CHAPTER III
EXPERIMENTAL

3.1 Materials

Poly(vinyl alcohol) (PVA) (degree of polymerization ≈1600 and degree of hydrolysis ≈97.5 to 99.5 mol%) was supplied from Fluka (Switzerland). Cellulose acetate (CA; acetyl content = 39.8%; M_w = 30 000 Da) was purchased from Sigma-Aldrich (Switzerland). Sodium salicylate (SS) was purchased from Carlo Erba (Italy). Diclofenac sodium(DS) was purchased from Tangyin Yongqi Chemical Industry(China). Naproxen (NAP) and indomethacin (IND) were donated from Pharmasant Laboratories (Thailand). These drugs are used in the symptomatic management of painful and inflammatory conditions. All-trans retinoic acid or vitamin A acid (Retin-A), a derivative of vitamin A or retinol, was purchased from Roche (Switzerland). Vitamin E or α-tocopherol (Vit-E) 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.

3.2 Methodology

3.2.1 Preparation of neat and drug-loaded electrospun PVA mats and as-cast PVA films

A weighed amount of PVA powder was dissolved in distilled water at 80°C for 3 hr to prepare a PVA solution at a fixed concentration of 10% w/v. After the solution was cooled down to room temperature, four different types of the model drugs were separately added into the PVA solution under constant stirring for 4 hr prior to electrospinning. Drugs were loaded at either 10 or 20 wt.% (based on the weight of PVA powder). Prior to electrospinning, the as-prepared solutions were measured for their viscosity, surface tension, and conductivity using a programmable

viscometer (Brookfield, DV-III), a drop-shape analyzer (KRÜSS, K10T), and a conduc tivity meter (Orion, 160), respectively. All the tests were carried out at 25°C and an average value for each solution was calculated from at least 3 measurements.

Electrospinning of the as-prepared solutions was carried out by connecting the emitting electrode of positive polarity from a high voltage DC power supply (D-ES30PN/M692, Gamma High Voltage Research, USA) to the solutions and the grounding electrode to a home-made rotating metal drum, used as the fiber-collecting device. The electrostatic field strength was fixed at 15 kV/15 cm. For morphological study, the collection time was about 5 min, while, for the rest of the experiments, the collection time was about 24 hr. The drum (outer diameter = 15 cm) was rotated at an angular velocity of about 50 to 65 rpm. The feed rate of the solutions was controlled to about 1 ml/hr using a syringe pump. A schematic photograph of electrospinning apparatus was shown in Figure 3.1. The drug-loaded PVA films were prepared by solution-casting technique from a PVA solution having a concentration of 7% w/v. The thickness of both the electrospun mats (for the mats that were electrospun for about 24 hr) and the as-cast films was controlled between 20 and 30 μm.

3.2.2 Preparation of neat and vitamin-loaded as-spun CA fiber mats and as-cast CA films

A weighed amount of CA powder was dissolved in 2:1 v/v acetone/DMAc to prepare a CA solution at a fixed 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. These mixtures were stirred under constant stirring for 3 h, after which time completely clear solutions were obtained. 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 was calculated from at least 3 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-ended gauge 20 stainless steel needle (OD = 0.91 mm), used as a 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 between the tip of the nozzle and the outer surface of the drum of 15 cm (i.e., the electrostatic field strength of 17.5 kV/15 cm) and the rotational speed of the rotating drum was set at 60 ± 5 rpm. The feed rate of the solutions was controlled at about 1 ml h^{-1} by means of a Kd Scientific syringe pump. For morphological observation of the as-spun products, the collection time was ~5 min, while, for the rest of the experiments, the collection time was ~9 h.

For comparison purposes, both the neat and the vitamin-loaded CA films were prepared by solution-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 controlled between 20 and 30 μm.

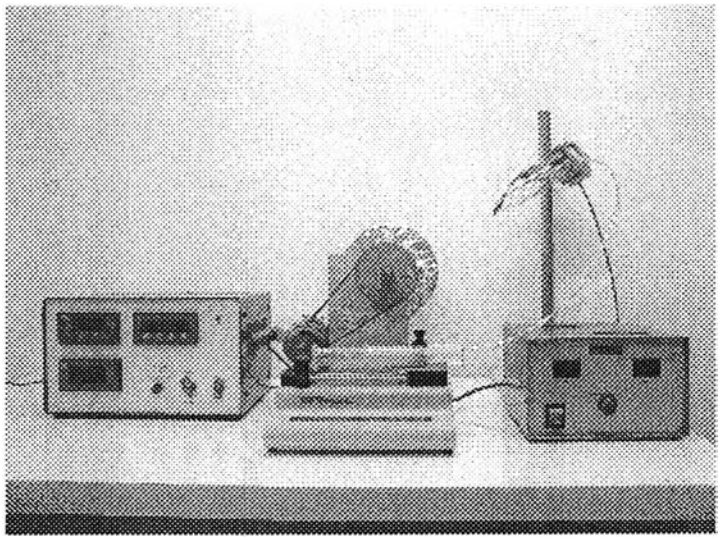

Figure 3.1 A schematic photograph of electrospinning apparatus.

3.2.3 Preparation of acetate buffer

Acetate buffer was chosen to simulate human skin pH condition of 5.5 and used as a releasing media. 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 aqueous sodium acetate solution. Finally, distilled water was then added into the solution up to the final volume.

3.3 Characterization and Testing

3.3.1 Scanning Electron Microscope (SEM)

The morphological appearance of both neat and drug-loaded electrospun mats was observed by a scanning electron microscope (SEM; JEOL JSM-5200). The electrospun mats were sputtered with a thin layer of gold prior to SEM observation. Based on these SEM images, the average diameter of the as-spun fibers and average size of the beads (if any) could be measured. The results were reported as average values from as least 100 measurements. The average number of beads per unit area (i.e. the bead density) of the beaded fibers was calculated from measurements on SEM images of 3500x magnification.

3.3.2 ^{1}H-nuclear magnetic resonance spectrometer (^{1}HNMR)

A ^{1}H-nuclear magnetic resonance spectrometer (^{1}HNMR;Bruker DRX400) was used to investigate the chemical integrity of the model drugs in the drug-loaded as-spun PVA mats (each sample weighed 2–3 mg), using deuterated dimethylsulfoxide (dDMSO) as solvent.

3.3.3 Differential Scanning Calorimeter (DSC)

A differential scanning calorimeter (DSC; Mettler Toledo 822e/400) was used to study thermal behavior of samples. The DSC thermogram (equilibratedwith an indium standard; each sample weighed 3–5 mg) was obtained during heating from 25 to 350 °C at a rate of 10 °C min^{-1} under nitrogen purge (60 ml min^{-1}).

3.3.4 Thermogravimetric Analyzer (TGA)

A thermogravimetric/differential thermal analyzer (TG/DTA; Perkin Elmer Pyris Diamond) was used to investigate thermal behavior of the PVA matrix, the drugs, and the drug-loaded as-spun PVA mats. The TGA thermogram was obtained during heating from 30 to 600 °C at a rate of 10 °C min^{-1} under nitrogen purge (200 ml min^{-1}).

3.3.5 UV-Spectrophotometer

A UV-spectrophotometer (Perkin Elmer, Lambda 10) at a wavelength of 296, 282, 272, and 321 for SS, DS, NAP, and IND, respectively, was used to determine the amount of drugs release and amount of drugs originally present in the as-spun PVA mats and the as-cast PVA films. These wavelength values correspond to the maximum wavelength (λ max) for each drug; however, for DS, we used the λmax for diclofenac. The predetermined calibration curve for each model drug was carefully established. These data were calculated to determine the accumulative amount of drugs released from the samples at each specified testing period. The experiments were carried out in triplicate and the results were reported as average values.

3.3.6 High Pressure Liquid Chromatograph (HPLC)

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 combination. 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.