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Electrospun cellulose acetate fiber mats containing curcumin and release characteristic of the herbal substance

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Abstract

Ultra-fine cellulose acetate (CA; $M_w \approx 30{,}000$ Da; degree of acetyl substitution ≈ 2.4) fiber mats containing curcumin from the plant *Curcuma longa* L., widely known for its anti-tumor, antioxidant, and anti-inflammatory properties, were fabricated, for the first time, from the neat CA solution (17% w/v in 2:1 v/v acetone/dimethylacetamide) containing curcumin in various amounts (i.e., 5–20 wt.% based on the weight of CA powder) by electrospinning. Incorporation of curcumin in the neat CA solution did not affect the morphology of the resulting fibers, as both the neat and the curcumin-loaded CA fibers were smooth. The average diameters of the curcumin-loaded CA fibers ranged between ~314 and ~340 nm. The integrity of the as-loaded curcumin in the curcumin-loaded CA fiber mats was intact as indicated by the ^{1}H nuclear magnetic resonance spectrometric results and the ability of the as-loaded curcumin in maintaining its free radical scavenging ability. Investigation of the release characteristic of curcumin from the curcumin-loaded CA fiber mats was carried out by the total immersion and the transdermal diffusion through a pig skin method in the acetate buffer solution containing Tween 80 and methanol or the B/T/M medium at 37 °C. In the total immersion method, almost all of the curcumin loaded in the curcumin-loaded CA fiber mat specimens was released into the medium (~90 to ~95%), while considerably lower values were obtained when the curcumin-loaded CA fiber mats were placed on top of a piece of pig skin. Lastly, the curcumin-loaded CA fiber mats were proven non-toxic to normal human dermal fibroblasts.

Keywords: Topical/transdermal drug delivery; Electrospinning; Cellulose acetate

1. Introduction

In recent years, much interest has been paid on fabricating ultra-fine fibers by a process commonly known as electrospinning (e-spinning). Due to the high surface area to volume or mass ratio of the obtained fibers, the potential for use of these fibrous materials in biomedical applications is in areas such as wound healing [1,2], tissue engineering [3–5], and drug delivery [6–10]. This process involves the application of a strong electrical potential to the end of a capillary containing a polymer liquid (i.e., solution or melt), causing an accumulation of charges on the surface of the liquid. When the voltage reaches a critical value where the Coulombic repulsion of the charges overcomes the surface tension of the polymer droplet at the tip of the capillary, a charged jet is ejected. Acceleration through the electric field causes the charged jet to thin down. Finally, ultra-fine fibers are collected on a grounded electrode, due to the evaporation or the cooling of the charged jet [11]. One of the advantages of the e-spinning process over the conventional film-casting technique is the highly porous nature of the electrospun (e-spun) fiber mats which exhibit much greater surface area that assumingly could allow drug molecules to diffuse out from the matrix much more conveniently [6,12], when these fibrous materials are used as carriers for delivery of drugs.

Cellulose acetate (CA) is the acetate ester of cellulose, the primary structural component of the cell wall of green plants

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and is one of the most common biopolymers on earth [13]. Cellulose acetate is manufactured by reacting cellulose with acetic anhydride using sulfuric acid as a catalyst. Liu and Hsieh [14] reported the preparation of ultra-fine CA fiber mats as well as regenerated cellulose membranes by e-spinning. They found that the most suitable solvent system for preparing the CA solutions for e-spinning was 2:1 v/v acetone/dimethylacetamide (DMAc), as this mixture 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 μm [14]. E-spinning of CA fibers from CA solutions in a new solvent system of acetone/water mixtures with the water content ranging between 10 and 15 wt.% and the deacetylation of the resulting CA fiber mats were investigated by Son et al. [15]. The average diameter of the obtained CA fibers was ~2 μm, with thinner fibers (~0.5 μm) being produced from basic solutions [15].

E-spun CA fiber mats have been explored as affinity membranes [16] antimicrobial membranes [17], three-dimensional (3D) structures resembling the urinary bladder matrix (UBM) [18], and drug-delivery membranes [9,10]. The affinity CA fibrous membranes were prepared from a CA solution in 3:1:1 v/v/v acetone/dimethylformamide (DMF)/trifluoroethylene (TFE) by e-spinning [16]. The membranes were subsequently heat-treated and later treated in a NaOH solution to obtain regenerated cellulose (RC) membranes, in which Cibacron Blue F3GA, a sulfonated triazine dye, was coupled on their surface [16]. The antimicrobial CA fibrous membranes were prepared from a CA solution in 80:20 w/w acetone/water containing $AgNO_3$ by e-spinning [17]. Ag^+ ions were photoreduced into Ag nanoparticles by irradiating the e-spun fibers with UV light [17]. The 3D structures resembling the UBM were fabricated from CA solutions in acetone by e-spinning under various solution and processing conditions [18]. Lastly, e-spun CA fiber mats were used as carriers for transdermal or topical delivery of model vitamins, i.e., *all-trans* retinoic acid or vitamin A acid (Retin-A) and α-tocopherol or vitamin E (Vit-E) [9], and four different types of model drugs, i.e., naproxen (NAP), indomethacin (IND), ibuprofen (IBU), and sulindac (SUL) [10]. CA solutions in 2:1 v/v acetone/DMAc were used as the base spinning solutions into which Retin-A and Vit-E in the amount of 0.5 and 5 wt.% (based on the weight of CA), respectively [9], and NAP, IND, IBU, and SUL in the amount of 20 wt.% (based on the weight of CA) were added [10].

Curcumin (see chemical structure in Fig. 1) is a naturally-occurring compound found in the plant *Curcuma longa* L. Its major constituents are curcuminoids, which are polyphenols normally existing in at least two tautomeric forms, keto and enol. The enol form is more energetically stable, both in the solid phase and in solution [19]. Curcumin is widely known for its anti-tumor, antioxidant, and anti-inflammatory properties [20–23]. It can enhance cutaneous wound healing in rats and guinea pigs. Sidhu et al. [24] have evaluated the efficacy of curcumin treatment by oral and topical applications on impaired wound healing in diabetic rats and genetically-diabetic mice using a full-thickness cutaneous punch wound model. Wounds of the animals treated with curcumin showed early re-epithelialization, improved neovascularization, increased migratory activity of various cells including dermal myofibroblasts, fibroblasts, and macrophages into the wound bed, and a higher collagen content [24]. Gopinath et al. [25] have incorporated curcumin into a collagen matrix. They found that the presence of curcumin helped to increase wound reduction, enhance cell proliferation, and provide efficient free radical scavenging activity [25].

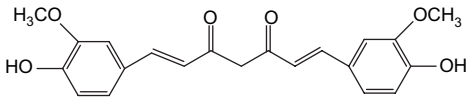


Fig. 1. Chemical structure of curcumin (keto form).

In the present contribution, curcumin was loaded into a CA solution which was later fabricated into ultra-fine fibers by e-spinning. The curcumin-loaded e-spun CA fiber mats were assessed for their potential use as carriers for topical or transdermal delivery of curcumin. Various properties (i.e., morphological, mechanical, swelling and weight loss, and cytotoxicity properties) of both the neat and the curcumin-loaded e-spun CA fiber mats as well as the release characteristic of curcumin from the curcumin-loaded e-spun CA fiber mats were investigated. Comparisons were made against the corresponding solvent-cast films. Both the chemical integrity and the antioxidant activity of the as-loaded curcumin in the curcumin-loaded e-spun CA fiber mats were also investigated.

2. Experimental details

2.1. 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). Curcumin (≥95.0% purity) was purchased from Fluka (Switzerland). Acetone (Carlo Erba, Italy), *N*,*N*-dimethylacetamide [DMAc, Labscan (Asia), Thailand], sodium acetate (Ajax Chemicals, Australia), and glacial acetic acid (Carlo Erba, Italy) were of analytical reagent grade and used without further purification.

2.2. Preparation of neat and curcumin-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. Curcumin-loaded CA solutions were prepared by dissolving curcumin powder in the amounts of 5, 10, 15, and 20 wt.% based on the weight of CA powder in the base CA solution. 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. All experiments were carried out at 25 °C. These mixtures were then e-spun under a fixed electric field of 17.5 kV/15 cm. The feeding rate of the solutions was controlled at

~ 1 ml h^{-1} by means of a Kd Scientific syringe pump. Unless otherwise noted, the collection time was ~ 18 h (resulting in the fiber mats of 90 ± 10 μm in thickness). For comparison purposes, both the neat and the curcumin-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 base CA solution that contained varying amounts of curcumin (5, 10, 15 and 20 wt.%). Unless otherwise noted, the thickness of the as-cast films was 90 ± 10 μm.

2.3. Characterization of neat and curcumin-loaded CA fiber mats and films

Morphological appearance of both the neat and the curcumin-loaded e-spun CA fiber mats and as-cast CA 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 observation under SEM. Diameters of the e-spun fibers were measured directly from SEM images using a SemAphore 4.0 software. A Bruker DRX400 ^{1}H nuclear magnetic resonance spectrometer (^{1}H NMR) was used to investigate the chemical integrity of curcumin in the curcumin-loaded e-spun fiber mat samples (2–3 mg), using deuterated dimethylsulfoxide (DMSO-d_6) as solvent. 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 curcumin-load e-spun CA fiber mats were tested on a Lloyd LRX universal testing machine (gauge length = 50 mm and crosshead speed = 20 mm min^{-1}). The specimens of $\sim 100 \pm 10$ μm in thickness (i.e., the collection time was ~ 24 h) were cut into a rectangular shape (10 mm × 100 mm).

The swelling and the weight loss behavior of both the neat and the curcumin-loaded e-spun CA fiber mats and as-cast films were measured in an acetate buffer solution (see below for the preparation of the acetate buffer solution) containing 0.5% v/v polysorbate 80 (hereafter, Tween 80) and 3% v/v methanol (hereafter, the B/T/M medium) at the physiological temperature of 37 °C for 48 h according to the following equations:

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

and

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

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

2.4. Release of curcumin from curcumin-loaded CA fiber mats and films

2.4.1. Preparation of acetate buffer

Acetate buffer was chosen to simulate the human skin pH condition of 5.5. 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 sodium acetate aqueous solution. Finally, distilled water was added into the solution to fill the volume.

2.4.2. Actual curcumin content

The actual amount of curcumin in the curcumin-loaded e-spun CA fiber mats and as-cast CA films was determined. Each specimen (circular disc; ~ 2.8 cm in diameter) was dissolved in 4 ml of 2:1 v/v acetone/dimethylacetamide (DMAc). After that, 0.5 ml of the solution was added into 8 ml of the acetate buffer solution and the actual amount of curcumin was measured by a Shimadzu UV-2550 UV–vis spectrophotometer at the wavelength of 426 nm. The actual amount of curcumin in the curcumin-loaded CA fiber mat and film samples was back-calculated from the obtained data against a predetermined calibration curve for curcumin.

2.4.3. Curcumin-release assay

The release characteristic of curcumin from the curcumin-loaded e-spun CA fiber mats and as-cast CA films was investigated by two types of the release assay, i.e., total immersion and transdermal diffusion through a pig skin method. Due to the solubility limitation of curcumin in the acetate buffer solution, the B/T/M releasing medium (96.5% v/v acetate buffer with 0.5% v/v Tween 80 and 3% v/v methanol) was used. Each specimen (circular disc; ~ 2.8 cm in diameter) was immersed in 30 ml of the medium at the physiological temperature of 37 °C. At a specified immersion or diffusion period ranging between 0 and 48 h (2880 min), either 1 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 curcumin-loaded fiber mat and film 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 curcumin in the sample solutions was determined using the UV–vis spectrophotometer at the wavelength of 426 nm. The obtained data were calculated to determine the cumulative amount of curcumin released from the specimens at each immersion or diffusion time point. The experiments were carried out in triplicate and the results were reported as average values.

2.5. Antioxidant activity

The antioxidant activity of curcumin loaded in both the curcumin-loaded e-spun CA fiber mats and as-cast CA films was

assessed with 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radicals, following the method of Blois [26]. Each specimen (circular disc; ~2.8 cm in diameter) was first dissolved in 4 ml of 2:1 v/v acetone/dimethylacetamide (DMAc) and treated with a methanolic solution of DPPH (100 μM) for 30 min at the physiological temperature of 37 °C. The free radical scavenging activity was determined photometrically in a microplate reader (Universal Microplate Analyzer, Model AOPUS01 and AI53601; Packard BioScience, USA) and the absorbance was measured at the wavelength of 550 nm. The antioxidant activity (%AA) of the as-loaded curcumin 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 curcumin), according to the following equation:

$$\%\mathrm{AA} = \frac{\left(A_{\mathrm{control}} - A_{\mathrm{sample}}\right)}{A_{\mathrm{control}}} \times 100, \quad (3)$$

where A_{control} and A_{sample} are the absorbance values of the testing solution without and with the presence of the as-loaded curcumin, respectively.

2.6. Indirect cytotoxicity evaluation

The indirect cytoxicity evaluation of both the neat and the curcumin-loaded e-spun CA fiber mats and as-cast CA films was conducted in adaptation from the ISO 10993-5 standard test method in a 96-well tissue-culture polystyrene plate (TCPS; Biokom Systems, Poland) using normal human dermal fibroblasts (NHDF; fourth passage) as reference. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, USA), supplemented by 10% fetal bovine serum (FBS; BIOCHROM AG, Germany), 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 and ~75 ± 5 μm in thickness) were sterilized by UV radiation for ~1 h and then were placed in wells of TCPS. Extraction media were prepared by immersing specimens in 150 μl of serum-free medium (SFM; containing DMEM, 1% L-glutamine, 1% lactabumin, and 1% antibiotic and antimycotic formulation) for one day. NHDF cells were separately cultured in wells of TCPS at 10,000 cells/well in serum-containing DMEM for 16 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 medium 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 μl/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 μl/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.

2.7. Statistical analysis

Data were presented as means ± 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.

3. Results and discussion

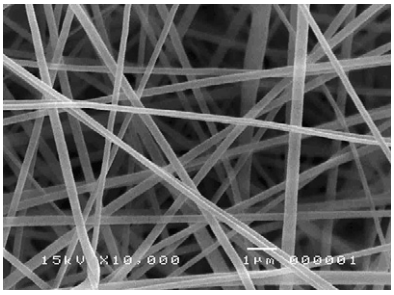
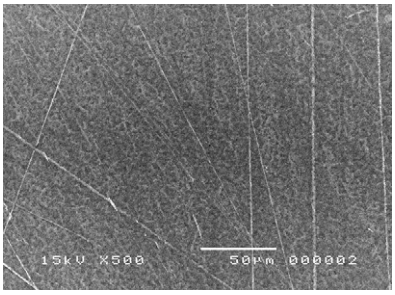
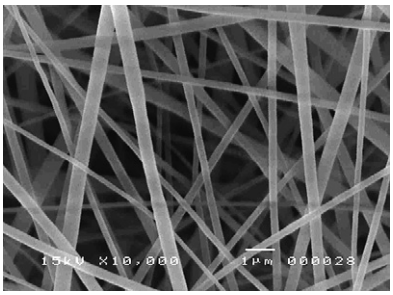
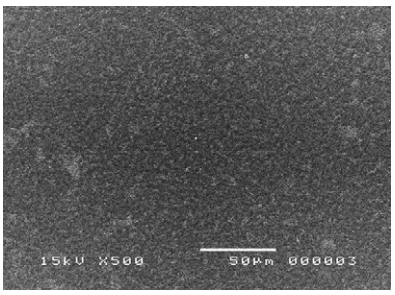
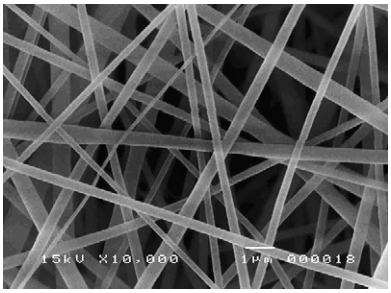
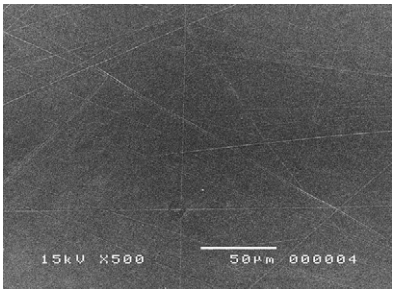
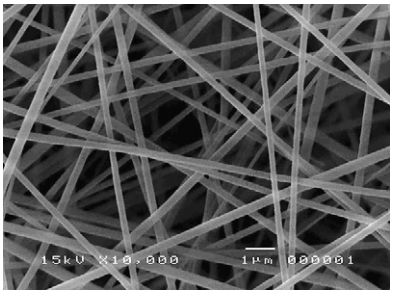
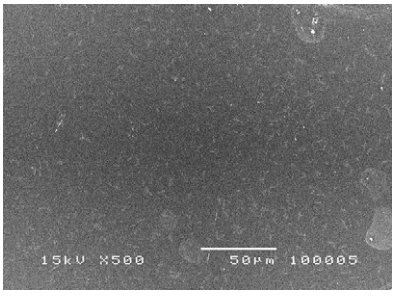
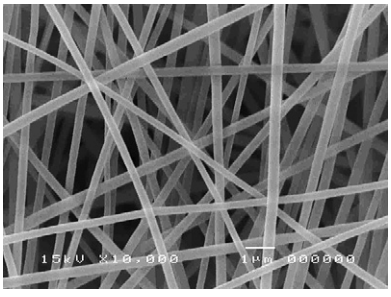
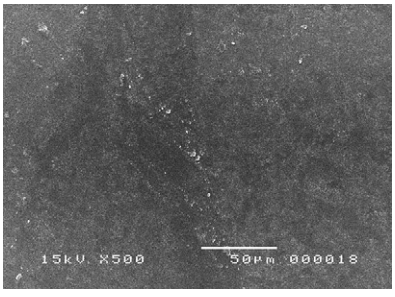
3.1. E-spinning of neat and curcumin-loaded CA solutions

Prior to e-spinning, both the neat and the curcumin-loaded CA solutions were measured for their shear viscosity and the conductivity, and the results are summarized in Table 1. The presence of curcumin in the base CA solution was responsible for the observed increase in the property values with increasing the curcumin content. 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 2. Clearly, cross-sectionally round fibers were obtained and no presence of any kind of curcumin aggregates was observed on the surface of these fibers, implying that the as-loaded curcumin was perfectly incorporated well within the fibers. The diameters of the neat CA fibers were ~300 ± 64 nm, while those of the curcumin-loaded CA fibers ranged between ~314 ± 60 and 340 ± 98 nm with no particular dependency on the initial amount of the as-loaded curcumin (see Table 1). Taepaiboon et al. [9] showed that incorporation of either Retin-A or Vit-E in the e-spun CA fibers did not affect their morphology, as the surface of the vitamin-loaded CA fibers was also smooth, and the average diameters of both the neat and the vitamin-loaded CA fibers ranged between 247 and 265 nm. Moreover, Tungprapa et al. [10] reported that the presence of NAP, IND, IBU, and SUL

Table 1
Shear viscosity and electrical conductivity of neat and curcumin-containing CA solutions ($n = 3$) as well as diameters of the individual fibers within the resulting electrospun fiber mats ($n \approx 100$)

Type of CA solution	Shear viscosity (mPa s)	Electrical conductivity (μS cm^{-1})	Fiber diameters (nm)
Neat	419 ± 1	8.31 ± 0.01	301 ± 64
With 5 wt.% curcumin	430 ± 1	8.53 ± 0.01	340 ± 98
With 10 wt.% curcumin	437 ± 1	8.89 ± 0.02	338 ± 85
With 15 wt.% curcumin	446 ± 1	9.31 ± 0.01	334 ± 49
With 20 wt.% curcumin	460 ± 1	9.80 ± 0.01	314 ± 60

Table 2
Selected scanning electron micrographs of neat and curcumin-loaded electrospun CA fiber mats and solvent-cast CA films at various curcumin contents

Curcumin content in CA solution (wt.%)	Materials	
	Curcumin-loaded electrospun CA fiber mats	Curcumin-loaded solvent-cast CA films
0		
5		
10		
15		
20		

Note: applied electric field = 17.5 kV/15 cm and the collection time = 18 h.

in the e-spun CA fibers also did not affect their morphology and the average diameters of both the neat and the drug-loaded CA fibers were in the range of 231–297 nm. Apparently, the results obtained in this work agreed well with these previous reports.

For comparison, both the neat and the curcumin-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 2. Evidently, the surface of the as-cast films was relatively smooth, indicating that the as-loaded curcumin was incorporated well within the films, as in the case of the curcumin-loaded CA fibers.

3.2. Chemical integrity of curcumin in curcumin-loaded CA fiber mats

Due to the application of a high electrical potential to the curcumin-containing CA solutions during e-spinning, it is questionable whether the chemical integrity of curcumin would be intact after such a treatment. To verify that, the curcumin-loaded e-spun CA fiber mats were dissolved in DMSO-d_6 and the resulting solutions were investigated by ^{1}H NMR. Solutions of both the neat e-spun CA fiber mat and curcumin in DMSO-d_6 were used as references. Fig. 2 shows ^{1}H NMR spectra of the as-received curcumin and the e-spun fiber mats that were obtained from both the base CA solution and the solution containing 5 wt.% curcumin. Evidently, the chemical integrity of the as-loaded curcumin was sustained after e-spinning, as the peaks corresponding to both CA and curcumin were observed in the ^{1}H NMR spectrum of the e-spun fiber mat that was obtained from the CA solution containing 5 wt.% curcumin. Though not shown, similar results were obtained for the ^{1}H NMR spectra of the e-spun fiber mats that were obtained from the CA solutions containing 10–20 wt.% curcumin, with the main difference being the observed increase in the intensity of the peaks corresponding to the as-loaded curcumin with increasing the amount of curcumin loaded in the solutions.

3.3. Mechanical integrity of neat and curcumin-loaded CA fiber mats and films

The mechanical properties in terms of the stress at maximum load, the strain at maximum load, the tensile strength, and the elongation at break of both the neat and the curcumin-loaded e-spun CA fiber mats were investigated and the results are summarized in Table 3. The stress at maximum load and the tensile strength for the neat e-spun CA fiber mats were 1.22 ± 0.01 and 0.15 ± 0.01 MPa, respectively, while the strain at maximum load and the elongation at break for the neat e-spun CA fiber mats were 18.1 ± 0.9 and 22.6 ± 0.3%, respectively. The initial addition of curcumin (i.e., at 5 wt.%) in the base CA solution caused both the stress at maximum load and the tensile strength of the resulting curcumin-loaded e-spun CA fiber mats to increase from that of the neat materials. With further increase in the curcumin content between 5 and 15 wt.% in the base CA solution, the property values of the obtained curcumin-loaded fiber mats increased monotonically with increase in the curcumin content to reach maximum values at the curcumin content of 15 wt.%. Further increase in the curcumin content to 20 wt.%, a decrease in the property values of the curcumin-loaded fiber mats was observed. On the other hand, both the strain at maximum load and the elongation at break of the fiber mats that were e-spun from the CA solutions containing 5–15 wt.% curcumin ranged between 15.8 ± 1.9 and 20.4 ± 1.2% and 21.4 ± 0.6 and 23.0 ± 0.4%, respectively. Only the fiber mats from the CA solutions containing 20 wt.% curcumin showed a significant decrease in the property values.

3.4. Swelling and weight loss behavior of neat and curcumin-loaded CA fiber mats and films

The neat and the curcumin-loaded e-spun CA fiber mats and corresponding as-cast films were further characterized to determine their swelling ability after submersion in the B/T/M medium at 37 °C for 48 h (see Fig. 3). The property value of the neat fiber mats was ~370%. A much greater value of 715% (in acetate buffer at 37 °C for 24 h) was reported by Tungprapa et al. [10] (for the neat CA fiber mats that were 20–35 μm in thickness with the average diameter of the individual fibers being ~230 nm). In comparison with that of the neat materials, the swelling ability of the e-spun fiber mats prepared from the CA solutions containing 5 and 10 wt.% curcumin was slightly lower (i.e., ~340 and ~355%, respectively), while that of the e-spun fiber mats prepared from the solutions containing 15 and 20 wt.% curcumin was essentially the same. On the other hand, the swelling ability of the corresponding solvent-cast films was much lower when compared with that of the e-spun fiber mats, with the property values of all of the film samples ranging between ~22 and ~26%.

The loss in the weight of the neat and the curcumin-loaded e-spun CA fiber mats and corresponding as-cast films after submersion in the B/T/M medium at 37 °C for 48 h was also investigated and the results are graphically shown in Fig. 3. Apparently, all of the fiber mat samples exhibited much greater weight loss in the testing medium than the corresponding film counterparts. Specifically, the property value of the neat fiber mats was ~13.5%, while that of the neat films was much lower at ~3.7%. Much lower values for the neat fiber mat and film samples (i.e., ~1.1 and ~0.6%, respectively) were reported by Tungprapa et al. [10]. The reason for the much greater weight loss of the materials observed in this work than that reported previously [10] is due possibly to the presence of methanol that enhances solubility of the materials in the testing medium. For the curcumin-loaded fiber mat samples, the property values ranged between 8.3 and 15.3%, while, for the curcumin-loaded film counterparts, the property values ranged between 3.8 and 5.0%. Again, the loss in the weight of both the curcumin-loaded fiber mats and films was found to increase with increasing the curcumin content in the base CA solutions.

Comparatively, all of the e-spun CA fiber mat samples exhibited much greater swelling and weight loss in the testing

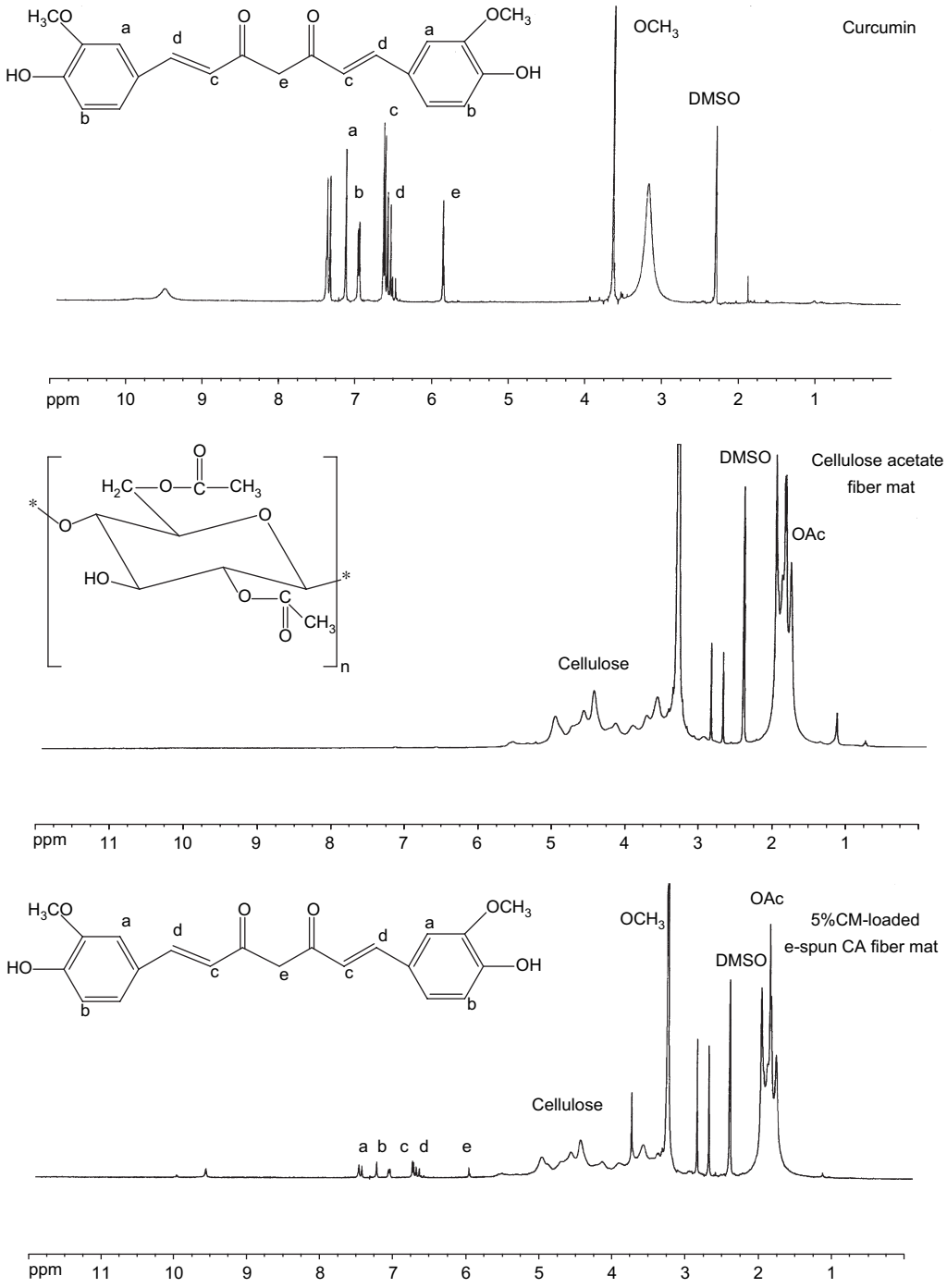


Fig. 2. ^{1}H nuclear magnetic resonance spectra of curcumin and electrospun CA fiber mats from the base CA solution and the solution containing 5% curcumin after being dissolved in DMSO-d_6. The spectra of the electrospun CA fiber mats from the solutions containing 10–20% curcumin were similar to that of the electrospun CA fiber mat from the solution that contained 5% curcumin.

Table 3
Mechanical integrity of neat and curcumin-loaded electrospun CA fiber mats ($n = 5$)

Type of electrospun CA fiber mats	Stress at maximum load (MPa)	Strain at maximum load (%)	Tensile strength (MPa)	Elongation at break (%)
Neat	1.22 ± 0.01	18.1 ± 0.9	0.15 ± 0.01	22.6 ± 0.3
With 5 wt.% curcumin	1.35 ± 0.09	15.8 ± 1.9	0.20 ± 0.04	21.4 ± 0.6
With 10 wt.% curcumin	1.63 ± 0.02	20.9 ± 2.0	0.28 ± 0.01	21.5 ± 0.3
With 15 wt.% curcumin	2.56 ± 0.09	20.4 ± 1.2	0.33 ± 0.02	23.0 ± 0.4
With 20 wt.% curcumin	1.78 ± 0.18	10.1 ± 1.4	0.22 ± 0.02	20.0 ± 2.5

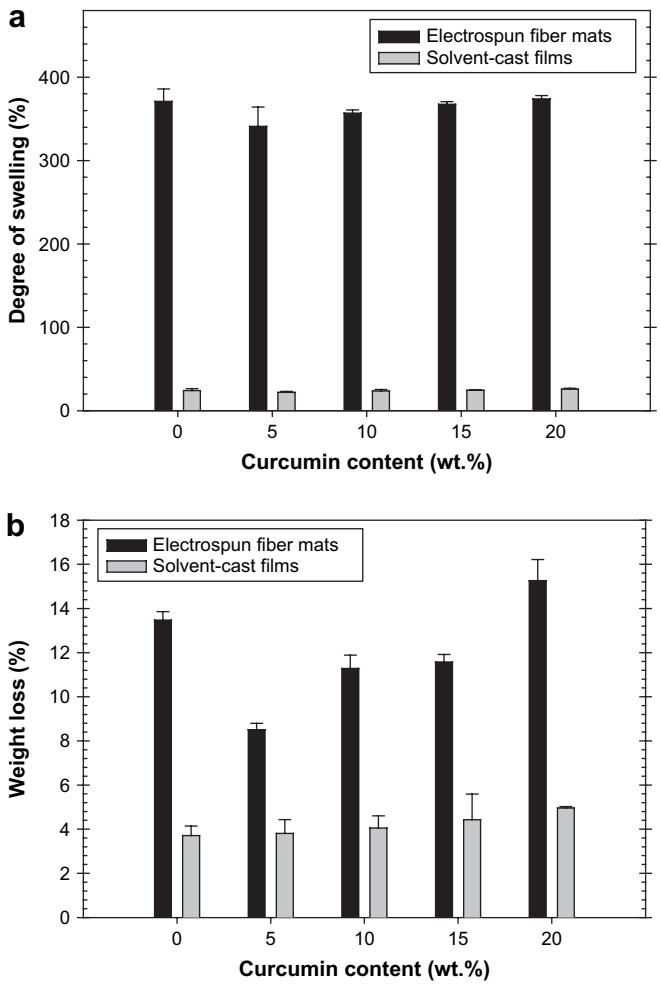


Fig. 3. (a) Degree of swelling and (b) weight loss of neat and curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films ($n = 3$).

medium than the corresponding film counterparts. This could be a result of the highly porous nature of the e-spun fiber mats that provides much greater surface area per unit volume or mass of the materials than the dense structure of the corresponding as-cast films. Though not shown, the physical integrity of the neat and the drug-loaded e-spun CA fiber mats was retained after submersion in the B/T/M medium at 37 °C for 48 h.

3.5. Release of curcumin from curcumin-loaded CA fiber mats and films

The actual amount of curcumin in the curcumin-loaded e-spun CA fiber mats and corresponding as-cast CA films needed to be determined prior to investigating the release characteristic of curcumin from these materials. Table 4 summarizes the actual amount of curcumin in these samples (reported as the percentage of the initial content of curcumin contained in both the spinning and the casting solutions). Evidently, the actual amount of curcumin in the curcumin-loaded fiber mat samples ranged between ~91 and ~102% (based on the weight of curcumin loaded in the solutions), while that in the curcumin-loaded film counterparts ranged between ~91 and ~95%. The discrepancy from the ideal value of 100% for these samples could be due to the inhomogeneous distribution of curcumin in different areas of the samples, which could be influenced by the fabrication techniques (e.g., the change in the local composition of the solutions during e-spinning and solvent-casting). These values were used as basis to arrive at the cumulative release of curcumin from these curcumin-loaded materials.

Table 4
Actual amount of curcumin incorporated in curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films ($n = 3$)

Initial amount of curcumin in either spinning or casting solution (wt.%)	Actual amount of curcumin based on the initial amount of curcumin loaded (%)	
	Curcumin-loaded electrospun CA fiber mats	Curcumin-loaded solvent-cast CA films
5	101.9 ± 0.8	90.8 ± 3.7
10	95.6 ± 2.5	93.6 ± 1.5
15	91.4 ± 0.4	93.9 ± 1.2
20	90.8 ± 0.4	94.9 ± 0.3

The release characteristic of curcumin from the curcumin-loaded e-spun CA fiber mats and corresponding as-cast CA films was carried out by the total immersion and the transdermal diffusion through a pig skin method. Both experiments were carried out using the acetate buffer solution containing 0.5% v/v Tween 80 and 3% v/v methanol (i.e., the B/T/M medium) at 37 °C. Previously, Taepaiboon et al. [9] used a B/T/M medium to study the release characteristics of Retin-A and Vit-E from the vitamin-loaded e-spun CA fiber mats and corresponding as-cast CA films. Here, the cumulative release profiles of curcumin from the curcumin-loaded fiber mats and films were reported in two different manners, i.e., as the percentage of the weight of curcumin released divided by the actual weight of the specimens and as the percentage of the weight of curcumin released divided by the actual weight of curcumin in the specimens.

In the total immersion method (see Fig. 4), when reported as the percentage of the weight of curcumin released divided by the actual weight of the specimens, both the curcumin-loaded fiber mat and film specimens showed a gradual increase in the amount of curcumin released from these materials. As expected, the maximum amount of curcumin released from these materials increased with increasing the initial amount of curcumin loaded in the spinning or the casting CA solutions. Specifically, for the fiber mats that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~4.4, ~8.0, ~11.0, and ~14.1% (i.e., mg of curcumin/mg of specimen × 100), respectively. Comparatively, much lower values were observed for the corresponding films, in which, for the films that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~0.1, ~0.3, ~0.5, and ~1.6%, respectively.

When reported as the percentage of the weight of curcumin released divided by the actual weight of curcumin in the specimens, both the curcumin-loaded fiber mat and film specimens

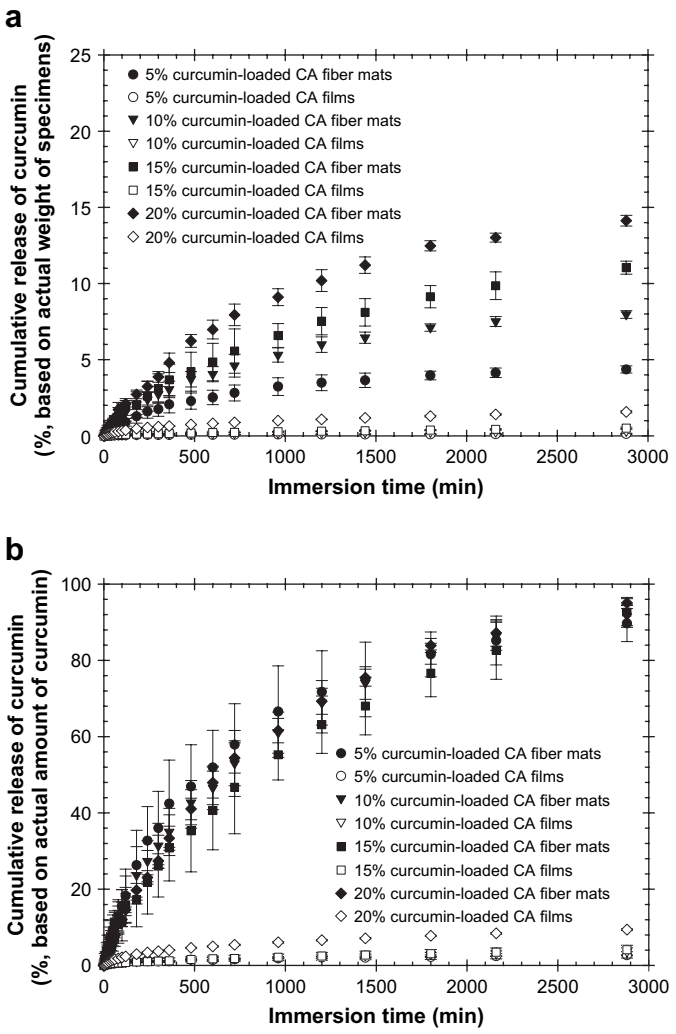


Fig. 4. Cumulative release profiles of curcumin from curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films reported as (a) the percentage of the weight of curcumin released divided by the actual weight of the specimens and (b) the percentage of the weight of curcumin released divided by the actual weight of curcumin present in the specimens by total immersion method in the B/T/M releasing medium (96.5% v/v acetate buffer with 0.5% v/v Tween 80 and 3% v/v methanol) at the physiological temperature of 37 °C ($n = 3$).

also showed a gradual increase in the amount of curcumin released from these materials and the maximum amount of curcumin released from these materials was also found to increase with increasing the initial amount of curcumin loaded in the spinning or the casting CA solutions. Specifically, for the fiber mats that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~89.7, ~91.6, ~92.6, and ~95.1%, respectively. Similarly, much lower values were also observed for the corresponding films, in which, for the films that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~2.7, ~3.6, ~4.1, and ~9.4%, respectively.

The results showed that almost all of the curcumin loaded in the curcumin-loaded fiber mat specimens was released into the medium, while only small amount of curcumin in the curcumin-loaded film counterparts was. The fact that the percentage of the weight of curcumin released from the curcumin-loaded fiber mat specimens was greater than that from the curcumin-loaded film counterparts could be due to a number of factors, e.g., the observed greater degree of swelling and the percentage of weight loss of all of the curcumin-loaded fiber mat specimens over those of the curcumin-loaded film counterparts and the hypothetically greater surface area of the curcumin-loaded fiber mat specimens over that of the curcumin-loaded film counterparts.

In the transdermal diffusion through a pig skin method (see Fig. 5), when reported as the percentage of the weight of curcumin released divided by the actual weight of the specimens, both the curcumin-loaded fiber mat and film specimens showed an initial burst release of curcumin, followed by

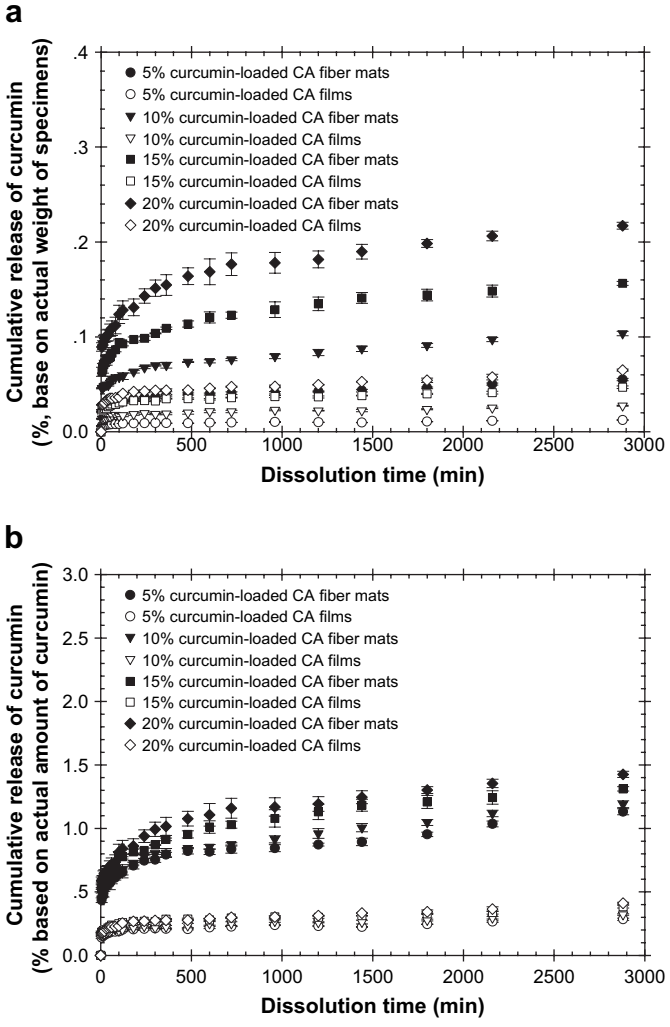


Fig. 5. Cumulative release profiles of curcumin from curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films reported as (a) the percentage of the weight of curcumin released divided by the actual weight of the specimens and (b) the percentage of the weight of curcumin released divided by the actual weight of curcumin present in the specimens by transdermal diffusion through a pig skin method in the B/T/M releasing medium (96.5% v/v acetate buffer with 0.5% v/v Tween 80 and 3% v/v methanol) at the physiological temperature of 37 °C ($n = 3$).

a gradual increase in the amount of the substance released from these materials. In a similar manner to what was observed by the total immersion method, the maximum amount of curcumin released from these materials also increased with increasing the initial amount of curcumin loaded in the spinning or the casting CA solutions. Specifically, for the fiber mats that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~0.05, ~0.10, ~0.16, and ~0.22%, respectively. Again, much lower values were observed for the corresponding films, in which, for the films that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~0.01, ~0.03, ~0.05, and ~0.07%, respectively.

When reported as the percentage of the weight of curcumin released divided by the actual weight of curcumin in the specimens, both the curcumin-loaded fiber mat and film specimens showed an initial burst release of curcumin, followed by a gradual increase in the amount of the substance released from these materials. In addition, the maximum amount of curcumin released from these materials also increased with increasing the initial amount of curcumin loaded in the spinning or the casting CA solutions. Specifically, for the fiber mats that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~1.13, ~1.19, ~1.31, and ~1.42%, respectively. Similarly, much lower values were also observed for the corresponding films, in which, for the films that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin, the maximum amount of curcumin released was ~0.28, ~0.32, ~0.38, and ~0.41%, respectively. Evidently, only small amount of curcumin from both types of the curcumin-loaded materials could diffuse into the medium solution through the pig skin.

3.6. Release kinetics of curcumin from curcumin-loaded CA fiber mats and films

The release kinetics of curcumin from a carrier can be characterized using an equation of the following form [27,28]:

$$\frac{M_t}{M_\infty} = kt^n, \quad \text{for} \quad \frac{M_t}{M_\infty} < 0.6, \tag{4}$$

where M_t is the cumulative amount of curcumin 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 curcumin that incorporates physical characteristics of the matrix/curcumin system as well as some physical contributions from the measurement method (viz. in the case of the transdermal diffusion through a pig skin method which involves the diffusion of curcumin through a pig skin).

For $n = 0.5$, the release mechanism can be described as Fickian diffusion [29]. For this mechanism, a straight line is expected when the fractional cumulative amount of curcumin released (i.e., M_t/M_∞) is plotted as a function of $t^{0.5}$. Here, only the release of curcumin from both the curcumin-loaded fiber mat and film specimens which was investigated by the transdermal diffusion through a pig skin method was analyzed. The results from the analyses (i.e., parameters k and r^2, which signifies the goodness of the fit) are summarized in Table 5. The rate parameter k for all of the curcumin-loaded CA fiber mat specimens ranged between 0.0020 and 0.0028 $s^{-0.5}$, while that for all of the curcumin-loaded CA film counterparts ranged between 0.0024 and 0.0036 $s^{-0.5}$. Recently, Taepaiboon et al. [9] reported that the rate parameter k for the release of Vit-E from the Vit-E-loaded CA fiber mat specimens 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 mat specimens was 0.0061 $s^{-0.5}$.

3.7. Antioxidant activity and indirect cytotoxicity evaluation

The antioxidant activity of the as-loaded curcumin in the curcumin-loaded e-spun CA fiber mats and corresponding as-cast CA films was investigated by the DPPH assay. The results of such analyses are summarized in Table 6. The antioxidant activity of curcumin relates to the ability of curcumin in de-activating the DPPH radicals, which could be detected photometrically. The antioxidant activity of the as-loaded curcumin in the fiber mats that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin was ~46, ~75, ~68, and ~66%, respectively, while that of the as-loaded curcumin in the films that were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin was ~85, ~64, ~92, and ~90%, respectively. Comparatively, the antioxidant activity of the as-loaded curcumin in the fiber mat specimens was greater than that of the as-loaded curcumin in the film counterparts. The reason for this is due to the fact that the weight of the film specimens (at equivalent curcumin loadings) was greater than that of the corresponding fiber mat ones (despite equivalent thicknesses). The obtained results confirm that the as-loaded curcumin still retained its

Table 5
Analyses of the release kinetics of curcumin from curcumin-loaded electrospun CA fiber mats and solvent-cast CA films based on the Fickian diffusion type of release mechanism ($n = 3$)

Type of sample	Rate parameter, k ($s^{-0.5}$)	r^2
Curcumin-loaded electrospun CA fiber mats		
With 5 wt.% curcumin	0.0028	0.97
With 10 wt.% curcumin	0.0020	0.93
With 15 wt.% curcumin	0.0027	0.98
With 20 wt.% curcumin	0.0025	0.96
Curcumin-loaded solvent-cast CA films		
With 5 wt.% curcumin	0.0036	0.96
With 10 wt.% curcumin	0.0027	0.88
With 15 wt.% curcumin	0.0028	0.93
With 20 wt.% curcumin	0.0024	0.92

Note: the experimental results were based on the transdermal diffusion through a pig skin method.

Table 6
Antioxidant activity of curcumin from curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films ($n = 5$)

Type of sample	%Inhibition
Curcumin-loaded electrospun CA fiber mats	
With 5 wt.% curcumin	46.1 ± 5.5
With 10 wt.% curcumin	74.8 ± 2.7
With 15 wt.% curcumin	68.4 ± 1.9
With 20 wt.% curcumin	65.7 ± 1.7
Curcumin-loaded solvent-cast CA films	
With 5 wt.% curcumin	85.4 ± 2.0
With 10 wt.% curcumin	64.4 ± 5.8
With 15 wt.% curcumin	92.0 ± 6.7
With 20 wt.% curcumin	89.5 ± 5.6

free radical scavenging ability, even after it had been subjected to a high electrical potential during e-spinning.

The potential for use of the curcumin-loaded e-spun CA fiber mats and corresponding as-cast CA films as topical/transdermal patches or wound dressings was assessed by investigating the cytotoxicity of these materials, using the neat CA fiber mats and films as an internal control group. The viability of the normal human dermal fibroblasts (NHDF) that were cultured with the extraction media from these materials in comparison with that of the cells that were cultured with fresh culture medium (i.e., control) is illustrated in Fig. 6. Clearly, both the neat CA fiber mats and films posed no threat to the cells, as the viability of the cells cultured with the extraction media from these materials was at similar level to that of the control (i.e., ~97 and ~107% for the fiber mats and films, respectively). The presence of curcumin in these materials caused a slight decrease in the viability of the cells. Specifically, the relative viability of the cells cultured with the extraction media from the fiber mat specimens which were prepared from the CA solutions containing 5, 10, 15, and 20 wt.% curcumin was ~93, ~84, ~90, and ~79%, respectively, while that of the corresponding film specimens was ~88, ~80, ~84, and ~86%, respectively. The relative viability of the cells at

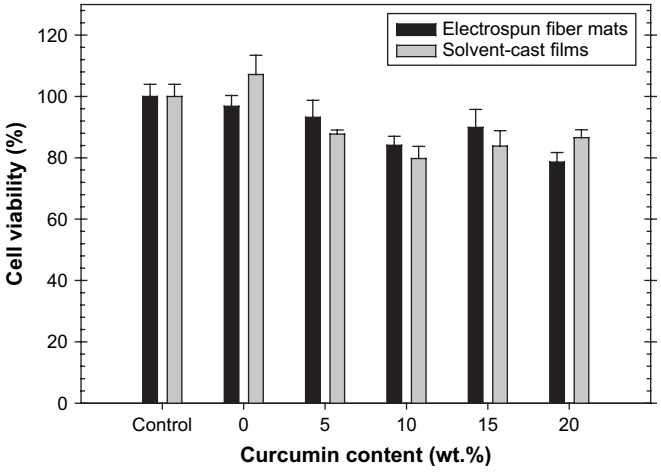


Fig. 6. Indirect cytotoxicity evaluation of neat and curcumin-loaded electrospun CA fiber mats and corresponding solvent-cast CA films in comparison with viability of the cells that were cultured with fresh culture medium ($n = 3$).

levels greater than 75% indicates low toxicity of these materials towards the skin cells.

4. Conclusions

In the present contribution, curcumin from the plant *C. longa* L., widely known for its anti-tumor, antioxidant, and anti-inflammatory properties, 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 various amounts (i.e., 5–20 wt.% based on the weight of CA powder). Both the neat and the curcumin-loaded CA solutions were e-spun into ultra-fine fibers under a fixed electric field of 17.5 kV/15 cm. The obtained fibers were smooth and, for the curcumin-loaded CA fibers, no curcumin aggregates were observed on the surface of the fibers, a result indicative of complete incorporation of curcumin within these fibers. The average diameter of the neat CA fibers was ~300 nm, while those of the curcumin-loaded ones were in the range of ~314 to ~340 nm. Chemical integrity of the as-loaded curcumin in the curcumin-loaded CA fiber mats was intact after the e-spinning process. The swelling and the weight loss of both the neat and the curcumin-loaded CA fiber mats in the acetate buffer solution containing Tween 80 and methanol (i.e., the B/T/M medium) were greater than those of the corresponding solvent-cast CA films. For the curcumin-loaded fiber mats and films, both the property values were found to increase with increasing the curcumin content in the base CA solutions.

Almost all of the curcumin loaded in the spinning or the casting solutions was incorporated within the curcumin-loaded fiber mat and film samples (i.e., ~91 to ~102%). The release characteristic of curcumin from the curcumin-loaded CA fiber mats and films was carried out by the total immersion and the transdermal diffusion through a pig skin method in the B/T/M medium at 37 °C. In the total immersion method, almost all of the curcumin loaded in the curcumin-loaded fiber mat specimens was released into the medium (i.e., ~90 to ~95%), while only small amount of curcumin in the curcumin-loaded film counterparts was (i.e., ~3 to ~9%). Considerably lower amounts of curcumin were released into the medium when the curcumin-loaded fiber mats and films were put on top of a piece of pig skin. The free radical scavenging ability of the as-loaded curcumin, even after it had been subjected to a high electrical potential during e-spinning, was retained. Finally, the potential for use of the curcumin-loaded e-spun CA fiber mats and corresponding as-cast CA films as topical/transdermal patches or wound dressings was assessed by investigating the cytotoxicity of these materials and the results showed that these materials posed no threat towards normal human dermal fibroblasts.

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