Pharmaceutical Development and Technology, 2013; 18(5): 1140–1147

ISSN 1083-7450 print/ISSN 1097-9867 online
DOI: 10.3109/10837450.2012.727004

informa
healthcare

RESEARCH ARTICLE

Fabrication and properties of capsicum extract-loaded PVA and CA nanofiber patches

Praneet Opanasopit[1], Warisada Sila-on[2], Theerasak Rojanarata[1], and Tanasait Ngawhirunpat[1]

[1]*Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand and* [2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand*

Abstract

The aim of this study was to prepare, characterize and evaluate electrospun polyvinyl alcohol (PVA) and cellulose acetate (CA) nanofibers loaded with capsicum extract (CE) for use in topical skin treatments. CE, 0.5, 1 or 2 wt %, was loaded into PVA and CA electrospun fiber mats. Various properties of the CE-loaded fiber mats as well as release and skin permeation were investigated. The average diameters of these fibers ranged from 251–368 nm. The release rate of capsaicin from CE-loaded as-spun PVA was faster than that of the CA fiber mats and increased as the CE content in CE-loaded as-spun PVA and CA increased. The release kinetics of the CA and PVA fibers followed the Higuchi equation. The percentages of CE that permeated the shed snake skin with PVA and CA fiber mats containing 2 wt % CE after 24 h were 60% and 20%, respectively. The results suggest a potential use of PVA and CA nanofibers being used to control skin permeation of capsicum extract. Our research suggests the potential application of CE-loaded PVA electrospun mats as transdermal drug delivery systems.

Keywords: Capsicum extract, electrospinning, cellulose acetate, polyvinyl alcohol

Introduction

Electrospinning has recently attracted a great deal of attention due to its ability to produce ultrafine fibers with average diameters in the sub-micrometer to nanometer range.[1,2] These nanofibers exhibit several interesting characteristics, including high surface areas, high mass to volume ratios, and small inter-fibrous pore sizes with high porosities, opening up a vast array of possibilities for surface functionalization.[3–5] These advantages make electrospun polymeric fibers good candidates for a wide variety of applications such as tissue-engineered scaffolds and drug delivery systems.[6–12] One of the obvious advantages of the electrospinning process compared to conventional film-casting techniques is the highly porous structure of electrospun fiber mats, which exhibit a much greater surface area that could potentially allow drug molecules to diffuse from the matrix more effectively.

Cellulose acetate (CA) is the acetate ester of cellulose, the primary structural component of the cell wall of green plants, and is one of the most common biopolymers on earth. The advantages of CA include its biocompatibility, biodegradability and regenerative ability. Electrospun CA fiber mats have been used as carriers for the transdermal or topical delivery of model vitamins,[13] drugs,[14,15] and herbal substances, (i.e., curcumin and asiaticoside) as a crude extract or pure substance.[16–18]

Capsaicin is a capsaicinoid compound that can be extracted from different types of peppers, including chili peppers. It is used for the temporary relief of minor muscle and joint pain associated with arthritis. The effectiveness is a result of the burning sensation that capsaicin causes; the nerves are overwhelmed by the additional influx of information and are unable to report pain for an extended period of time. Capsaicin is now available in topical dosage forms such as creams, gels and dermal patches. However, very few data for the development of nanofiber mats containing capsaicin is available in the literature. Our previous study demonstrated the

Address for Correspondence: Praneet Opanasopit, Ph.D., Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand. Tel: 66-34-255800 Fax: 66-34-255801. E-mail: praneet@su.ac.th; opraneet@hotmail.com

(Received 02 July 2012; revised 21 August 2012; accepted 22 August 2012)

successful fabrication of electrospun polyvinyl alcohol (PVA) nanofibers containing capsaicin, which is a pure compound.[19] PVA is a water-soluble, non-toxic, biodegradable, and biocompatible synthetic polymer with the ability to form fibers possessing properties that are particularly amenable to the electrospinning process. Therefore, PVA is well-suited as a topical carrier for many drugs.[20–22] We were interested in developing mats of electrospun PVA, and comparing them to CA nanofibers, as capsaicin carriers for delivery to the skin. In the present contribution, capsicum extract (CE) was used instead of the pure compound capsaicin. CE was loaded into either CA or PVA solution which was later fabricated into ultra-fine fibers by electrospinning. The CE-loaded electrospun CA and PVA fiber mats were assessed for their potential as carriers for the topical or transdermal delivery of capsaicin. Various properties (i.e., morphological, swelling, and cytotoxicity properties) of CE-loaded electrospun CA and PVA fiber mats as well as the release and skin permeation characteristics of capsaicin from the CE-loaded electrospun CA or PVA fiber mats were investigated and compared using shed snake skin.

Materials and methods

Materials

Cellulose acetate (CA; white powder; Mw ≈30,000 Da; acetyl content = 39.7 wt %; and degree of acetyl substitution ≈2.4), capsaicin standard, *N,N*-dimethylacetamide (DMAc; ≥ 99.5%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (Saint Louis, MO, USA). Capsicum extract (CE; hexane extract of *Capsicum frutescens* and *Capsicum annum*, capsaicin content 6.66% by HPLC) was purchased from Thai-China Flavors and Fragrances Industry Co., Ltd. (Thailand). Polyvinyl alcohol (PVA; white powder; degree of polymerization 1600; and degree of hydrolysis ≈97.5 to 99.5 mol%) was supplied from Fluka (Switzerland). Acetone was purchased from Carlo Erba (Italy). Normal human foreskin fibroblast (NHF) cells were obtained from the American Type Culture Collection (ATCC), Rockville, MD (USA). Dimethyl sulfoxide (DMSO) was obtained from BDH Laboratories (UK). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, and penicillin-streptomycin were purchased from Gibco BRL Rockville, MD (USA). All other reagents and solvents were commercially available and of analytical grade.

Preparation of blank and CE-loaded CA or PVA fiber mats

A weighed amount of PVA powder was dissolved in distilled water at 80°C for 3 h to prepare a PVA solution at a fixed concentration of 10% w/v. After the solution was cooled to room temperature (25°C), capsicum extract (CE), 0.5, 1 and 2 wt % based on the dry weight of PVA, was added into the PVA solution under constant stirring for 4 h.[19] When using CA, 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.[16] CE-loaded CA solutions were prepared by dissolving CE, 0.5, 1 and 2 wt % based on the weight of CA powder, in the CA base solution. Prior to electrospinning, the viscosities and conductivities of the as-prepared solutions were measured using a Brookfield DV-III programmable viscometer (Brookfield Engineering Laboratories, USA) and an Orion 160 conductivity meter (Orion Research Incorporated, USA), respectively. The electric potential was fixed at 15 kV and 17.5 kV for PVA and CA, respectively. The nanofibers were collected as-spun on an aluminum sheet that was wrapped on a rotating collector. The solution was electrospun at room temperature (25°C), and the collection distance was fixed at approximately 15 cm. The solution feed was controlled, at a feeding rate of 0.2 $mL \cdot h^{-1}$, using a syringe pump (Model: NE-300, New Era Pump Systems Inc). The thickness of both types of electrospun mats was set by electrospinning for approximately 24 h.

Morphology study

The morphological appearances of both the blank and the CE-loaded as-spun PVA and CA fiber mats were observed by a JEOL JSM-5200 scanning electron microscope (SEM), JEOL, Japan. Each of the fiber mat samples was sputtered with a thin layer of gold prior to SEM observation. Based on these SEM images, the average diameter of the PVA electrospun mats was measured, and the average values were determined from at least 100 measurements.

Mechanical integrity study

The mechanical integrity was measured in terms of the maximum tensile strength of both the blank and CE-loaded electrospun PVA and CA mats (0.5, 1 and 2% wt) using a texture analyzer (Stable Micro System, Surrey, England). Each specimen was cut into a rectangular shape (10 × 40 mm). The results were reported as the average values ($n = 10$).

Swelling and weight loss study

The swelling and weight loss behaviors of both the blank and CE-loaded electrospun PVA or CA mats (0, 0.5, 1 and 2 wt %) were measured in pH 7.4 phosphate buffer solution at room temperature (25°C) for 5 min and 24 h, respectively, according to Equations 1 and 2.

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

Where M is the weight of each sample after submersion in the buffer solution for 5 min, M_d is the dry weight of the sample after submersion in the buffer solution for 5 min.

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

Where, M_d is the dry weight of the sample after submersion in the buffer solution for 24 h, M_i is the initial dry

weight of the sample, and M_r is the weight of capsicum extract that was released from the sample.

Actual drug content analysis

The actual amount of capsaicin in the CE-loaded as-spun PVA and CA fiber mats (cut into circular discs of ~1.5 cm in diameter) was quantified by dissolving each sample in 4 mL of dimethylsulfoxide (DMSO). Then, 0.5 mL of the solution was added to 8 mL of phosphate buffer solution. A dilute capsaicin-containing solution was used to measure the drug amount using a spectrofluorometer (Perkin Elmer, USA) with an excitation wavelength of 281 nm and emission wavelength of 312 nm. The amount of drug originally present in the as-spun PVA or CA mats was then calculated from the obtained data using a predetermined calibration curve. The results are reported as the averages from at least five measurements. The entrapment efficiency (%) and capsaicin content were calculated using equations 3 and 4, respectively.

$$\text{Entrapment efficiency}\ (\%) = \left(M_c / M_t\right) \times 100 \quad (3)$$

Where M_c is the amount of capsaicin embedded in fibers, and M_t is the theoretical amount of capsaicin (obtained from the feeding conditions) incorporated into fibers.

$$\text{Capsaicin content} = M_c\,(\text{mg}) / M_f\,(\text{g}) \quad (4)$$

Where M_c is the total amount of capsaicin embedded in fibers, and M_f is the total amount of fibers.[23]

In vitro release of capsaicin from CE-loaded PVA or CA fiber mats

The *in vitro* release experiments were performed using a Franz diffusion cell (MatTek Corporation, USA). A dialysis membrane (Spectra/Por® 12,000–14,000 MWCO, Spectrum Laboratories, USA) was mounted between the donor and receptor phases of the Franz diffusion cell. The temperature of the receptor solution was maintained at 32°C using a water jacket connected to a water bath. The receptor phase had a volume of 4.0 mL and an effective diffusion area of 1.28 cm^2. Each of the dry CE-loaded as-spun fiber mat samples (cut into circular discs of ~1.5 cm in diameter) was placed on cellophane membrane, which in turn was placed on top of phosphate buffer solution on the Franz diffusion cell. *In vitro* release proceeded for 2 h. A portion (0.3 mL) of the receiver solution was withdrawn and replaced with the same volume of acetate buffer solution to maintain a constant volume. The amount of capsaicin in the sample solution was determined using a spectrofluorometer (Perkin Elmer, USA) with an excitation wavelength of 281 nm and an emission wavelength of 312 nm. These data were carefully calculated to determine the amount of capsaicin cumulatively released from both types of CE-loaded fiber mats.

Skin permeation of capsaicin from CE-loaded PVA and CA fiber mats

The method for the percutaneous absorption study followed Test Guideline 428 of the Organization for Economic Cooperation and Development (OECD).[24] Shed snake skin of *Naja kaouthia* was used as a model membrane for the skin permeation study because of its similarity to human skin regarding lipid content and skin permeability.[25] Shed snake skin was donated by the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. The thickness of the shed snake skin was approximately 0.02–0.03 mm. The skin was soaked in pH 7.4 phosphate buffer for 1 h to equilibrate. The *in vitro* permeation experiments were performed using a Franz diffusion cell (MatTek Corporation, USA). The shed snake skin was mounted between the donor and receptor phases of the Franz diffusion cell. The temperature of the receptor solution was maintained at 37°C using a water jacket connected to a water bath. The receptor phase had a volume of 4.0 mL and an effective diffusion area of 1.28 cm^2. Each of the dry CE-loaded as-spun fiber mat samples (cut into circular discs of ~1.5 cm in diameter) was placed on a piece of shed snake skin, which was placed on top of the pH 7.4 phosphate buffer solution on the Franz diffusion cell. Skin permeation proceeded for 24 h. A portion (0.3 mL) of the receiver solution was withdrawn and replaced with the same volume of phosphate buffer solution to maintain a constant volume. The amount of capsaicin in the sample solution was determined using high-performance liquid chromatography (HPLC) and calculated using the predetermined calibration curve for capsaicin. These data were carefully calculated to determine the cumulative amount of capsaicin that permeated through the shed snake skin over each time period. The experiments were performed in triplicate.

HPLC analysis

A Perkin Elmer Series 2000 HPLC (Perkin Elmer, USA) was used to quantify the amount of capsaicin in the sample solutions. Chromatographic separation of capsaicin was achieved using a Waters symmetry C18 column (Water, USA, particle size = 5 μm; column dimension = 4.6; 150 mm) at 1 mL/min. The mobile phase was a mixture of acetonitrile: water (53:47). The injection volume was 25 μL. The detection was performed using a fluorescence detector with an excitation wavelength of 281 nm and an emission wavelength of 312 nm. All of the sample solutions were filtered through a polytetrafluoroethylene (PTFE) filter (average pore size = 0.45 μm) prior to injection. The range for the calibration curve of capsaicin was 1–40 μg/mL.

Cytotoxicity evaluation

The cytotoxicity of the nanofiber mats was evaluated based on a procedure adapted from the ISO10993-5 standard test method (indirect contact).[26] The CE- loaded nanofiber mats were sterilized by UV radiation for 1 h.

The mats were then extracted in serum-free medium (SFM; containing DMEM, 1% L-glutamine, 1% lactalbumin and 1% antibiotic and antimycotic formulation) in an incubator for 24 h to produce extraction media of varying concentrations (CE = 0–400 μg/ml, or blank fiber = 0–10 mg/mL). Normal human foreskin fibroblast (NHF) cells were plated in 90 μL of DMEM, supplemented with 10% FBS, at a density of 8000 cells/well in 96-well plates. When the cultures reached confluence (typically 48 h after plating), the extraction media to be tested at varying concentrations was replaced, and the cells were re-incubated for 24 h. After treatment, the extraction solutions were removed. Finally, the cells were incubated with 100 μL of a MTT-containing medium (1 mg/mL) for 4 h. The medium was removed, the cells were rinsed with PBS (pH 7.4), and the formazan crystals that formed in the living cells were dissolved in 100 μL DMSO per well. Cell viability (%) was calculated based on the absorbance at 550 nm using a microplate reader. The viability of non-treated control cells was arbitrarily defined as 100%.

Statistical analysis

All experimental measurements were performed in triplicate. All results are expressed as the means ± S.D. The data were analysed by one-way ANOVA followed by a least significant difference (LSD) *post hoc* test. Differences of $p < 0.05$ were considered statistically significant.

Results and discussion

Morphology of blank and CE-loaded PVA or CA fiber mats

All of the 10% w/v PVA and 17% w/v CA solutions were prepared by dissolution in water and 2:1 acetone/DMAc, respectively. The viscosity and conductivity of the blank and 0.5, 1, and 2 wt % CE-loaded solutions in these solvents were measured and are listed in Table 1. With increasing CE concentration, the conductivity and viscosity of both the PVA and CA solutions slightly increased. The as-prepared 10% w/v PVA solution in distilled water was electrospun under an electrostatic field strength of 15 kV/15 cm, whereas the 17% w/v CA solution in 2:1 v/v acetone/DMAc was electrospun under an electrostatic field strength of 17.5 kV/15 cm; both were spun for 24 h. A selected SEM image of the obtained fibers (0.5, 1, and 2 wt % CE-loaded PVA and CA electrospun fibers) is shown in Table 2. The obtained fibers possessed round cross-sections and smooth surfaces. The average diameters of the blank 10% w/v PVA and 17% w/v CA fibers ($n = 100$) were 136.6 ± 5.7 nm and 317 ± 42 nm, respectively. Due to the smoothness of the resulting fibers, the 10% w/v PVA solution and 17% w/v CA were used as the base solution into which various amounts of CE were added. Based on the information summarized in Table 2, the addition of CE to the PVA and CA solutions affected the morphological appearance and size of the resulting fibers. The SEM images of the CE-loaded as-spun PVA and CA fibers revealed no presence of crystals or other types of extract aggregates on the surface of the fibers. This implies that the CE was dispersed on a molecular level within the electrospun fiber. However, a bead-on-string structure was obtained at 2% CE-loaded as-spun PVA. The bead-on-string structure may be due to an increase in the conductivity that could override the decrease in the surface tension, which could destabilize the jet and result in beaded fibers. In comparison, the fibers fabricated from PVA showed bead-on-string structures to a greater extent than those from CA. The average diameter of the CE-loaded fibers increased as the CE concentration increased from 0.5 to 2% w/v. The observed increase in the diameters of CE-loaded PVA and CA fibers should be due to the increased viscosity of the CE-containing PVA and CA solutions compare to those of the blank PVA and CA solutions. This result was described in a previous study, which revealed that the average diameter of the fibers increased because the higher viscosity of solutions with increased drug concentrations resulted in stretching from the electrostatic force during electrospinning.[20]

Table 1. Conductivity and viscosity of blank and CE-loaded CA or PVA solutions.

	Conductivity (μS/cm)		Viscosity (mPas)	
Sample	10% PVA	17% CA	10% PVA	17% CA
Blank	734 ± 1.5	666 ± 2.5	443.1 ± 23.5	746.1 ± 33.0
0.5% CE	722 ± 2.5	1097 ± 2.1	431.9 ± 4.6	1070.3 ± 18.1
1% CE	734 ± 9.2	1097 ± 0.40	504.0 ± 9.2	1041.0 ± 42.2
2% CE	741 ± 5.5	1330 ± 0.01	579.4 ± 3.4	828.1 ± 6.3

Tensile strength of blank and CE-loaded PVA or CA fiber mats

Regarding the tensile strength, the mechanical integrities of the blank and the CE-loaded as-spun PVA and CA fiber mats were investigated. The thicknesses of the PVA and CA fiber mats ranged from 34 to 54 μm and from 100 to 165 μm, respectively (Table 3). The average tensile strengths for all of the PVA fiber mats ranged from 2–3.2 MPa, while the average tensile strength for all of the CA fiber mats ranged from 6.0–9.8 MPa. The much greater tensile strengths of the CA fiber mats compared to that of the PVA as-spun fiber mats might be due to the much greater thickness of the CA fibers. The presence of CE at the concentrations tested here was accompanied by slightly increased thickness as well as increased tensile strength. This finding suggests that if the electrospinning time were fixed at 24 h, CE-loaded CA as-spun fiber mats would be superior to the corresponding CE-loaded PVA as-spun fiber mats because of the dramatic improvement in flexibility and increased thickness (Table 4).

Swelling and weight loss behavior

Figure 1 shows the degree of swelling and the weight loss of blank and CE-loaded as-spun PVA and CA mats after immersion in phosphate buffer solution at room temperature (25°C) for 5 min and 24 h, respectively. In each of the sample types investigated, the swelling of the CA electospun mats was greater than that of

Table 2. The scanning electron microscopy (SEM) images (1000×) and the diameter of PVA or CA fibers and 0.5, 1 and 2 wt.% capsicum extract (CE)-loaded electrospun CA or PVA fiber mats.

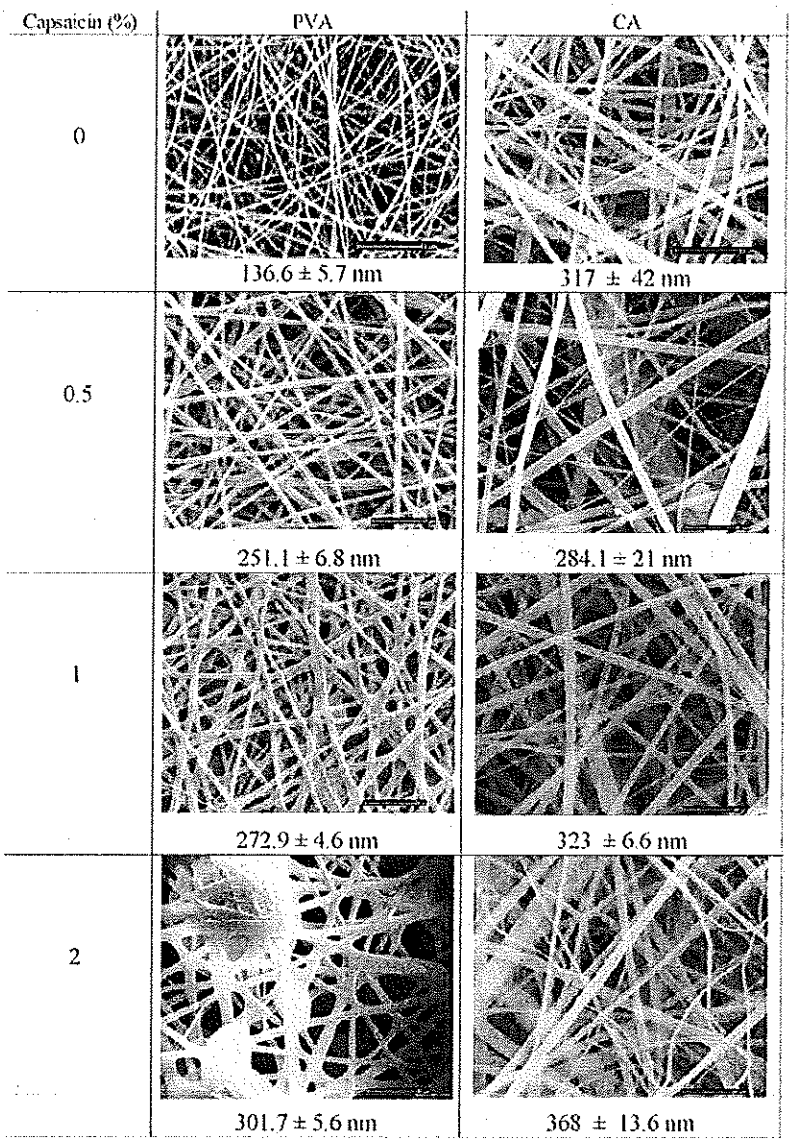

Table 3. The thickness and tensile strength of blank- and CE-loaded CA or PVA fibers.

	Thickness (μm)		Tensile strength (MPa)	
Samples	10% PVA	17% CA	10% PVA	17% CA
Blank	35.0±1.0	114.3±1.5	2.1±1	6.8±1
0.5% CE	34.3±4.0	100.3±2.1	2.0±2	6.0±1
1% CE	39.3±5.9	155.3±5.8	2.3±4	9.3±3
2% CE	54.7±1.5	165.0±8.5	3.2±5	9.8±1

Table 4. The entrapment efficiency (EE) percentage and capsaicin content of CE-loaded electrospun PVA and CA mats.

	PVA fiber mats		CA fiber mats	
Samples	% EE	CE content (mg/g)	% EE	CE content (mg/g)
0.5% CE	91.29±1.10	4.3±0.6	80.21±2.03	3.5±0.1
1% CE	79.94±1.41	9.7±0.7	71.61±4.96	7.2±0.5
2% CE	59.88±0.79	18.5±0.4	64.96±3.14	12.6±0.6

the PVA electospun mats, which could be due to the greater thickness of these electrospun mats (Table 3). A slightly higher degree of swelling was found as the % wt of CE in CE-loaded as-spun PVA and CA mats increased (Figure 1a). In comparison with that of the blank materials, the swelling ability of the as-spun fiber mats prepared from the polymer solutions containing 0.5 and 1 wt % CE was slightly lower, while that of the as-spun fiber mats prepared from the solutions containing 2 wt % CE was not significantly different. These results are consistent with our previous results in the curcumin-loaded as-spun CA fiber mats.[16]

The weight loss of the blank and CE-loaded as-spun PVA and CA mats after immersion in phosphate buffer solution for 24 h was also investigated, and the results are shown in Figure 1b. All PVA fiber mat samples exhibited much greater weight loss in the testing medium than the CA fiber mats. Specifically, the weight loss of the PVA blank fiber mats was 24.2%, while that of the CA blank fiber mats was much lower at 8.5%. For the CE-loaded PVA fiber mat samples, the weight loss ranged between 28.1 and 40.4%, while for the CE-loaded CA fiber mat samples, the weight loss ranged between 9.1 and 11.0%. Again, the weight loss of both the CE-loaded PVA and CA fiber mats was found to increase with increasing CE content in the polymer solutions. These results are consistent with our previous results in meloxicam-loaded electrospun PVA, where greater weight losses were observed at higher % wt amounts of meloxicam.[21]

Capsicum extract content

The actual amount of the capsicum extract incorporated into the CE-loaded as-spun PVA and CA fiber mats was determined prior to investigating their release and skin permeation characteristics. The entrapment efficiency percentages of CE-loaded as-spun PVA and CA fiber mat samples were determined to be approximately 59.88–91.29% and 64.96–80.21%, respectively. The CE contents of the PVA and CA nanofiber mats were 4.3–18.5 mg/g fibers and 3.5–12.6 mg/g fiber, respectively. A greater CE content was observed in the PVA fiber mats compared to that of the CA as-spun fiber mats. It is possible that the loading of capsicum extract in CA–based electrospun organic solvent was limited. Additionally, due to the smoothness of the surface of the CE-loaded as-spun PVA and CA fibers (Table 2), CE may also have been encapsulated within the fibers.

In vitro release

The release rates of capsaicin from the CE-loaded as-spun PVA and CA fiber mats were determined. Figure 2 shows the release profiles of CE. The percentage of CE release shows a good linear correlation with the square root of time. These results indicate that the capsaicin in the donor system is dissolved, and a concentration gradient exists. Equation 5 is used to describe the release of CE.

$$M_t = kt_{1/2} \quad (5)$$

Where M_t is the amount of drug released at a given time, and k is the release rate. Eq. 1 could be used for elucidating the release kinetics of the CE from the as-spun PVA and CA fiber mats. The cumulative amount of CE release from the CE-loaded as-PVA electrospun at 60 min was approximately 100% for the mats loaded with 0.5, 1 and 2% wt of CE; the amount of CE released from the CE-loaded CA electrospun at 24 h was approximately 79.55%, 73.6% and 76% for the mats loaded with 0.5, 1 and 2% wt of CE, respectively. The release rates for CE-loaded PVA electrospun samples at 0.5, 1 and 2 wt % were 7.80, 11.49 and 13.01% $min^{-1/2}$, respectively, whereas the release rates for CE-loaded as-spun CA electrospun

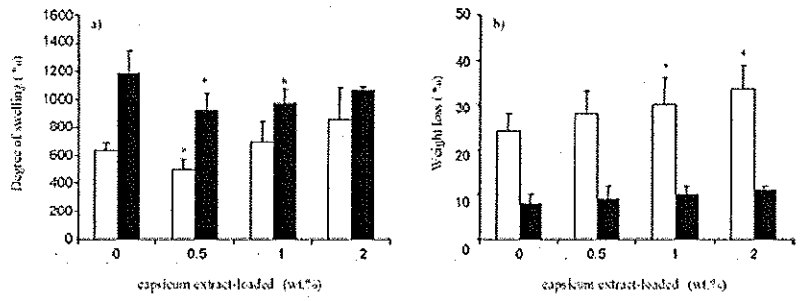

Figure 1. (a) Degree of swelling and (b) weight loss of (■) electrospun PVA fiber mats and (□) electrospun CA fiber mats after immersion in phosphate buffer solution at room temperature (25°C) for 5 min and 24 h, respectively. Each point represents the mean ± S.D. of three experiments. (*) indicates $p < 0.05$.

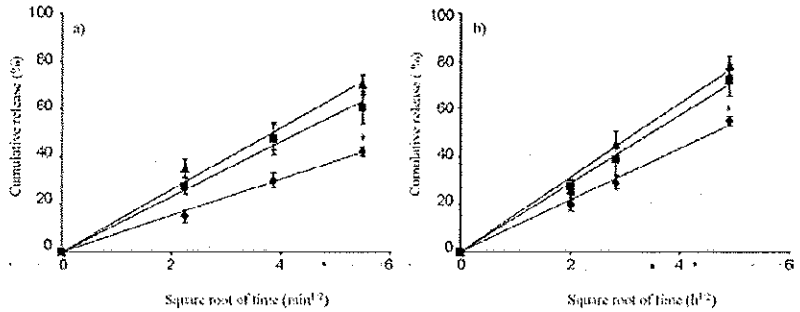

Figure 2. Release profile of CE from (a) CE-loaded electrospun PVA mats and (b) CE-loaded electrospun CA mats at (♦) 0.05%, (■) 1%, and (▲) 2% by weight of polymer. Each point represents the mean ± S.D. of three experiments. (*) indicates $p < 0.05$.

samples were 10.86, 15.59, and 16.12% $h^{-1/2}$, respectively. These results might be due to the large amount of water dissolved in PVA electrospun samples that is in contrast to the CA electrospun samples. Capsaicin can be released easily from the PVA-based nanofiber mats, and the capsaicin release patterns may be explained by polymer erosion.[27] An increase in the capsaicin content in the polymer matrix resulted in a reduction of the relative amount of polymer acting as a diffusion barrier, which resulted in the increased capsaicin release for PVA.

Skin permeation

The skin permeation characteristics of capsaicin from the CE-loaded as-spun PVA and CA fiber mats were determined based on transdermal diffusion through shed snake skin. Figure 3 shows the permeation profiles of capsaicin across shed snake skin. The percentage of capsaicin that permeated shows a good linear correlation with the square root of time (R^2 value higher than 0.98). These results indicate that the capsaicin in the donor system is dissolved, and a concentration gradient exists. The cumulative amount of capsaicin that permeated through the skin of the CE-loaded PVA fibers (at 24 h) was approximately 60% for the mats loaded with 2% wt of CE, whereas the amount that permeated through the skin from the CE-loaded CA fibers (at 24 h) was approximately 20%. CE-loaded as-spun PVA mats exhibited significantly higher total amounts of permeated capsaicin and higher skin permeation rates of capsaicin when compared with the CE-loaded as-spun CA mats. These data might be the result of the highly erosive nature of the PVA mat, which also contributed to another observed behavior – a high susceptibility for release in aqueous media. As soon as the PVA fibers began to swell, molecules of capsaicin were solvated and leached out from the fibers. The release amounts and release rates of capsaicin from the CE-loaded as-spun PVA mats were approximately 60 times higher than those for the CE-loaded CA mats, which might enable a higher amount of capsaicin to permeate the skin. Zeng et al.[28] reported that bovine serum albumin (BSA) was rapidly released from as-spun PVA/BSA fibers during the first 2 h and that dissolution of PVA fibers was a factor contributing to the release characteristics of the fiber.

Cytotoxicity

The cytotoxicity of the capsicum extract (CE), blank-electrospun PVA mats, blank-electrospun CA mats, and 0.5–2 wt % CE-loaded electrospun PVA and CA mats were investigated by an MTT assay. The IC_{50} values of the blank-electrospun PVA and CA mats are expressed as concentrations in mg/ml of fibers, whereas those for the CE and CE-loaded electrospun PVA and CA mats are expressed as concentrations in μg/mL of extract as shown in Table 5. The results of the CE and CE-loaded electrospun PVA and CA mats showed a concentration-dependent cytotoxicity in NHF cells when incubated for 24 h, as the IC_{50}s were 218.12 and 191.29–209.94 μg/mL, respectively. There was a little decrease in the cell viabilities when the NHF cells were incubated with CE-loaded electrospun PVA and CA mats compared to those with CE. However, the blank electrospun PVA mats and CA mats did not remarkably change the viabilities of the NHF cells even after 24 h of incubation.

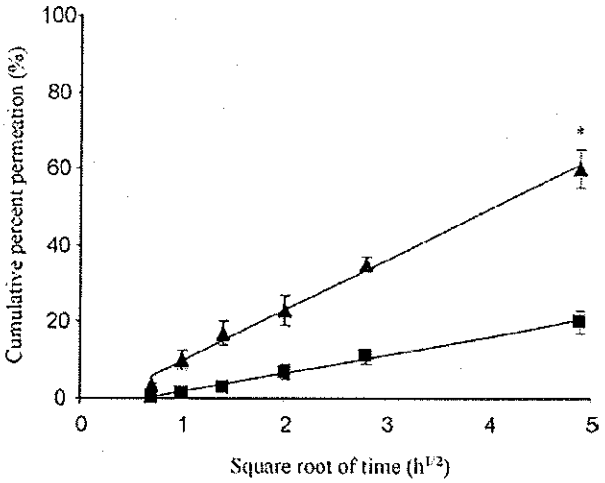

Figure 3. Skin permeation profile of CE permeated from (▲) 0.2% CE-loaded electrospun PVA mats and (■) 0.2% CE-loaded electrospun CA mats. Each point represents the mean ± S.D. of three experiments. (*) indicates $p < 0.05$.

Table 5. IC_{50} values of the capsicum extract (CE), blank-electrospun PVA mats, blank-electrospun CA mats, 0.5–2 wt % CE-loaded electrospun PVA and CA mats with CE concentrations from 0 to 400 μg/mL and incubating with NHF cells for 24 h.

	IC_{50} (μg/mL)		
Samples	CE	PVA fiber mats	CA fiber mats
CE	218.12±3.07	-	
Blank	-	>10 mg/mL	>10 mg/mL
0.5% CE	-	191.29±6.10	194.56±4.06
1% CE	-	209.94±7.41	189.67±3.07
2% CE	-	199.88±4.79	200.55±5.16

Conclusion

Capsicum extract (CE)-loaded nanofibers were successfully formed through electrospinning. The as-spun fibers had average diameters ranging between 251 to 368 nm. Capsaicin from CE-loaded as-spun PVA had a significantly higher release rate and greater skin permeation than capsaicin from CE-loaded as-spun CA. These results suggest the potential utilization of CE-loaded electrospun PVA mats as transdermal therapeutic agents.

Declaration of interest

The authors would like to acknowledge financial support from the Silpakorn University Research and Development Institute (SURDI 54/01/25) and Thailand

Research Funds (RSA 5280001). The authors report no conflicts of interest.

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