CHAPTER VIII
ELECTROSPUN DOXYCYCLINE-LOADED POLY(ε-CAPROLACTONE)/POLY(3-HYDROXYBUTYRATE-*CO*-3-HYDROXYVALERATE) COMPOSITE FIBROUS SUBSTRATES AS WOUND DRESSINGS

8.1 Abstract

Doxycycline hyclate (DOXY) incorporated into blend solutions in different ratios (e.g. 75/25, 50/50 and 25/75, (w/w)) of poly(ε-caprolactone) (PCL) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) were fabricated by using an electrospinning technique. Although incorporation of DOXY made the diameter of the DOXY-loaded PCL/PHBV fibrous substrates slightly smaller, the surface topography of those substrates was not affected, as both of the neat and the DOXY-loaded PCL/PHBV fibers remained uniform and smooth. *In vitro* drug release studies suggested an initial rapid release of DOXY followed by a plateau after 36 h, which could be tailored by the PCL/PHBV blend ratio. Sustainable antibacterial activity of the DOXY-loaded PCL/PHBV fibers was apparent against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*. The capability of this antibacterial effect indicated their potential use not merely as a drug carrier but as a wound dressing as well.

(**Key-words**: Electrospinning; Fibrous substrate; PCL; PHBV; Doxycycline)

8.2 Introduction

Numerous processes have been developed and used in preparing polymeric nano- and submicrofibers for biomedical applications (Yang, 2004; Wan, 2003; Chuysinuan, 2009). Electrospinning, a simple and cost-effective technique, is one of the straightforward ways utilized for fabricating fibrous materials, which have large surface-area-to volume, flexible surface functionality, and superior mechanical performance (Cui *et al.*, 2007). On account of the ability to incorporate a wide range

of drugs in an amorphous or solid solution form within the fiber as well as the interconnecting porous structure with high permeability, electrospun fibers have also been attracting much attention as appropriate delivery devices for wound repair and drug delivery system (Kim *et al.*, 2004).

Aliphatic polyesters and their copolymers, which have been electrospun into nanofiber mats, meet several controlled released criteria such as biocompatibility, biodegradability, and high efficacy of drug loading (Chuysinuan, 2009; Kim, 2004; Travis, 2008). Controlled release of tetracycline hydrochloride from poly(lactic acid) (PLA), poly(ethylene-co-vinyl acetate) (PEVA), and their blend was explored. The release profiles suggested relatively smooth release of drug over 5 days (Kenawy *et al.*, 2002). Similar time-dependent kinetics with a fast release in an early period, followed by a very reduced release of diclofenac drug were obtained from poly(ε-caprolactone) (PCL) and the composite PCL/Hydrotalcite-like clay (Tammaro *et al.*, 2009). Poly(3-hydroxybutyrate) (PHB) nanofibers were also electrospun to encapsulate Kanamycin sulphate within a sandwich structure. The drug demonstrated more than 95% release within 8 h, resulting in the inhibition of Staphylococcus aureus growth (Naveen *et al.*, 2010). To achieve sustained-release characteristics of drugs, drug incorporation within blended polymer-based e-spun fiber mats together with adjusting the blending ratio of polymers has been further developed (Han, 2009; Suganya, 2011).

Poly(ε-caprolactone) (PCL) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), their blends, and their composites are very prestigious for providing good support in biomedical fields (Yasin, 1992; Yu, 2011; Meng, 2008). Owing to their nontoxic biodegradation products, suitable degradation rate, excellent tissue compatibility, and good solubility in a wide range of organic solvents, both PCL and PHBV have been highly used as a bone scaffolding material (K-hasuwan, 2011; Sombatmankhong, 2006; Catledge, 2007). Nonetheless, incorporating drugs into the blend of PCL and PHBV have not been reported yet. It has therefore been challenged to apply electrospun PCL/PHBV blend fiber mats as a drug deliver carrier.

Doxycycline (DOXY), a semi-synthetic tetracycline, is bacteriostatic against most of the pathogenic bacteria isolated from inflamed joints of horses and cattle

(Ensink, 1993; Pereira, 1995). It has well known for treating periodontal diseases, such as juvenile and refractory marginal periodontitis, as well as ocular surface diseases, such as recurrent epithelial erosions and sterile corneal ulcerations effectively by inhibiting the activity of matrix metallo-proteinase (MMPs) both *in vitro* and *in vivo* (Tamimi, 2008; Obaidat, 2010; Ramamurthy, 2002; Dursun, 2001). It has also been documented to be chondroprotective in human clinical studies (Sreekanth *et al.*, 2004). Treatment with doxycycline was approved to promote wound healing by reducing inflammation and protease activity (Anumolu *et al.*, 2010).

In the present study PCL, PHBV, and their blends (e.g. 25/75, 50/50 and 75/25, w/w) e-spun fiber mats were fabricated to encapsulate doxycycline hyclate (DOXY) for potential use as drug carriers. The morphological, structural, and mechanical characterizations of all e-spun mats were examined. The release behaviors of DOXY from the fibers were investigated by the total immersion method in a phosphate buffer. *In vitro* antibacterial activity was also tested against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*.

8.3 Experimental

8.3.1 Materials

Poly(ε-caprolactone) (PCL; M_w = 80,000 g·mol^{-1}), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV; M_w = 680,000 g·mol^{-1}), and Doxycycline hyclate (DOXY) were purchased from Sigma-Aldrich, USA. Chloroform, Methanol, and Phosphate buffered saline (10× solution) were purchased from Fisher Scientific, USA. All other chemicals were of analytical grade and used without further purification.

8.3.2 Electrospinning

Blend solutions of PCL, PHBV, and their blends (25/75, 50/50 and 75/25, w/w) at 10 wt% were prepared in chloroform. Around 4 wt% DOXY was solubilized in a small amount of methanol and added to the polymer solutions. The resulting solutions became yellowish but still clear. Electrospinning of these

solutions was carried out using a typical method. The experimental set-up consisted of a syringe and needle, power supply (model CZE1000R, Spellman), syringe pump (KD Scientific), and a variable speed, rotating, stainless steel grounded counter-electrode drum. Briefly, each of the solutions was loaded into a 5 mL syringe with a blunt-tip 18-guage needle (0.8 mm OD) and placed 15 cm away from the leading face of the rotating collection target. The solutions were electrified by applying a positive voltage (15 kV) to the syringe needle by means of an alligator clamp. The solutions were delivered through syringe pumps with the mass flow rate of 1.0 mL·h^{-1}. All experiments were carried out at room temperature and relative humidity of 20%.

8.3.3 Characterization

The morphology of the obtained electrospun fibrous substrates was observed by scanning electron microscope (SEM; JSM-6510, JEOL). The average fiber diameter of the individual fibers was also determined from various positions of at least five different SEM images ($n \geq 50$). Moreover, fiber thicknesses were measured by means of a digital micrometer (Mitutoyo, 0.001 mm resolution) to be in the range of 90–100 μm after spinning for 1 h.

The incorporation of DOXY in the polymeric fibers was confirmed by a Fourier transform infrared spectrometer in attenuated total reflection mode (ATR-FTIR; Nicolet NEXUS 870) at a resolution of 4 cm^{-1} over a wavenumber range of 500 to 4000 cm^{-1}.

The melting, crystallization, and formation of a crystalline structure were investigated by differential scanning calorimetry measurement using a thermal analyzer (TA Instruments DSC Q2000) at a heating rate of 10 °C·min^{-1} from -90 °C to 180 °C. The crystallinity degree (X_c) of PCL and PHBV was calculated from the melting enthalpies (ΔH_m) using the following equation:

$$X_c\,(\%) = \frac{\Delta H_m}{\Delta H_m^0}\frac{1}{\xi} \times 100, \qquad (1)$$

where ΔH_m is the melting enthalpy of the polymer, ΔH_m^0 is the melting enthalpy of fully crystalline polymers, 139.5 J/g for PCL and 109 J/g for PHBV, and ξ is the weight factor of the considered polymer (Del Gaudio *et al.*, 2011).

The water retention of all fiber mats was assessed in a phosphate buffer saline (pH 7.4) at the physiological temperature of 37 °C at various submersion time intervals. The measurements were carried out in pentuplicate within a total submersion period of 36 h. The water retention was calculated according to the following equation:

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

where M is the weight of each specimen after submersion in the medium at each submersion time point and M_d is the weight of the specimen in its dry state after submersion in the medium at each submersion time point.

The mechanical integrity, in terms of Young's modulus, tensile strength, and strain at break, was carried out on dog-bone fibrous mats cut out by means of a stainless steel die (gauge length 25 mm and width 10 mm), according to ASTM D638-V. The mechanical tests were performed by a Zwick/Roell universal testing machine with 500 N load cell and 10 mm/min crosshead speed at ambient conditions. Ten specimens for each sample type were considered.

8.3.4 *In Vitro* Drug Release Assay

8.3.4.1 Actual Drug Content Determination

The actual content of DOXY in the fiber mats was quantified by first dissolving each specimen (square disc; 6 mg) in 10 mL chloroform, then adding a small amount of ethanol in the solution. After that, 1 mL of the solution was added into 9 mL of phosphate buffer saline. The actual amount of the as-loaded DOXY was then quantified with a Lambda 800 UV-Vis spectrometer at the wavelength of 365 nm against a pre-determined calibration curve of DOXY in the

phosphate buffer saline. The solutions of the drug-free fiber mats in chloroform that had been diluted with the phosphate buffer saline were used as blanks. The measurements were carried out in pentuplicate.

8.3.4.2 DOXY-Releasing Assay

Electrospun fiber mats (square disc; 6 mg) were immersed in 10 mL of the phosphate buffer saline solutions at the physiological temperature of 37 °C. 1 mL of samples was taken from the medium after 0, 1, 2, 3, 4, 7, 10, 13, 16, 24 and 36 h, and then the same volume of fresh release medium solution was added as replacement. The content of DOXY in the medium was measured by a UV-Vis spectrometer (Lambda 800, Perkin Elmer) at the wavelength of 365 nm through the use of a pre-determined standard calibration curve. The measurements were carried out in pentuplicate at each time point. The results were presented in term of cumulative release as a function of release time. The cumulative release was calculated according to the following equation:

$$Cumulative\ release\ amount\ (\%) = \frac{M_t}{M_\infty} \times 100, \qquad (3)$$

where M_t is the amount of DOXY released at time t and M_∞ is the actual amount of DOXY found in the electrospun fibers.

8.3.5 Antibacterial Evaluation

The antibacterial activity of the electrospun fibrous substrates was tested against Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 2297) and Gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC 933). The assessment was conducted based on the disc diffusion method of the US Clinical and Laboratory Standards Institute (CLSI). Both the drug-free and DOXY-loaded PCL/PHBV fibrous substrates were cut into circular discs (15 mm in diameter) and placed on Difco™ Mueller Hinton agar in a Petri dish, and then incubated at 37 °C for 24 h. If inhibitory concentrations were reached, there would be no growth of the microbes,

which could be seen as a clear zone around the disc specimens. The zone was then recorded as an indication of inhibition against the microbial species. Triplicate experiments were conducted and the results were reported as average values.

8.4 Results and Discussion

8.4.1 Morphology of Drug-Free and DOXY-Loaded Fibrous Substrates

The representative SEM images of electrospun fibrous substrates are shown in Figure 8.1. The individual fibers possess the common features of being randomly aligned and partially conglutinated. Both the drug-free and the DOXY-loaded fibers appeared to be smooth with no drug crystals detected on the polymer surface, suggesting good and homogeneous dispersion of DOXY in the electrospun fibers. However, it should be noted that the incorporation of DOXY made the fiber diameter slightly smaller. Average fiber diameters of the drug-free and the DOXY-loaded fibers were in the range of 1.8–4.6 μm and 1.0–3.4 μm, respectively. A reasonable explanation is that the addition of DOXY, which was used in a salt form, disturbed the polymer solution by slightly decreasing the viscosity and shearing strength, lowered the surface tension, and therefore enhanced the bending instability (Zeng, 2003; Qin, 2007). Moreover, it seemed that the PCL/PHBV blend ratios significantly influenced the morphology of the obtained fibers. In the PCL/PHBV 75/25 w/w fibers, some spindle-like structures were evident. When PCL and PHBV were equally blended (PCL/PHBV 50/50 w/w), the most consistent and uniform fiber morphology was observed. This could be resulted from the substantial suitability of the polymer solution used for electrospinning (Han *et al.*, 2009). When the content of PHBV was raised up to 75% (PCL/PHBV 25/75 w/w), fused-fiber structures were noticed; but the fibers were still continuous and smooth.

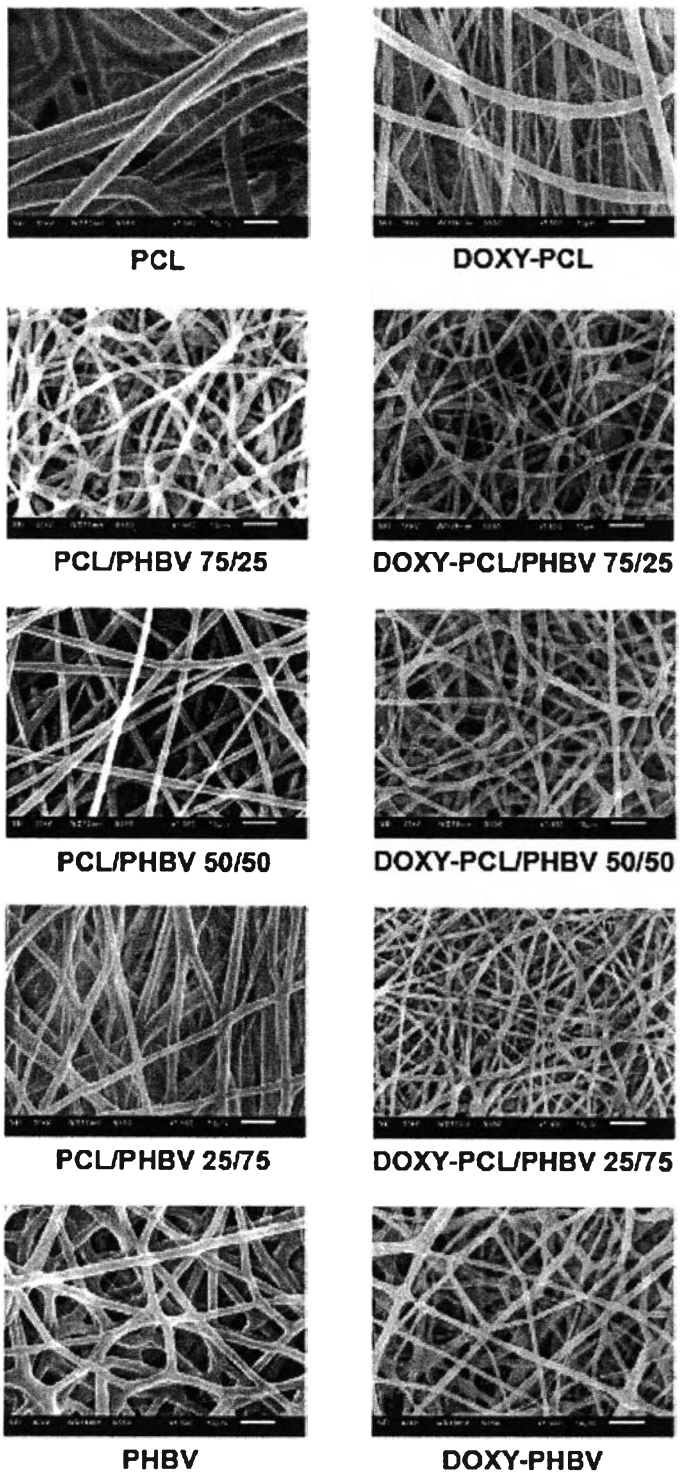

Figure 8.1 SEM images (scale bar = 10 μm and magnification = 1500 ×) of drug-free and DOXY-loaded electrospun fibrous substrates.

8.4.2 Characterization of Drug-Free and DOXY-Loaded Fibrous Substrates

ATR-FTIR spectra for PHBV, PCL, PCL/PHBV 50/50, DOXY-loaded PCL/PHBV 50/50, and DOXY were demonstrated in Figure 8.2. The peak at 1724 cm^{-1} corresponded to the C=O vibration of ester was observed in all of the fibrous spectra. The IR of PCL/PHBV blend carries features of neat PCL and neat PHBV spectra, confirming the presence of both PCL and PHBV within the blend fibers (K-hasuwan *et al.*, 2011). The IR spectrum of DOXY carries the aromatic hydrocarbons at 1600-1585 cm^{-1}, the carbon-carbon stretching vibrations in the aromatic ring at 1500-1400 cm^{-1}, and the amide group at 1650 cm^{-1} (Tamimi *et al.*, 2008). The IR spectrum of DOXY-loaded PCL/PHBV shows all features of the neat PCL, neat PHBV, and DOXY, especially the absorption peaks from 1560 to 1650 cm^{-1} appearing to be the main evidence for the existence of the drug in the PCL/PHBV blend.

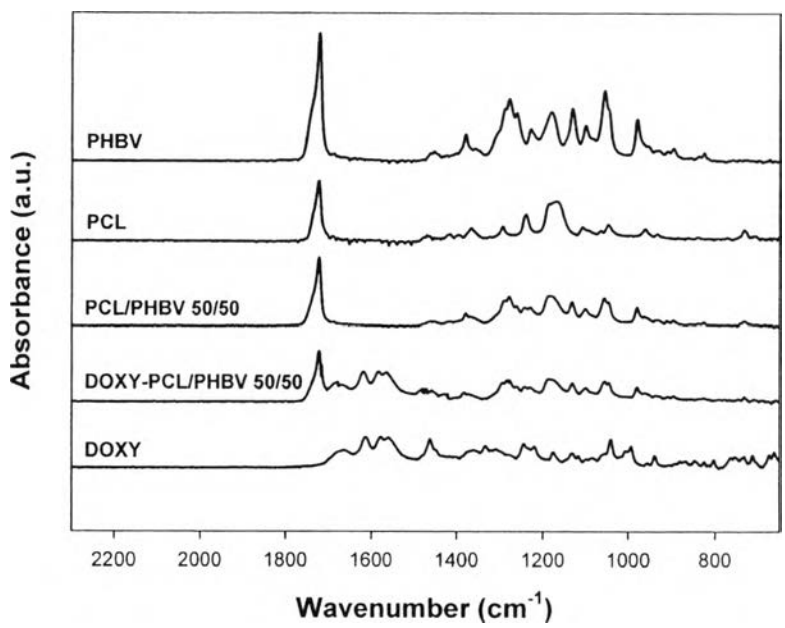

Figure 8.2 ATR-FTIR spectra of neat PHBV, neat PCL, PCL/PHBV 50/50, DOXY-loaded PCL/PHBV 50/50, and DOXY.

Owing to the similar results of both drug-free and DOXY-loaded fibrous substrates, only the results of thermal properties (see Table 8.1 and Table 8.2) and DSC thermograms (see Figure 8.3) of the drug-free fibers were reported. The melting temperatures of PCL and PHBV were 55.0 °C and 154.8 °C, respectively. In the case of PCL/PHBV blends, two single melting peaks in the range of 53.7–55.0 °C and 154.8–156.9 °C associated respectively to the PCL and PHBV component were detected, indicating two immiscible components in the blends. The degree of crystallinity was calculated from the melt endotherms and normalized with respect to the content of each component. The degree of crystallinity of the PCL component in PCL/PHBV blends was significantly lower than that of the neat fiber, while the degree of crystallinity of the PHBV component in the blends was significantly higher than that of the neat one. On the other hand, the crystallization temperature of the PCL and PHBV component in the blends decreased with respect to the neat PCL and the neat PHBV, respectively. These results suggested that the two immiscible polymers interacted to some degree in the nucleation and growth of crystalline structures because of the deactivation of heterogeneity (Del Gaudio *et al.*, 2011).

Table 8.1 Melting temperature (T_m) and melting enthalpy (ΔH_m) of electrospun fibrous substrates

Sample	$T_{m(PCL)}/T_{m(PHBV)}$ (°C)	$\Delta H_{m(PCL)}/\Delta H_{m(PHBV)}$ (J/g)
PCL	55.0	59.4
PCL/PHBV 75/25	54.3/155.5	42.4/17.5
PCL/PHBV 50/50	53.7/155.7	20.2/32.8
PCL/PHBV 25/75	54.9/156.9	13.6/47.3
PHBV	154.8	55.3

Table 8.2 Crystallization temperature (T_c), crystallization enthalpy (ΔH_c), and crystallinity degree (X_c) of electrospun fibrous substrates

Sample	$T_{c(PCL)}/T_{c(PHBV)}$ (°C)	$\Delta H_{c(PCL)}/\Delta H_{c(PHBV)}$ (J/g)	$X_{c(PCL)}/X_{c(PHBV)}$ (%)
PCL	31.1	68.1	42.6
PCL/PHBV 75/25	25.7/88.5	50.8/26.6	n/a
PCL/PHBV 50/50	23.5/92.1	23.0/43.9	n/a
PCL/PHBV 25/75	20.4/93.1	19.9/54.7	n/a
PHBV	95.0	56.4	50.7

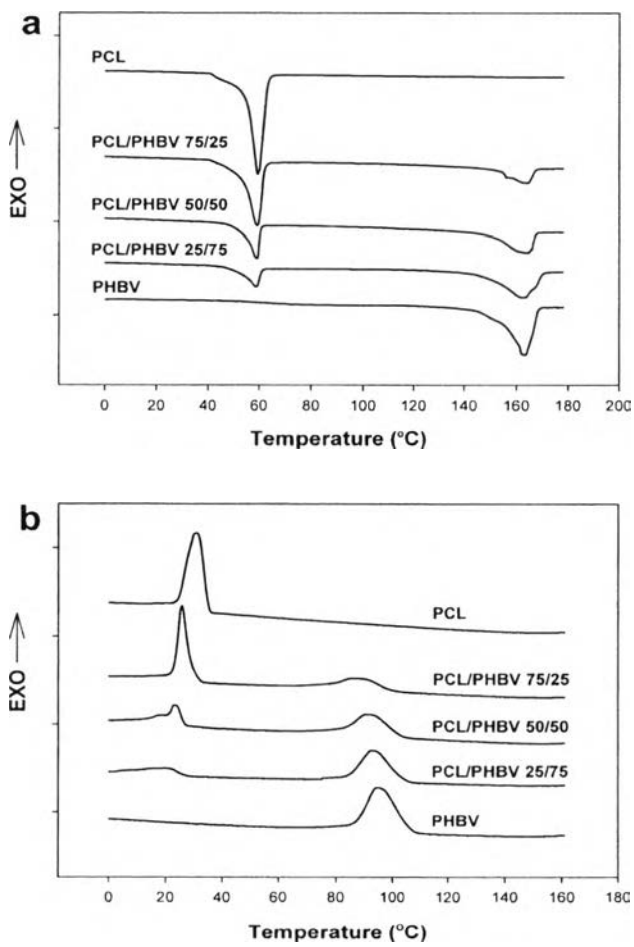

Figure 8.3 DSC thermograms of electrospun fibrous substrates, (a) heating and (b) cooling scan.

Both the drug-free and the DOXY-loaded fibrous substrates were further characterized for their water retention behavior upon submersion in phosphate buffer saline solution (pH 7.4) at the physiological temperature of 37 °C as a function of the submersion time (see Figure 8.4). At any given time point, the water retention of all fiber mats increased with an initial increase in the submersion time and became saturated with the medium after they had been submerged for 24 h. Simultaneously, it was noticed that the amount of retained water in all blend fibers, at any given time point, was higher with respect to the neat ones; both drug-free and DOXY-loaded PCL/PHBV 50/50 showed the greatest amount of retained water. The water retention reached a maximum of around 250% for the drug-free and 270% for the DOXY-loaded PCL/PHBV 50/50, around 220% for the drug-free and 240% for the DOXY-loaded PCL/PHBV 75/25, and around 190% and 170% in the case of the drug-free and the DOXY-loaded PCL/PHBV 25/75, respectively. Apparently, the amount of retained water depends not only upon the polymer blend ratio, but also upon the presence of the drug. When an appropriate salt form of the drug was added, the charge density was increased; and hence, the conductivity of the polymer solution was increased as well (Stanger *et al.*, 2009). The increase in the conductivity led to higher amount of water retention (Jang *et al.*, 2006). Porous nature would be another factor contributing to the different water retention behavior of the fiber mats. Water could be absorbed and retained within the porous structure of the fibers held by the capillary action (Chuysinuan *et al.*, 2009). Therefore, it could be assumed that the blend fibers might have more porosity or pore volume than the neat ones, ranking in a descending order as: PCL/PHBV 50/50, PCL/PHBV 75/25, and PCL/PHBV 25/75, respectively.

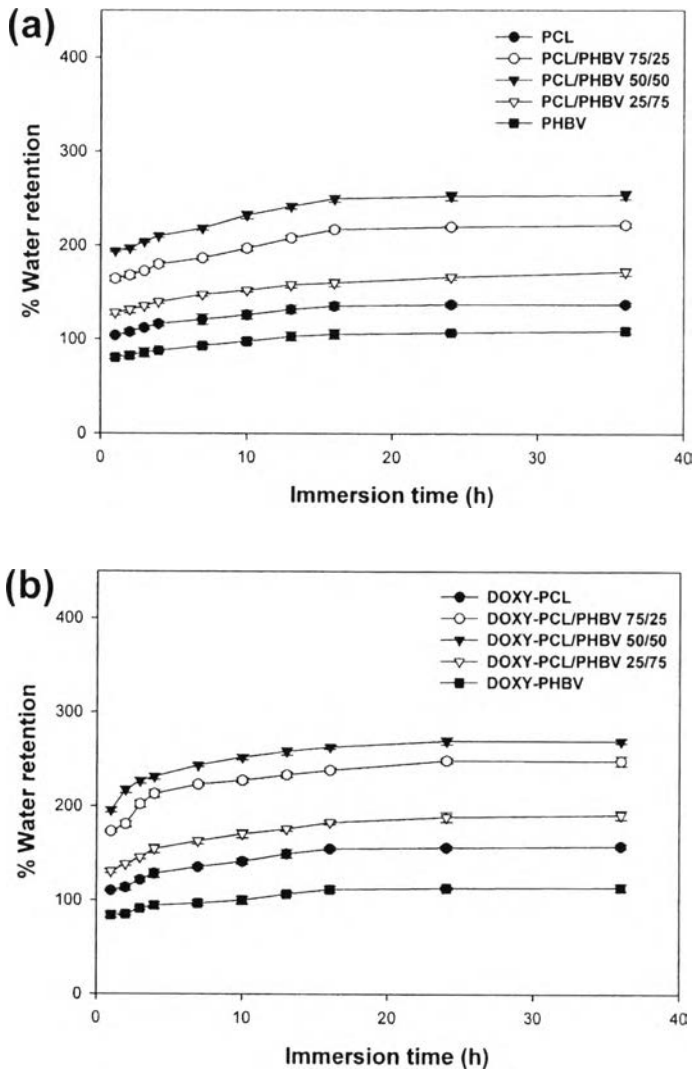

Figure 8.4 Water retention behavior of (a) the drug-free and (b) the DOXY-loaded fibrous substrates immersed in phosphate buffer saline solution pH 7.4 at 37 °C for various time intervals (n = 5).

Due to the similarity of mechanical properties of both drug-free and DOXY-loaded fibrous substrates, merely mechanical assessments of the drug-free fibrous substrates are presented. Stress-strain behavior of the fiber mats shown in Figure 8.5 has a shape characteristic with a linear elastic region followed by a period of plastic deformation until failure. The stress-strain curves were used to evaluate tensile strength, Young's modulus, and elongation at break values (see Table 8.3).

Regarding the neat polymer, PCL exhibited the lowest tensile strength and Young's modulus, as well as the largest elongation at break, while PHBV showed the lowest deformation at break. For the blends, the tensile strength value was found to be highest in PCL/PHBV 75/25, followed by that value of PCL/PHBV 50/50. The improvement in the tensile strength might be attributed to the fusing between the fiber junctions (Zhang *et al.*, 2006) and the fiber packing density (Del Gaudio *et al.*, 2011). Although all PCL/PHBV blends had higher Young's modulus values than those of the neat PCL, these blends still had those values lower than those of the neat PHBV. The elongation at break, however, increased with the PCL content.

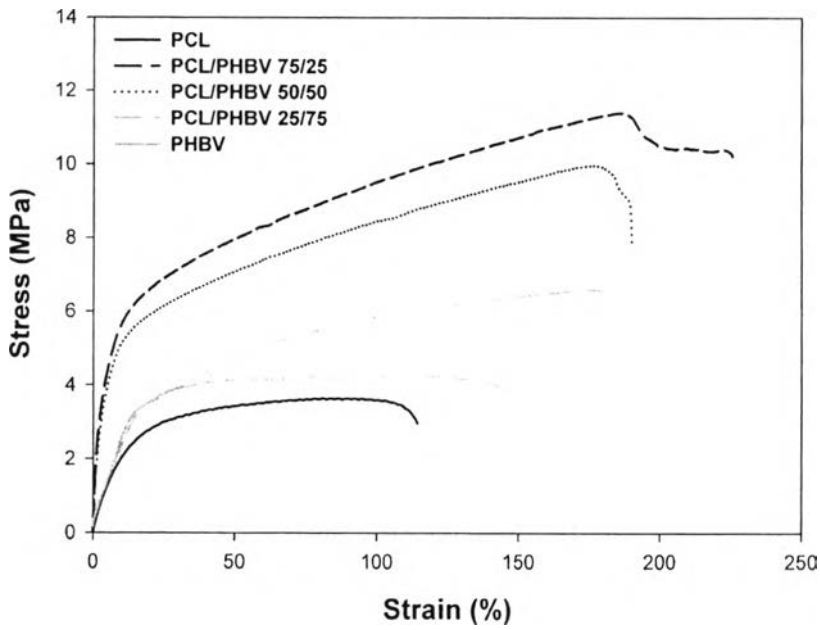

Figure 8.5 Stress-strain curves of the drug-free PCL/PHBV electrospun fibrous substrates.

Table 8.3 Mechanical integrity of the drug-free PCL/PHBV electrospun fibrous substrates

Sample	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation at Break (%)
PCL	3.3 ± 0.5	107.0 ± 2.1	307.4 ± 39.2
PCL/PHBV 75/25	5.4 ± 0.3	183.9 ± 3.5	225.8 ± 52.7
PCL/PHBV 50/50	5.1 ± 0.3	173.0 ± 4.7	190.0 ± 41.5
PCL/PHBV 25/75	3.6 ± 0.2	115.1 ± 3.8	136.3 ± 25.6
PHBV	3.7 ± 0.1	280.8 ± 4.1	90.2 ± 22.4

8.4.3 Drug-Loaded Determination and *In Vitro* Drug Release Studies

Prior to determining the release characteristic of DOXY from the DOXY-loaded fibrous substrates, the calibration curve of DOXY in the medium and the actual amount of DOXY within the fibers need to be investigated. The calibration curve of DOXY in the medium was [abs] = 9.491[c] + 0.039 (r^2 = 0.990), where [abs] is UV absorbance value and [c] is DOXY concentration in mg·mL^{-1}. The theoretical contents of DOXY in the DOXY-loaded fiber were 3.93, 3.84, 3.74, 3.67, and 3.60 wt% for DOXY-PCL, DOXY-PCL/PHBV 75/25, DOXY-PCL/PHBV 50/50, DOXY-PCL/PHBV 25/75, and DOXY-PHBV, respectively (based on the weight of the fiber mats). After the drug assay experiments, the actual contents of DOXY within the fiber mats were 2.93 ± 0.10, 3.53 ± 0.10, 3.51 ± 0.10, 3.11 ± 0.19, and 2.62 ± 0.16 wt% for DOXY-PCL, DOXY-PCL/PHBV 75/25, DOXY-PCL/PHBV 50/50, DOXY-PCL/PHBV 25/75, and DOXY-PHBV, respectively (based on the weight of the fiber mats). These values corresponded to 75 ± 2, 92 ± 3, 94 ± 2, 85 ± 5, and 73 ± 3 % (based on the weight of DOXY initially contained in the spinning solutions). The actual amounts of DOXY in the DOXY-PCL/PHBV blend fibers were more than 80%, indicating better compatibility between the drug and the matrix system. The deviation from the ideal value of 100% would be owing to the inhomogeneous distribution of DOXY in different parts of the obtained fibrous substrates.

The cumulative release of DOXY from all electrospun fibrous substrates as a function of the submersion time was demonstrated in Figure 8.6. The cumulative amount of the released DOXY increased rather rapidly during the first 16 h of the submersion time. Further increase in the submersion time resulted in a gradual increase in the cumulative amount of DOXY released to eventually assume a plateau value at 36 h of the submersion time. An initial fast release is possibly because drug molecules dispersing close to the surface of polymer fibers and absorbing near the surface would diffuse out quickly in initial time. The slower release might be ascribed to the drug being encapsulated in the inner core of the fiber matrix, which would absolutely need a long distance to diffuse through, and therefore take longer time to be released. Furthermore, owing to the decline of the diffusion driving force induced by the reduction of the drug molecules inside the inner space, the release rate became slower and slower (Han *et al.*, 2009). For the DOXY-PCL/PHBV 50/50 fibrous substrates at any given submersion time point, the cumulative amount of the released DOXY was the greatest, followed by those released from DOXY-PCL/PHBV 75/25, DOXY-PCL/PHBV 25/75, DOXY-PCL, and DOXY-PHBV. After 36 h the fibrous substrates that had been submerged in the medium, the released values could finally reach to about 67, 56, 41, 31 and 25% for DOXY-PCL/PHBV 50/50, DOXY- PCL/PHBV 75/25, DOXY-PCL/PHBV 25/75, DOXY-PCL, and DOXY-PHBV (equivalent to about 2.4, 2.0, 1.3, 0.9, and 0.7 wt%, based on the weight of the fibrous substrates, respectively).

The resulting cumulative amounts of released DOXY could be correlated to the water retention behavior of the DOXY-loaded fibrous substrates that showed the greatest values in DOXY-PCL/PHBV 50/50, followed by those in DOXY-PCL/PHBV 75/25, DOXY-PCL/PHBV 25/75, DOXY-PCL, and DOXY-PHBV, respectively. Due to the higher retained water, the macromolecular mobility increases, leading to higher drug mobility (Siepmann *et al.*, 2007).

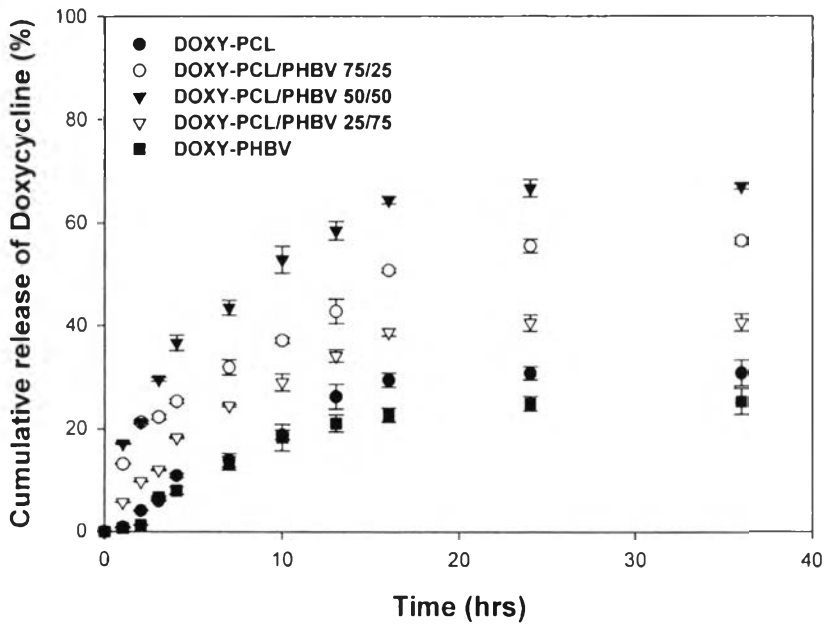

Figure 8.6 Cumulative release of DOXY from DOXY-loaded electrospun fibrous substrates, in terms of the percentage of the weight of DOXY released divided by the actual weight of DOXY in the samples, as a function of submersion time in phosphate buffer saline solution, at physiological temperature of 37 °C ($n = 5$).

8.4.4 Antibacterial Activity

The antibacterial activity of DOXY-loaded fibrous substrates was assessed against two typical pathogenic bacteria *P. aeruginosa* and *S. aureus* to evaluate their biomedical application potentials. The activity of all drug-free fibrous substrates against these bacteria was used as control. Table 8.4 summarizes the average lengths of the inhibition zones (measured from the edge of the samples to the edge of the clear zone) for all of the substrates investigated. As reported in Table 8.4, all of the neat fibrous substrates showed no activity against the tested bacteria. For the DOXY-loaded specimens, inhibition zones were evident. The inhibition zone lengths of the DOXY-loaded PCL/PHBV 50/50 against both types of bacteria were the greatest (i.e., 6.5 mm for *S. aureus* and 3.0 mm for *P. aeruginosa*), followed by those of the DOXY-loaded PCL/PHBV 75/25. These results were consistent with the results on the release of DOXY, as the DOXY-loaded PCL/PHBV 50/50 had the

greatest cumulative amount of released DOXY, followed by the DOXY-loaded PCL/PHBV 75/25. Additionally, it should be noted that the *S. aureus* was more sensitive to DOXY than *P. aeruginosa* in this experiment. This result could be explained from the difference in the cell wall structures of bacteria. A major structural difference between Gram-negative bacteria and Gram-positive bacteria is that the cell wall of the former is overlaid with an outer membrane that consists of lipopolysaccharide, and this region offers a supplementary barrier that inhibits the penetration of antimicrobial agents (Tan and Obendorf, 2007).

Table 8.4 Average lengths of the inhibition zones (measured from the edge of the samples to the edge of the clear zones) for all fibrous substrates

Sample	Inhibition Zone Length (mm) *S. aureus*	Inhibition Zone Length (mm) *P. aeruginosa*
DOXY-PCL : PCL	2.5 : 0	1.2 : 0
DOXY-PCL/PHBV 75/25 : PCL/PHBV 75/25	5.5 : 0	2.5 : 0
DOXY-PCL/PHBV 50/50 : PCL/PHBV 50/50	6.5 : 0	3.0 : 0
DOXY-PCL/PHBV 25/75 : PCL/PHBV 25/75	3.0 : 0	1.5 : 0
DOXY-PHBV : PHBV	2.5 : 0	1.0 : 0

8.5 Conclusions

DOXY regarded as an effective drug associated with infected wounds was successfully loaded in PCL, PHBV, and PCL/PHBV (i.e., 75/25, 50/50 and 25/75, (w/w)) fibrous substrates using electrospinning technique. The obtained fibrous substrates were smooth and no evidence of any kind of aggregate was observed on the surface of the DOXY-loaded fibrous substrates. The average diameters of the drug-free and the DOXY-loaded fibers were in the range of 1.8–4.6 μm and 1.0–3.4 μm, respectively. Almost all of the DOXY contained in the spinning solutions was retained within the obtained fibrous substrates (i.e., 73 to 94% on average). The

cumulative amount of DOXY released from the DOXY-loaded fibrous substrates increased rather rapidly during the first 16 h of the submersion time, while it increased more slowly as the submersion time increased further. The cumulative amount of DOXY released from the DOXY-PCL/PHBV 50/50 fibrous substrate was the greatest, followed by those released from the DOXY-PCL/PHBV 75/25 and the DOXY-PCL/PHBV 25/75 fibrous substrates, respectively. The potential use of the DOXY-loaded fibrous substrates as wound dressings was assessed by antibacterial activity against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*. The results showed that the DOXY-loaded fibrous substrates appeared to be effective against the two pathogens.

8.6 Acknowledgments

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