CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Polymer Chemicals

1. Poly(d,l-lactide) (PDLLA)
2. Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV)
3. poly(ethylene glycol) (PEG)
4. Silver nitrate ($AgNO_3$)
5. Gram negative bacteria *Escherichia coli* (E.coli)
6. Gram-positive bacteria *Staphylococcus aureus* (S.aureus)

3.1.2 Solvents

1. Chloroform 99.9% purity
2. *n*-hexane
3. *N*,*N*-Dimethyl formamide (DMF)
4. Phosphate buffered saline
5. Nutrient broth
6. Agar

3.2 Equipment

1. Small-scale melt spinning machine
2. Thermogravimetric/differential thermal analyzer (TGA/DTA)
3. Scanning Electron microscope (SEM)
4. Universal testing machine (UTM)
5. Mettler Toledo/DSC822 Differential Scanning Calorimeter (DSC)
6. Transmission electron microscopy (TEM)
7. UV-visible absorption
8. Atomic absorption spectrometer (AAs).
9. 37°C incubators

3.3 Methodology

3.3.1 Preparation of Polymer Blend

3.3.1.1 *Preparation of Materials*

PDLLA and PHBV were dried in vacuum at 50°C for 48 hours and stored under vacuum in desiccators.

3.3.1.2 *Blending of PDLLA/PHBV Polymer*

A mixture of PDLLA/PHBV was dissolved in hot chloroform at 50°C for 2 hours to produce PDLLA/PHBV pre-blends of 10%, 20%, 30%, 40%, and 50% w/w of PHBV, respectively. After that, the polymer samples were precipitated by dropping t *n*-hexane into the PDLLA/PHBV solutions and then blended samples were dried under vacuum at 50°C for 48 hours and was stored under vacuum in desiccators until spinning.

3.3.1.3 *Blending of PDLLA/PHBV/PEG1000 Polymer*

The suitable composition of PDLLA/PHBV blend and PEG1000 at 10%, 20%, 30% and 40% w/w of PDLLA/PHBV blends were dissolved in the hot chloroform solvent at 50°C for 2 hours. After that, the polymer samples were precipitated by dropping the *n*-hexane into PDLLA/PHBV/PEG solution and then the blended samples were dried under vacuum at 50°C for 48 hours and was stored under vacuum in desiccators until spinning.

3.3.2 Preparation of Fibers Containing Silver nanoparticles

3.3.2.1. *Finding the Suitable Component of Polymer Blends without Silver Nanoparticles to Spinning Fibers*

All of composition of PDLLA/PHBV was spun in the same conditions and then tensile strength and toughness were measured to find the suitable blend compositions. After that, that suitable blend composition was blended with various percent weight of PEG1000 by using the same method mentioned above to find the suitable blend composition of PDLLA/PHBV/PEG to spin the fiber containing silver nanoparticles.

3.3.2.2 *Preparation of Polymer Blends Containing Silver Nanoparticles*

The suitable PDLLA/PHBV/PEG1000 blend polymers containing silver nanoparticles were prepared by adding silver nitrate as 0.2%, 0.3% and 0.4% w/w into PDLLA/PHBV/PEG1000 mixture after dissolving $AgNO_3$ in 50/50 chloroform-to-dimethylformamide ratio and then stirring for 24 hours to produce silver nanoparticles. Finally, the blended sample containing various silver nanoparticles contents were precipitated by using *n*-hexane.

3.3.2.3 *Spinning of the Fibers without and Containing Silver Nanoparticles*

The PDLLA/PHBV fibers were spun at 180°C, 1.5 mm min^{-1} of piston velocity and 20 m $min^{-1}$of take up speed by using a melt spinning machine from Venture & Consultancy Bradford Ltd. The minimal dead volume within the system enable sample sizes of as small as 10g to be processed satisfactorily. The water cooling bath is maintained at a temperature of 30°C, while the vertical distance or air gap between a 1 mm single-hole spinneret and the surface of the water cooling bath is kept constant at 2 cm.

The PDLLA/PHBV/PEG and PDLLA/PHBV/PEG/Silver nanoparticle were spun by using the same melt spinning machine and same conditions as above with spinning temperature of 170°C.

Flow diagram to produce fibers containing silver nanoparticles is shown below

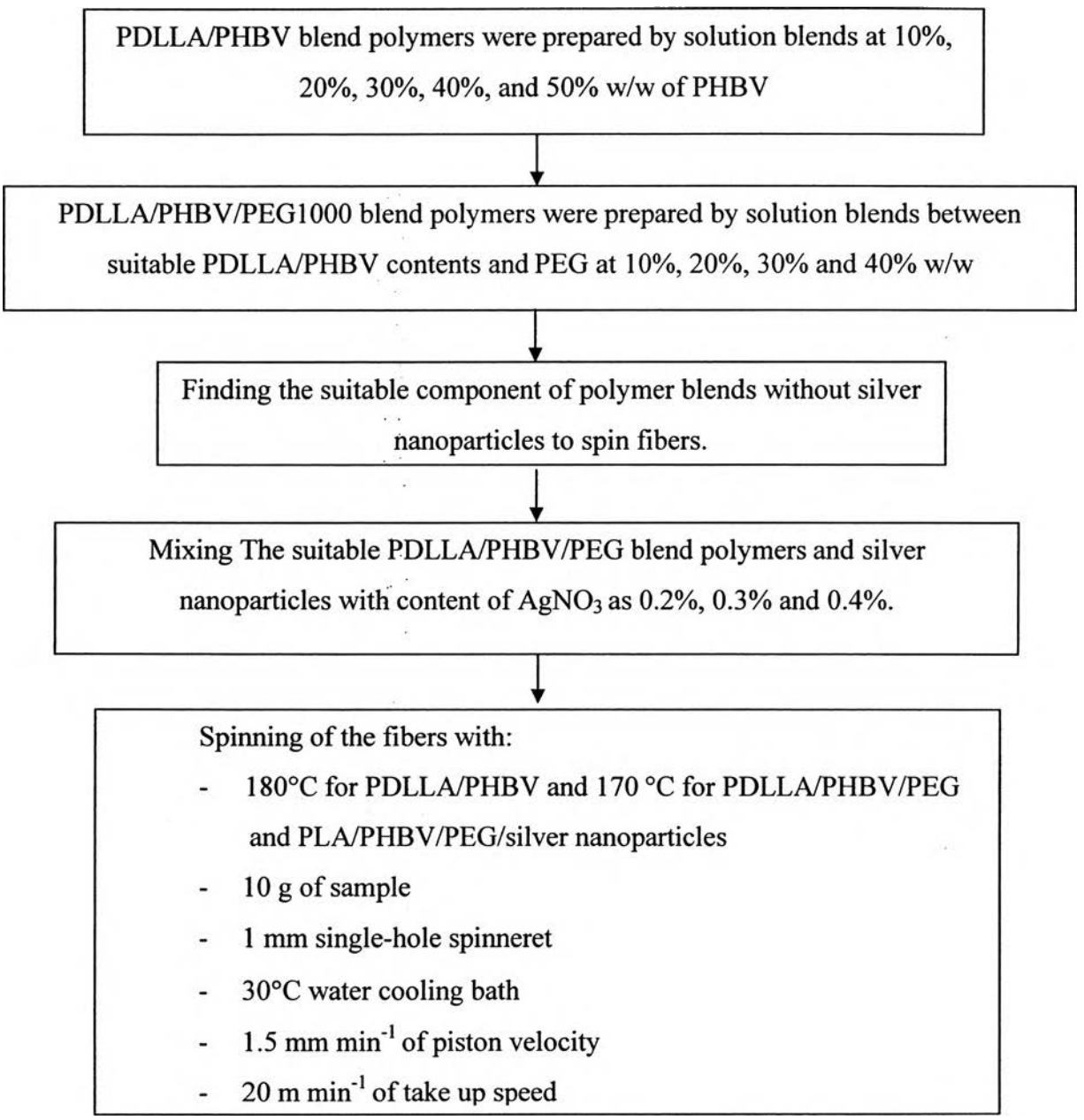

Figure 3.1 Preparation of fibers containing silver nanoparticles diagram.

3.3.3 Measurements

3.3.3.1 *Blending Polymer*

a) *Thermal Stability*

The decomposition temperatures (T_d) for PDLLA and their blends were measured with a Mettler Toledo Thermogravimetric Analyzer/ Differential Scanning Calorimeter (TGA/DSC) within the temperature range from 25°C to 500°C. The heating rate during thermal scanning is fixed at 10 °C min^{-1}.

b) *Miscibility of Polymer blends, Crystallization and Melting behavior*

Differential Scanning Calorimeter (DSC) is used to record the non-isothermal cold and melt-crystallization exotherms as well as the subsequent melting endotherms for PDLLA and their blends. The experiments started with heating PDLLA and their blends from 25°C to a fusion temperature of 200°C at a rate of 20°C min^{-1} for a melt-annealing period of 10 min to reset previous thermal histories, after which the samples were taken out and immediately quenched in liquid nitrogen to attain the completely amorphous state of the samples. To observe the non-isothermal cold-crystallization and subsequent melting behavior, each sample was reheated again with DSC from -60 to 200°C at a rate of 10°C min^{-1}. After that, the sample was cooled to -60°C at a cooling rate of 10°C min^{-1} to observe the non-isothermal melt-crystallization behavior.

3.3.3.2 *Fibers containing Silver nanoparticles*

a) Producing Silver Nanoparticles from Silver Nitrate in Polymer Blends Mixture

UV-visible absorption spectra are used to observe the reduction of the silver ions to metallic silver nuclei or silver nanoparticles. The intensity of the surface plasmon resonance absorption at 416 nm was observed with time. Moreover, Transmission Electron Microscopy (TEM) shows the TEM images of the silver nanoparticles after the preparation of the solution to observe their average diameter and their size distribution in polymer blend mixture

b) Morphology of the Fibers Containing Silver Nanoparticles

The morphology and silver nanoparticles distribution on surface of fibers were investigated by JEOL JSM-5200 Scanning Electron Microscopy (SEM)

c) Mechanical Properties of the Fibers Containing Silver Nanoparticles

The mechanical properties are observed by the tensile test using a instron testing machine. The tests were carried out at cross-head speed 60 mm min^{-1}. The results obtained were averaged over ten samples for each condition. The reports showed the physical break stress, initial E-modulus and elongation.

3.3.4 Antimicrobial Activity Testing

3.3.4.1 *Preparation of bacteria*

E. coli and S. aureus bacteria were grown in nutrient broth in shake flasks at 37°C in incubator for 7 hours prior to harvesting. After the incubation, the concentration of the bacteria was abjusted to approximately 10^8 CFU.

3.3.4.2 *Silver ion release*

The fibers containing silver nanoparticles were cut which weighed approximately 0.04 g. The in vitro release of silver ions were carried out in 5 mL of 0.02 mol L^{-1} phosphate buffered saline (PBS, pH= 7.4). The samples are incubated at 37°C under oscillation of 50 round min^{-1}. The silver ions concentration in the solution released is measured every 24 hours over 18-day test period by an atomic absorption spectrometer (AAs).

3.3.4.3 *The Agar diffusion test*

This method is used to observe antibacterial efficiency that will be performed in nutrient broth medium solid agar petri dish. Starting with inoculating E. coli and S. aureus bacteria into growth medium. Approximately 10^8 CFU of each micro organism will be spread on agar plates. After that, the fibers containing silver nanoparticles will be cut into 1.0 cm in many pieces and then placed on E. coli and S. aureus cultured agar plates. The zone of inhibition will be then measured clear zone.