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Research paper

Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E

Pattama Taepaiboon [a], Uracha Rungsardthong [b], Pitt Supaphol [a,*]

[a] *Technological Center for Electrospun Fibers and The Petroleum and Petrochemical College, Chulalongkorn University, Pathumwan Bangkok 10330, Thailand*
[b] *National Nanotechnology Center Thailand Science Park, K long Luang, Phathumthani 12120, Thailand*

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Abstract

The present contribution reports the use of mats of electrospun cellulose acetate (CA; acetyl content = 39.8%; $M_w = 30{,}000$ Da) nanofibers as carriers for delivery of the model vitamins, all-trans retinoic acid or vitamin A acid (Retin-A) and α-tocopherol or vitamin E (Vit-E). The amounts of Vit-E and Retin-A loaded in the base CA solution [17% w/v in 2:1 v/v acetone/*N*,*N*-dimethylacetamide (DMAc)] were 5 and 0.5 wt% (based on the weight of CA), respectively. Cross-sectionally round and smooth fibers were obtained. The average diameters of these fibers ranged between 247 and 265 nm. The total immersion of the vitamin-loaded as-spun CA fiber mats in the acetate buffer solutions containing either 0.5 vol % Tween 80 or 0.5 vol % Tween 80 and 10 vol % methanol was used to arrive at the cumulative release of the vitamins from the fiber mat samples. The same was also conducted on the vitamin-loaded solution-cast CA films for comparison. In most cases, the vitamin-loaded as-spun fiber mats exhibited a gradual and monotonous increase in the cumulative release of the vitamins over the test periods (i.e., 24 h for Vit-E-loaded samples and 6 h for Retin-A-loaded ones), while the corresponding as-cast films exhibited a burst release of the vitamins.

Keywords: Electrospinning; Nanofibers; Cellulose acetate; Transdermal drug delivery; Dermal drug delivery

1. Introduction

Electrospinning is an interesting process for fabricating ultrafine fibers with average diameters in sub-micrometer down to nanometer range. In this process, a continuous strand of a polymer liquid (i.e., solution or melt) was drawn through a spinneret by a high electrostatic force to deposit randomly on a grounded collector as a non-woven mat. These fibers exhibit several interesting characteristics, for example, a high surface area to mass or volume ratio, a small inter-fibrous pore size with high porosity, vast possibilities for surface functionalization, etc. [1,2]. These advantages render electrospun polymeric fibers good candidates for a wide variety of applications, including filters [3], composite reinforcements [4,5], drug carriers [6–10], and tissue-engineered scaffolds [11–13]. Additionally, a method for preparing twisted ultrafine fiber from electrospun fiber mat has recently been introduced [14].

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 [15]. CA has been fabricated as semi-permeable membranes for separation processes and fibers and films for biomedical applications. Electrospinning of 5 and 8 wt% CA solutions in acetone produced short and beaded fibers with diameters being ~1 μm [16]. An improvement in the

* Corresponding author. Technological Center for Electrospun Fibers and The Petroleum and Petrochemical College, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand. Tel.: +66 22184131; fax: +66 22154459.
E-mail address: pitt.s@chula.ac.th (P. Supaphol).

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electrospinning of CA was achieved when 2:1 v/v acetone/dimethylacetamide (DMAc) was used as the solvent system [17]. This mixture allowed the resulting 12.5–20 wt% CA solutions to be continuously spun into fibers with diameters ranging between ~100 nm and ~1 μm. CA solutions in a mixture of acetone/water with the water content in the range of 10–15 wt% could also be spun into ultrafine fibers [18]. Furthermore, electrospinning of a CA solution in acetone/water in an acidic condition produced larger fibers, while that of the solution in a basic condition produced much finer ones [18]. In addition, 3:1:1 v/v/v acetone/dimethylformamide (DMF)/trifluoroethylene (TFE) could be used to prepare a CA solution that resulted in the electrospun fibers with diameters ranging from ~200 nm to ~1 μm [19].

So far, electrospun CA fiber mats have been explored as affinity [19] and antimicrobial [20,21] membranes. The affinity CA fibrous membranes [19] were prepared from 0.16 g ml^{-1} CA (acetyl content = 40%; M_w = 29,000 Da) solution in 3:1:1 v/v/v acetone/DMF/TFE by electrospinning (applied electrical potential = 25 kV; collection distance = 15 cm; polarity of emitting electrode = positive; solution flow rate = 4 ml h^{-1}). The membranes were heat-treated at 208 °C for 1 h and later treated with 0.1 M NaOH solution in 4:1 v/v water/ethanol for 24 h to obtain regenerated cellulose (RC) membranes. Cibacron Blue F3GA, a sulfonated triazine dye, was then covalently coupled onto the surface of RC membranes. The capture capacity of the membranes for bovine serum albumin (BSA) was reported to be 13 mg g^{-1}. On the other hand, the antimicrobial CA fibrous membranes [20,21] were prepared from 10 wt% CA (acetyl content = 39.8%; M_w = 30,000 Da) solution in 80:20 w/w acetone/water containing $AgNO_3$ in the amount of 0.01–0.5% (based on the weight of CA) by electrospinning (applied electrical potential = 17 kV; collection distance = 10 cm; polarity of emitting electrode = positive; solution flow rate = 3 ml h^{-1}). Ag+ ions were photo-reduced into Ag nanoparticles, an active antimicrobial agent, by irradiating the as-spun fibers (average diameters = 610–1910 nm) with UV light (the maximum wavelength = 245 or 365 nm) that resulted in the Ag nanoparticles with the average diameters ranging between 3 and 21 nm.

As mentioned, many of the electrospun polymeric fiber mats have been developed as carriers for delivery of drugs [6–10]. Recently, we reported the preparation of poly(vinyl alcohol) (PVA; degree of polymerization ≈ 1600; degree of hydrolysis = 97.5–99.5%) nanofiber mats containing four different types of model drugs (i.e., sodium salicylate, diclofenac sodium, naproxen, and indomethacin) by electrospinning (applied electrical potential = 15 kV; collection distance = 15 cm; polarity of emitting electrode = positive; solution flow rate = 1 ml h^{-1}) [9]. The release characteristics of the drugs from the drug-loaded fiber mats were compared to those of the corresponding solution-cast films and the results indicated that the drug-loaded as-spun fiber mats showed much better release characteristics of the model drugs than the drug-loaded as-cast films. CA in the form of solution-cast membranes has been explored as carriers for transdermal delivery of scopolamine base [22], which is structurally similar to the neurotransmitter acetylcholine and acts by blocking the muscarinic acetylcholine receptors [23], but no known reports related to the use of CA in the form of electrospun fiber mats as carriers for delivery of drugs or other related substances have yet been available in the open literature,

It is of our interest to develop mats of electrospun CA nanofibers as carriers for delivery of some vitamins to the skin. Usually, vitamins are applied to the skin in the form of topical creams, lotions, or ointments. Here, vitamin E or α-tocopherol (Vit-E; see Fig. 1) and all-trans retinoic acid or vitamin A acid (Retin-A; see Fig. 1), a vitamin A or retinol derivative, were selected as the model vitamins, due to their benefits in cosmetics. Retin-A, a naturally occurring derivative of vitamin A, is a lipid-soluble substance, known to be used for the treatment of acute promyelocytic leukemia, acne, and other skin disorders, and it is believed to help slow skin aging, remove wrinkles, or reduce hyper-pigmentation due to photo-aging [24,25]. Vit-E, also a lipid-soluble vitamin, is known for its potent antioxidant ability, owing to the presence of a hydroxyl group on its chromanol ring which can readily donate a proton to reduce free radicals (viz. free radicals can cause cell damage that may contribute to the development of cardiovascular disease and cancer) [26–29].

Based on the above-mentioned reasons, the main objectives of the present contribution were to fabricate mats of electrospun CA nanofibers that contained either Retin-A or Vit-E, and to investigate the *in vitro* release characteristics of these substances from the vitamin-loaded as-spun CA fiber mats in comparison with those from the corresponding solution-cast CA films.

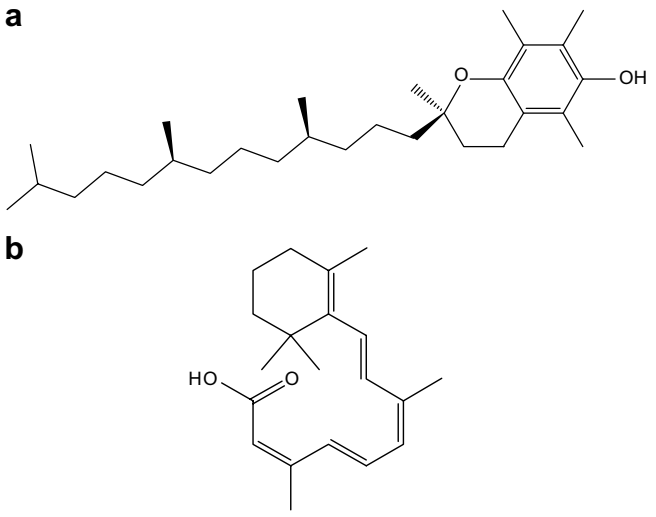

Fig. 1. Chemical structures of (a) vitamin E or α-tocopherol (Vit-E) and (b) all-trans retinoic acid or vitamin A acid (Retin-A).

2. Experimental

2.1. Materials

Cellulose acetate (CA; white powder; $M_w = 30{,}000$ Da; acetyl content = 39.7 wt%; degree of acetyl substitution ≈ 2.4) was purchased from Sigma–Aldrich (Switzerland). All-trans retinoic acid or vitamin A acid (Retin-A; purity = 99%), a derivative of vitamin A or retinol, was purchased from Roche (Switzerland). Vitamin E or α-tocopherol (Vit-E; purity = 98%) was purchased from Sigma–Aldrich (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 vitamin-loaded CA fiber mats and films

A weighed amount of CA powder was dissolved in 2:1 v/v acetone/DMAc to obtain a CA solution at a concentration of 17% w/v. Vitamin-loaded CA solutions were prepared by dissolving the same amount of CA powder and Retin-A or Vit-E in the amount of 0.5 or 5 wt% based on the weight of CA powder, respectively, in the acetone/DMAc mixture. The initial amounts of Retin-A and Vit-E in the drug containing CA solutions were based on the amounts of 0.1 and 1 wt% stated in the literature [29,30]. These mixtures were stirred into clear solutions under constant stirring for 3 h. Prior to electrospinning, the as-prepared solutions were measured for their viscosity and conductivity using a Brookfield DV-III programmable viscometer and a Orion 160 conductivity meter, respectively. The measurements were carried out at 25 °C and average values for each solution were calculated from at least three measurements.

Electrospinning of the as-prepared solutions was carried out by connecting the emitting electrode of positive polarity from a Gamma High-Voltage Research ES30PN/M692 high voltage DC power supply to the solutions contained in a standard 50-ml syringe, the open end of which was attached to a blunt gauge-20 stainless steel needle (OD = 0.91 mm), used as the nozzle, and the grounding electrode to a home-made rotating metal drum (OD = 9 cm), used as the fiber collection device. A fixed electrical potential of 17.5 kV was applied across a fixed distance of 15 cm between the tip of the nozzle and the outer surface of the drum (i.e., the electrostatic field strength of 17.5 kV/15 cm) and the rotational speed of the rotating drum was 60 ± 5 rpm. The feed rate of the solutions was controlled at ~1 ml h^{-1} by means of a Kd Scientific syringe pump. Electrospinning was carried out in room conditions (i.e., temperature = 26 ± 1 °C; relative humidity = 71 ± 3%). For morphological observation of the as-spun products, the collection time was ~5 min, while, for the rest of the experiments, it was ~9 h.

For comparison, both the neat and the vitamin-loaded CA films were prepared by solvent-casting technique from 4% w/v CA solution in 2:1 v/v acetone/DMAc and the same solution that contained either 0.5 wt% of Retin-A or 5 wt% of Vit-E, respectively. The thicknesses of both the as-spun CA fiber mats (collection time ≈ 9 h) and the as-cast CA films were between 20 and 30 μm.

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

Morphological appearance of both the neat and the vitamin-loaded as-spun CA fiber mats and as-cast CA films was observed by a JEOL JSM-5200 scanning electron microscope (SEM). Each of the fiber mat and the film samples was sputtered with a thin layer of gold prior to SEM observation. Diameters of the individual fibers in the as-spun fiber mats were measured directly from the SEM images using a SemAphore 4.0 software ($n \geqslant 50$). The mechanical integrity in terms of the tensile strength and the strain at maximum of both the neat and the vitamin-loaded as-spun CA fiber mats and as-cast CA films was investigated using a Lloyd LRX universal testing machine at room conditions. Each specimen was cut into a rectangular shape (10 × 100 mm). The crosshead speed and the gauge length were 20 mm min^{-1} and 50 mm, respectively. The results were reported as average values ($n = 7$).

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

2.4.1. Actual vitamin content

The actual amount of vitamins in the vitamin-loaded as-spun CA fiber mats and as-cast CA films (cut into circular discs of ~2.8 cm in diameter) was quantified by dissolving each sample in 10 ml of 2:1 v/v acetone/DMAc. Each of the obtained solutions was measured for the amount of the dissolved vitamins using a high performance liquid chromatography (HPLC) (see later). The amount of vitamins originally present in the as-spun CA fiber mats and the as-cast CA films was back-calculated from the obtained data against a predetermined calibration curve for each vitamin. The results were reported as average values ($n = 5$).

2.4.2. 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 about 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 the volume.

2.4.3. Preparation of releasing media

Due to the solubility limits of both vitamins in the acetate buffer solution, two types of releasing media were

prepared. The first releasing medium was prepared by adding 0.5 vol % of a non-ionic surfactant, polysorbate 80 (hereafter, Tween 80), to the acetate buffer solution to help solubilize vitamins from the vitamin-loaded samples. This medium is hereafter referred to as B/T. The other releasing medium was prepared by adding 0.5 vol % of Tween 80 and 10 vol % of methanol in the acetate buffer solution. This medium is hereafter referred to as B/T/M.

2.4.4. Stability of vitamins

Stability of Retin-A and Vit-E in the B/T/M medium was evaluated at 37 °C by varying the aging period of each vitamin in the medium. The test solution was prepared by dissolving an amount of either Retin-A or Vit-E in a measured quantity of the B/T/M medium. At a specified period ranging between 0 and 24 h (1440 min), 0.5 ml of the test solution was withdrawn (i.e., sample solution) and the amount of the vitamin that remained detectable in the sample solution was determined using HPLC (see later) against the predetermined calibration curve for each vitamin. The experiments were carried out in duplicate and the results were reported as average values.

2.4.5. Vitamin-release assay

Total immersion method was used to study the cumulative release profiles of vitamins from vitamin-loaded as-spun CA fiber mats and as-cast CA films. Based on this technique, each of the vitamin-loaded as-spun fiber mat or as-cast film samples (cut into circular discs of ~2.8 cm in diameter) was immersed in 20 ml of either B/T or B/T/M releasing medium at 37 °C. At a specified immersion period between 0 and 24 h (1440 min) for Vit-E and 0 and 6 h (360 min) for Retin-A, 0.3 ml of the test medium was withdrawn (i.e., sample solution) and an equal amount of the fresh medium was refilled. The amount of vitamins in the sample solution was determined using HPLC (see later) against the predetermined calibration curve for each vitamin. These data were carefully calculated to determine the cumulative amount of vitamins released from the samples at each specified immersion period. The experiments were carried out in triplicate and the results were reported as average values.

2.4.6. HPLC analysis

A Perkin Elmer Series 2000 HPLC was used to quantify the amount of vitamin in a sample solution. Chromatographic separation of each vitamin was achieved using a water symmetry® C_8 column (particle size = 5 μm; column dimension = 3.9 × 150 mm) operating at 1 ml min^{-1}. The mobile phases for Vit-E and Retin-A separations were 25:25:1 and 45:45:10 v/v/v acetonitrile/methanol/distilled water, respectively. The injection volume was 100 μl. A UV detector for Vit-E and Retin-A was set at (λ_{max}) 295 and 325 nm, respectively. All of the sample solutions were filtered through a polytetrafluoroethylene (PTFE) filter (average pore size = 0.45 μm) prior to injection. After injection, Vit-E and Retin-A were separated out at elution periods of 4.8–5.3 and 1.6–1.8 min, respectively. The calibration curve was conducted separately for each vitamin-releasing medium. The calibration curve for Vit-E and Retin-A was in the range of 5–20 and 0.3–1.4 μg ml^{-1}, respectively.

3. Results and discussion

3.1. Morphology of neat and vitamin-loaded CA fiber mats and films

The as-prepared 17% w/v CA solution in 2:1 v/v acetone/DMAc was electrospun under an applied electrostatic field strength of 17.5 kV/15 cm. A selected SEM image of the obtained fibers is shown in Fig. 2a. Cross-sectionally round fibers with smooth surface were obtained. The average diameter of these fibers ($n \geqslant 50$) was determined to be 265 ± 39 nm. Due to the smoothness of the resulting fibers, the 17% w/v CA solution was used as the base solution, into which Vit-E and Retin-A were individually added. For Vit-E, about 5 wt% based on the weight of CA powder was added, while, for Retin-A, about 0.5 wt% was added. These values were about 5× the amount of the substances generally used in commercial cosmetic products [29,30].

After complete dissolution of vitamins, the obtained solutions were measured for their viscosity and conductivity, as summarized in Table 1. The property values for the neat CA solution are also listed for comparison. The conductivity values of the vitamin containing solutions were not much different from that of the neat solution, which could be attributed to the fact that both vitamins contained no ionizable groups (see Fig. 1). On the other hand, the addition of these small molecular weight substances caused the viscosity of the resulting solutions to decrease from that of the neat solution, especially for the solution containing Vit-E that showed the larger decrease (viz. a direct result of the greater amount of such a substance in the solution), likely a result of the drag-reducing effect of these substances in the solution. Electrospinning of the vitamin containing solutions was straightforward. Selected SEM images of the fibers obtained from the solutions containing Vit-E and Retin-A are shown in Fig. 2b and c, respectively. Again, cross-sectionally round fibers with smooth surface were obtained. The average diameters for these vitamin-loaded fibers ($n = 50$) were 253 ± 41 and 247 ± 31 nm, respectively.

Both the neat and the vitamin containing CA solutions were also fabricated into films by solvent-casting technique. The surface morphology of the obtained films is shown in Fig. 3. Films having rather rough surfaces, with the roughness dimensionality being in the vicinity of ~1 μm, were obtained from the neat solution and the solution containing 0.5 wt% of Retin-A (see Fig. 3a and c). Interestingly, a bumpy structure was observed on the surface of the film obtained from the solution containing 5 wt% of Vit-E (see Fig. 3b), which is believed to be a result of the phase separation of the three components during the

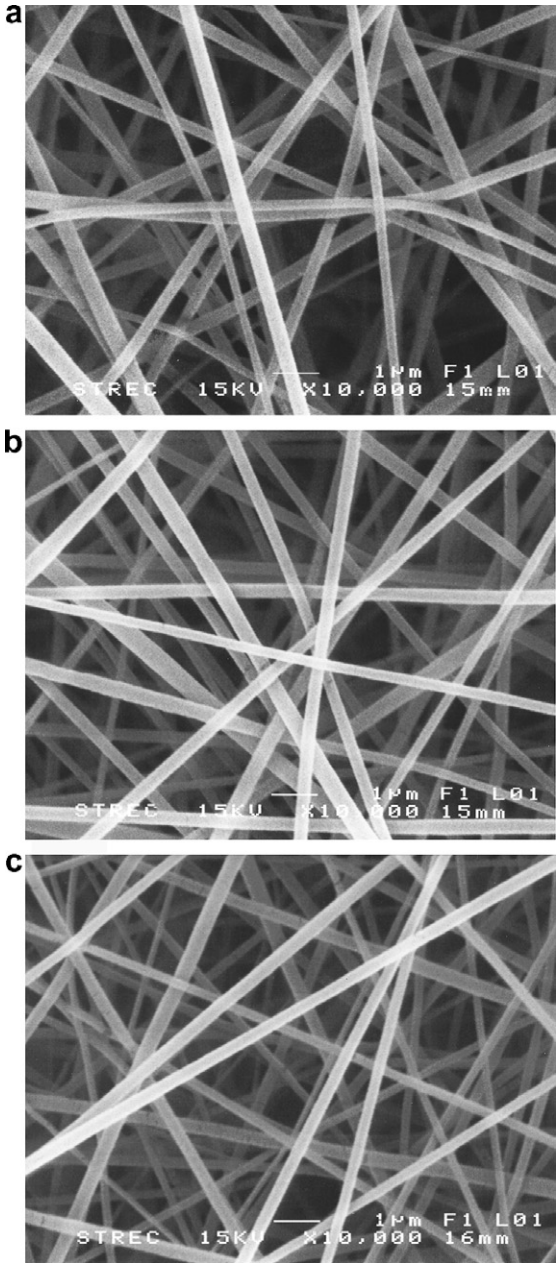

Fig. 2. Selected scanning electron micrographs (10,000×) of as-spun CA fiber mats from (a) neat 17% w/v CA solution in 2:1 v/v acetone/DMAc and from the CA solutions that contained (b) 5 wt% of Vit-E and (c) 0.5 wt% of Retin-A. The electrostatic field strength was 17.5 kV/15 cm and the collection time was 5 min. The average diameters for these as-spun fibers ($n = 50$) were 265 ± 39, 253 ± 41, and 247 ± 31 nm, respectively.

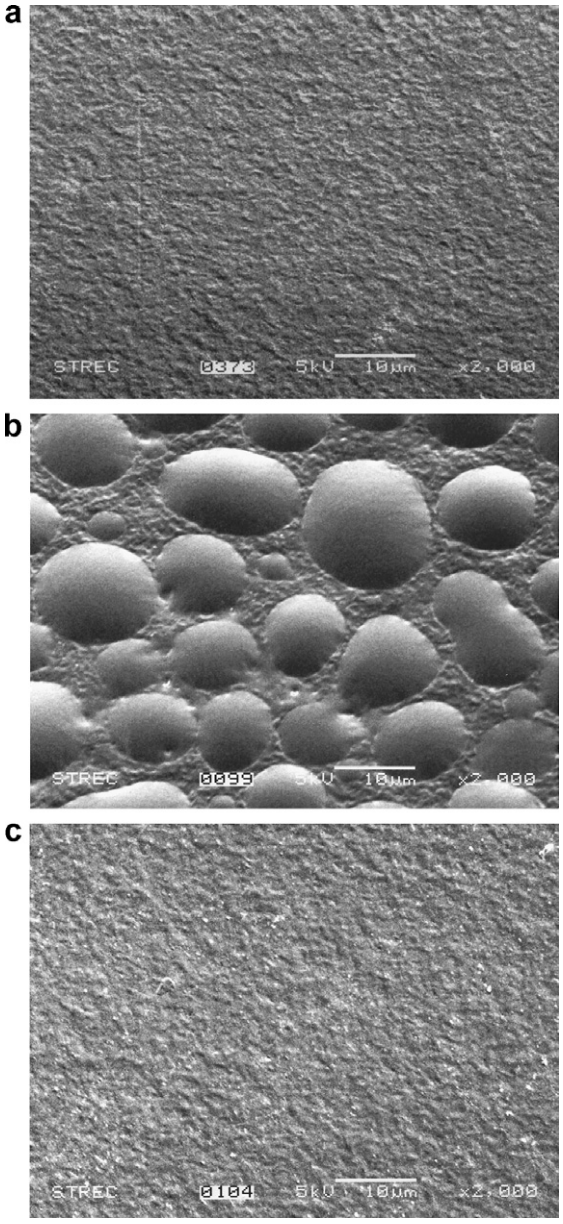

Fig. 3. Selected scanning electron micrographs (2000×) of as-cast CA films from (a) neat 4% w/v CA solution in 2:1 v/v acetone/DMAc and from the CA solutions that contained (b) 5 wt% of Vit-E and (c) 0.5 wt% of Retin-A.

Table 1
Some properties of neat and vitamin containing CA solutions

Type of CA solution	Viscosity (mPa s)	Conductivity (mS cm^{-1})
Neat	465 ± 2.6	4.01 ± 0.04
With 5 wt% vitamin E (Vit-E)	453 ± 1.5	3.92 ± 0.04
With 0.5 wt% retinoic acid (Retin-A)	460 ± 3.0	4.03 ± 0.02

'drying' of the film, and it was further postulated that the Vit-E-rich phase was the cause for the formation of these bumps. The sizes of these bumps ranged from ~2 to ~15 μm.

3.2. Tensile properties of vitamin-loaded CA fiber mats and films

The mechanical integrity in terms of the tensile strength and the strain at maximum of the neat and the vitamin-loaded as-spun CA fiber mats and as-cast CA films was investigated and the results are graphically shown in Fig. 4. The thicknesses of these fiber mats and films ranged

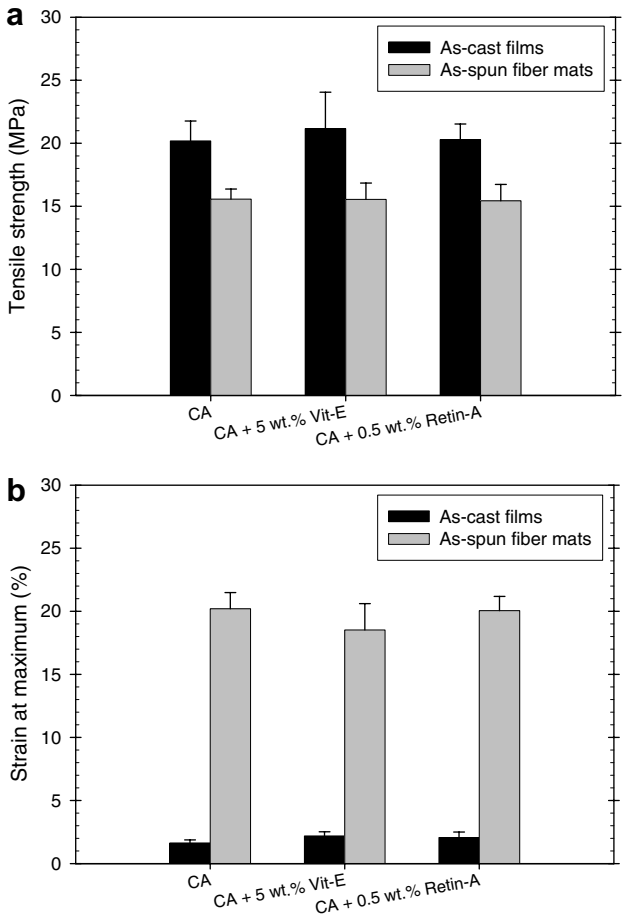

Fig. 4. Mechanical properties of vitamin-loaded as-spun CA fiber mats and corresponding as-cast CA films: (a) tensile strength (MPa) and (b) strain at maximum (%).

between 20 and 30 μm. The tensile strength for all the m mats was in the range of 15.4–15.6 MPa, with the average value being 15.5 MPa, while that for all of the as-cast films was in the range of 20.2–21.2 MPa, with the average value being 20.5 MPa. The much greater tensile strength of the as-cast films in comparison with that of the as-spun fiber mats was also reflected in the observed lower strain at maximum of the films in comparison with that of the fiber mats. Specifically, the strain at maximum for all the as-cast films was in the range of 1.6–2.2%, with the average value being 2.0%, while that for all of the as-spun fiber mats was in the range of 18.5–20.2%, with the average value being 19.6%.

The results demonstrated that the presence of vitamins had no obvious effect on the tensile properties of both the as-spun CA fiber mats and the as-cast CA films. Moreover, the as-cast films showed slightly greater tensile strength than the as-spun fiber mats, while the strain at maximum of the as-spun fiber mats was about 10× as much as that of the as-cast films. This finding suggests that, if the vitamin-loaded as-spun fiber mats are to be developed as transdermal or dermal patches, there is an advantage over the corresponding as-cast films on the dramatic improvement in the flexibility of the obtained patches.

3.3. Actual vitamin content in vitamin-loaded CA fiber mats and films

The actual amount of the vitamins incorporated in the vitamin-loaded as-spun CA fiber mats and as-cast CA films was determined prior to the investigation on their release characteristics. Table 2 summarizes the actual amount of the vitamins in these samples (reported as the percentage of the initial content of the vitamins contained in both the spinning and the casting solutions). Evidently, the actual amount of Vit-E in the Vit-E-loaded as-spun fiber mat and as-cast film samples was determined to be ~83% and ~78%, respectively. On the other hand, the actual amount of Retin-A in the Retin-A-loaded as-spun fiber mat and as-cast film samples was much lower at ~45% and ~53%, respectively. The much less amount of Retin-A incorporated in both the fiber mat and the film samples, in comparison with the relative amount of Vit-E in the Vit-E-loaded samples, could be due partly to the poor stability of Retin-A [30,31] as there is a possibility for Retin-A to lose its characteristics during the electrospinning, due to the high electrical potential that was applied to the solution. Additionally, due to the smoothness of the surface of the vitamin-loaded as-spun CA fibers (see Fig. 2b and c), it is postulated that both vitamins were encapsulated well within the fibers.

3.4. Stability of vitamins in releasing medium

Prior to the release studies, the stability of the vitamins in the releasing conditions was investigated. The B/T/M medium was chosen as the model releasing medium. Vit-E and Retin-A powder in an exact amount were separately added in the B/T/M medium to prepare the test solutions of known concentrations (i.e., 0.052 mg ml^{-1} for Vit-E containing solution and 0.023 mg ml^{-1} for Retin-A containing solution). The test solutions were incubated at 37 °C for 24 h. At a certain period of time between 0 and 24 h (1440 min), 0.5 ml of the test solutions was taken out (i.e., sample solutions) and the amount of the vitamins in the sample solutions was determined. Fig. 5 shows the amount of vitamins, reported as the percentage of the initial amount of the vitamins, in the test solutions. Appar-

Table 2
Actual amount of vitamins incorporated in vitamin-loaded as-spun CA fiber mats and corresponding as-cast CA films

Type of vitamin	Actual amount of vitamin based on the initial amount of the vitamin loaded (%)	
	Vitamin-loaded as-spun CA mats	Vitamin-loaded as-cast CA films
Vitamin E (Vit-E)	82.9 ± 2.2	78.4 ± 1.4
Retinoic acid (Retin-A)	44.5 ± 1.1	52.7 ± 2.1

The initial amounts of Vit-E and Retin-A loaded in the spinning and the casting solutions were 5% and 0.5% (based on the weight of PVA), respectively.

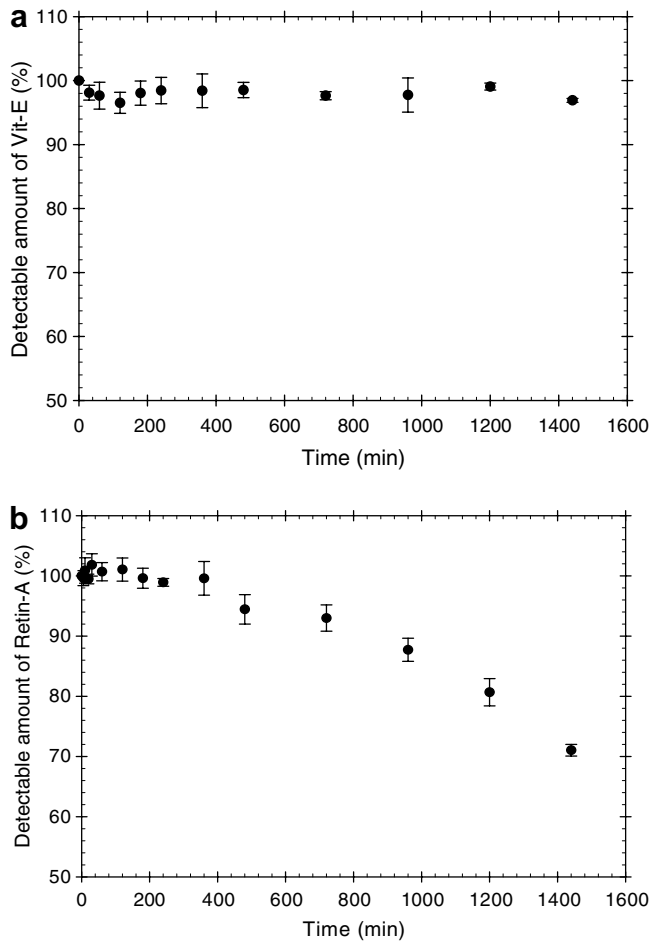

Fig. 5. Stability of (a) Vit-E and (b) Retin-A in the acetate buffer solution containing 0.5 vol % of Tween 80 and 10 vol % of methanol at 37 °C (i.e., B/T/M medium).

ently, the amount of Vit-E in the sample solutions remained close to its initial value in the test solution, with the lowest average value being ~97% (see Fig. 5a). On the other hand, the amount of Retin-A in the sample solutions remained close to its initial value during the first 6 h (360 min), after which time the amount of Retin-A started to decrease from ~94% at 8 h (480 min) to ~71% at 24 h (1440 min) (see Fig. 5b). Evidently, Vit-E remained stable in the B/T/M medium throughout the 24-h period, while Retin-A only did so during the first 6-h period. Even though the stability of Retin-A in the releasing medium was poor, the results did not imply the poor instability of Retin-A when it is incorporated within the CA matrix.

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

The release characteristics of the vitamins from the vitamin-loaded as-spun CA fiber mats and as-cast CA films were carried out by the total immersion method. The experiments were carried out in the acetate buffer solution that contained either 0.5 vol % Tween 80 (i.e., the B/T releasing medium) or 0.5 vol % Tween 80 and 10 vol % methanol (i.e., the B/T/M releasing medium) at the physiological temperature of 37 °C. The cumulative amount of the vitamins released (reported as the percentage of the actual amount of vitamins present in the vitamin-loaded samples; see Table 2) from the vitamin-loaded samples is illustrated in Fig. 6. It should be noted the cumulative amount of the vitamins released from the vitamin-loaded samples at the skin temperature of 32 °C was practically similar to that shown in Fig. 6.

In the B/T medium, it is obvious from Fig. 6a that Vit-E released from the Vit-E-loaded as-cast film samples exhibited a burst release, while that from the as-spun fiber mat ones did not. Specifically, Vit-E released from the as-cast film samples showed the burst release during the first 20 min and reached a plateau at the maximum release of ~20% with further increase in the immersion time. On the other hand, the Vit-E-loaded as-spun fiber mat samples showed a gradual increase in the cumulative release of Vit-E over the 24 h testing period. At 24 h, the maximum amount of Vit-E released from the as-spun fiber mat samples was ~52%. After the immersion in the B/T medium for 24 h, the morphology of the Vit-E-loaded as-cast film (see Fig. 7a) was roughly the same as that of the film prior to the drug release assay (see Fig. 3b). As mentioned previously that the formation of the bumpy structure on the surface of the Vit-E-loaded as-cast CA films was likely a result of the formation of the Vit-E-rich phase, the burst release of Vit-E from the as-cast film could be a result of the quick release of Vit-E from these bumps. Similarly, the morphology of the Vit-E-loaded as-spun fiber mat (see Fig. 8a), after the immersion in the B/T medium for 24 h, was essentially the same as that of the fiber mat prior to the drug release assay (see Fig. 2b).

With regard to Retin-A, the Retin-A-loaded as-cast film samples exhibited a smoother burst release than the Vit-E-loaded as-cast film samples, while the Retin-A-loaded as-spun fiber mat samples showed a gradually increasing release profile in a manner similar to the Vit-E-loaded as-spun fiber mat samples. Specifically, Retin-A released from the as-cast film samples increased rather rapidly during the first 30 min and reached a plateau at the maximum release of ~57% with further increase in the immersion time. On the other hand, the Retin-A-loaded as-spun fiber mat samples exhibited a gradual increase in the cumulative release of Retin-A over the 6 h testing period, with the maximum amount of Retin-A released at 6 h being ~34%. The fact that the Retin-A-loaded as-cast film samples exhibited a burst release, while the as-spun fiber mat samples did not, could be a result of the phase separation that caused Retin-A to locate close to the surface of the films (viz. the 'drying' of the film was much slower than that of the fiber mat) and the fact that the Retin-A-loaded as-cast film samples exhibited the much greater release of Retin-A than the as-spun fiber mat samples could be a result of the greater initial content of Retin-A in the films (i.e., ~53% in Retin-A-loaded as-cast films versus ~45% in Retin-A-loaded as-spun fiber mats), hence the greater driving force for dif-

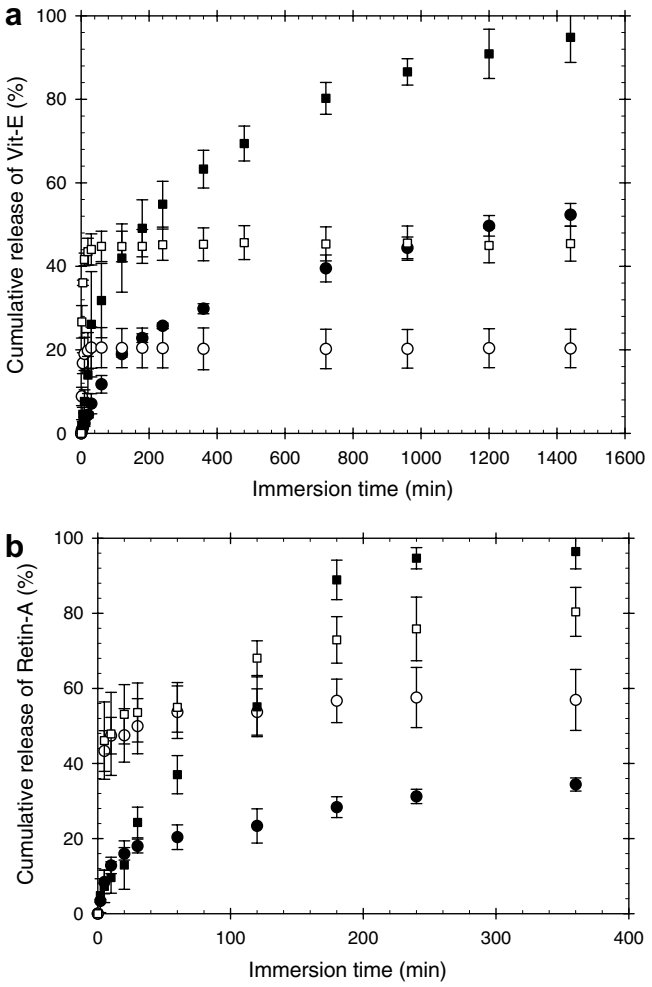

Fig. 6. Profiles of (a) Vit-E (immersion period = 0–1440 min) or (b) Retin-A (immersion period = 0–360 min) released from (●) vitamin-loaded as-spun CA fiber mats and (○) corresponding as-cast CA films in B/T medium and (■) vitamin-loaded as-spun CA fiber mats and (□) corresponding as-cast CA films in B/T/M medium.

fusion. In addition, the morphology of the Retin-A-loaded fiber mat (see Fig. 8c) and the corresponding film (not shown), after the immersion in the B/T medium for 6 h, was practically the same as that of the samples prior to the drug release assay (see Figs. 2c and 3c, respectively).

In the B/T/M medium, it is apparent from Fig. 6b that Vit-E released from the Vit-E-loaded as-cast film samples exhibited a burst release, while that from the as-spun fiber mat ones did not. Specifically, Vit-E released from the as-cast film samples also showed the burst release during the first 20 min and reached a plateau at the maximum release of ~45% with further increase in the immersion time. On the other hand, Vit-E released from the Vit-E-loaded as-spun fiber mat samples increased gradually and monotonously with increasing the immersion time to reach a maximum value of ~95% at 24 h. After the immersion in the B/T/M medium for 24 h, the morphology of the Vit-E-loaded as-cast film (see Fig. 7b) was roughly similar to that of the film prior to the drug release assay (see Fig. 3b). Again, the bumpy structure on the surface of

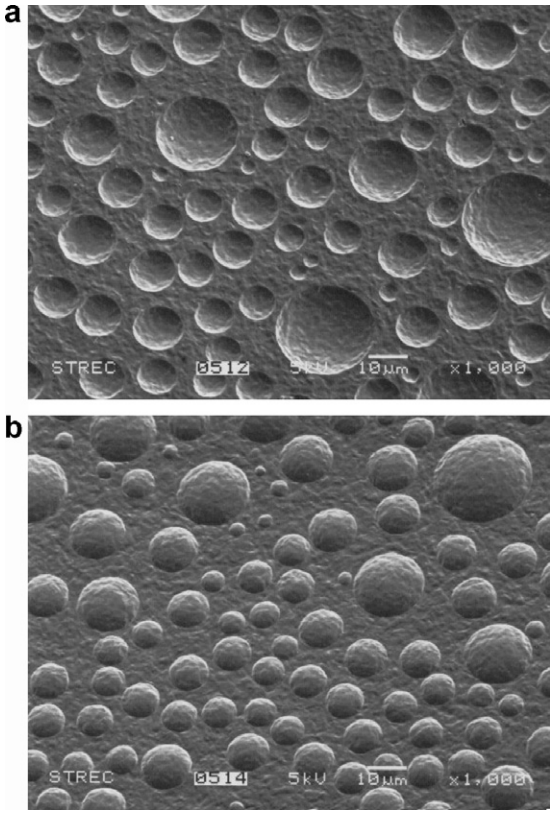

Fig. 7. Selected scanning electron micrographs (1000×) of Vit-E-loaded as-cast CA films after immersion in (a) B/T or (b) B/T/M medium for 24 h.

the Vit-E-loaded as-cast films could be the cause for the burst release of Vit-E from the films. On the other hand, while it was not obvious in the case of the Vit-E-loaded as-cast film (see Fig. 7b), it is obvious that the Vit-E-loaded as-spun fiber mat partially lost its fibrous structure (see Fig. 8b), most likely a result of the partial dissolution of the fibers in methanol, which is likely the reason for the observed greater release of Vit-E from both types of samples in this medium in comparison with that of the samples in the B/T medium (see Fig. 6a).

With regard to Retin-A, it is evident from Fig. 6b that Retin-A released from the Retin-A-loaded as-cast film samples exhibited a burst release during the first 5 min of immersion in the medium and gradually increased with further increase in the immersion time to reach a maximum value of ~80% at 6 h. On the other hand, Retin-A released from the Retin-A-loaded as-spun fiber mat samples showed a steady increase in the release characteristic with increasing immersion time. At 6 h, the maximum release of Retin-A from the fiber mat samples was close to 100% (i.e., ~96%). Interestingly, in the B/T medium, Retin-A released from the Retin-A-loaded as-cast film samples was greater than that from the corresponding as-spun fiber mat ones at any given immersion time. In the B/T/M medium, Retin-A released from the Retin-A-loaded as-cast film samples was also greater than that from the corresponding as-spun fiber mat samples at an immersion time lower than

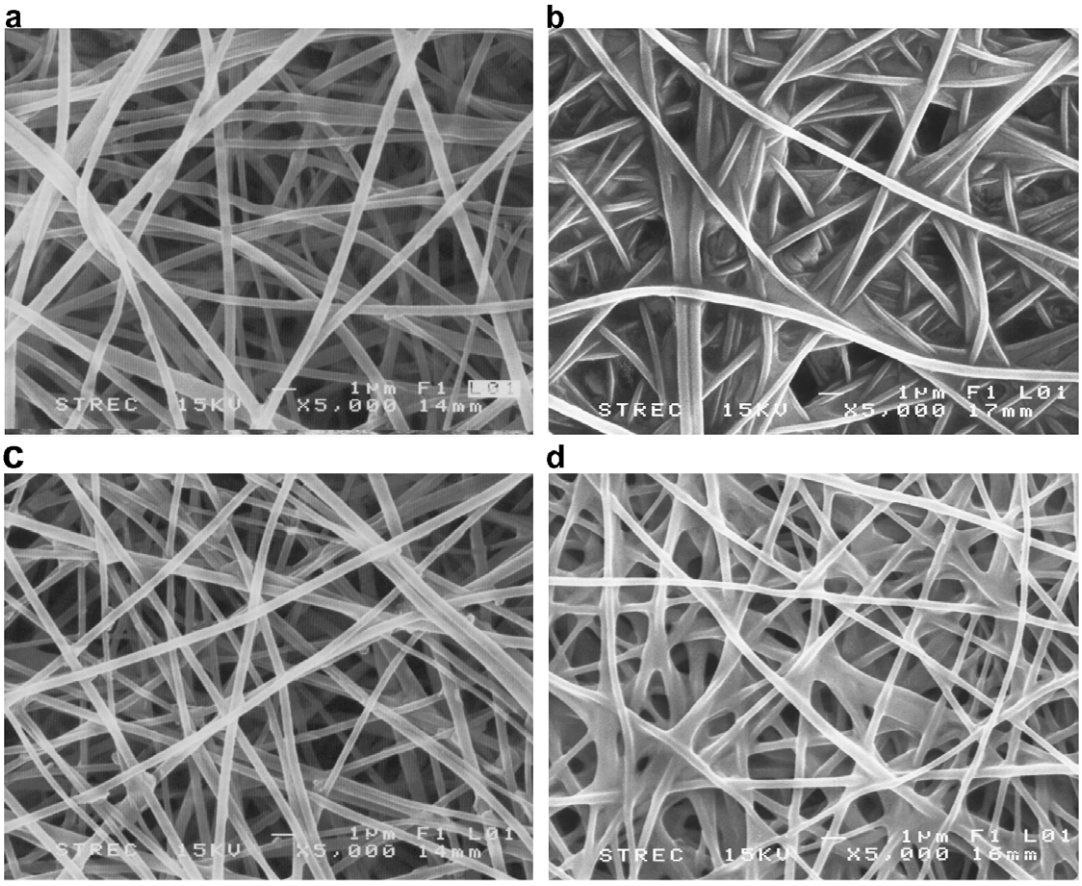

Fig. 8. Selected scanning electron micrographs (5000×) of Vit-E-loaded as-spun CA fiber mats after immersion in (a) B/T or (b) B/T/M medium for 24 h and Retin-A-loaded as-spun CA fiber mats after immersion in (c) B/T or (d) B/T/M medium for 6 h.

about 150 min, after which time Retin-A released from the Retin-A-loaded as-spun fiber mat samples became greater. The likely reason for such observation is the partial dissolution of both types of samples in the B/T/M medium. Since the Retin-A-loaded as-cast fiber mat exhibited a much greater surface area than the corresponding as-cast film, the dissolution should occur in a greater extent (see Fig. 8d), causing Retin-A to leash out more readily at a long immersion time (i.e., >∼150 min).

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

The release kinetics of a drug from a carrier is often characterized using an equation of the following form [32,33]:

$$\frac{M_t}{M_\infty} = kt^n \text{ for } \frac{M_t}{M_\infty} < 0.6, \quad (1)$$

where M_t is the cumulative amount of drugs released at an arbitrary time t, M_∞ is the cumulative amount of drugs 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 the drugs that incorporates physical characteristics of the matrix/drug system as well as some physical contributions from the measurement method.

For $n = 0.5$, the release mechanism can be described as Fickian diffusion [34]. For Fickian diffusion, a straight line is expected when the fractional cumulative amount of drug released (i.e. M_t/M_∞) is plotted as a function of $t^{0.5}$. Due to the initial burst release of the vitamins from vitamin-loaded as-cast CA films, only the release of the vitamins from vitamin-loaded as-spun CA fiber mats could be analyzed for their release kinetics using Eq. (1), indicating the Fickian diffusion type of the release mechanism of these vitamins from the vitamin-loaded as-spun CA fiber mats in both types of releasing media. The results from the analyses (i.e. parameters k and r^2, which signifies the goodness of the fit) are summarized in Table 3. Apparently, the rate parameter k for Vit-E-loaded as-spun CA fiber mat samples was 0.0039 and 0.0049 $s^{-0.5}$ in B/T and B/T/M media, respectively, while that for Retin-A-loaded as-spun CA fiber mat samples it was 0.0128 and 0.0061 $s^{-0.5}$ in B/T and B/T/M media, respectively.

4. Conclusions

Vitamin-loaded cellulose acetate (CA; acetyl content = 39.8%; $M_w = 30{,}000$ Da) mats of electrospun nanofibers

Table 3
Analyses of the release kinetics of vitamins from vitamin-loaded as-spun CA fiber mats based on the Fickian diffusion type of release mechanism

Type of sample	Rate parameter k ($s^{-0.5}$)	r^2
Vit-E-loaded as-spun CA fiber mat		
In B/T medium	0.0039	0.98
In B/T/M medium	0.0049	0.99
Vit-A-loaded as-spun CA fiber mat		
In B/T medium	0.0128	0.98
In B/T/M medium	0.0061	0.98

were successfully fabricated by electrospinning. All-trans retinoic acid or vitamin A acid (Retin-A), a derivative of vitamin A or retinol, and α-tocopherol or vitamin E (Vit-E), were used as the model vitamins. 17% w/v CA solution in 2:1 v/v acetone/*N*,*N*-dimethylacetamide (DMAc) was used as the base spinning solution, into which Vit-E or Retin-A in an amount of 5 or 0.5 wt% (based on the weight of CA) was added to prepare the vitamin-loaded spinning solutions. The as-spun fibers from these solutions were found to be cross-sectionally round with smooth surface, with their average diameters ranging between 247 and 265 nm. The mechanical integrity in terms of the tensile strength and the strain at maximum of these as-spun fiber mats was evaluated in comparison with that of the corresponding solution-cast films. It was observed that the as-cast films exhibited slightly greater tensile strength than the as-spun fiber mats, while the strain at maximum of the as-spun fiber mats was about 10× as much as that of the as-cast films.

The actual contents of Vit-E and Retin-A loaded within the as-spun fiber mats were ~83% and ~45%, respectively, while those within the as-cast films were ~78% and ~53%, respectively. The stability of both vitamins in the acetate buffer solution containing 0.5 vol % Tween 80 and 10 vol % methanol (i.e., the B/T/M medium) was evaluated and the results showed that Vit-E was stable in the B/T/M medium throughout the 24-h period, while Retin-A only did so during the first 6-h period. In the B/T medium, the Vit-E-loaded and the Retin-A-loaded as-cast films exhibited a burst release during the first 20 and 30 min, respectively, to reach a plateau at the maximum release of Vit-E and Retin-A at ~20% and ~57%, respectively, with further increase in the immersion time, while the Vit-E-loaded and the Retin-A-loaded as-spun fiber mats exhibited a gradual increase in the cumulative release during the 24 and the 6 h testing period, respectively, to reach the maximum release of Vit-E and Retin-A at ~52% and ~34%, respectively. On the other hand, in the B/T/M medium, the Vit-E-loaded as-cast films exhibited a burst release during the first 20 min, respectively, to reach a plateau at the maximum release of Vit-E at ~45, while Retin-A released from the Retin-A-loaded as-cast films exhibited a burst release during the first 5 min and increased gradually to reach the maximum value of ~80%. Again, a gradual and monotonous increase in the amount of Vit-E and Retin-A was observed from the vitamin-loaded as-spun fiber mats, with the maximum release of Vit-E and Retin-A at 24 and 6 h, being ~95% and ~96%, respectively.

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References

[1] J. Doshi, D.H. Reneker, Electrospinning process and applications of electrospun fibers, J. Electrostat. 35 (1995) 151–160.
[2] J.M. Deitzel, J. Kleinmeyer, D. Harris, N.C. Beck Tan, The effect of processing variables on the morphology of electrospun nanofibers and textiles, Polymer 42 (2001) 261–272.
[3] P.W. Gibson, H.L. Schreuder-Gibson, D. Rivin, Electrospun fiber mats: transport properties, AIChE J. 45 (1999) 190–195.
[4] M.M. Bergshoef, G.J. Vancso, Transparent nanocomposites with ultrathin, electrospun nylon-4,6 fiber reinforcement, Adv. Mater. 11 (1999) 1362–1365.
[5] J.S. Kim, D.H. Reneker, Mechanical properties of composites using ultrafine electrospun fibers, Polym. Composite 20 (1999) 124–131.
[6] E.R. Kenawy, G.L. Bowlin, K. Mansfield, J. Layman, D.G. Simpson, E.H. Sanders, G.E. Wnek, Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend, J. Control. Release 81 (2002) 57–64.
[7] X.H. Zong, K. Kim, D.F. Fang, S.F. Ran, B.S. Hsiao, B. Chu, Structure and process relationship of electrospun bioabsorbable nanofiber membranes, Polymer 43 (2002) 4403–4412.
[8] J. Zeng, L. Yang, Q. Liang, X. Zhang, H. Guana, X. Xua, X. Chen, X. Jing, Influence of the drug compatibility with polymer solution on the release kinetics of electrospun fiber formulation, J. Control. Release 105 (2005) 43–51.
[9] P. Taepaiboon, U. Rungsardthong, P. Supaphol, Drug-loaded electrospun mats of poly(vinyl alcohol) fibres and their release characteristics of four model drugs, Nanotechnology 17 (2006) 2317–2329.
[10] F. Ignatious, L. Sun, A. Craig, D. Crowe, T. Ho, M. Millan, Amorphous pharmaceutical compositions, U.S. Patent Application 20060083784 (2006).
[11] P. Wutticharoenmongkol, N. Sanchavanakit, P. Pavasant, P. Supaphol, Novel bone scaffolds of electrospun polycaprolactone fibers filled with nanoparticles, J. Nanosci. Nanotech. 6 (2006) 514–522.
[12] J. Yuan, G. Kaustabh, Z.S. Xiao, L. Bingquan, C.S. Jonathan, D.P. Glenn, A.F.C. Richard, H.R. Miriam, Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds, Biomaterials 27 (2006) 3782–3792.
[13] C. Meechaisue, R. Dubin, P. Supaphol, V.P. Hoven, J. Kohn, Electrospun mat of tyrosine-derived polycarbonate fibers for potential use as tissue scaffolding material, J. Biomater. Sci. Polym. Ed. 17 (2006) 1039–1056.
[14] X. Li, Twisted ultra-fine nanometer fiber film material and preparing method thereof, Chinese Patent Application 200310106517 (2003).
[15] Anonymous, Cellulose acetate, <http://en.wikipedia.org/wiki/Cellulose_acetate>, 2006.
[16] R. Jaeger, M.M. Bergshoef, C. Martin i Batlle, H. Schoenherr, G.J. Vansco, Electrospinning of ultra thin polymer fibers, Macromol. Symp. 127 (1998) 141–150.

[17] H. Liu, Y.L. Hsieh, Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate, J. Polym. Sci. Polym. Phys. 40 (2002) 2119–2129.
[18] W.K. Son, J.H. Youk, T.S. Lee, Y.H. Park, Electrospinning of ultrafine cellulose acetate fibers: studies of a new solvent system and deacetylation of ultrafine cellulose acetate fibers, J. Polym. Sci. Polym. Phys. 42 (2004) 5–11.
[19] Z. Ma, M. Kotaki, S. Ramakrishna, Electrospun cellulose nanofiber as affinity membrane, J. Membr. Sci. 265 (2005) 115–123.
[20] W.K. Son, J.H. Youk, T.S. Lee, W.H. Park, Preparation of antimicrobial ultrafine cellulose acetate fibers with silver nanoparticles, Macromol. Rapid Commun. 25 (2004) 1632–1637.
[21] W.K. Son, J.H. Youk, W.H. Park, Antimicrobial cellulose acetate nanofibers containing silver nanoparticles, Carbohydr. Polym. 65 (2006) 430–434.
[22] F.J. Wang, Y.Y. Yang, X.Z. Zhang, X. Zhu, T.S. Chung, S. Moochhala, Cellulose acetate membranes for transdermal delivery of scopolamine base, Mater. Sci. Eng. C20 (2002) 93–100.
[23] Anonymous, Scopolamine, <http://en.wikipedia.org/wiki/Scopolamine>, 2006.
[24] Anonymous, Retinoic acid, <http://en.wikipedia.org/wiki/Retinoic_acid>, 2006.
[25] S. Cho, L. Lowe, T.A. Hamilton, G.J. Fisher, J.J. Voorhees, S. Kang, Long-term treatment of photoaged human skin with topical retinoic acid improves epidermal cell atypia and thickens the collagen band in papillary dermis, J. Am. Acad. Dermatol. 53 (2005) 769–774.
[26] Anonymous, Tocopherol, <http://en.wikipedia.org/wiki/Vitamin_E>, 2006.
[27] R. Wolf, D. Wolf, V. Ruocco, Vitamin E: the radical protector, J. Eur. Acad. Dermatol. Venereol. 10 (1998) 103–117.
[28] S.S. Shapiro, C. Saliou, Role of vitamins in skin care, Nutrition 17 (2001) 839–844.
[29] J.Y. Lin, M.A. Selim, C.R. Shea, J.M. Grichnik, M.M. Omar, N.A. Monteiro-Riviere, R. Pinnell, UV photoprotection by combination topical antioxidants vitamin C and vitamin E, J. Am. Acad. Dermatol. 48 (2003) 866–874.
[30] L.H. Kligman, Effects of all-trans-retinoic acid on the dermis of hairless mice, J. Am. Acad. Dermatol. 15 (1986) 779–785.
[31] R. Gatti, M. Gioia, V. Cavrini, Analysis and stability study of retinoids in pharmaceuticals by LC with fluorescence detection, J. Pharm. Biomed. Anal. 23 (2000) 147–159.
[32] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release-I. Fickian and anomalous release from swellable devices, J. Control. Release 5 (1987) 37–42.
[33] N.A. Peppas, A.R. Khare, Preparation, structure and diffusional behavior of hydrogels in controlled release, Adv. Drug Deliv. Rev. 11 (1993) 1–35.
[34] G. Verreck, I. Chun, J. Rosenblatt, J. Peeters, A. van Dijck, J. Mensch, M. Noppe, M.E. Brewster, Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer, J. Control. Release 92 (2003) 349–360.