/M medium, the maximum amounts of AC released from the films (i.e., at 1440 min after immersion) were ~0.8 and ~1.0% (based on the weight of the specimens), which corresponded to ~3.9 and ~8.4% of all of the AC that was originally present in these specimens, respectively. On the other hand, in the P/B/M medium, the maximum amounts of AC released from the films (i.e., at 1440 min after immersion) were ~2.5 and ~2.3% (based on the weight of the specimens), which corresponded to ~11 and ~18% of all of the AC that was originally present in these specimens, respectively.

In the transdermal through a pig skin method, much lower amounts of AC were released from both types of the tested materials, with those of the AC released from the PAC- and the CACE-loaded e-spun CA fiber mats being greater than those of the AC released from the corresponding as-cast films (see Figures 5.5 and 5.6). Specifically, in the A/B/M medium, the maximum percentage of AC released from the PAC- and the CACE-loaded e-spun CA fiber mats and the corresponding as-cast films (i.e., at 1440 min after immersion) was ~0.82, ~1.91, ~0.25, and ~0.19% (based on the actual amount of AC being present in the specimens), which corresponded to ~0.20, ~0.26, ~0.05, and ~0.02% (based on the weight of the specimens), respectively. Relatively greater amounts of AC could be released from these materials when they were in contact with the P/B/M medium. Specifically, the maximum percentage of AC released from the PAC- and the CACE-loaded e-spun CA fiber mats and the corresponding as-cast films (i.e., at 1440 min after immersion) was ~2.6, ~10, ~0.47, and ~0.92% (based on the actual amount of AC being present in the specimens), which corresponded to ~0.69, ~1.3, ~0.11, and ~0.12% (based on the weight of the specimens), respectively.

The fact that the amounts of AC released from the herb-loaded e-spun CA fiber mats was greater than that from the corresponding as-cast films could be due to a number of factors, e.g., the observed greater water retention and weight loss of all of the herb-loaded fiber mat specimens over those of the film counterparts and the hypothetically greater surface area of the fiber mats specimens over that of the films (Suwantong, 2007). Additionally, the observed greater amounts of the AC released from both types of the tested materials in the P/B/M medium than those of the AC released in the A/B/M medium should be due to the observed greater values of both the water retention and the weight loss of these materials when they were submerged in the P/B/M medium (see Figure 5.2).

5.4.4 Release Kinetics of Asiaticoside from Herb-Loaded CA Fiber Mats and Films

The release kinetics of AC from either type of the herb-loaded materials can be characterized by an equation of the following form (Philip, 1987; Peppas, 1993):

$$\frac{M_t}{M_\infty} = kt^n, \text{ for } \frac{M_t}{M_\infty} < 0.6, \quad (5.3)$$

where M_t is the cumulative amount of AC released at an arbitrary time t , M_∞ is the cumulative amount of the substance released at an infinite time, n is an exponent characterizing the mechanism with which the release kinetics can be described, and k is the rate of release of AC that incorporates physical characteristics of the matrix/herb system as well as some physical contributions from the measurement method.

For $n = 0.5$, the release mechanism can be described as the Fickian diffusion (Verreck, 2003). In this type of diffusion, a straight line is expected when the fractional cumulative amount of AC released (i.e., M_t/M_∞) is plotted as a function of $t^{0.5}$. Here, only the release of AC from both the PAC- and the CACE-loaded e-spun CA fiber mats and the corresponding as-cast films as determined by the total immersion method in the A/B/M and the P/B/M medium was analyzed. The results of the analyses are summarized in Table 5.4. Apparently, the rate parameter k for the release of AC from the PAC-loaded e-spun CA fiber mats in the A/B/M and the P/B/M media was 0.0051 and 0.0159 $s^{-0.5}$, respectively, while that for the release of

AC from the CACE-loaded e-spun CA fiber mats was 0.0134 and 0.0264 $s^{-0.5}$, respectively. On the other hand, the k values for the release of the AC from the PAC-loaded as-cast CA films in the A/B/M and the P/B/M media were 0.0119 and 0.0025 $s^{-0.5}$, respectively, while those for the release of AC from the CACE-loaded as-cast CA films were 0.0049 and 0.0082 $s^{-0.5}$, respectively. Taepaiboon *et al.* (2007) reported that the k value for the release of Vit-E from the Vit-E-loaded CA fiber mats in a B/T/M medium was 0.0049 $s^{-0.5}$, while that for the release of Retin-A from the Retin-A-loaded CA fiber mats was 0.0061 $s^{-0.5}$. Recently, Suwantong *et al.* (2007) showed that the k values for the release of curcumin from the curcumin-loaded CA fiber mats in another B/T/M medium ranged between 0.0020 and 0.0028 $s^{-0.5}$, while those for all of the curcumin-loaded CA films ranged between 0.0024 and 0.0036 $s^{-0.5}$.

5.4.5 Indirect Cytotoxicity Evaluation

The potential for use of both the PAC- and the CACE-loaded e-spun CA fiber mats and the corresponding as-cast films as topical/transdermal patches or wound dressings was assessed by evaluating the cytotoxicity of these materials, using the neat CA fiber mats and films as internal control. The viability of the normal human dermal fibroblasts (NHDF) that had been cultured with the extraction media from these materials in comparison with that of the cells that had been cultured with fresh culture medium (i.e., control) is illustrated in Figure 5.7. Three extraction ratios of the extraction media (i.e., 10, 5, and 0.5 $mg \cdot mL^{-1}$) were investigated. Apparently, both the neat CA fiber mats and films were non-toxic to the cells, as the viability of the cells that had been cultured with extraction media from these materials at all extraction ratios investigated ranged between ~87 and ~109% (relative to the viability of the cells that had been cultured with fresh culture medium). At the lowest extraction ratio investigated (i.e., 0.5 $mg \cdot mL^{-1}$), all of the herb-loaded materials appeared to be non toxic to the cells, with the viability of the cells ranging between ~86 and ~92%. At greater extraction ratios investigated (i.e., 5 and 10 $mg \cdot mL^{-1}$), while the PAC-loaded materials were non-toxic to the cells, as the viability of the cells was found to range between ~86 and ~98%, the CACE-loaded materials appeared to be toxic to the cells, as the viability of the cells was low (i.e., ranging between ~39 and ~55%). It is assumed that the toxicity of the CACE-loaded

materials should be a result of other triterpenoid compounds that are present in the extract.

5.5 Conclusions

In the present contribution, asiaticoside (AC) from the plant *Centella asiatica* L., regarded as the most active compound associated with the healing of wounds, was added to the neat cellulose acetate (CA; $M_w \approx 30,000$ Da; degree of acetyl substitution ≈ 2.4) solution (17% w/v in 2:1 v/v acetone/dimethylacetamide) in the form of pure substance (PAC) or a crude extract (CACE) at 40 wt.% based on the weight of CA powder. E-spinning of the as-prepared solutions was carried out at a fixed electric field of 17.5 kV/15 cm. The obtained fibers were smooth, without the presence of any kind of aggregation on their surface. The average diameters of the neat, the PAC-, and the CACE-loaded e-spun CA fibers were 301, 485, and 545 nm, respectively. The water retention and the weight loss of both the neat and the herb-loaded CA fiber mats in acetate or phosphate buffer solution containing methanol (hereafter, A/B/M or P/B/M medium) were greater than those of the corresponding as-cast films. Interestingly, both types of materials exhibited greater water retention and weight loss upon submersion in the P/B/M medium.

The release characteristics of AC from both the PAC- and the CACE-loaded e-spun CA fiber mats and the corresponding as-cast films were tested in the A/B/M or the P/B/M medium at either the skin or the physiological temperature of 32 or 37 °C, respectively, by two types of the release assays, i.e., the total immersion and the transdermal diffusion through a pig skin methods. In the total immersion method, the maximum amounts of the AC released from the PAC- and the CACE-loaded e-spun CA fiber mats into the A/B/M medium were ~ 24 and $\sim 10\%$ (based on the weight of the specimens), while those of the AC released into the P/B/M medium were ~ 26 and $\sim 12\%$, respectively. Significantly lower amounts of AC were released into both types of the releasing medium, when the herb-loaded e-spun CA fiber mats were placed on top of a piece of pig skin. In comparison with the amounts of AC released from the herb-loaded e-spun CA fiber mats, those of the AC released from the corresponding as-cast films were much lower. Finally, the potential for use of the PAC- and the

CACE-loaded e-spun CA fiber mats and the corresponding as-cast films as topical/transdermal or wound dressing patches was assessed by investigating the cytotoxicity of these materials against normal human dermal fibroblasts. The results showed that only the extraction media from both the CACE-loaded fiber mats and films at the extraction ratios of 5 and 10 mg·mL⁻¹ were toxic to the cells.

5.6 Acknowledgments

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Supplementary data

Supplementary data associated with this research work can be found in the online version, at doi:10.1016/j.polymer.2008.07.020.

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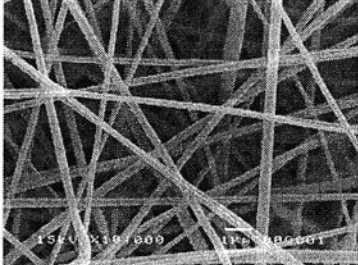
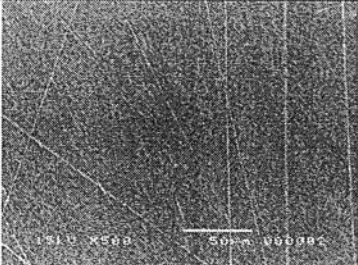
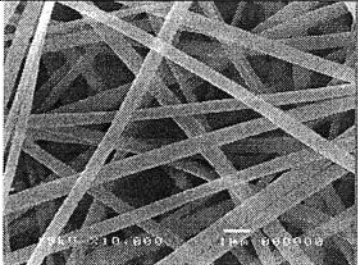
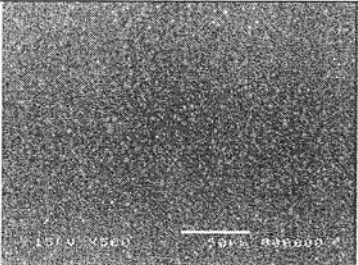
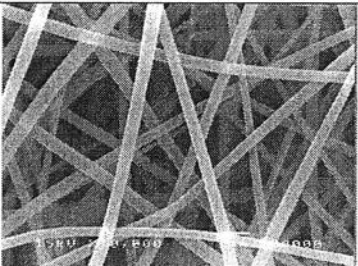
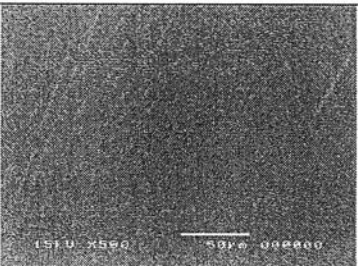
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Table 5.1 Shear viscosity and electrical conductivity of neat, PAC-, and CACE-containing CA solutions ($n = 3$) as well as diameters of the individual fibers of the resulting electrospun fiber mats ($n \approx 100$)

Type of CA solution	Shear viscosity (mPa s)	Electrical conductivity ($\mu\text{S cm}^{-1}$)	Fiber diameters (nm)
Neat	419 ± 1	8.31 ± 0.01	301 ± 64
with 40 wt.% PAC	487 ± 1	3.81 ± 0.01	485 ± 91
with 40 wt.% CACE	530 ± 1	4.62 ± 0.02	545 ± 96

Note: PAC = pure asiaticoside; CACE = *Centella asiatica* crude extract

Table 5.2 Selected scanning electron micrographs of neat, PAC-, and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films

Type of materials	Electrospun fiber mats*	Solvent-cast films
Neat CA		
CA + 40 wt.% PAC		
CA + 40 wt.% CACE		

* applied electric field = 17.5 kV/15 cm; collection time = 18 h

Table 5.3 Actual amount of AC in PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast CA films as determined in either A/B/M or P/B/M medium ($n = 3$)

Type of materials	Actual amount of AC based on the initial amount of the AC loaded (%)	
	Electrospun CA fiber mats	Solvent-cast CA films
In A/B/M medium		
with 40 wt.% PAC	84.0 ± 2.3	69.7 ± 3.6
with 40 wt.% CACE	79.6 ± 0.9	72.3 ± 2.3
In P/B/M medium		
with 40 wt.% PAC	92.4 ± 0.9	79.0 ± 3.6
with 40 wt.% CACE	75.4 ± 4.6	74.1 ± 6.2

Table 5.4 Analyses of the release kinetics of AC from PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films based on the Fickian diffusion type of the release mechanism ($n = 3$)

Type of sample	Rate parameter, k ($s^{-0.5}$)	r^2
PAC-loaded e-spun CA fiber mats:		
In A/B/M medium	0.0051	0.96
In P/B/M medium	0.0159	0.87
CACE-loaded e-spun CA fiber mats:		
In A/B/M medium	0.0134	0.88
In P/B/M medium	0.0264	0.98
PAC-loaded as-cast CA films:		
In A/B/M medium	0.0119	1.00
In P/B/M medium	0.0025	0.84
CACE-loaded as-cast CA films:		
In A/B/M medium	0.0049	0.94
In P/B/M medium	0.0082	0.96

Note: the experimental results were based on the total immersion method.

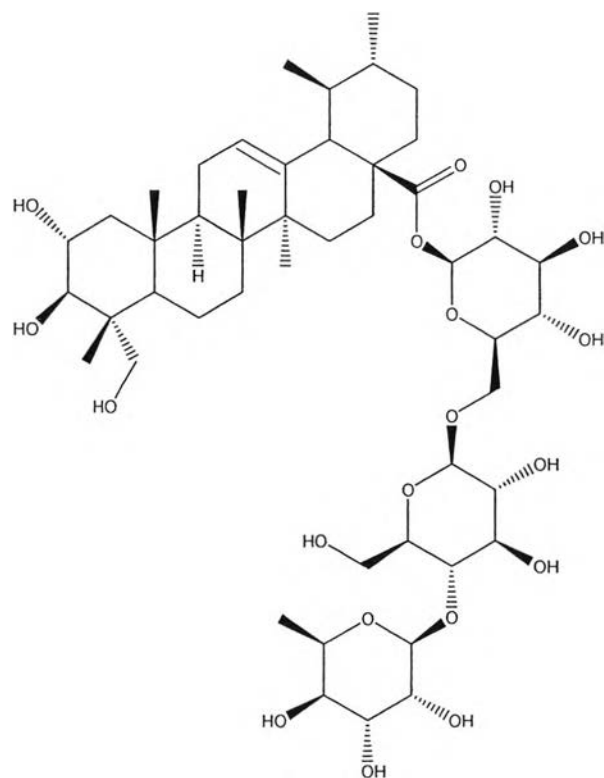


Figure 5.1 Chemical structure of asiaticoside (AC).

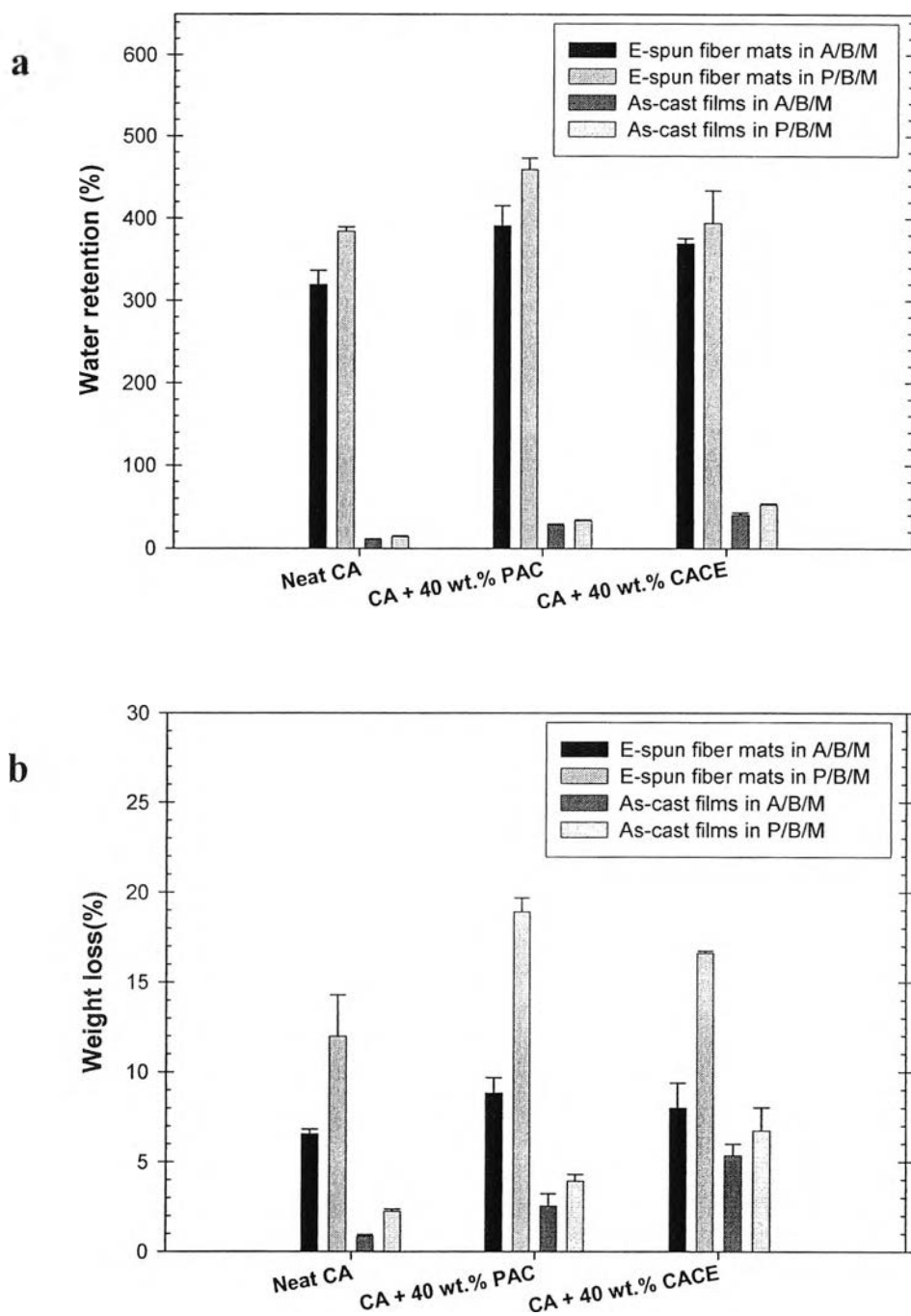


Figure 5.2 (a) Water retention and (b) weight loss behavior of neat, PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films in two types of medium, i.e., acetate or phosphate buffer solutions containing 10% v/v methanol ($n = 3$).

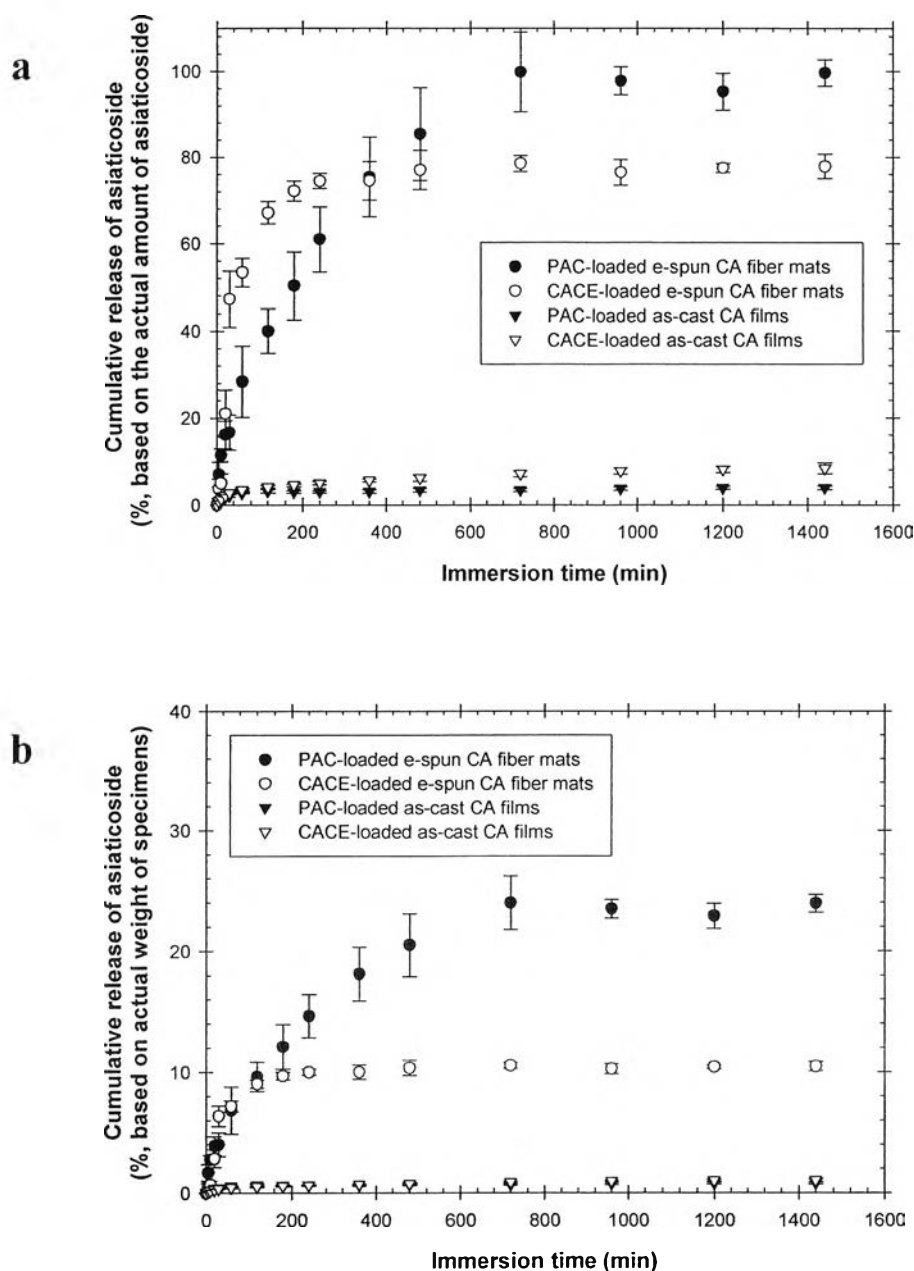


Figure 5.3 Cumulative release profiles of AC from PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films reported as (a) the percentage of the weight of AC released divided by the actual weight of AC in the specimens and (b) the percentage of the weight of AC released divided by the actual weight of the specimens, as determined by total immersion method in A/B/M medium (90% v/v acetate buffer with 10% v/v methanol) at the skin temperature of 32°C ($n = 3$).

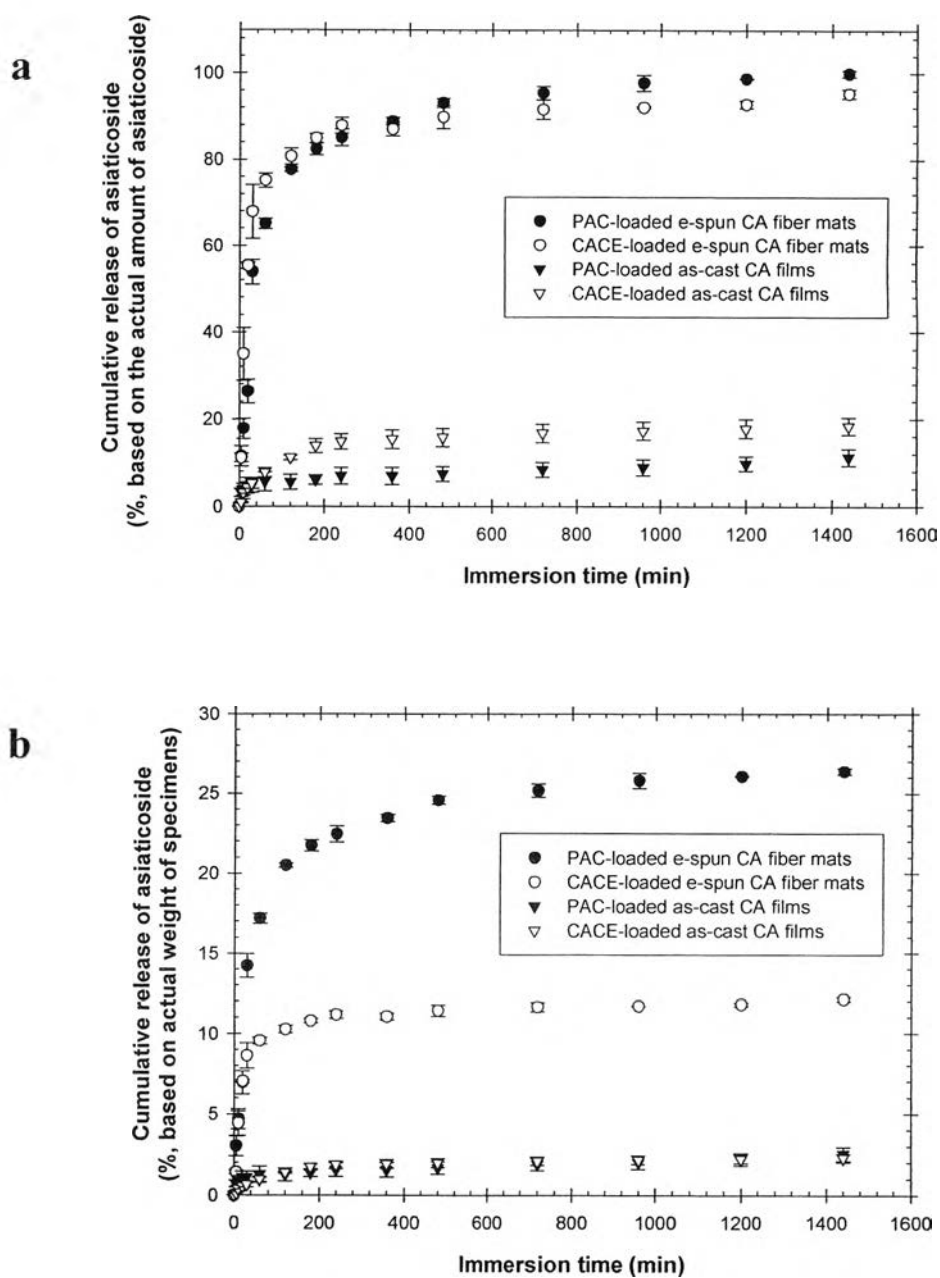


Figure 5.4 Cumulative release profiles of AC from PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films reported as (a) the percentage of the weight of AC released divided by the actual weight of AC in the specimens and (b) the percentage of the weight of AC released divided by the actual weight of the specimens, as determined by total immersion method in P/B/M medium (90% v/v phosphate buffer with 10% v/v methanol) at the physiological temperature of 37°C ($n = 3$).

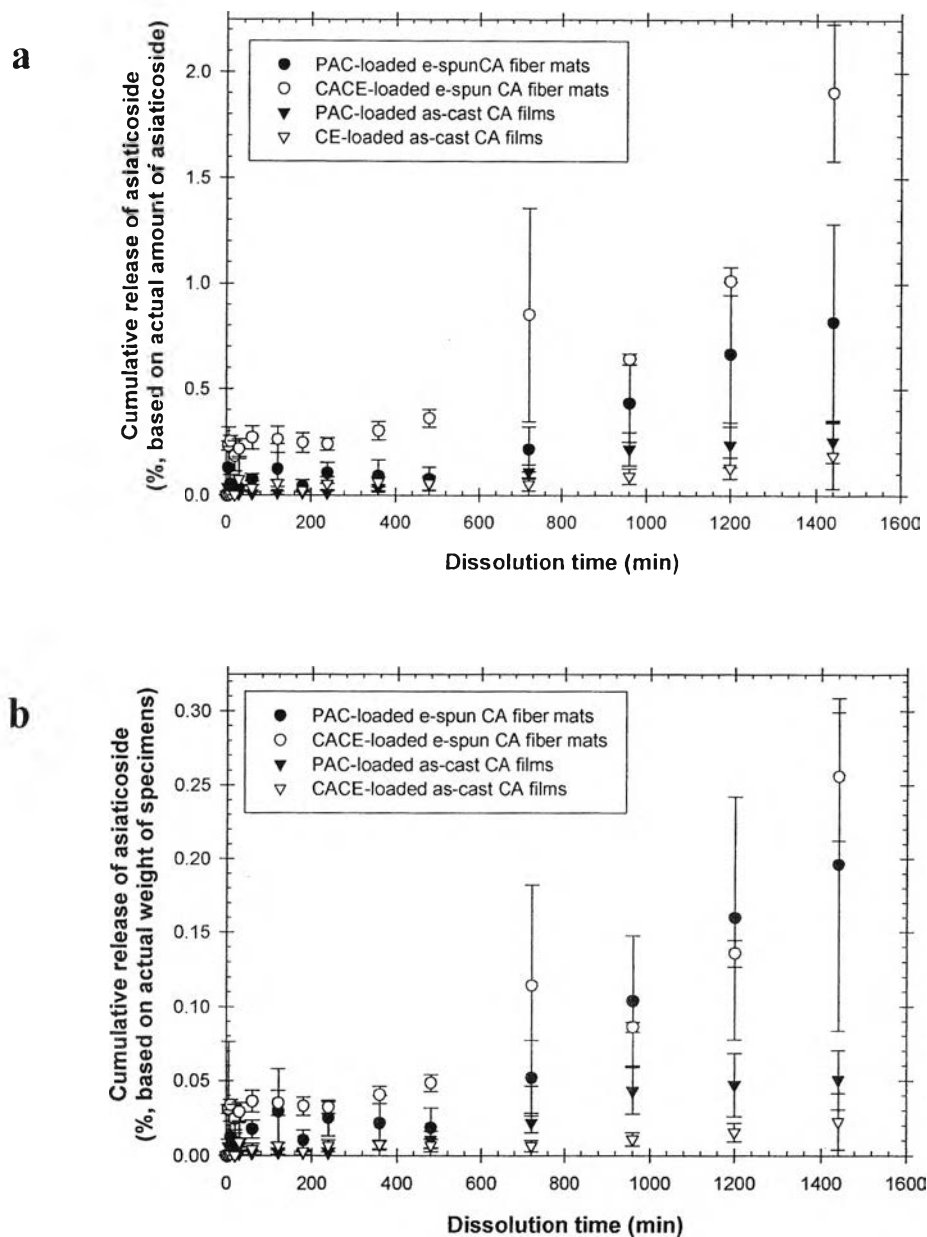


Figure 5.5 Cumulative release profiles of AC from PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films reported as (a) the percentage of the weight of AC released divided by the actual weight of AC in the specimens and (b) the percentage of the weight of AC released divided by the actual weight of the specimens, as determined by transdermal diffusion through a pig skin method in A/B/M medium (90% v/v acetate buffer with 10% v/v methanol) at the skin temperature of 32°C ($n = 3$).

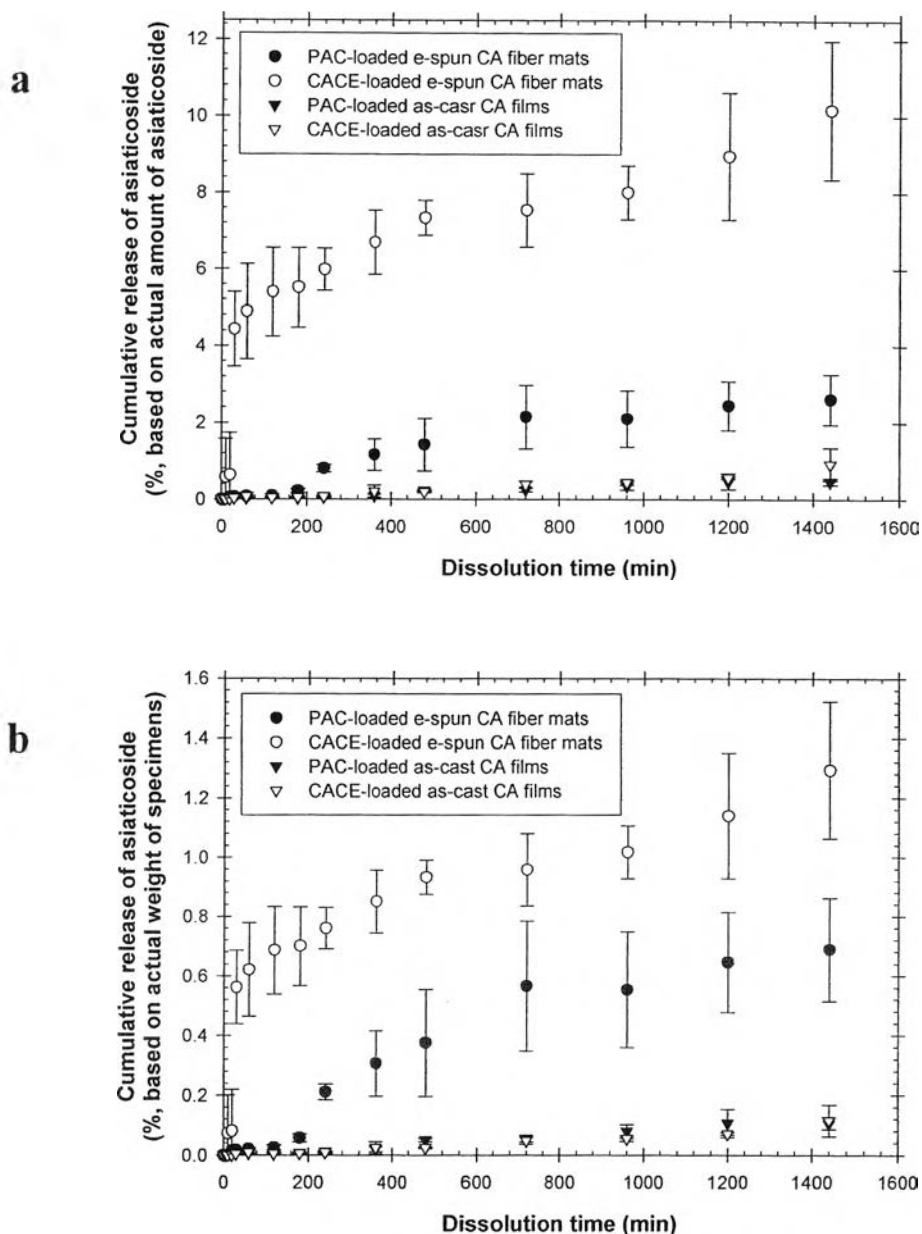


Figure 5.6 Cumulative release profiles of AC from PAC- and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films reported as (a) the percentage of the weight of AC released divided by the actual weight of AC in the specimens and (b) the percentage of the weight of AC released divided by the actual weight of the specimens, as determined by transdermal diffusion through a pig skin method in P/B/M medium (90% v/v phosphate buffer with 10% v/v methanol) at the physiological temperature of 37°C ($n = 3$).

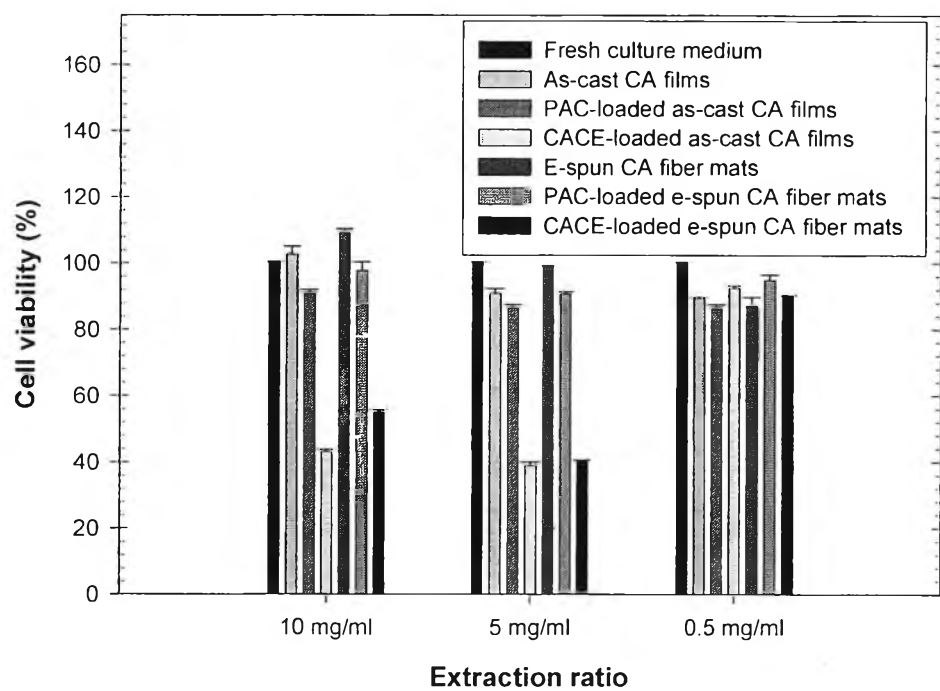


Figure 5.7 Indirect cytotoxicity evaluation of neat, PAC-, and CACE-loaded electrospun CA fiber mats and corresponding solvent-cast films in comparison with viability of normal human dermal fibroblasts (NHDF) that had been cultured with fresh culture medium ($n = 3$).