CHAPTER III
ELECTROSPUN GELATIN FIBER MATS CONTAINING
A HERBAL—*CENTELLA ASIATICA*—EXTRACT AND RELEASE
CHARACTERISTIC OF ASIATICOSIDE

3.1 Abstract

Ultrafine gelatin (type A, porcine skin, ~180 Bloom) fiber mats containing a methanolic crude extract of *Centella asiatica* (L.) Urban, a medicinal plant widely known for its traditional medical applications including wound healing ability, were fabricated from the neat gelatin solution (22% w/v in 70 vol.% acetic acid) that contained the crude extract (mCA) in various amounts (i.e., 5-30 wt.% based on the weight of gelatin powder) by electrospinning. Incorporation of mCA in the neat gelatin solution had no obvious effect on both the morphology and the size of the mCA-loaded gelatin fibers obtained, as both of the neat and the mCA-loaded gelatin fibers were smooth and the average diameters of these fibers were found to be in the range of 226 to 232 nm. The cross-linked mCA-loaded e-spun gelatin fiber mat from the neat gelatin solution that contained 30 wt.% of mCA was further investigated for the release characteristic of asiaticoside, identified as the most active compound associated with the healing of wounds, in two different types of releasing medium, i.e., acetate buffer and the buffer containing 10 vol.% of methanol, based on thin-layer chromatography (TLC)-densitometry technique. It was found that, based on the unit weight of actual amount of asiaticoside present in the specimens, the total amount of asiaticoside released from the fiber mat specimens was lower than that from the film counterparts, while, based on the unit weight of the specimens, an opposite trend was observed.

(**Key-words**: topical drug delivery; electrospinning; nanofibers; gelatin; herbal extract; asiaticoside)

3.2 Introduction

Gelatin is a natural biopolymer, prepared by partial hydrolysis of collagens, the most abundant structural proteins found in skin, tendon, cartilage, bones, and connective tissues of animals such as bovines, porcines, and pisces (http://en.wikipedia.org/wiki/Gelatin). Depending on the method in which collagens are pre-treated, two different types of gelatin with different characteristics can be produced (Young, S., et al., 2005). There exist three common processes, i.e., acidic, alkaline, and enzymatic pre-treatment processes, used to convert collagens into gelatin (http://en.wikipedia.org/wiki/Gelatin). Acidic treatment is most suitable for less fully cross-linked collagens found in pig or fish skins, while alkaline treatment is suitable for more complex collagens found in bovine hides (http://en.wikipedia.org/wiki/Gelatin). The gelatin obtained from acid-treated collagens is called type-A gelatin, while that obtained from alkali-treated ones is called type-B gelatin (http://en.wikipedia.org/wiki/Gelatin). Due to the difference in the nature of the acidic and the alkaline pre-treatment processes, the resulting gelatin products are different both in their molar mass and, specifically, in their electrical property, i.e., with gelatins from alkali-treated collagens possessing a greater proportion of carboxyl groups which render their negatively charged and lower their isoelectric points compared to those from acid-treated ones (Young, S., et al, 2005). In contrast to the two pre-treatment processes, enzymatic treatment is relatively new, but it gives gelatin products with greater yield and purity in a relatively shorter time (http://en.wikipedia.org/wiki/Gelatin).

Because of its relatively low cost and its excellent biocompatibility and biodegradability in physiological environments, gelatin is commonly used in food, photographical, cosmetic, pharmaceutical, and medical applications (http://en.wikipedia.org/wiki/Gelatin). Gelatin can be fabricated in many forms, depending on its functional utilization, such as films (Jongjareonrak, A., et al, 2006), micro- or nanoparticles (Huss, F.R.M., et al, 2007; Vandervoort, J. and Ludwig A. 2004), and dense or porous hydrogels (Liu, T.Y., et al, 2006; Liu, J., et al, 2007). Gelatin in the form micro- and nanofibers can also be fabricated by gel and electrospinning (e-spinning) techniques, respectively (Fukae, R., et al, 2005; Huang,

Z.M., *et al*, 2004; Ki, C.S., *et al*, 2005; Li, M., *et al*, 2005; Li, M., *et al*, 2006; Choktaweesap, N., *et al*, 2007). Due to the unique characteristics of electrospun (e-spun) fibers (e.g., high surface area to volume or mass ratio, high density of pores in sub-micrometer length scale of the obtained as-spun non-woven mat, and flexibility for special functionalizations), e-spun gelatin fibers are of interest here. Solvents suitable for preparing an electrospinnable gelatin solution are 2,2,2-trifluoroethanol (TFE) (Huang, Z.M., *et al*, 2004), 98% formic acid (Ki, C.S., *et al*, 2005), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (Li, M., *et al*, 2005; Li, M., *et al*, 2006), and glacial acetic acid (Choktaweesap, N., *et al*, 2007). The average diameters of e-spun smooth or beaded gelatin fibers were reported to be 100-1900 nm (type-A gelatin; porcine skin; 5-15% w/v in TFE) (Huang, Z.M., *et al*, 2004), 70-170 nm (type-A gelatin; porcine skin; 7-12 wt.% in 98% formic acid) (Ki, C.S., *et al*, 2005), 80-490 nm (type-B gelatin; bovine skin; 2-8.3% w/v in HFP) (Li, M., *et al*, 2005), 800 nm (type-B gelatin; bovine skin; 8% w/v in HFP) (Li, M., *et al*, 2006), 210-840 nm (type-A gelatin; porcine skin; 15-29% w/v in glacial acetic acid) (Choktaweesap, N., *et al*, 2007). To improve the stability of e-spun gelatin fibers in an aqueous medium, these fibers can be further cross-linked with hexamethylene diisocyanate (HDMI; 10 vol.% in isopropanol for 1 h at room temperature) (Li, M., *et al*, 2005), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC; 0.2% in 90% ethanol for up to 2 h at room temperature) (Li, M., *et al*, 2006), and glutaraldehyde vapor (25% aqueous solution for >2 days, followed by exposure in a fume hood for 2 h and curing at 100°C for 1 h) (Zhang, Y.Z., *et al*, 2006).

Due to the aforementioned characteristics of e-spun fibers, the proposed use for e-spun polymeric fibers are in areas such as filters (Gibson, P.W., *et al*, 1999), composite reinforcements (Bergshoef, M.M. and Vancso, G.J., 1999), carriers for delivery of drugs (Kenawy, E.R., *et al*, 2002; Taepaiboon, P., *et al*, 2006 and Taepaiboon, P., *et al*, 2007), scaffolds for cell and tissue culture (Li, M., *et al*, 2005; Li, M., *et al*, 2006; Liao, S., *et al*, 2006; Wutticharoenmongkol, P., *et al*, 2006; Ji, Y., *et al*, 2006 and Meechaisue, C., *et al*, 2006), and wound dressings (Rho, K.S., *et al*, 2006 and Hong, K.H., *et al*, 2007). In tissue engineering and wound care applications, rapid and proper healing is important in the treatment of wounds. In case of severe cases of burns and trauma, the application of a dressing material on

the wounds achieves the functions of the natural skin by protecting the area from the loss of fluid and proteins and preventing infection from microbial invasion, which may lead to complications during treatment. The invention of a bi-layer wound dressing, consisting of a silicone membrane attached to an inner layer of collagen/chondroitin-6-sulfate sponge, by Yannas I.V., *et al.* (Yannas, I.V. and Burke J.F., 1980 and Yannas, 1980) has paved the way to a now-accepted concept on wound management. Gelatin, in the form of solvent-cast sheet or freeze-dried sponge hydrogels, has been developed as wound dressings (Ulubayram, K., *et, al,* 2001; Miyashi, M., *et, al,* 2005 and Huang, S., *et, al,* 2006). To increase the rate of wound-healing, epidermal or basic fibroblast growth factors (EGF or bFGF, respectively), known to promote the proliferation of almost all cells associated with wound healing, were added either in their free form or within gelatin microspheres (Young, S., *et al.,* 2005; Ulubayram, K., *et, al,* 2001; Miyashi, M., *et, al,* 2005; Huang, S., *et, al,* 2006). The use of microspheres as carriers for sustained release of these substances is necessary as these substances can diffuse away from the wound site very rapidly or even become decomposed by proteases secreted by injured tissues (Young, S., *et al.,* 2005; Ulubayram, K., *et, al,* 2001; Huang, S., *et, al,* 2006).

In Japan, the use of recombinant human bFGF in clinical treatments of some chronic wounds is a common practice (Miyashi, M., *et, al,* 2005), but, due to limited accessability and relatively high cost of this substance, the clinical use of this substance only becomes available to limited number of patients in developing countries. However, the use of medicinal herbs as alternative medicine in these countries is very common. Among the various herbs, extracts from *Centella asiatica* (L.) Urban also known as Asiatic Pennywort or Buabok (in Thai) (see supplementary data (available at stacks.iop.org/Nano/19/015102)) 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, T., 1988). Among the four major triterpenoid components of *Centella asiatica* (i.e., asiatic acid, asiaticoside, madecassic acid, and madecassoside), asiaticoside (see Figure 3.1), a trisaccharide triterpene, has been identified as 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, A., *et, al,* 1999) and increased proliferation and production of types I and III pro-collagen mRNA and protein levels of human dermal fibroblasts (Lu, L., *et, al,* 2004) in response to the presence of this substance. It was suggested, however, that the sugar portion of asiaticoside is not the essential pharmacophore for biological activity, but enhances the wound healing effect of asiatic acid in topical application, and could be greatly simplified without the loss of biological activity (Shim, P.J., *et, al,* 1996).

The aim of the present contribution is to investigate the potential for use of e-spun gelatin fiber mats as carriers for topical delivery of a methanolic crude extract of *Centella asiatica* (mCA). The release characteristic of asiaticoside from e-spun mCA-loaded gelatin fiber mats was investigated by total immersion method in an acetate buffer and the buffer that contained 10 vol.% of methanol, in comparison with that from corresponding solvent-cast films.

3.3 Experimental Details

3.3.1 Materials

Gelatin powder (type A, porcine skin, ~180 Bloom) was purchased from Sigma (USA). Buthanol, dichloromethane, hexane, and methanol (analytical reagent grade) were purchased from Labscan (Asia) (Thailand). Glutaraldehyde (50 vol.% aqueous solution) and asiaticoside (98.5% purity) were purchased from Fluka (Switzerland). Sodium acetate (Ajax Chemicals, Australia) and glacial acetic acid (Carlo Erba, Italy) were of analytical reagent grade and used without further purification.

3.3.2 Extraction of *Centella Asiatica*

Leaves from the plant were collected from Min Buri, one of the 50 districts in the City of Bangkok, Thailand. The leaves (~65 kg) were cleaned, dried, and finally ground into powder (~5 kg). The dried powder was first extracted by hexane. The solid residue from the hexane extraction was pressed to remove as much solvent as possible prior to further extraction in dichloromethane. The solid residue from the dichloromethane extraction was also pressed to remove as much solvent as possible prior to final extraction in methanol. After ridding as much

methanol from the supernatant liquid as possible using a home-made evaporator, a pasty substance (~780 g) with dark green/brown in color was obtained.

3.3.3 Sample Preparation

A weighed amount of gelatin powder was dissolved in 70 vol.% acetic acid aqueous solution (diluted from glacial acetic acid with distilled water) to prepare a gelatin solution at a fixed concentration of 22% w/v (Choktaweesap, N., *et al,* 2007). The methanolic crude extract of *Centella asiatica* (mCA) was then added into the gelatin solution under constant stirring for 15 min. Different amounts of the herbal extract, i.e., 5-30 wt.% based on the weight of gelatin powder, were loaded in the gelatin solution. Prior to e-spinning, the neat gelatin solution was measured for its shear viscosity and conductivity by Brookfield DV-III programmable rheometer and a Orion 160 conductivity meter, respectively.

The neat and the mCA-loaded gelatin solutions were e-spun by loading each of the as-prepared solutions in a standard 10-mL glass syringe, the open end of which was attached with a blunt gauge-20 stainless steel hypodermic needle (OD = 0.91 mm), used as the nozzle. Both the syringe and the needle were tilted about 45°C from a horizontal baseline. Either a piece of aluminum (Al) sheet wrapped around a rigid plastic sheet or metal grids (3 cm × 10 cm) placed around a rotating cylinder (OD = 7 cm; ~40-50 rpm) was used as the collecting device. A Gamma High-Voltage Research ES30P-5W DC power supply (Florida, USA) was used to charge the solution by attaching the emitting electrode of positive polarity to the nozzle and the grounding one to the collecting device. A fixed electrical potential of 15 kV was applied across a fixed distance between the tip of the needle and the outer surface of the collecting device (i.e., collection distance, measured at right angle to the surface of the collecting device) of 20 cm. The e-spun fiber mats were collected, 'dried' in a vacuum oven for 24 h, and stored in a desiccator for further use.

For comparison purposes, the neat and the mCA-loaded gelatin films were also prepared by solvent-casting technique from 5% w/v gelatin solution in 70 vol.% acetic acid and the neat gelatin solution that contained 30 wt.% of the herbal

extract. A polystyrene box was used as the mold and the as-cast films were left to 'dry' at room condition for 24 h and later in a vacuum oven for another 24 h.

3.3.4 Cross-linking with Glutaraldehyde Vapor

The neat and the mCA-loaded gelatin fiber mats and films were cross-linked with glutaraldehyde vapor by incubating both the fiber mat and film samples in glass jars saturated with the vapor from the 50 vol.% glutaraldehyde aqueous solution for various exposure time intervals, ranging between 0 and 3 h. The temperature of the jars was equilibrated in a water bath at 37°C. After cross-linking, both the fiber mat and film samples were placed in a vacuum oven at room temperature for 24 h and later at 110°C over night to remove as much amount of unreacted glutaraldehyde as possible. The samples were kept in a desiccator for further use.

To assess the extent of cross-linking at various exposure times, specimens of both the neat gelatin fiber mat and film samples (circular discs of ~1.65 cm in diameter cut from the as-prepared fiber mat and film samples) were weighed and then submerged in distilled water for 24 h at room temperature. The degree of swelling and the weight loss of these specimens were determined according to the following equations:

$$\text{Degree of swelling (\%)} = \frac{M - M_d}{M_d} \times 100, \tag{1}$$

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

where M is the weight of each specimen after submersion in distilled water for 24 h, M_d is the weight of the specimen in its dry state after submersion in distilled water for 24 h, and M_i is the initial weight of the specimen in its dry state.

3.3.5 Morphological Observation

Morphological appearance of the neat and the mCA-loaded e-spun gelatin fiber mats both before and after cross-linking was observed by a JEOL JSM-5200 scanning electron microscopy (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 the SEM images using

SemAphore 4.0 software, from which at least 100 measurements for each sample type were analyzed to obtain an average value along with the standard deviation.

3.3.6 Release of Asiaticoside from mCA-loaded Fiber Mats and Films

3.3.6.1 Preparation of Acetate Buffer

To prepare 1000 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 into the solution to fill the volume. The pH of the as-prepared acetate buffer solution was 5.5.

3.3.6.2 Asiaticoside-release Assay

The release characteristic of asiaticoside from mCA-loaded gelatin fiber mat and film specimens was determined by total immersion method (Taepaiboon, P., *et al*, 2006 and Taepaiboon, P., *et al*, 2007). Two types of releasing medium were used: (1) the acetate buffer and (2) the acetate buffer that contained 10 vol.% methanol. Methanol was added to the acetate buffer to enhance the solubility of asiaticoside in the resulting releasing medium. Briefly, the specimens, cut from the as-prepared fiber mat and film samples into circular discs of ~2.8 cm in diameter by a home-made hole-puncher, were immersed in 2 ml of the acetate buffer solution at the physiological temperature of 37°C. At a specified immersion period ranging between 0 and 7 d, 200-300 µL of the buffer solution was taken out (i.e., the sample solution), and an equal amount of fresh buffer solution was refilled. The amount of asiaticoside in the sample solution was determined by thin-layer chromatography (TLC)-densitometry technique. The experiments were carried out in triplicate and the results were reported as average values.

3.3.6.3 TLC-densitometry Technique

The standard asiaticoside and the methanolic crude extract of *Centella asiatica* (mCA) were dissolved in the acetate buffer at a concentration of 0.5 and 5 mg·mL, respectively. About 2-8 µL of the standard asiaticoside solution and about 2 µL of the mCA solution were applied on 10 × 20 cm silica gel 60 F254-covered aluminum plates (Merck, Germany) by means of a Linomat 5 CAMAG applicator (Switzerland). About 5-10 µL of the sample solutions from the release

assay at different immersion periods were applied on the plates as previously mentioned. TLC separations were performed on these plates. The mobile phase used was 80:10:10 v/v/v buthanol/water/methanol mixture. The developed plates were 'dried' at room temperature and the amount of asiaticoside on the plates was quantified by first dipping the plates in 10% sulfuric acid-containing ethanol solution for ~10 s, incubating the plates at 100-105°C for ~45 min, and finally performing the densitometric measurement of the bands associated with the presence of asiaticoside by a CAMAG TLC Scanner 3 (Switzerland).

3.4 Results and Discussion

3.4.1 Effect of Storage Time

Among the various properties, the shear viscosity of the spinning solution is the most contributing factor, influencing both morphology and size of the e-spun fibers. Mit-uppatham, C., *et al.* (Mit-uppatham, C., *et al,* 2004) showed that the morphological appearance of e-spun polyamide-6 (PA-6) fibers changed from discrete beads, to beaded fibers, and finally to smooth fibers when the shear viscosity of the PA-6 solutions increased, with the diameters of the fibers (for beaded fibers, only the diameters of the fiber segments between beads were accounted for) being found to increase with increasing the shear viscosity of the solutions. They also found that, at a given concentration, fibers obtained from PA-6 of higher molecular weights appeared to be larger in diameter, but it was observed that the average diameters of the fibers from PA-6 of different molecular weights had a common relationship with the solution viscosities which could be approximated by an exponential growth equation (Mit-uppatham, C., *et al,* 2004).

Recently, Chuangchote, S., *et al.* (Chuangchote, S., *et al,* 2007) reported that the shear viscosity of the base PVA solution (i.e., 10% w/v in reverse osmotic water) decreased from 810 mPa·s to about 600 mPa·s upon sonication, while the conductivity was not affected. The reduction in the shear viscosity was responsible for the observed decrease in the average diameter of the e-spun PVA fibers from ~290 nm to ~170 nm [i.e., for a fixed applied electrostatic field strength

(EFS) of 15 kV/15 cm] (Chuangchote, S., *et al*, 2007). Because Ki, C.S., *et al.* (Ki, C.S., *et al*, 2005) showed that the shear viscosity of the gelatin solutions in 98% formic acid decreased monotonically over the 30 h-period after preparation, with the extent of the reduction in the shear viscosity of the solutions increasing with increasing the solution concentration, and it was postulated to be a result of the hydrolysis of gelatin molecules in such a strong acid, it is necessary to determine whether or not such a reduction in the shear viscosity of the gelatin solution in 70 vol.% acetic acid would occur, since, despite the relative simplicity of the e-spinning technique, the applicability of the technique requires that the properties of a spinning solution do not change appreciably with time.

Here, both the shear viscosity and the conductivity of the neat gelatin solution (i.e., 22% w/v in 70 vol.% acetic acid) were measured at 1, 2, 3, 4, 5, 12, and 24 h after preparation (see supplementary data (available at stacks.iop.org/Nano/19/015102)). A slight reduction in the shear viscosity of the solution was observed (i.e., from ~462 mPa·s at 1 h of storage time to ~447 mPa·s at 24 h of storage time), while a slight increase in the conductivity of the solution was noted (i.e., from ~1260 μS·cm^{-1} at 1 h of storage time to ~1270 μS·cm^{-1} at 24 h of storage time). To investigate the effect of storage time on electrospinnability of the gelatin solution and morphology and size of the e-spun fibers, the neat gelatin solution was e-spun at 2.5, 4, 7.5, 12, 17, and 24 h after preparation onto an aluminum sheet wrapped around a stationary rigid plastic sheet at a fixed EFS of 15 kV/20 cm for a fixed collection time of 5 min. The storage time had no obvious effect on the morphology and the size of the e-spun gelatin fibers, as the fibers appeared to be smooth and the average diameters of these fibers were found to range between ~221 and ~234 nm (see supplementary data (available at stacks.iop.org/Nano/19/015102)). It can be inferred from the obtained results that appreciable degradation of gelatin molecules in 70 vol.% acetic acid was not occurred as in the case of gelatin in 98% formic acid (Ki, C.S., *et, al*, 2005) (cf. $K_{a,\text{acetic acid}} \approx 1.8 \times 10^{-5}$ versus $K_{a,\text{formic acid}} \approx 1.7 \times 10^{-4}$).

3.4.2 Effect of Cross-linking

Since gelatin is a water-soluble material, an e-spun gelatin fiber mat can either partially or completely dissolve to lose its fibrous structure when coming into contact with an aqueous medium or partially dissolve to lose its fibrous structure when being exposed to a high humidity ambient, e.g., 80-90%, for a certain period of time (Zhang, Y.Z., et al, 2006). To extend its use in applications that require a contact with an aqueous medium or an exposure to a high humidity, cross-linking is necessary. Among the various chemical cross-linking systems used to cross-link gelatin (e.g., glutaraldehyde (Vandervoort, J. and Ludwig, A., 2004; Zhang, Y.Z., et al, 2006; Ulubayram, K., *et al*, 2001; Miyashi, M., *et al*, 2005 and Huang, S., *et al*, 2006), genipin (Liu, T.Y., *et, al,* 2006), EDC/N-hydroxysuccinimide (NHS) (Liu, J., *et al*, 2007), HDMI (Li, M., *et al*, 2005), EDC (Li, M., *et al*, 2006)), glutaraldehyde seems to be the most widely-used system, likely a result of its relatively low cost. Nevertheless, conventional cross-linking by way of immersing a fabricated form of gelatin in an aqueous solution of glutaraldehyde is not applicable with an e-spun gelatin fiber mat, as the material would lose its fibrous structure when it comes into contact with the cross-linking medium (see supplementary data (available at stacks.iop.org/Nano/19/015102)).

Recently, Zhang *et al.* (Zhang, Y.Z., *et al,* 2006) showed that exposing an e-spun gelatin (Type A, porcine skin, ~300 Bloom) fiber mat (~100 μm in thickness) to saturated vapor from 25 vol.% glutaraldehyde aqueous solution for more than ~2 d was proven to be an efficient method for cross-linking the fiber mat. Here, the e-spun gelatin fiber mats were also cross-linked by treating the fiber mats in a chamber filled with saturated vapor from 50 vol.% glutaraldehyde for various exposure time intervals, ranging from 0 to 3 h (180 min). To remove as much amount of the unreacted glutaraldehyde from the fiber mats as possible, the fiber mats were later placed in a vacuum oven at room temperature for 24 h and later at 110°C over night. To assess the degree of cross-linking, cross-linked e-spun gelatin fiber mat specimens were investigated for their swelling and weight loss behavior after submersion in distilled water for 24 h at room temperature. These specimens were cut from the fiber mat (~70-90 μm in thickness) that was e-spun from the neat

gelatin solution (i.e., 22% w/v in 70 vol.% acetic acid) on metal grids (3 cm × 10 cm) placed around the rotating cylinder at a fixed EFS of 15 kV/20 cm for a fixed collection time of 15 h. Comparisons were also made against solvent-cast gelatin film specimens (~100-120 μm in thickness).

Table 3.1 shows selected SEM images of the e-spun gelatin fiber mat specimens that were cross-linked with glutaraldehyde vapor for various exposure time intervals before and after the swelling and the weight loss studies. Evidently, increasing the exposure time in the chamber caused adjacent fiber segments to fuse to one another, a result of the partial dissolution of the fiber segments when they came into contact with the moisture-rich vapor from the aqueous solution of glutaraldehyde. A similar observation was reported for the cross-linking of the e-spun poly(vinyl alcohol) (PVA) fibers containing sodium salicylate as the model drug with the glutaraldehyde vapor (Taepaiboon, P., *et al,* 2007). In addition, the fiber mat specimens, after cross-linking, changed their color from white to pale yellow and slightly shrunk from their original dimensions. The change in color of cross-linked gelatin is caused by the formation of aldimine linkages (-CH=N-) between the free amino groups of lysine or hydroxylysine amino acid residues of the protein and the aldehyde groups of glutaraldehyde (Olde Damink, L.H.H., *et al,* 1995; Akin, H. and Hasirci, N., 1995). Without cross-linking, submersion of the e-spun gelatin fiber mat specimen in distilled water for 24 h completely destroyed its fibrous structure. Evidently, treating the fiber mat specimens in the cross-linking chamber for a short exposure time, i.e., lower than ~30 min, was insufficient to maintain the fibrous structure of these specimens. Further increasing the exposure time interval over ~30 min in the cross-linking chamber caused the fiber mat specimens to maintain their fibrous structure, despite a strong evidence of a high degree of shrinkage of these specimens after the swelling and the weight loss studies.

Figure 3.2 shows the degree of swelling and the weight loss of the cross-linked fiber mat and film specimens after submersion in distilled water for 24 h as a function of the exposure time in the cross-linking chamber. Despite losing its fibrous structure through partial dissolution, the neat e-spun gelatin fiber mat specimen showed a very high degree of swelling at ~1150%, while that of the cross-

linked e-spun fiber mat specimens was found to gradually decrease from ~360% at the exposure time of 5 min to ~250% at the exposure time of 180 min. On the contrary, the neat gelatin film specimen and the film specimens that were treated in the cross-linking chamber shorter than ~30 min could not maintain their physical form, as they partially dissolved and/or disintegrated after submersion in distilled water. Increasing the exposure time in the cross-linking chamber for more than or equal to 30 min caused the film specimens to be able to maintain their physical form and the degree of swelling of these film specimens was found to gradually decrease from ~610% at the exposure time of 30 min to ~290% at the exposure time of 180 min. In the same manner, the neat e-spun gelatin fiber mat specimen showed a high percentage of weight loss at ~41%, while that of the cross-linked e-spun fiber mat specimens was found to gradually decrease from ~26% at the exposure time of 5 min to ~15 at the exposure time of 15 min to reach a plateau value in the range of 12-13% over the exposure time intervals of 30-180 min. On the other hand, the degree of swelling of the cross-linked gelatin film specimens was found to gradually decrease from ~35% at the exposure time of 30 min to ~20% at the exposure time of 180 min. Evidently, the greater degree of swelling and the percentage of weight loss of the film specimens over those of the fiber mat counterparts, at a given exposure time, suggested that the degree of cross-linking of the fiber mat specimens was greater than that of the film counterparts, most likely a result of the greater surface area of the fiber mat specimens over that of the film counterparts.

3.4.3 *Centella Asiatica* Crude Extract (mCA)-loaded Gelatin Fiber Mats and the Release of Asiaticoside

Methanolic crude extract of *Centella asiatica* (mCA) in the amount of 5-30 wt.% based on the weight of gelatin powder was added in the neat gelatin solution (i.e., 22% w/v in 70 vol.% acetic acid) to prepare the mCA-loaded gelatin solutions. Both the neat and the mCA-loaded gelatin solutions were e-spun onto an aluminum sheet wrapped around a stationary rigid plastic sheet at a fixed electrostatic field strength of 15 kV/20 cm over a fixed collection time of 5 min. Selected SEM images of the neat and the mCA-loaded gelatin fiber mats are shown in Figure 3.3. The addition of mCA had no obvious effect on both the morphology and the size of the mCA-loaded gelatin fibers as both of the neat and the mCA-

loaded gelatin fibers were smooth and the diameters of these fibers were found to be in the same range (i.e., 226 ± 52 to 232 ± 49 nm; see supplementary data (available at stacks.iop.org/Nano/19/015102)).

The fiber mat from the gelatin solution containing 30 wt% of mCA was chosen for further investigating the release characteristic of asiaticoside. The mCA-loaded gelatin fiber mat (~180 – 220 μm in thickness) was e-spun onto metal grids (3 cm × 10 cm) placed around the rotating cylinder at a fixed EFS of 15 kV/20 cm for a fixed collection time of 48 h. Comparisons were also made against the mCA-loaded gelatin film (~90–120 μm in thickness) prepared by the solvent-casting technique. It should be noted that the addition of mCA also did not affect the morphology of the mCA-loaded gelatin films, as both the neat and the mCA-loaded gelatin films were smooth (see supplementary data (available at stacks.iop.org/Nano/19/015102)). Both the mCA-loaded fiber mat and film samples were cross-linked with glutaraldehyde vapor for 1 h to improve their stability in the releasing medium. Prior to investigating the release characteristic of asiaticoside from the mCA-loaded fiber mat and film specimens (disc shape; 2.8 cm in diameter; cut from the mCA-loaded e-spun fiber mat and solvent-cast film samples), the content of asiaticoside in mCA and the mCA-loaded fiber mat and film samples needed to be determined. TLC–densitometric analyses revealed that the amount of asiaticoside in mCA was ~17 ± 2% and the amount of asiaticoside in the mCA-loaded fiber mat and film samples in comparison with that in the spinning and the casting solutions was ~99 and 87%, respectively. These values were used to arrive at the cumulative release of asiaticoside from the mCA-loaded specimens.

Examples of the TLC-densitometric chromatograms are given as supplementary data (available at stacks.iop.org/Nano/19/015102). For the particular pair of stationary/mobile phases, the retention factor (Rf) of asiaticoside was observed at ~0.78. Since the majority of the peaks, including that of asiaticoside, observed on the chromatograms of the mCA solution in the acetate buffer solution and those of the mCA that was released from both the fiber mat and film specimens was practically similar, the chemical integrity of the extract should still be intact. To

arrive at the cumulative release of asiaticoside, only the area under the peak associated with asiaticoside at $Rf \approx 0.78$ was investigated.

Figure 3.4 shows cumulative release profiles of asiaticoside from the mCA-loaded gelatin fiber mat and film specimens reported as the percentage of the weight of asiaticoside released divided by the actual weight of asiaticoside present in the specimens in two different types of releasing medium, i.e., acetate buffer and 90:10 v/v acetate buffer/methanol, at the physiological temperature of 37°C. In the acetate buffer, the percentage of asiaticoside released from both the fiber mat and film specimens increased rather rapidly during the first 24 h (i.e., from ~18% at the immersion time of 5 min to ~50% at 24 h for the fiber mat specimens and from ~32% at the immersion time of 5 min to ~69% at 24 h for the film counterparts) and increased rather slightly afterward (i.e., to reach the total amount of asiaticoside released from the fiber mat and film specimens on day 7 of ~56 and ~77%, respectively). In the acetate buffer/methanol, a much greater amount of asiaticoside was released from both types of specimens, at any given immersion time, with the total amount of asiaticoside released from the fiber mat and the film specimens on day 7 being ~71 and ~80%, respectively).

The fact that the percentage of the weight of asiaticoside released from the mCA-loaded solvent-cast gelatin film specimens in any type of releasing medium was greater than that from the mCA-loaded e-spun gelatin fiber mat counterparts could be due to a number of factors, e.g., the observed greater degree of swelling and the percentage of weight loss of the neat gelatin films over those of the neat gelatin fiber mats (see Figure 3.2) and the difference in the total amount of asiaticoside present in the fiber mat and the film specimens. The latter factor was caused by the difference in the amount of asiaticoside in the fiber mat and film specimens in comparison with that in the spinning and the casting solutions (i.e., ~99 and ~87%, respectively) and to the difference in the actual weight of the fiber mat and film specimens [i.e., despite the same diameter of the specimens, the differences in the thickness and the morphology of the fiber mat and film specimens caused the weight of the film specimens (i.e., ~50 mg) to be greater than that of the fiber mat counterparts (i.e., ~60 mg)].

To assess whether the actual weight of the specimens had a profound effect on the release profiles of asiaticoside, the weight of asiaticoside released from the mCA-loaded gelatin fiber mat and film specimens was divided by the actual weight of the specimens and the results are shown in Figure 3.5 Evidently, by normalizing the weight of asiaticoside released from the fiber mat and film specimens by the actual weight of the specimens, the amount of asiaticoside released from the fiber mat specimens in any type of the releasing medium was greater than that from the film counterparts. Even though the appearance of the release profiles was similar to those shown in Figure 3.4, the total amount of asiaticoside released from these specimens was clearly different. Specifically, the total amount of asiaticoside released from the fiber mat and film specimens in the acetate buffer on day 7 was ~97 and ~77 ng of asiaticoside/mg of specimen, respectively, while that in the acetate buffer/methanol was ~122 and ~79 ng of asiaticoside/mg of specimen, respectively. For both types of plots, the fact that the release of asiaticoside from the fiber mat and film specimens in the acetate buffer/methanol was greater than that in the acetate buffer is due to the fact that methanol is the good solvent for asiaticoside.

3.5 Conclusion

A crude extract from *Centella asiatica* (L.) Urban, a medicinal plant widely known for its traditional medical applications including wound healing ability, in the methanol fraction (mCA) was added to the neat gelatin (type A, porcine skin, ~180 Bloom) solution (22% w/v in 70 vol.% acetic acid) in various amounts (i.e., 5-30 wt.% based on the weight of gelatin powder). Both the neat and the mCA-containing gelatin solutions were fabricated into ultrafine fibers by electrospinning under a fixed electrostatic field strength of 15 kV/20 cm. The storage time after the preparation of the neat gelatin solution had no obvious effect on both the morphology and the diameters of the electrospun (e-spun) fibers, as the fibers appeared to be smooth and the average diameters of these fibers were found to range between ~221 and ~234 nm. The stability in an aqueous medium of the e-spun gelatin fiber mats was improved by cross-linking with glutaraldehyde vapor (i.e., exposing the mats in a chamber saturated with the vapor from the 50 vol.% glutaraldehyde aqueous solution

for various exposure time intervals, ranging between 0 and 3 h at 37°C, followed by incubating in a vacuum oven at room temperature for 24 h and later at 110°C over night). Incorporation of mCA in the neat gelatin solution had no obvious effect on both the morphology and the size of the mCA-loaded gelatin fibers obtained, as both the neat and the mCA-loaded gelatin fibers were smooth and the average diameters of these fibers were found to in the range of 226 to 232 nm. The cross-linked mCA-loaded e-spun gelatin fiber mat (from the neat gelatin solution containing 30 wt.% of mCA and exposed in the cross-linking chamber for 1 h) was finally investigated for the release characteristic of asiaticoside, identified as the most active compound associated with the healing of wounds, in two different types of releasing medium, i.e., acetate buffer and the buffer containing 10 vol.% of methanol, based on thin-layer chromatography (TLC)-densitometry technique. It was found that, based on the unit weight of actual amount of asiaticoside present in the specimens, the total amount of asiaticoside released from the fiber mat specimens was lower than that from the film counterparts, while, based on the unit weight of the specimens, an opposite trend was observed.

3.6 Acknowledgments

3.7 Supplementary Data

Supplementary data associated with this research work can be found in the online version, at stacks.iop.org/Nano/19/015102.

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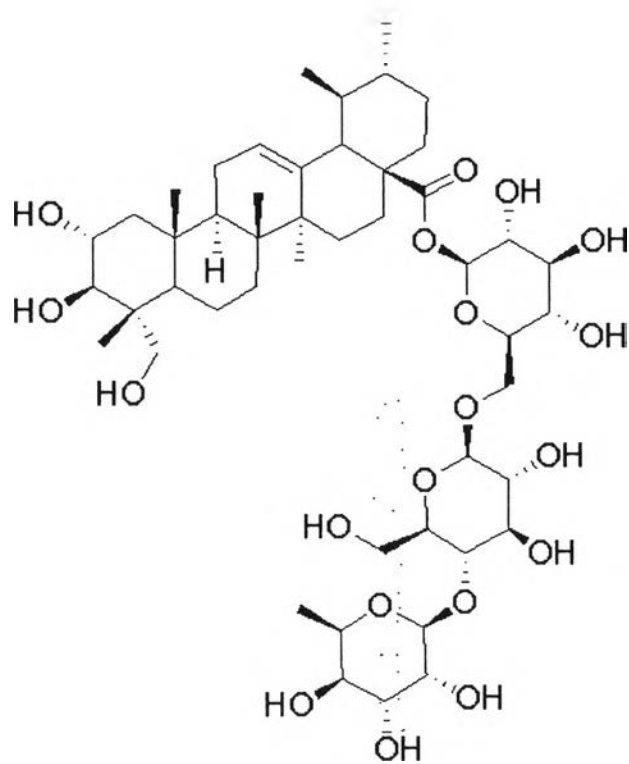
Figure 3.1 Chemical structure of asiaticoside.
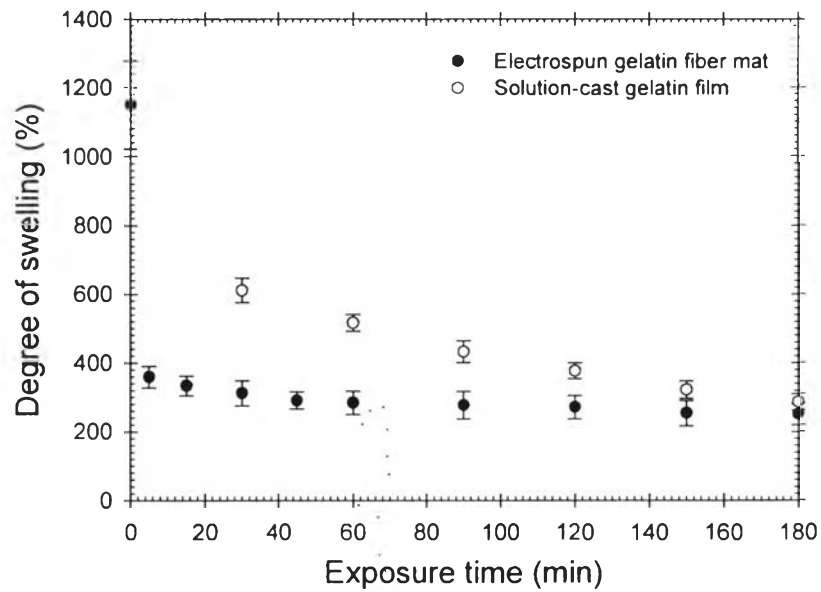
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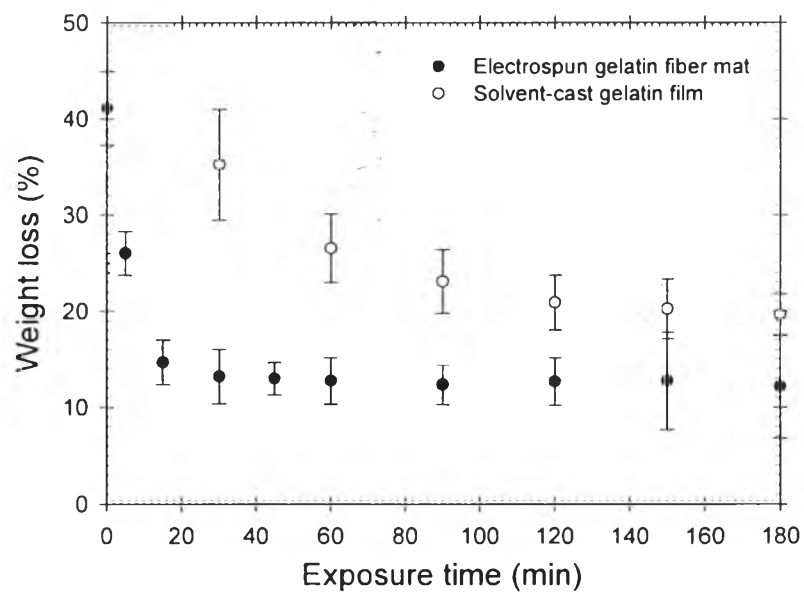
(b)

Figure 3.2 (a) Swelling and (b) weight loss of electrospun gelatin fiber mat specimens (~70-90 μm in thickness) and solvent-cast film specimens (~100-120 μm in thickness) after submersion in distilled water at room temperature for 24 h as a function of the exposure time in the cross-linking chamber containing saturated vapor from 50 vol.% glutaraldehyde aqueous solution.
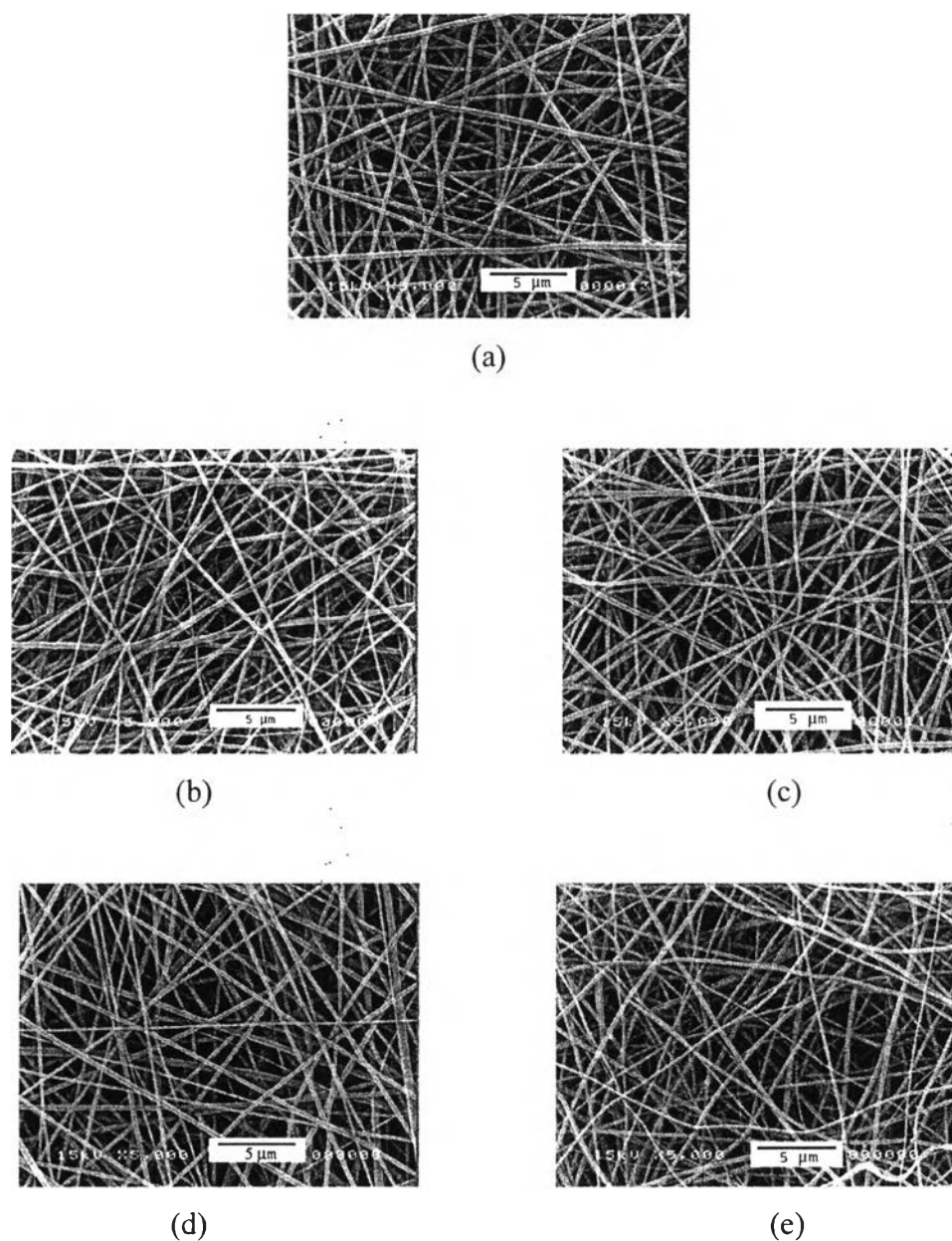
Figure 3.3 Selected SEM images of electrospun fiber mats from (a) the neat gelatin solution (i.e., 22% w/v in 70 vol.% acetic acid) and the ones that contained methanolic crude extract of *Centella asiatica* (mCA) in the amount of (b) 5, (c) 10, (d) 20, and (e) 30 wt.% (based on the weight of gelatin powder). These fibers were electrospun onto an aluminum sheet wrapped around a stationary rigid plastic sheet at a fixed electrostatic field strength of 15 kV/20 cm over a fixed collection time of 5 min.
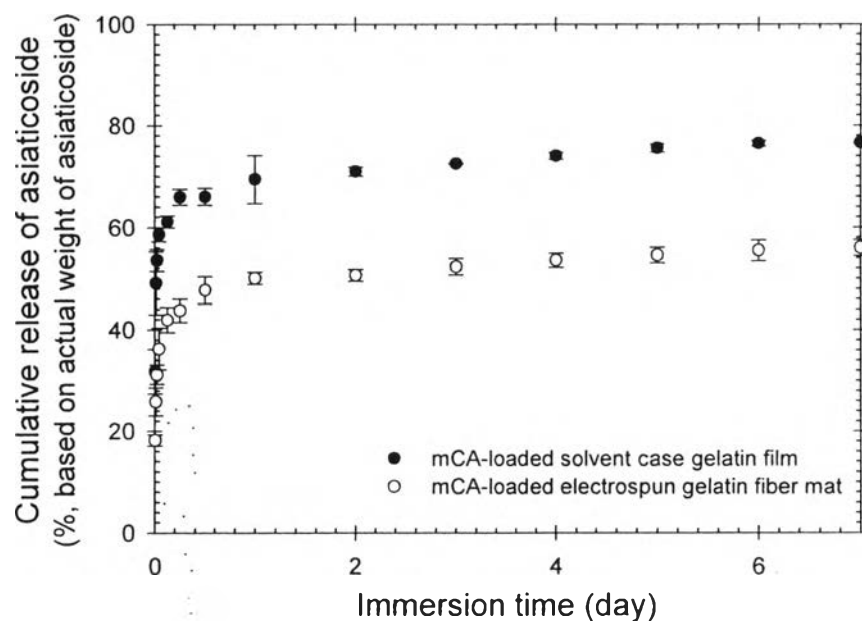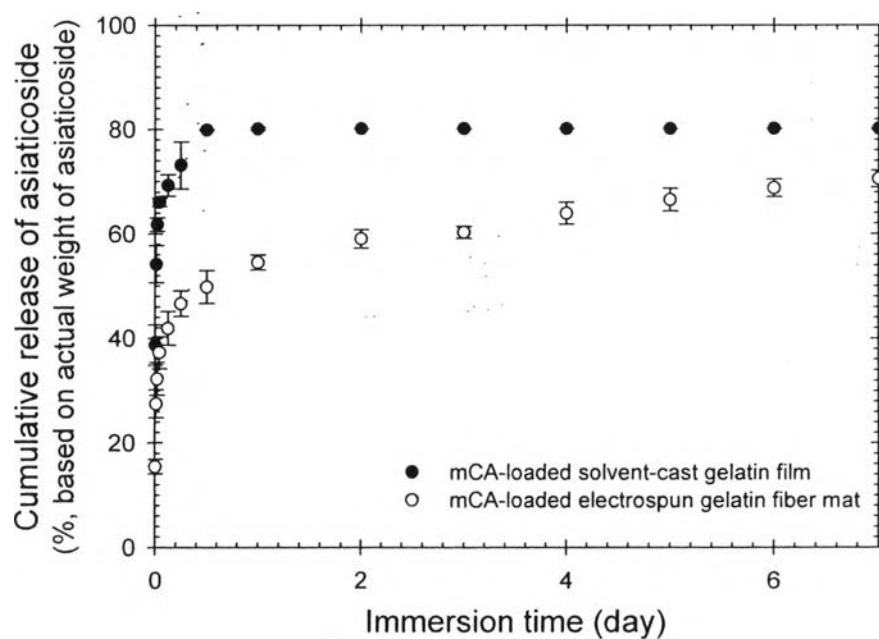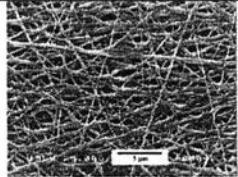
Figure 3.4 Cumulative release profiles of asiaticoside from mCA-loaded gelatin fiber mat and film specimens reported as the percentage of the weight of asiaticoside released divided by the actual weight of asiaticoside present in the specimens in two different types of releasing medium, i.e., (a) acetate buffer and (b) 90:10 v/v acetate buffer/methanol, at the physiological temperature of 37 °C.
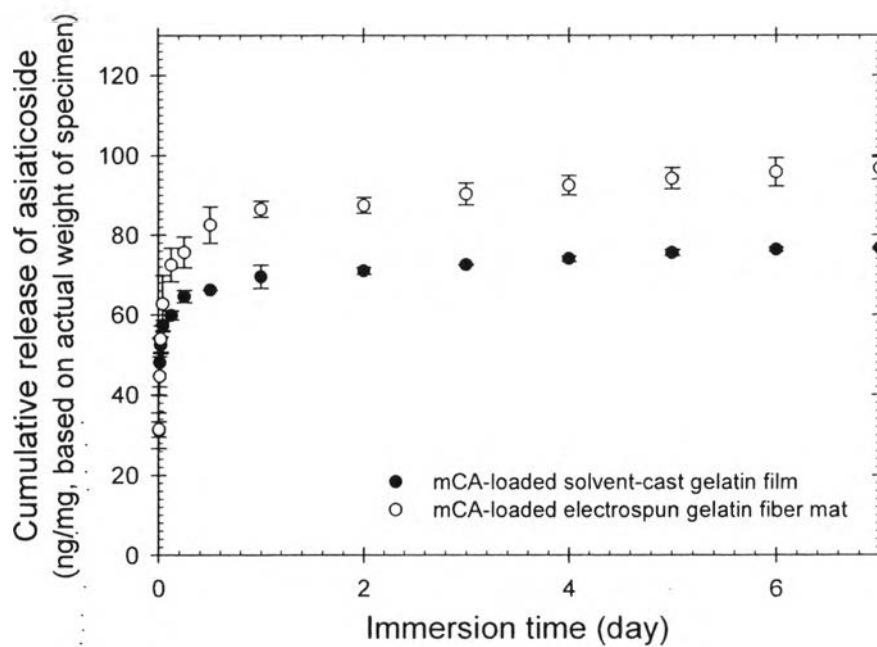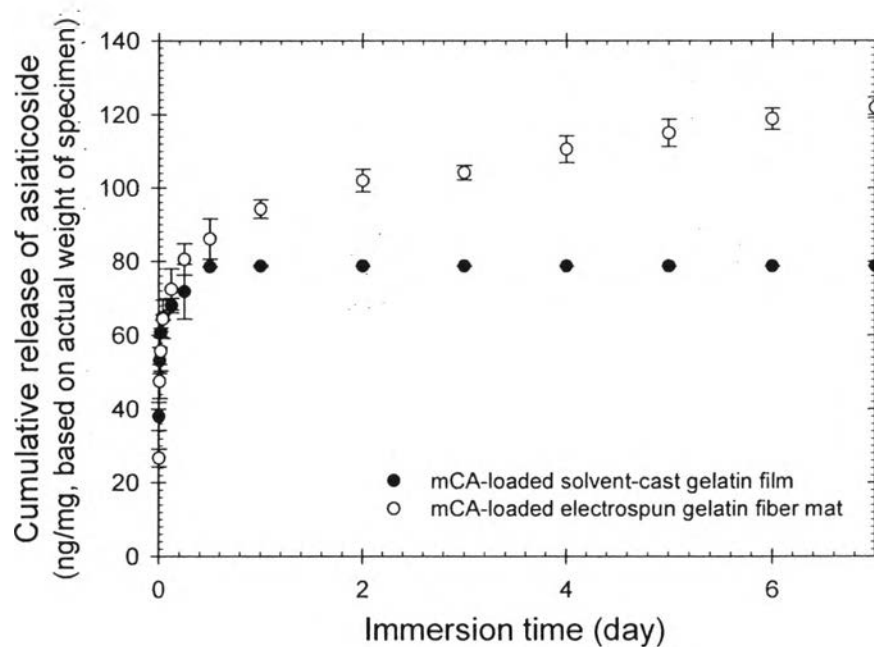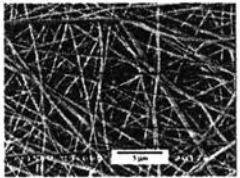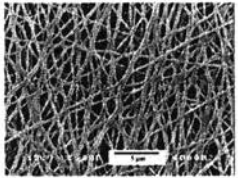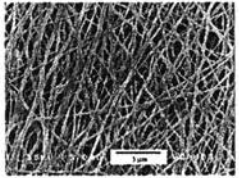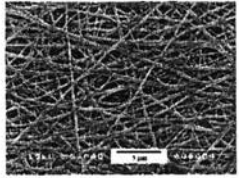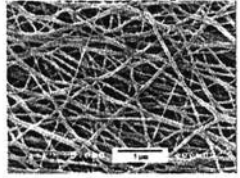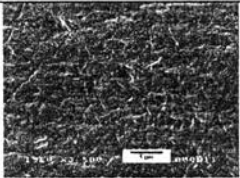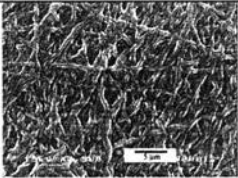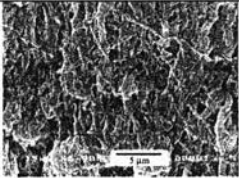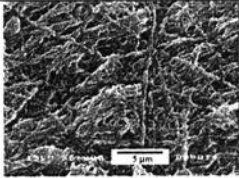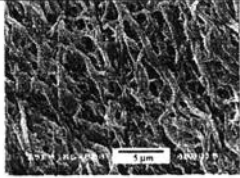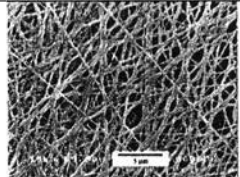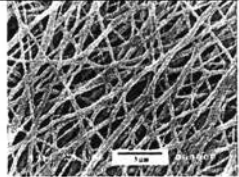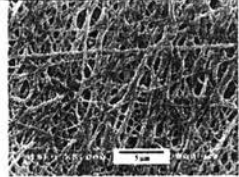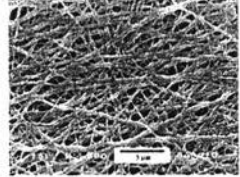
Figure 3.5 Cumulative release profiles of asiaticoside from mCA-loaded gelatin fiber mat and film specimens reported as the weight of asiaticoside released divided by the actual weight of the specimens in two different types of releasing medium, i.e., (a) acetate buffer and (b) 90:10 v/v acetate buffer/methanol, at the physiological temperature of 37 °C.

Table 3.1 Selected SEM images of the e-spun gelatin fiber mat specimens that were cross-linked with glutaraldehyde vapor for various exposure time intervals before and after the swelling and the weight loss studies (see **Figure 3.2**).

	Cross-linking time (min)				
	Neat	5	15	30	45
Before swelling and weight loss studies					
After swelling and weight loss studies					
	Cross-linking time (min)				
	60	90	120	150	180
Before swelling and weight loss studies					
After swelling and weight loss studies					