CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 L-lactide Monomer ((3*S*)-*cis*-3,6-Dimethyl-1,4-dioxane-2,5-dione) (99.5% in water)

L-lactide was supplied by Bio Invigor Corporation Co., Ltd.

3.1.2 Silk Sericin

The silk sericin powder was supported by Chul Thai Silk Co., Ltd.

3.1.3 Catalyst

2-ethylhexanoic acid Tin (II) ($Sn(Oct)_2$) was purchased from Sigma Aldrich Chemical.

3.1.4 Lithium Chloride (LiCl)

Lithium chloride (LiCl) was supplied from Fluka.

3.1.5 Reactants for Polybenzoxazine Synthesis

Bisphenol-A (97% purity) was supplied from Sigma Aldrich Chemical. 1,6-Diaminohexane (99% purity) was purchased from Fluka. And Paraformaldehyde (98.7%, purify) was supplied from Merck.

3.1.6 Marl Filler

The marl, or marly limestone, used is a product from Lopburi Province, Thailand.

3.1.7 Modifying Agents

The stearic acid ($CH_3(CH_2)_{16}COOH$) and the silane coupling agent—(3-aminopropyl)trimethoxysilane, $NH_2(CH_2)_3Si(OCH_3)_3$—were purchased from Sigma Aldrich Chemical.

3.1.8 Solvents

Toluene (AR grade), dimethylsulfoxide (DMSO, AR grade), ethyl acetate (AR grade), and 1,4-dioxane (AR grade) were supplied from Lab Scan Co., Ltd. Tetrahydrofuran (THF, HPLC grade) was purchased from Burdick&Jackson supplier (B&J). Methyl and Ethyl alcohol were purchased from Italmar.

All chemicals are used without further purification.

3.2 Instruments

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra of sericin, lactide, sericin-g-PLA copolymers, polybenzoxazine precursors, and the composites were obtained on a Nicolet Nexus 670 FT-IR spectrometer in the frequency range of 4000-400 cm^{-1} with 32 scans at a resolution of 4 cm^{-1}. A KBr pellet technique was applied in the preparation of powder samples. The presence of functional groups of polymers were investigated by using FTIR.

3.2.2 Proton Nuclear Magnetic Resonance (^{1}H NMR)

Proton Nuclear Magnetic Resonance spectra were recorded on Varian Inova 500 NMR spectrometer working at 500 MHz for proton. The preparation of silk sericin and graft copolymer samples were carried out by dissolving it in D_2O in 5 mm NMR tubes at the room temperature. The sample concentration was about 1.0wt%, while the PLA and PBZ samples were dissolved in $CDCl_3$ in 5 mm NMR tubes at the room temperature (1.0wt%). All peaks referenced relative to tetramethylsilane (TMS).

3.2.3 Thermogravimetric Analysis (TGA)

TG-DTA curves were collected on a Mettler Toledo TGA instrument. The samples were loaded on the platinum pan and heated from 50°C to 850°C at a heating rate of 10°C/min under N_2 flow of 50 mL/min.

3.2.4 Differential Scanning Calorimetry (DSC)

DSC analyses were carried out using a Perkin-Elmer DSC 7 instrument. The sercin, lactide and graft copolymer samples were first heated from 30°C to 200°C and cooled down at a rate of 10°C/min under a N_2 atmosphere with a flow rate of 20 ml/min. The sample was then reheated to 200°C at the same rate. For polybenzoxazine, polybenzoxazine–marl composite, and the biocomposite samples were heated from 30°C to 400°C at a rate of 10°C/min under a N_2 atmosphere with a flow rate of 20 ml/min.

3.2.5 Scanning Electron Microscope (SEM)

Scanning electron microscopy was performed on JEOL JSM-5410 Model with 15 kV to observe the morphology on the surface fracture of the samples. Bonding between filler and matrix, and dispersion of filler in the matrix are determined. The specimens were fractured in liquid nitrogen, dried in the oven at the temperature about 60°C, and coated with gold under vacuum before observation to make them electrically conductive. Elemental analysis on the surface of marl particles was also made by EDX analysis to determine composition of marl particles.

3.2.6 Gel Permeation Chromatrography (GPC)

Gel Permeation chromatrography was carried out Shimadzu LC-10A dvp instrument to observe molecular weight of synthesized polymer. The system was operated in THF and water as the mobile phase for PBZ and graft copolymer, respectively using Waters Styragel THF (7.8*600 mm) Column, which was supplied in THF, PL aquagel-OH 50, 8 μm (7.5*300 mm) Column, which was supplied in water and RID-10A detector. The THF and water solvents were filtrated with MN 615 with 155 mm diameter filter paper under vacuum. The crude polymers were dissolved in THF and water at the concentration of 0.5wt% and then filtrated with 0.45 mm diameter filter before injecting into the column. The experimental conditions were 40°C column temperature, 1 ml/min flow rate, and 30 min run time. Molecular weight distributions (MWD) of PBZ were calculated employing a polystyrene calibration, whereas MWD of graft copolymer were calculated employing a PL Pollutant Calibration kit (PL).

3.2.7 Mechanical Testing

3.2.7.1 Instron Universal Testing Machine

Flexural test was performed using Instron/4206 Universal Testing Machine. Flexural specimens were prepared according to ASTM D790M. An Instron Model 4206 fitted with a 5 kN static load cell and a standard 3-point bending fixture. Flexural test specimens with dimensions of 60×10×3 mm^3 were tested for each material using Series IX control and analysis software. Samples were flexed until breakage at a rate 1.28 mm/min using a support span of 48 mm. Flexural strain was calculated based upon crosshead movement. The flexural stress, modulus, and strain were reported from at least five average values.

3.2.7.2 Impact Testing

The sheets of sample were cut into the specimen shape following the ASTM D256 (notched IZOD type) with dimensions of 64×12.7×3.2 mm^3, then the impact strength were tested by the ZWICK impact testing machine with the pendulum load of 2.7 J. The impact strength was reported from at least five average values.

3.2.8 Dynamic Mechanical Analysis (DMA)

Dynamic mechanical spectra were obtained with a dynamic-mechanical analyzer NETZSCH DMA 242 instrument to measure the glass transition temperature (T_g) of polybenzoxazine and their composites. The specimens with dimensions of approximately 60×10×3 mm^3 were tested in a rectangular torsion fixture. A sinusoidal shear strain of 0.1% was applied at a frequency 1 Hz during each temperature sweep experiment. Linearity of the chosen shear strain was verified by a strain sweep prior to each experiment. Measurement were collected every 3°C as the samples were heated at a rate of approximately 2°C/min from -100°C to well above the glass transition of each material.

3.2.9 X-ray Diffraction (XRD)

X-ray diffractometer (XRD) was used to investigate the crystal structure of graft copolymer. X-ray diffraction patterns were measured on a Bruker AXS Model D8 Advance. The X-ray beam was Ni-filtered Cu K_α (λ = 0.154 nm), and the radiation operated at a tube voltage of 40 kV and a tube current of 30 mA. The powder samples were observed on the 2θ range of 2–80 degree with a scan speed 2 degree/min and a scan step 0.01 degree.

3.2.10 Barbender Mixer W50

The graft copolymer was prepared using a barbender mixer W50 with the operating temperatures at 130°C. The screw speed was maintained at 50 rpm. For ring-opening polymerization of lactide in bulk phase, mixture of lactide monomer, marl filler, and sericin protein were mixed together (in the ratio of 1:2:4 (sericin:LA:marl filler)) with using $Sn(Oct)_2$ as a catalyst were placed in the chamber used as polymerization reactor. Firstly, 26 g marl and $Sn(Oct)_2$ were added to the

chamber mixer and mixed for 5 min. Then the 6.5 g of silk sericin and 13 g of lactide monomer were added and mixed further for 30 min.

3.2.11 Compression Molding Machine

Polybenzoxazine-marl composite and the biocomposite samples were fabricated by a compression press (Wabash, model V50H-18-CX) with the condition temperature, as shown in Table 3.1. The thin compressed sheets, with 3 mm thickness, were cut into the specific size for investigating the mechanical properties.

Table 3.1 Temperature program for compression molding process of polybenzoxazine, polybenzoxazine–marl composites, and the biocomposites

Temperature (°C)	Time (min)	Applied load (20 ton)
80	15	-
80	15	+
100	15	+
120	15	+
150	30	+
180	30	+
210*	60	+

Note : *The last curing temperature for the biocomposite was lower than 210°C due to avoid the degradation of the graft copolymer.

3.3 Methodology

3.3.1 Synthesis of Aliphatic Diamine Based on a Polybenzoxazine Precursor via a Quasi-Solventless Method

The polybenzoxazine precursor (Figure 3.1) was synthesized by reacting bisphenol-A (6 mmol) with paraformaldehyde solution (24 mmol) in 1,4-dioxane in an ice bath. The mixture was stirred continuously before a solution of 1,6-Diaminohexane (6 mmol) in 1,4-dioxane was added slowly. The mixture was stirred continuously until the solution formed into gel. Then the reaction temperature was raised to 80–90°C; the mixture was then kept at that temperature and stirred continuously for 1 h until a clear homogeneous viscous liquid was obtained. This viscous product was washed by cool methyl alcohol to eliminate any unreacted formaldehyde at least three times and dried with the rotary evaporator to obtain a yellow viscous polybenzoxazine precursor. The chemical structure of the precursors was confirmed by FTIR and ^{1}H NMR.

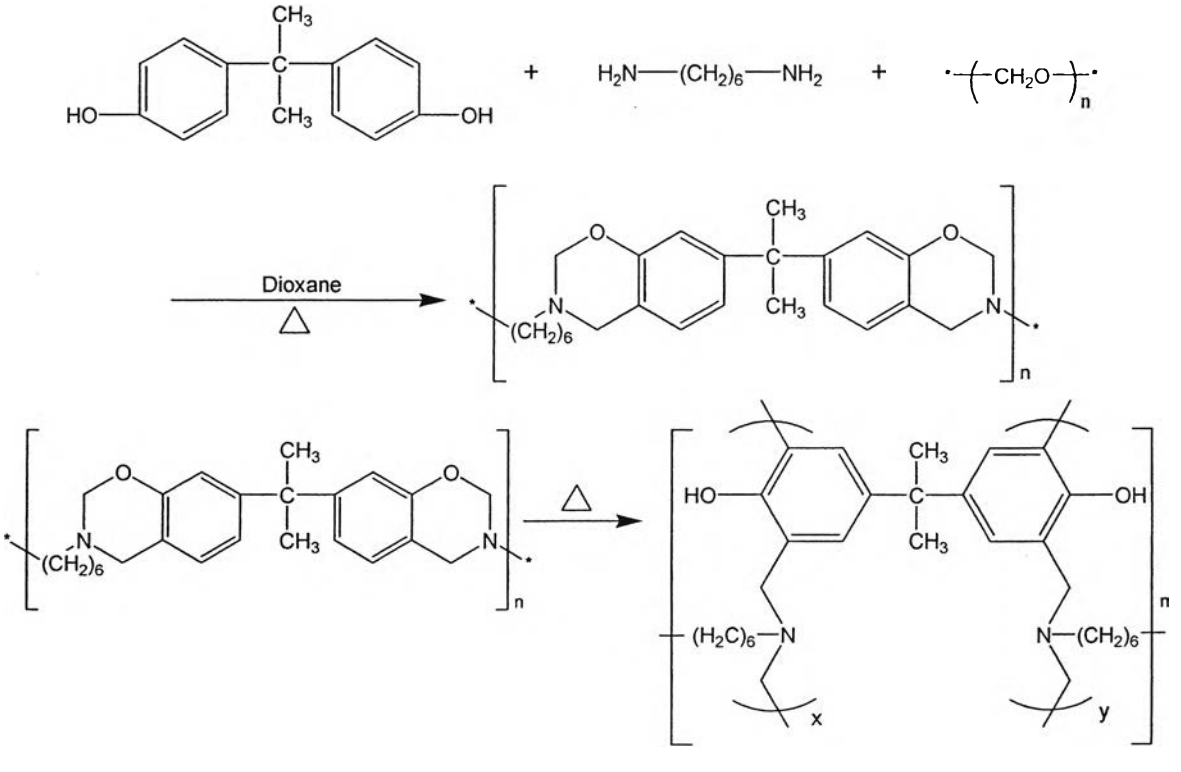

Figure 3.1 Schematic diagram of synthesis reaction of polybenzoxazine precursors based on bisphenol-A, 1,6-diaminohexane, and paraformaldehyde.

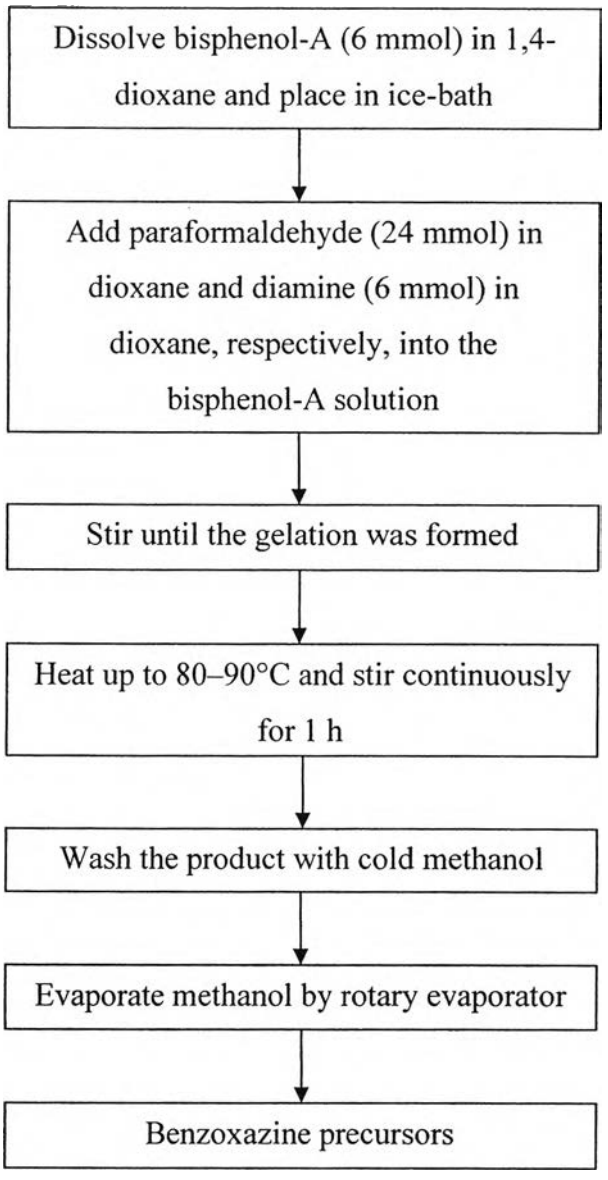

Figure 3.2 Benxozaxine precursor preparation.

3.3.2 Preparation of Polybenzoxazine–Marl Composites

Marl was pulverized and sieved through 325-hole mesh. The obtained marl was dispersed in 1,4-dioxane and stirred by homogenizer at a speed 8000 rpm for 30 min. The homogenized marl solution was added to the synthesized polybenzoxazine precursor at 80–90°C to obtain the composition of marl in polybenzoxazine between 10–50 wt%. The solutions were then mixed and stirred for1 h; a homogeneous solution was obtained after thorough mixing.

3.3.3 Fabrication of Polybenzoxazine and Polybenzoxazine–Marl Composite

Due to the high curing temperature of polybenzoxazine, the compression molding technique was chosen to fabricate the specimens. The mixture was preheated at 80–100°C for solvent removal followed by step-cure in a compression molder. All samples were polymerized without adding any catalysts. The curing condition was given in Table 3.1.

3.3.4 Surface Modification of a Marl Filler

3.3.4.1 Silane Treatment

The surface modification of marl with silane coupling agent—(3-aminopropyl)trimethoxysilane—was carried out in solution. An aqueous ethyl alcohol solution (95% ethanol/ 5%water) was prepared, and the silane coupling agent (1 wt% filler) was added to solution. The solution was then mixed for 15 min to ensure the hydrolyzation of silane to give a silanol. Then the marl, which was pulverized and sieved through 325-hole mesh, was added to silane/water mixture stirred for 2 h. Afterwards, the solution was dried by using a rotary evaporator at 60°C under vacuum for 1 h to ensure no agglomeration of marl particles occur. The filler was further dried in an oven at 70°C for 24 h. The treated marl was pulverized and sieved through 325-hole mesh again before the preparation of the polybenzoxazine–marl composites and the biocomposite (Leong *et al.,* 2005).

3.3.4.2 Stearic Acid Treatment

Marl was pulverized and sieved through 325-hole mesh; subsequently the filler was loaded into a stirrer. The filler treated with stearic acid (1wt% filler) was suspended in toluene, a good solvent of the coupling agents, at approxomately 360 ml per 100 g filler. The solution was stirred continuously for 24 h

with the vigorous stirring (about 1000 rpm) to reduce particle agglomeration. When the setting reaction time elapsed, the solution was dried by using a rotary evaporator at 60°C under vacuum for 1 h. The filler was further dried in an oven at 70°C for 24 h. The treated marl was pulverized and sieved again, like silane coupling agent treatment. The functional groups analysis and morphology were studied with FTIR and SEM, respectively (Wang *et al.*, 2003).

3.3.5 Preparation of Polybenzoxazine–Modified Surface Marl

The modified surface marl was dispersed in 1,4-dioxane and stirred by homogenizer at a speed of 8000 rpm for 30 min. The homogenized marl solution was added to the synthesized polybenzoxazine precursor to obtain the 20 wt% marl–polybenzoxazine composite, which was the concentration resulted in a good mechanical properties (as a result from 3.3.2). The solutions were then mixed and stirred for 1 h at 80–90°C for solvent removal, then a homogeneous solution was obtained after thorough mixing. The composite was fabricated by compression molding technique with the condition shown in Table 3.1.

3.3.6 Synthesis of Silk Sericin Protein–Polylactide Graft Copolymer

The polymerizations were carried out under magnetic stirring for 24 h in dimethyl sulfoxide at 130°C. The sericin protein was dried in a vacuum oven at 70°C for 24 h, after that it was dissolved in 10 ml of 1 M LiCl/DMSO by heating at 60°C for 60 min under nitrogen atmosphere. $Sn(Oct)_2$, which used as a catalyst for the ring-opening polymerization of lactide was added dropwise to the solution. The mixture was then stirred continuously for 4 h at the same temperature under nitrogen atmosphere. A solution of lactide in 10 ml DMSO was then placed into the sericin mixture (in the weight ratio of 20:80 (sercin/LA)). It was placed into a preheated oil bath, which was controlled at 130°C by a thermostat, stirred by a magnetic stirrer, and kept under nitrogen atmosphere. The reaction was allowed to proceed for 24 h. After 24 h had passed, the mixture became dark brown. The reactor was then cooled to room temperature. The mixture was precipitated by cold water (200 ml), and filtered out with a Buchner funnel for chemical analysis by FTIR spectrometer.

3.3.7 Synthesis of Silk Sericin Protein–Polylactide Graft Copolymer with a Marl Filler by Using Brabender Mixer W50

To conduct grafting from technique and ring-opening polymerization of lactide in bulk phase, the lactide monomer, marl filler, and sericin protein (at a weight ratio of sericin:LA:marl filler of 1:2:4) using $Sn(Oct)_2$ as a catalyst. The mixture was placed in a mixer chamber which was used as a polymerization reactor. First, 26 g marl and $Sn(Oct)_2$ were added to the chamber mixer and were mixed for 5 min at 100°C. Then the 6.5 g of silk sericin and 13 g of lactide monomer were added and mixed for a further 30 min. The processing conditions included a rotor speed of 50 rpm, an operating temperature of 130°C, and a monomer-to-catalyst ratio of 0.1 wt%. The resulting polymers received from a Brabender Mixer were purified by soxhlet extraction with ethyl acetate at the temperature about 190°C for 3 h to remove lactide monomer and polylactide homopolymer (without separated marl). After that the resulting yields were dried in vacuum oven at the temperature of 60°C before the chemical structure characterization of the graft copolymer was confirmed by Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^{1}H NMR).

3.3.8 Preparation of the Biocomposite Material for Using as a Soft Splint from Sericin-g-PLA Blended with Polybenzoxazine

The graft copolymer filled with modified surface marl, which prepared in a brabender mixer, was dispersed in 1,4-dioxane and stirred by homogenizer at a speed of 8000 rpm for 30 min. The homogenized solution was added to the synthesized polybenzoxazine precursor at 80–90°C to obtain the composition of graft copolymer in polybenzoxazine between 10–50 wt% and a homogeneous solution was obtained after thorough mixing. The mixture was preheated at 80–100°C for solvent removal followed by step-cure in a compression molding. The cure temperatures for the compression molding of the biocomposites were 80, 100, 120, 150, 180, and 200°C. The biocomposite sheets were cut into the specific sizes for characterization the thermal and mechanical properties.

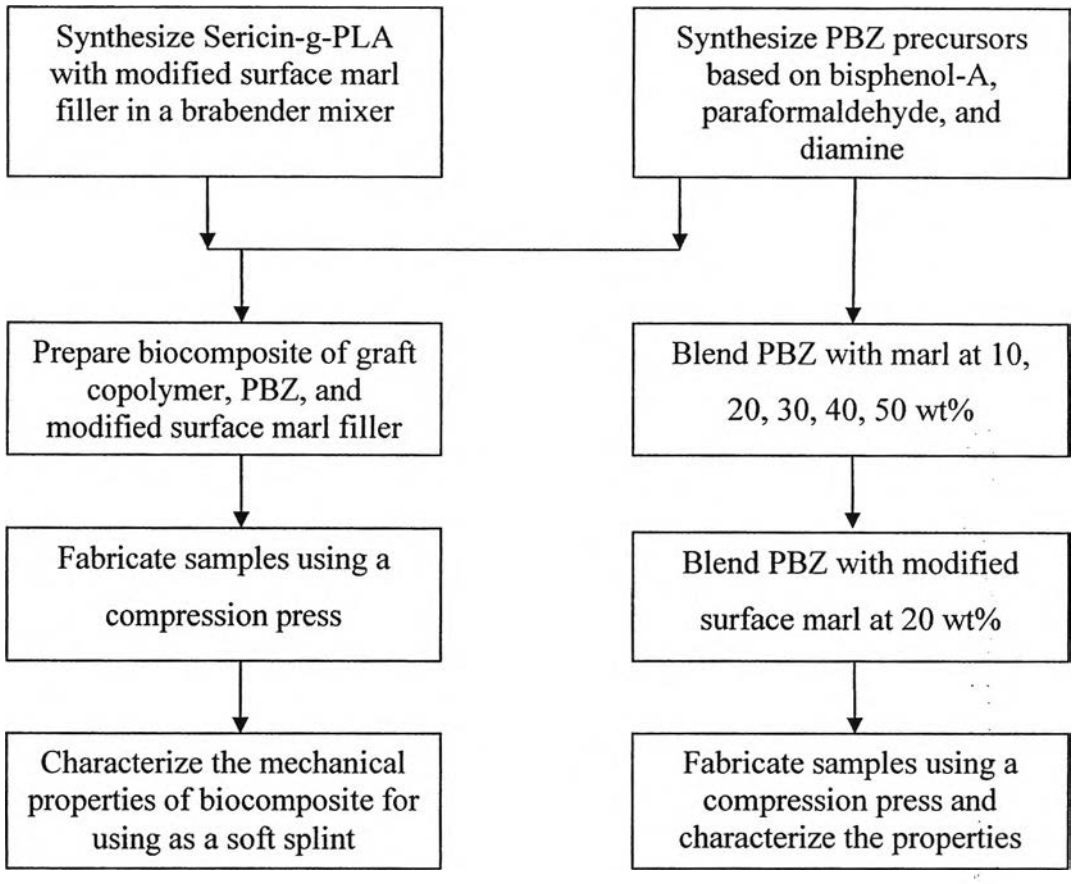

Figure 3.3 Preparation of Sericin-g-PLA, benzoxazine, and modified surface marl biocomposite to be used as a soft splint.