CHAPTER III

GALLIC ACID-LOADED ELECTROSPUN POLY(L-LACTIC ACID) FIBER MATS AND THE RELEASE CHARACTERISTIC OF GALLIC ACID

3.1 Abstract

Ultra-fine fiber mats of poly(L-lactic acid) containing gallic acid were prepared by electrospinning from gallic acid-containing PLLA solution in 7:3 v/v dichloromethane (DCM)/N,N-dimethylformamide (DMF). The amount of the as-loaded gallic acid was 40% w/w (based on the weight of PLLA in the solution) or 28.6 wt. % (based on the weight of the resulting fiber mats). Both the neat and the gallic acid-loaded PLLA fibers were smooth, with the average diameters of 965 and 843 nm, respectively. No aggregates of gallic acid were observed on the fiber surface and the actual amount of gallic acid in the gallic acid-loaded PLLA fiber mats, determined in the acetate buffer, the citrate-phosphate buffer, and the normal saline, was 26.3, 27.1, and 24.6 wt.-%. The cumulative amount of gallic acid released from the gallic acid-loaded PLLA fiber mats was greatest in the normal saline, followed by those in the citrate-phosphate and the acetate buffer, respectively. Lastly, the free radical scavenging activity, based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, of the as-loaded and the as-released gallic acid remained active.

(**Key-words**: Ultra-fine fiber mats; electrospinning; nanofibers; poly(L-lactic acid); gallic acid)

3.2 Introduction

Electrospinning has been recognized as an efficient method for the fabrication of submicron-sized fibers with a large surface area to volume ratio.[1] It is well-accepted in the researchcommunitydue to its simplicity, adaptability, and inexpensive tooling costs. In this process, a polymer liquid (i.e., melt or solution) in a container with a small opening (used as the nozzle) is charged with a high electrical potential across a finite distance between the nozzle and a grounded collection device. When the electric field increases beyond a critical value such that the electrical forces overcome the surface tension of the polymeric liquid droplet located

at the tip of the nozzle, a charged jet is ejected from the apex of the conical droplet.[1] As the jet accelerates to the collector, it either cools down (in case of the melt) or dries out (in case of the solution) to obtain ultra-fine fibers in the form of a nonwoven fabric on the collector. Due to interesting characteristics of the electrospun nonwoven fabrics (e.g., high surface area to mass or volume ratio, high porosity, etc.), they have been proposed as candidates for various biomedical applications, such as tissue engineering,[2] wound dressing,[3,4] and carriers for delivery of drugs and other therapeutic agents.[5-7]

Poly(L-lactic acid) (PLLA), a polymer derived from L-lactic acid or L-2-hydroxypropionic acid, is classified as a biocompatible and biodegradable polyester.[8,9] Its monomer is effectively derived from sugar-based biomass through bacterial fermentation. High-molecular weight PLLA can be synthesized in a single step by a direct condensation of the monomer or in two steps by a ring opening polymerization of L-lactide cyclic dimer. Due to its biocompatibility, PLLA has been fabricated into various forms, e.g., films for potential for uses as semi-permeable membranes for separation processes and fibers for biomedical applications.[8,9] PLLA can be and have been fabricated into ultra-fine fibers by electrospinning.[10-15] Various solvent systems have been used to prepare electrospinnable PLLA solutions: they are, for examples, 3:2 w/w methylene chloride/dimethylformamide (DMF),[10] chloroform,[11] 1,1,3,3-hexafluoro-2-propanol (HFIP),[12] chloroform, dichloromethane (DCM), or tetrahydrofuran (THF),[13] 9:1 v/v DCM/DMF, [14] and 3:1 v/v chloroform/acetone[15]. The proposed uses for these electrospun PLLA fibers are, for instance, as carriers for delivery of drugs, [10, 11] substrates for bone cell culture,[12] anti-bacterial membranes,[14] and substrates for biosensor assemblies.[15]

As mentioned, a number of electrospun polymeric fiber matrices have been developed as carriers for delivery of drugs and other therapeutic agents.[5-7,10,11] Recently, electrospun cellulose acetate (CA) fiber mats have been developed as carriers for topical and/or transdermal delivery of various types of drugs and other therapeutic agents.[16-19] Taepaiboon *et al.*[16] prepared electrospun CA fiber mats containing all-trans retinoic acid or vitamin A acid (Retin-A) and α-tocopheral or vitamin E (Vit-E) from CA solutions in 2:1 v/v acetone/dimethylacetamide (DMAc) containing Retin-A or Vit-E in the amount of 0.5 and 5% w/w(based on the weight of

CA), respectively. Electrospun CA fiber mats containing four different types of nonsteroidal anti-inflammatory drugs (NSAIDs), i.e.,naproxen(NAP), indomethacin (IND), ibuprofen (IBU), and sulindac (SUL), were prepared by Tungprapa *et al.* [17] from CA solutions in 2:1 v/v acetone/DMAc that contained NAP, IND, IBU, or SUL in the amount of 20%w/w (base on the weight of CA). Suwantong *et al.* [18] prepared electrospun CA fiber mats containing 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% w/w based on the weight of CA). In a subsequent work, electrospun CA fiber mats containing asiaticoside (AC) from the plant Centella asiatica L., in the form of either pure substance (PAC) or a crude extract (CACE), were prepared by Suwantong *et al.* [19] from CA solutions in 2:1 v/v acetone/DMAc that contained PAC or CACE in the amount of 40%w/w (based on the weight of CA). Here, PLLA in the form of electrospun fiber mats was used as the carrier for topical and/or transdermal delivery of gallic acid (3,4,5-trihydroxybenzoic acid; see Figure 1). Gallic acid is a naturally occurring polyphenol found in a variety of plants either in its free or bound form, e.g., gallotannins (viz. ester products of gallic acid and glucose).[20,21] It is commonly found in a variety of fruits and vegetables, such as grapes, cherry, tea leaves, and longan seeds.[21] Gallic acid has been shown to exhibit antioxidant, anti-inflammatory, anticarcinogenic, and antifungal properties.[22] It has been shown to exhibit melanogenesis inhibitory action as it showed a strong antityrosinase activity (IC_{50} = 3.59 × 10^{-6} M) and effectively suppressed murine tyrosinase action and the amount of melanin.[23] It was shown to down-regulate reactive species (RS) generation and enhanced the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio of B16 melanoma cells. Based on these results, gallic acid could be used as an effective skin-lightening agent and an effective antioxidant for the protection of the skin.[23] The antioxidant activity of gallic acid was investigated in vivo using senescence-accelerated mice (SAM) and the results showed that the oral administration of the herbal substance helped reverse the reduced catalase (CAT) and the glutathione peroxidase (GPx) activities and, simultaneously, helped reduce the lipid peroxidation level in the SAM model.[24] The aim of the present contribution was to investigate the potential for use of the electrospun PLLA fiber mats as carriers for topical and/or transdermal delivery of

gallic acid. The release characteristic of gallic acid from the gallic acid-loaded electrospun PLLA fiber mats was investigated by the total immersion method in three different types of releasing medium, i.e., an acetate buffer, a citrate-phosphate buffer, and a normal saline. The antioxidant activity of gallic acid in the gallic acid loaded PLLA fiber mats was also evaluated based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

3.3 Experimental

3.3.1 Materials

Poly(L-lactic acid) (PLLA; M_n = 200 000 g·mol^{-1}, intrinsic viscosity = 0.332 dL·g^{-1}) was obtained from Nature Works (USA). Gallic acid was obtained from Fluka (Switzerland). DCM, DMF, and methanol were purchased from Labscan (Asia) (Thailand). Glacial acetic acid was obtained from Carlo Erba (Italy). Sodium acetate and disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) were purchased from Ajax Chemicals (Australia). Citric acid monohydrate and trifluoroacetic acid were purchased from Sigma-Aldrich (USA). All of these chemicals were of analytical reagent grade and used without further purification.

3.3.2 Preparation of Neat and Gallic Acid-Loaded Poly(L-lactic acid) Fiber Mats

A weighed amount of PLLA powder was dissolved in 7:3 v/v DCM/ DMF to prepare the base PLLA solution at a fixed concentration of 10% w/v. Gallic acid-containing PLLA solutions were prepared by dissolving gallic acid powder in the amount of 40% w/w (based on the weight of PLLA powder) in the base PLLA solution. Prior to electrospinning, the as-prepared solutions were characterized for their conductivity using a SUNTEX conductivity meter. The measurements were carried out in triplicate at 25 ± 1 °C. These solutions were then electrospun under a fix electric field of 20 kV/18 cm, using a Gamma High Voltage Research D-ES30PN/M692 dc power supply, onto an aluminum (Al) sheet wrapped around a rotating cylinder (width and OD of the cylinder = 15 cm; rotational speed = 50 rpm), used as the collector. Unless otherwise noted, the collection time was 12 h (resulting in the fiber mats of ca. 400 μm in thickness).

3.3.3 Characterization of Neat and Gallic Acid-Loaded Poly(L-lactic acid) Fiber Mats

The morphology of the obtained electrospun fiber mat, was investigated using a JEOL JSM-6400 scanning electron microscope. The size of the individual fibers was determined from specimens cut from randomly selected areas of three different fiber mats. Each specimen was coated with a thin layer of gold using a JEOL JFC-1100E sputtering device prior to observation by Scanning Electron Microscopy (SEM). Diameters of these fibers were measured directly from 5000 × SEM images using a SemAphore 4.0 software. More than 50 individual fibers were measured for their diameters. Mechanical properties in terms of stress at maximum load, strain at maximum load, tensile strength, and elongation at break of both the neat and the gallic acid-load electrospun PLLA fiber mats were tested on a Lloyd LRX universal testing machine (gauge length = 50 mm and crosshead speed = 100 mm · min^{-1}). The specimens were cut into rectangular shape of 10×100 mm^2 and the measurements were carried out on ten separate specimens. The bulk densities of the fiber mats were investigated by a Sartorious YDK 01 density measurement kit, according to the following equation:

$$\rho_S = \frac{w_a \rho_w}{0.99983(w_a - w_w)} + 0.0012 \quad (1)$$

where W_a is the weight of each fiber mat specimen that is measured in air, ρ_w is the density of water (i.e., 0.99648 g·cm^{-3}@ 27.2 °C), and W_w is the weight of each fiber mat specimen that is measured in water. The measurements were carried out in pentuplicate.

The water retention of the gallic acid-loaded electrospun fiber mats was assessed in three different types of medium, i.e., an acetate buffer, a citrate-phosphate buffer, and a normal saline (see below for the preparation of these media), at the skin temperature of 32 °C at various time points upon their submersion in each respective medium. The measurements were carried out in pentuplicate within a total submersion period of 48 h. The water retention was calculated according to the following equation:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100, \quad (2)$$

where W is the weight of each specimen after submersion in each respective medium for a certain period of time and M_d is the weight of the specimen after submersion in its dry state.

3.3.4 Release of Gallic Acid From Gallic Acid-Loaded Poly(L-lactic acid) Fiber Mats

3.3.4.1 Preparation of Acetate Buffer, Citrate-Phosphate Buffer and Normal Saline

While acetate and citrate phosphate buffer solutions (pH 5.5) were chosen to simulate the human skin pH condition, normal saline (pH 7.0) was chosen as a reference solution as it is medically used in intravenous drips for patients who cannot take fluid orally. To prepare 1000 mL of the acetate buffer solution, 150 g of sodium acetate was dissolved in 250 mL of distilled water. Exactly 15mL of glacial acetic acid was then added very slowly into the sodium acetate aqueous 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. To prepare 1000 mL of the citratephosphate buffer solution, 18.1 g of $Na_2HPO_4 \cdot 7H_2O$ was dissolved in 100 mL of distilled water and 9.42 g of citric acid was added into the solution. Finally, distilled water was added into the solution to fill the volume. TFA was used to adjust the pH of the solution to 5.5. Lastly, the normal saline was prepared by dissolving 9 g of NaCl in 1 000mL of distilled water.

3.3.5 Actual Gallic Acid Content

To determine the actual content of gallic acid in the gallic acid loaded PLLA fiber mats, each specimen (circular disc; ca. 2.8 cm in diameter) was first dissolved in 10mL of 7:3 v/v DCM/DMF. Later, 1mL of the solution was added into 9mL of each of the three releasing media. The actual amount of the as-loaded gallic acid was then quantified with a Shimadzu UV-2550 UV-Vis spectrophotometer at the wavelength of 259 nm against a predetermined calibration curve of gallic acid in

each medium. The solutions of the neat PLLA fiber mats in 7:3 v/v DCM/DMF that had been diluted with each of the three releasing media were used as blanks. The measurements were carried out in pentuplicate.

3.3.6 Gallic Acid-Releasing Assay

The release characteristic of gallic acid from the gallic acid-loaded PLLA fiber mat was investigated by total immersion method in each of the three releasing media. Each specimen (circular disc; ca. 2.8 cm in diameter; cut from randomly selected areas of three different fiber mats) was submerged in 30mL of each medium at the skin temperature of 32 °C. At a specified submersion time point ranging between 0 and 48 h (2880 min), 1mL of the medium solution was withdrawn (hereafter, a sample solution) and an equal amount of the fresh medium was refilled. The amount of gallic acid in the sample solutions was determined using the UV-Vis spectrophotometer at a wavelength of 259 nm. The obtained data were calculated to determine the cumulative amount of gallic acid released from the specimens at each submersion time point. The actual content of the as-loaded gallic acid in the fiber mat specimens, as determined in each of the three releasing media, was used as the base value to calculate the cumulative amount of gallic acid released from the specimens. The measurements were carried out in triplicate.

3.3.7 Antioxidant Activity

The antioxidative activity of the as-loaded gallic acid in the gallic acid-loaded PLLA fiber mats was assessed by the 1,1-diphenyl-2- picryldrazyl (DPPH) radical scavenging assay. The procedure was modified from a method proposed by Robert *et al.*[25] Briefly, each specimen (circular disc; ca. 2.8 cm in diameter; cut from randomly selected areas of three different fiber mats) was first dissolved in 10 mL of 7:3 v/v DCM/DMF. An aliquot of the sample solution was then diluted with 100 mL methanol. Later, 1mL of the solution was mixed with 3 mL of 100×10^{-3} M DPPH solution and the resulting solution was incubated for 30 min at room temperature in darkness. The absorbance of the final solution was recorded spectrophotometrically at the wavelength of 517 nm.

The antioxidant activity of gallic acid that had been released from the gallic acid-loaded PLLA fiber mats were also investigated by the DPPH assay. Specimen (circular disc; ca. 2.8 cm in diameter; cut from randomly selected areas of three different fiber mats) was submerged in each of the three releasing media and the released solutions were collected at 6, 12, 24, and 48 h after submersion. An aliquot of each of these solutions was then diluted with 5 mL of the respective releasing medium. Later, 1mL of the solution was then mixed with 3 mL of 100×10^{-3} M DPPH solution. After the resulting solution had been incubated in darkness for 30 min at room temperature, the absorbance was recorded spectrophotometrically at a wavelength of 517 nm.

The antioxidant activity (%AA) of both the as-loaded and the as-released gallic acid was expressed as the percentage of DPPH that was decreased in comparison with that of the control condition (i.e., the testing solution without the presence of the as-loaded or the as-released gallic acid), according to the following equation:

$$\%AA = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 , \qquad (3)$$

where $A_{control}$ and A_{sample} represent the absorbance values of the testing solution without and with the presence of the as-loaded or the as-released gallic acid, respectively. The measurements were carried out in triplicate.

3.4 Results and Discussion

3.4.1 Electrospinning of Neat and Gallic Acid-Loaded Poly(L-lactic acid) Solutions

Prior to electrospinning, both the neat and the gallic acid containing PLLA solutions were measured for their conductivity. While the neat PLLA solution exhibited the conductivity value of 2.8 ± 2.2 $\mu S.cm^{-1}$, the addition of gallic acid to the neat PLLA solution increased the conductivity of the resulting solution dramatically to 14.6 ± 1.3 $\mu S.cm^{-1}$. Such an increase in the conductivity should be

due to the dissociation of gallic acid into ionic species. Electrospinning of these solutions was straightforward. Representative SEM images of the neat and the gallic acid-loaded PLLA fiber mats are shown in Figure 3.2 Smooth fibers with rather flat cross-sections were the common features. As with the gallic acid-loaded PLLA fiber mats, no evidence of any kind of aggregates was observed on the surface of the fibers, suggesting that the as-loaded gallic acid was incorporated well within the fibers. The observed increase in the conductivity of the spinning solution in response to the presence of gallic acid should be the main cause for the diameters of the gallic acid-loaded PLLA fibers that were thinner than those of the neat ones (i.e., 843 ± 2 vs. 965 ± 3 nm). The increase in the number of charged species, in response to the increase in the conductivity, in the gallic acid-containing solution results in an increase in the charge density on the surface of and/or within the ejected polymer jet and the increased charge density imposes greater elongation and thinning forces on the jet as it travels through the electric field onto the collector, hence the formation of smaller fiber diameters.

3.4.2 Mechanical Integrity and Bulk Densities of Neat and Gallic Acid-Loaded Poly(L-lactic acid) Fiber Mats

The mechanical properties and the bulk density of both the neat and the gallic acid-loaded PLLA fiber mats were investigated and the results are summarized in Table 3.1 For these investigations, the thickness of both the neat and the gallic acid-loaded PLLA fiber mats was 412 ± 9 μm (i.e., collection time =12 h). The stress at maximum load and the tensile strength of the neat PLLA fiber mats were 1.9 ± 0.4 and 0.23 ± 0.04 MPa, respectively, while the strain at maximum load and the elongation at break of the materials were very similar at about 80%. The incorporation of gallic acid into the PLLA fibers obviously weakened the fibers, with both the stress at maximum load and the tensile strength of the gallic acid-loaded PLLA fiber mats decreasing to 1.4 ± 0.2 and 0.18 ± 0.05 MPa, respectively. The decrease in the stiffness of the fibers was coupled with the observed decrease in both the strain at maximum load and the elongation at break (i.e., about 41 and 57%, respectively). To better compare certain mechanical properties of two different materials, normalization of the property values with the bulk densities of the

materials is a common practice. Here, the bulk densities of both the neat and the gallic acid-loaded PLLA fiber mats were determined to be 0.0496 ± 0.0057 and 0.0847 ± 0.0117 $g \cdot cm^{-3}$, respectively. It is hypothesized that the presence of gallic acid was responsible for the bulk density value of the gallic acid loaded PLLA fiber mats that was greater than that of the neat ones. After normalizing the stress at maximum load and the tensile strength of both the neat and the gallic acid-loaded PLLA fiber mats with the corresponding density values, it is obvious that the specific property values of the neat fiber mats were far greater than those of the gallic acid-loaded ones (see Table 3.1).

3.4.3 Water Retention Behavior of Gallic Acid-Loaded Poly(L-lactic acid) Fiber Mats

The gallic acid-loaded PLLA fiber mats were further characterized for their water retention behavior upon submersion in the acetate buffer, the citrate-phosphate buffer, or the normal saline at the skin temperature of 32 °C as a function of the submersion time (see Figure 3.3). For a given type of the releasing medium, the water retention of the gallic acid-loaded PLLA fiber mats increased with an increase in the submersion time. At any given time point, the water retention of the fibrous materials upon their submersion in the normal saline was the greatest, followed by those in the citrate-phosphate and the acetate buffer solutions, respectively. Specifically, at 30 min after submersion, the water retention of the fibrous materials in the normal saline was 60%, while, in the citrate-phosphate and the acetate buffer solutions, they were 39 and 23%, respectively. Finally for the specimens that had been submerged in the normal saline, the citrate-phosphate buffer, and the acetate buffer for 48 h, the property values increased to 348, 347, and 74%.

Due to the highly porous nature of the fiber mats, the amount of water absorbed within the fibers and that retained within the porous structure, held by the capillary action, should contribute to the total amount of water retained within the fiber mats (hence, the water retention). In addition, the release of the as-loaded gallic acid from the fiber mats could also influence their water retention behavior. Based on these postulations, a number of factors may have contributed to the different water

retention behavior of the gallic acid-loaded PLLA fiber mats in each of the three media investigated, as shown in Figure 3.3. The most likely factor should be due to the difference in the chemical potential of water molecules in the three media. Because the three media exhibit different ionic strength values (i.e., 0.120, 0.432, and 1.366 M for the normal saline, the citrate-phosphate buffer, and the acetate buffer, respectively), the chemical potential of water molecules in these media could be ranked as follows (in a descending order): the normal saline, the citrate-phosphate buffer, and the acetate buffer, respectively. In addition, the formation of the interfacial double layers of ions that are present in the media on the surface of the individual fibers may also contribute to the additional reduction in the chemical potential of the water molecules adjacent to the surface of the fibers. The reduction in the chemical potential of the water molecules acts to reduce the ability of the water molecules to diffuse into the fibers (i.e., less absorption).As a result, the ability of the water molecules to diffuse into the fibers should be greatest in the normal saline, followed by those in the citrate-phosphate and the acetate buffer solutions, respectively, which agreed well with the water retention behavior of the gallic acid-loaded PLLA fiber mats observed in these media. Moreover, the pH of the releasing media may have contributed to the different water retention behavior of the gallic acid-loaded PLLA fiber mats. At the acidic pH condition of both the acetate and the citrate-phosphate buffer solutions (i.e., 5.5) and at the neutral pH condition of the normal saline, the dissociation of gallic acid into ionic species should occur more readily in the normal saline than in the acetate and the citrate-phosphate buffer solutions (viz. pK_a of gallic acid = 4.41 @ 25 °C). Based on this, the amount of gallic acid that could diffuse out from the fibers should be greater in the normal saline than in the other two media (see later). Furthermore, Rosenberg et al.[26] observed an unexpected increase in the water content of drug-loaded polycaprolactone (PCL) matrices after all of the drug had been released. They related such the phenomenon to the semi-crystalline nature of PCL that stabilized the voids that were created upon the release of the drug.[26] We believe that these phenomena could be additional factors contributing to the greatest water retention of the gallic acid-loaded PLLA fiber mats observed in the normal saline.

3.4.4 Release of Gallic Acid from Gallic Acid-Loaded Poly(Llactic acid) Fiber Mats

Prior to investigating the release characteristic of gallic acid from the gallic acid-loaded PLLA fiber mats, the calibration curve of gallic acid in each of the three releasing media and the actual amount of gallic acidwithin thematerials needed to be determined. The calibration curves of gallic acid in the three media were as follows: let [abs] = UV absorbance value and [c] = gallic acid concentration in mg · mL^{-1} ; in acetate buffer: [abs] = 53.937[c]-0.0168 (r^2 = 0.999), in citrate phosphate buffer: [abs] = 53.835[c] - 0.0385 (r^2 = 0.997), and in normal saline: [abs] = 57.903[c] - 0.0592 0.0592 (r^2 = 0.988). It should be noted that the theoretical content of gallic acid in the gallic acid-loaded PLLA fiber mats was 40% w/w (based on the weight of PLLA powder) or 28.57wt.-% (based on the weight of the fiber mats). After the drug assay experiments, the actual contents of gallic acid within the fiber mat specimens, as determined in the acetate buffer, the citrate-phosphate buffer, and the normal saline, were 26.29±1.13, 27.05±1.29, and 24.62±1.88 wt.-% (based on the weight of the fiber mat specimens), respectively. These values corresponded to 92±4, 95±5, and 86±7% (based on the weight of gallic acid initially contained in the spinning solutions). The deviation from the ideal value of 100% should be due to the inhomogeneous distribution of gallic acid in different parts of the obtained electrospun fiber mats. The average value in each respective medium was later used to calculate the cumulative amount of gallic acid released fromthese gallic acid-loadedmaterials in that particular medium.

Figure 4.4 shows the cumulative release of gallic acid from the gallic acid-loaded PLLA fiber mats in each of the three releasing media as a function of the submersion time. In any given medium, the cumulative amount of the released gallic acid increased rather rapidly with an initial increase in the submersion time. Further increase in the submersion time resulted in a gradual increase in the cumulative amount of gallic acid released to finally assume a plateau value at the longest submersion time investigated (i.e., @ 2880 min). At any given submersion time point, the cumulative amount of gallic acid released into the normal saline was the greatest, followed by those released into the citrate-phosphate and the acetate buffer solutions, respectively. Specifically, at 30 min after submersion, the cumulative

amount of gallic acid released into the normal saline was 21% (equivalent to about 5.1 wt.-%, based on the weight of the fiber mat specimens), while they were about 11% in the acetate and the citrate-phosphate buffer solutions (equivalent to about 2.6 and 2.9 wt.-%, based on the weight of the fiber mat specimens, respectively). Such values finally increased to about 90, 70, and 32 % after the specimens had been submerged in the normal saline, the citrate-phosphate buffer, and the acetate buffer for 48 h (equivalent to about 22.3, 18.8, and 7.2 wt. %, based on the weight of the fiber mat specimens, respectively). Two possible reasons are postulated for such an observation. The first relates to the water retention behavior of the gallic acid-loaded fiber mats that showed the greatest value in the normal saline, followed by those in the citrate-phosphate and the acetate buffer solutions, respectively. The second relates to the fact that dissociation of gallic acid into ionic species could occur more readily in the normal saline than in the other two media. This is due possibly to the difference between the pK_a value of gallic acid and the pH of the normal saline (i.e., 4.41 vs. 7.0) that is greater than those of the other two media (i.e., 4.41 vs. 5.5). Moreover, at pH 7.0 of the normal saline, the dissociation of the carboxylic acid chain ends of PLLA into carboxylate groups (and protons) could additionally help promote the diffusion of gallic acid (i.e., the gallate ions) out of the fibers (i.e., due to electrostatic repulsion), hence the observed greatest amount of gallic acid released from the gallic acid-loaded fibrous materials.

The kinetics for the release of gallic acid from the gallic acid-loaded PLLA fiber mats can be characterized using an equation of the following form:[27,28]

$$\frac{M_t}{M_\infty} = kt^n, \tag{4}$$

where M_t is the cumulative amount of gallic acid released at an arbitrary time t, M_∞ is the cumulative amount of the substance released at an infinite time (here, @ 2880 min), n is an exponent characterizing the mechanism with which the release kinetics can be described, and k is the rate of the release of gallic acid that incorporates physical characteristics of the matrix/gallic acid system as well as some physical contributions from the measurement method. The release kinetics is taken as the normal Fickian diffusion when n = 0.45, Case II diffusion when n = 1.0 and a

mixture between Fickian and Case II diffusion when n falls between 0.5 and 1.0. The last case is sometimes categorized as non-Fickian or anomalous diffusion.

According to Equation (4), by performing a plot between ln (M_t/M_∞) and ln (t), a linear relationship is expected, from which the diffusion exponent, n, and the rate constant, k, are taken as the value of the slope and the anti-logarithmic value of the y-intercept, respectively. Based on such plots for the cumulative release of gallic acid from the gallic acid-loaded PLLA fiber mats in each of the three media, two linear regions were obtained. Only the initial region was used in the analyses to obtain the kinetics values. Table 3.2 summarizes the values of the kinetics parameters obtained and the range of the submersion time points used in the analyses. The diffusion exponent values signifying the mechanism for the release of gallic acid from the gallic acid-loaded PLLA fiber mats in the acetate buffer, the citrate-phosphate buffer and the normal saline are 0.45, 0.73 and 1.58, respectively, while the rate parameter values are 7.648×10^{-2} $min^{-0.45}$, 1.855×10^{-2} $min^{-0.73}$ and 6.657×10^{-4} $min^{-1.58}$, respectively. According to the values of n, the release of gallic acid from the gallic acid-loaded PLLA fiber mats in the acetate buffer occurred through the normal Fickian diffusion mechanism, while, in the citrate-phosphate buffer and the normal saline, it can be categorized as anomalous and Case II diffusion mechanisms, respectively.

3.4.5 Antioxidant Activity of Gallic Acid

The antioxidant activity of the as-loaded gallic acid in the gallic acid-loaded PLLA fiber mats was first determined by the DPPH assay. DPPH· is a stable free radical, exhibiting a characteristic UV absorption at 517 nm, and has been widely used to test the antioxidant activity of various substances. [29-31] Gallic acid, which acts as a donor of a hydrogen atom or an electron, can transform a DPPH radical into its reduced form DPPH·H, [32] as illustrated in Scheme 1. The antioxidant activity of the as-loaded galli acid in the gallic acid-loaded PLLA fiber mats was 85.4±1.3%. The result confirmed that the as-loaded gallic acid retains its free radical scavenging ability, despite its being subjected to a high electrical potential during the fabrication of the gallic acid-loaded fiber mats. As an affidavit of the potential for use of the gallic acid loaded PLLA fiber mats as an effective carrier for topical and/

or transdermal delivery of gallic acid, the antioxidant activity of gallic acid that had been released into each of the three releasing media was investigated. The released medium solutions were collected at four different time points, i.e., at 6, 12, 24, and 48 h, after the fiber mat specimens had been submerged in the media. The amounts of the as-released gallic acid ranged between 35.3 ± 2.1 and 77.2 ± 3.2% in the normal saline, 30.1 ± 3.4 and 53.4 ± 3.6% in the citrate-phosphate buffer, and 13.6 ± 0.5 and 19.9 ± 2.2% in the acetate buffer (see Figure 3.5a). Even though these released amounts were lower than those reported in Figure 3.4, a similar conclusion could still be made from the obtained results. In the releasing assay, the sample solutions were withdrawn at different time points and an equal amount of the fresh medium was immediately put in. In the antioxidant assay however, none of the fresh medium was put in. These two different methods would result in the different concentrations of gallic acid in the releasing medium solutions at an arbitrary time point, hence the difference in the concentration driving forces.

The antioxidant activity of the as-released gallic acid was finally assessed with the DPPH assay (see Figure 3.5b). The antioxidant activity of gallic acid that had been released into the normal saline ranged between 32.8 ± 5.6 and 78.7 ± 0.6%, while those of gallic acid that had been released into the citrate-phosphate and the acetate buffer solutions ranged between 19.6 ± 4.2 and 33.4 ± 3.4% and 17.0 ± 6.2 and 25.4 ± 3.5%, respectively. Apparently, the antioxidant activity of the as-released gallic acid is in excellent agreement with the amounts of the as-released gallic acid, as shown in Figure 3.5a, in that it was the greatest in the normal saline, followed by those in the citrate-phosphate and the acetate buffer solutions, respectively.

3.5 Conclusion

In the present contribution, gallic acid, widely known for its antioxidant, antiinflammatory, anticarcinogenic, antifungal, and antityrosinase activities, was added to the neat PLLA solution (10% w/v in 7:3 v/v DCM/DMF) at 40% w/w (based on the weight of PLLA powder). Both the neat and the gallic acid-containing PLLA solutions were fabricated into ultra-fine fibers by electrospinning under a fixed electric field of 20 kV/18 cm. The obtained fibers were smooth and no evidence of any kind of aggregate was observed on the surface of the gallic acid-

loaded PLLA fibers. The average diameters of the neat and the gallic acid-loaded PLLA fibers were 965 and 843 nm, respectively. The water retention behavior of the gallic acid-loaded PLLA fiber mats in the acetate buffer, the citrate-phosphate buffer, and the normal saline increased with an increase in the submersion time and the property value in the normal saline was the greatest, followed by those in the citrate-phosphate and the acetate buffer solutions, respectively. Almost all of gallic acid contained in the spinning solutions [i.e., 40% w/w (based on the weight of PLLA powder) or 28.57 wt.-% (based on the weight of the obtained electrospun fiber mats)] was retained within the obtained fibers (i.e., 86 to 95% on average, as determined in three different media). The cumulative amount of gallic acid released from the gallic acid-loaded PLLA fiber mats into each medium increased rapidly during the initial increase in the submersion time, while it increased more slowly as the submersion time increased further. In general agreement with the water retention results, the cumulative amount of gallic acid released into the normal saline was the greatest, followed by those released into the citrate-phosphate and the acetate buffer solutions, respectively. Lastly, the free radical scavenging ability of gallic acid released into each medium related strongly to its contents in the medium.

3.6 Acknowledgments

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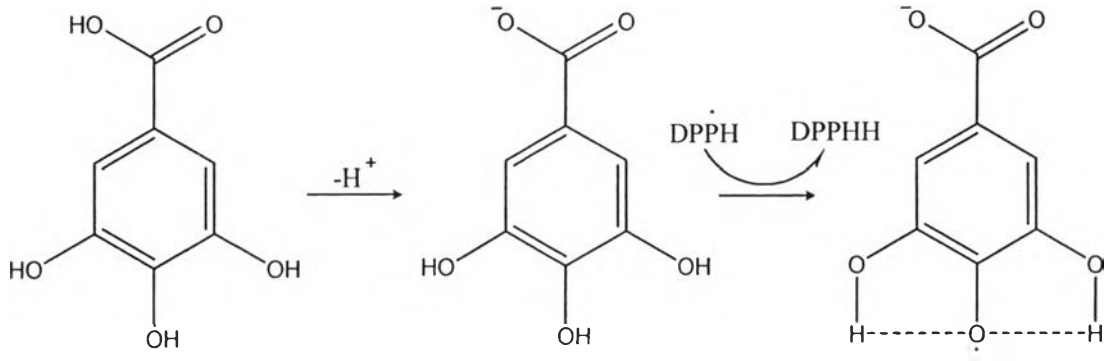

Scheme 3.1 DPPH radical-scavenging mechanism of gallic acid.

Table 3.1 Mechanical properties of neat and gallic acid-loaded electrospun PLLA fiber mats (n = 10)

Property	Neat PLLA fiber mats	Gallic acid-loaded PLLA fiber mats
Stress at maximum load (MPa)	1.9 ± 0.4	1.4 ± 0.2
Specific stress at maximum load ($MPa \cdot cm^3 \cdot g^{-1}$)	38.3±8.1	16.5±2.4
Strain at maximum load (%)	81 ± 4	41 ± 2
Tensile strength (MPa)	0.23 ± 0.04	0.18 ± 0.05
Specific tensile strength ($MPa \cdot cm^3 \cdot g^{-1}$)	4.64±0.80	2.13±0.59
Elongation at break (%)	82 ± 3	57 ± 4
Bulk density ($g \cdot cm^{-3}$)	0.0496±0.0057	0.0847±0.0117

Table 3.2 Values of kinetics parameters obtained from and the range of submersion time points used in the analyses for the releasing mechanisms of gallic acid from gallic acid-loaded electrospun PLLA fiber mats in three different types of releasing medium

Releasing medium	Range (min)	n	k (min-n)	r^2
Acetate buffer	10-30	0.45	7.648×10^{-2}	0.97
Citrate-phosphate buffer	10-50	0.73	1.855×10^{-2}	0.98
Normal saline	10-40	1.58	6.657×10^{-4}	0.99

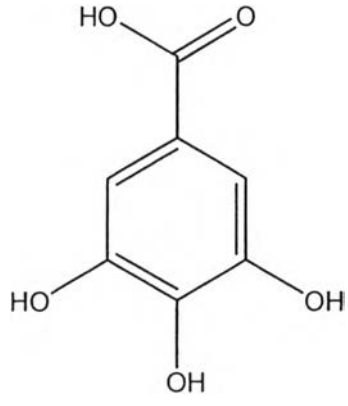

Figure 3.1 Chemical structure of gallic acid (3,4,5-trihydroxybenzoic acid).

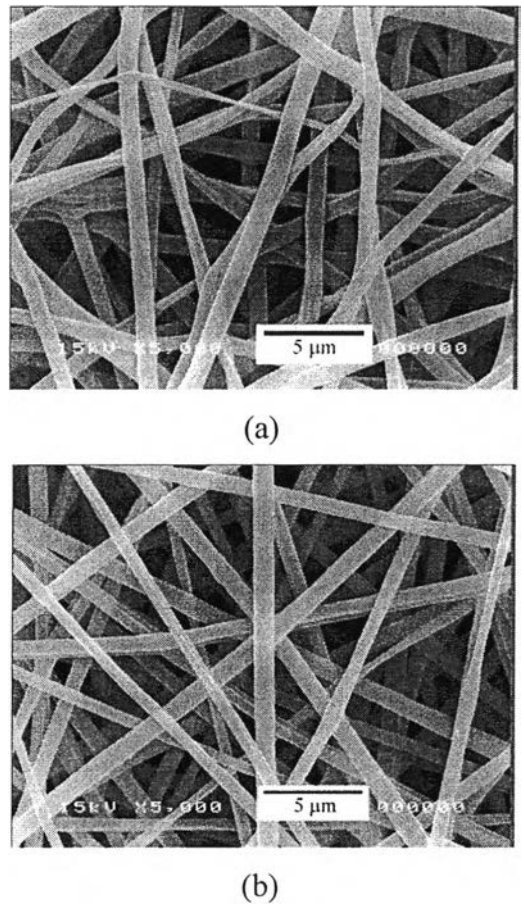

Figure 3.2 Representative scanning electron micrographs of (a) neat and (b) gallic acid-loaded electrospun PLLA fiber mats.

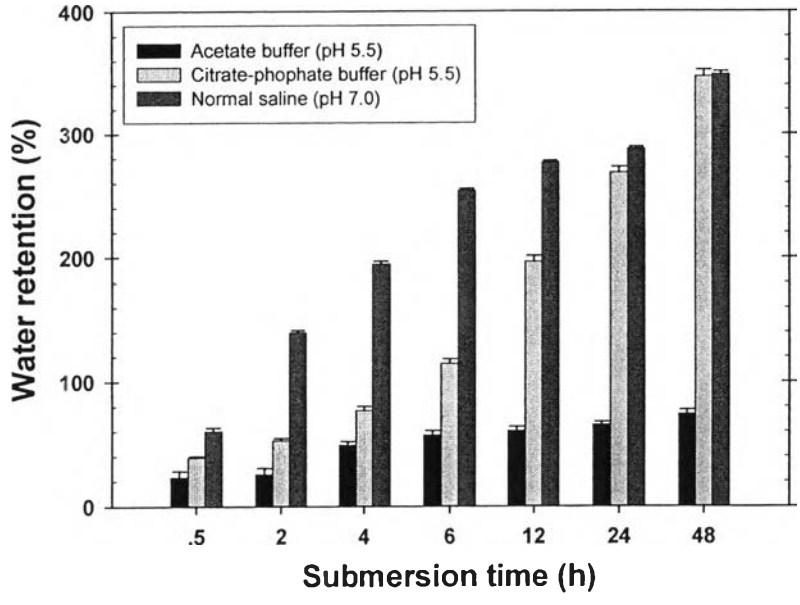

Figure 3.3 Water retention behavior of gallic acid-loaded electrospun PLLA fiber mats ($n = 3$).

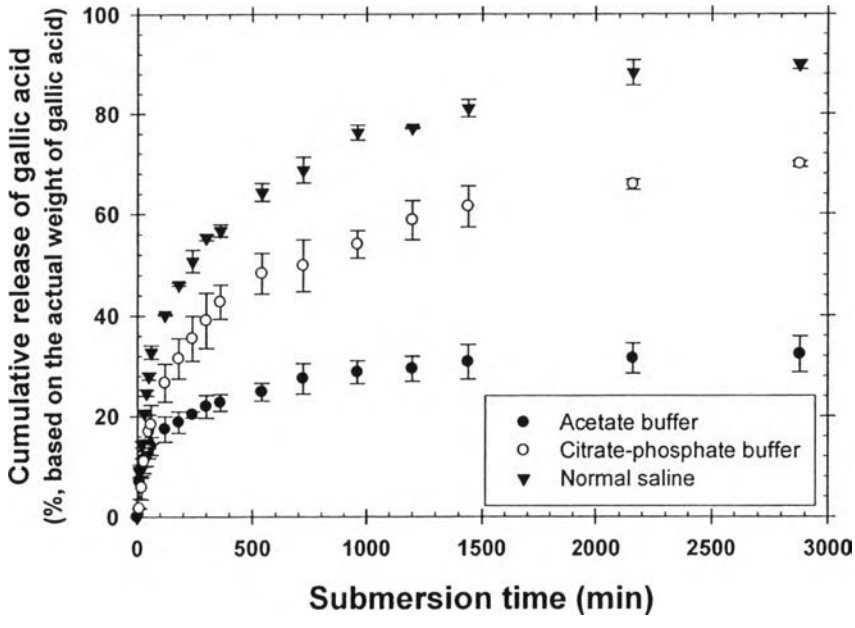

Figure 3.4 Cumulative release of gallic acid from gallic acid-loaded electrospun PLLA fiber mats, in terms of the percentage of the weight of gallic acid released divided by the actual weight of gallic acid in the specimens, as a function of submersion time in three different types of releasing medium, i.e., acetate buffer, citrate-phosphate buffer and normal saline, at skin temperature of 32 °C (n = 3).

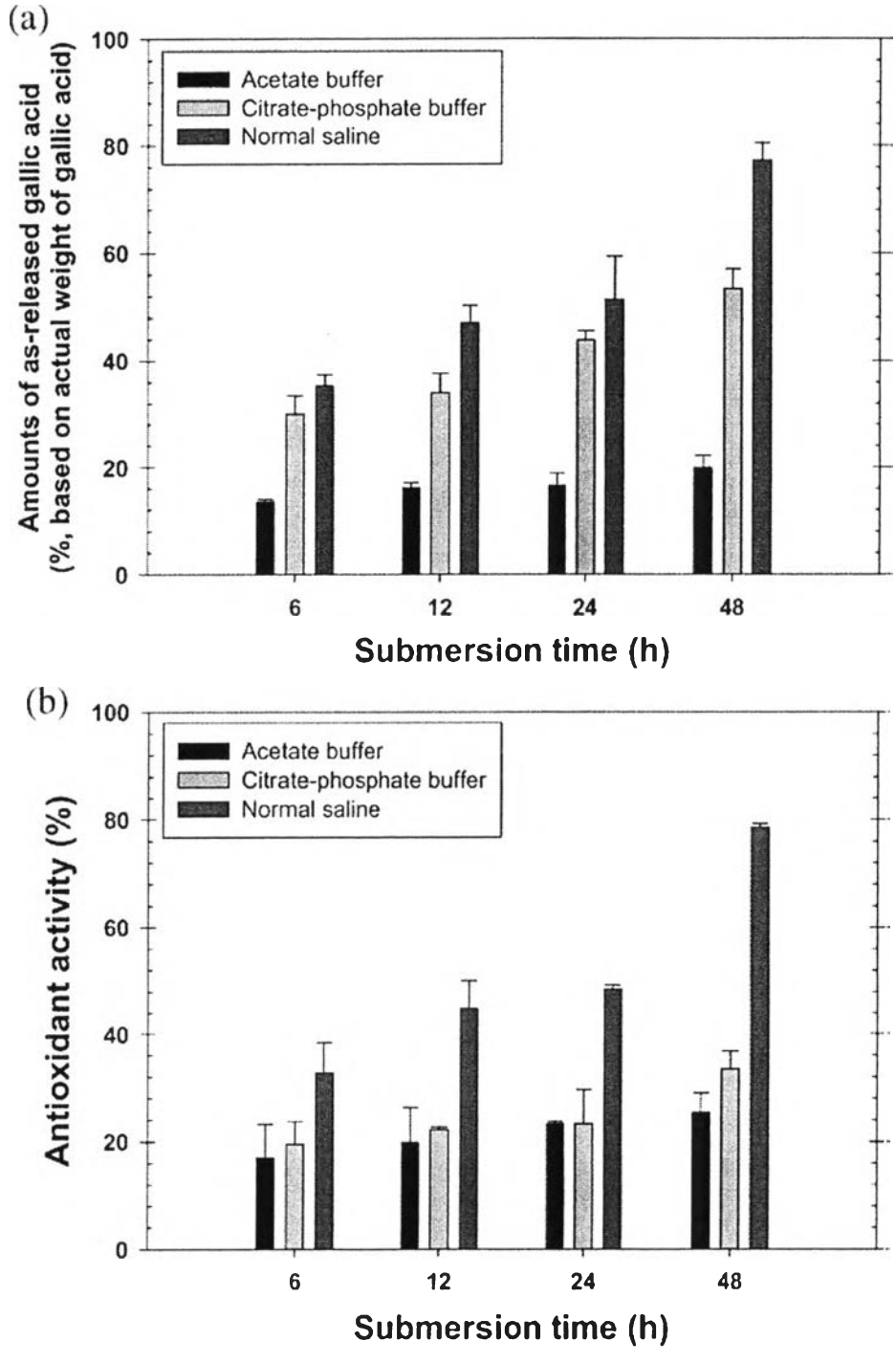

Figure 3.5 (a) Amounts of gallic acid that had been released into each of the three releasing media of acetate buffer, citrate phosphate buffer, and normal saline at skin temperature of 32 ^{0}C, reported in terms of the percentage of the weight of gallic acid released divided by the actual weight of gallic acid in the specimens (n=3), and (b) the antioxidant activity of the as released gallic acid (n=3).