CHAPTER IV

EXPERIMENTAL WORK

The experimental work can be divided into four parts : (i) materials used in the experiments, (ii) PHB characterizations, (iii) effects of the sample storage time on mechanical properties of PHB and (iiii) effects of modifying agents on the properties of PHB

4.1 Materials

4.1.1 PHB

There are two types of PHB used in this work. The first type of PHB was purchased from Biomer company, Krailling, Germany. This type of PHB will be noted as "Biomer" throughout this work. The second type of PHB was acquired by fermentation and purification at Biochemical Engineering Laboratory, Faculty of Engineering, Chulalongkorn University. This type of PHB will be noted as "f-PHB" throughout this work. The details of PHB fermentation and purification are given in appendix C.

After fermentation and purification process, f-PHB is white power and ready for further experiments. f-PHB used in this work is divided into 2 parts, each of which comes from the different batch of purification noted as "f-PHB1" and "f-PHB2", respectively.

Biomer "as-received" is an additives-free white powder with 98.5% purity. The "as-received" Biomer was pretreated as follows. The solution of six grams of Biomer in 50 ml of chloroform was filled in the flask connected to a condenser chamber. The condenser chamber was chilled with a continuous water stream in order to condense the chloroform. The solution was heated to 80°C for 10 minutes and cooled down to room temperature for 10 minutes. The solution was stirred at 300 rpm along the heating-cooling cycle. Six cycles were needed to treat the solution. After treating the solution

was filtered with 0.5 mm mesh seive to remove the undissolved or coagulated Biomer. The filtered solution was poured into the petridish and left in the fume cupboard overnight to evaporate chloroform. After chloroform is completely evaporated, Biomer is ready for further experiments.

4.1.2 Modifying Agents

There are three modifying agents used in this work including: Propylene Glycol, Polypropylene Glycol and Epoxidized Soybean Oil. Propylene Glycol was kindly supplied by Sunny World Co., Ltd. Polypropylene Glycol was kindly supplied by Pacific Plastics (Thailand) Co., Ltd. Epoxidized Soybean Oil was kindly supplied by Nics Chem Co., Ltd. Fundamental characteristics of the modifying agents provided by the supplier company are summarized in Table 4.1. Structural formulas of propylene glycol, polypropylene glycol and epxidized soybean oil are shown in Figure 4.1, 4.2 and 4.3, respectively.

Table 4.1 Fundamental characteristics of modifying agents provided by supplier company

	Propylene Glycol	Polypropylene Glycol	Epoxidized Soybean Oil
Abbreviation	PG	PPG	ESO
Appearance	Clear oily liquid	Viscous colorless liquid	Yellowish oily liquid
Molecular Weight (g/mol)	76.09	4000	About 1000
Boiling Point (^{o}C)	188.2	Not available	150.2
Specific gravity	1.0352-1.0364	1.005	0.982-1.002
Volatile Matter (%)	Not available	Not available	0.1

Figure 4.1 Structural formula of propylene glycol (PG)

$$HO(CH_2CH(CH_3)O)_nH$$

where "n" represents the average number of oxypropylene units.

Figure 4.2 Structural formula of polypropylene glycol (PPG)

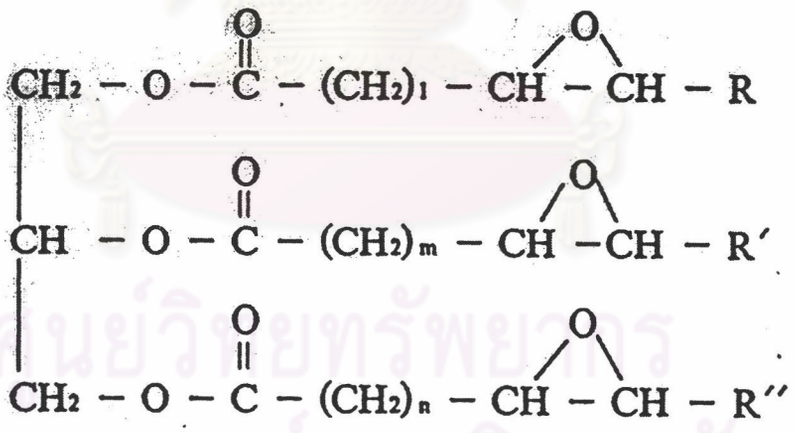

Figure 4.3 Structural formula of epoxidized soybean oil (ESO)

4.2 PHB Characterization

In order to assure that f-PHB which was acquired by the fermentation of the bacterial strain *Alcaligenes eutrophus* is PHB and to characterize its properties and characteristics, IR spectroscopy, molecular weight measurement, and thermal properties measurement of f-PHB were proceeded. The detail of characterization is presented as follows:

4.2.1 IR Spectroscopy

Specimen preparation and condition of measurement

Three PHB samples are characterized by Fourier Transform Infrared Spectroscopy (FT-IR) technique. All spectra of Biomer, f-PHB1 and f-PHB2 were recorded with a "Perkin-Elmer 1760X" instrument (at Scientific and Technological Research Equipment Center, Chulalongkorn University). The specimens were prepared by KBr pellets method. The KBr pellets were prepared by mixing about 1 to 10 mg of sample with 100 mg of IR spectroscopic grade KBr. The spectra was measured and recorded in the range of 4000 to 400 cm^{-1}.

4.2.2 Molecular weight measurement

Specimen preparation and condition of measurement

Molecular weight and molecular weight distribution of five samples of f-PHB were determined by gel permeation chromatography (GPC) technique. All fives f-PHB samples were randomly selected from different batch of purification of f-PHB. The GPC technique was carried out on a "Water 150-CV" instrument (at National Metal Materials Technology Center) at 30°C. The instrument is composed of two columns of PL-Gel 10μ MIXED-B and refractive index detector. Chloroform was used as eluent at a flow

rate of 1.0mL/min. Sample injection volumes of 100 μL was used in the measurement. Polystyrene standards with low polydispersity were used to generate a calibration curve.

4.2.3 DSC Measurement

Specimen Preparation and condition of measurement

The melting temperature (T_m), glass transition temperature (T_g), degree of crystallinity and heat of fusion (ΔH_f) of Biomer, f-PHB1 and f-PHB2 were measured by DSC technique, using a "NETZSCH DSC 2000" (at Scientific and Technological Research Equipment Center, Chulalongkorn University). About 15 mg of sample was heated from room temperature to 200°C at the heating rate of 10°C/min. After holding at 200°C for 1 minute, The sample was cooled down to -50°C with rapid rate of cooling. In the second heating, the sample was heated again to 200°C at the heating rate of 10°C/min. All of the experiments were performed under a nitrogen atmosphere to prevent oxidative degradation. Melting temperature and heat of fusion were determined from DSC endothermic peak in the first heating. Degree of crystallinity based on a value of 146 J/g as heat of fusion at 100% crystallinity of PHB [Bibers *et al.*, 1999], can be calculated by the following equation

$$\text{Degree of crystallinity of sample} = \frac{\Delta H_f \text{ sample} \times 100}{\Delta H_f \text{ 100\% crystalline poly}(\beta\text{-hydroxybutyrate})} \quad (4.1)$$

4.3 Blend preparation

Five blend systems of polymer and modifying agents which were studied in this work are summarized in Table 4.2. Each blend system was prepared at various concentration of modifying agents: 0%, 10%, 20%, 30%, 40% and 50% by weight. The blends of PHB/modifying agent were prepared by dissolving the proper amount of PHB (summarized in table 4.3) in 30 ml of chloroform. The PHB solution was heated at 50°C

for 1 hour with 100 rpm stir rate. The proper amount of modifying agent was added to the PHB solution to make a desired blend concentration and a continuous stirring at room temperature is needed. After the homogeneous solution was formed, the solution was further stirred at 100 rpm for 4 hrs to complete the polymer chain relaxation process. The homogeneous solution was poured into a clean glass sheet and then swept on the glass by the film applicator to achieve the film with a uniform thickness around 0.12-0.15 mm. The swept solution was covered to reduce the evaporation rate of chloroform and avoid contaminants. Film of PHB/modifying agent blend was obtained after leaving the solution overnight to complete the evaporation process.

Table 4.2 Five PHB/modifying agent blend systems studied in this work

	Biomer	f-PHB1	f-PHB2
PG	×		
PPG	×	×	
ESO	×		×

Table 4.3 The concentration and composition of PHB/modifying agent systems

System	% by weight of modifying agent	Concentration (g/ 30 ml of chloroform)	Composition (g)		Gap of the film applicator (mm)
			Polymer	Modifying agent	
Pure Biomer	0	6	6	-	1.0
Biomer + PG					
Biomer/10PG	10	6.5	4.85	0.65	1.0
Biomer/20PG	20	6.75	5.40	1.35	1.0
Biomer/30PG	30	6.10	4.27	1.83	1.0
Biomer/40PG	40	6.00	3.60	2.40	1.0
Biomer/50PG	50	9.00	4.50	4.50	0.6

Table 4.3 The concentration and composition of polymer/modifying agent system (cont.)

System	% by weight of modifying agent	Concentration (g/ 30 ml of chloroform)	Composition (g)		Gap of the film applicator (mm)
			Polymer	Modifying agent	
Biomer + PPG					
Biomer/10PPG	10	6.00	5.40	0.60	1.0
Biomer/20PPG	20	6.25	5.00	1.25	1.0
Biomer/30PPG	30	6.25	4.375	1.875	1.0
Biomer/40PPG	40	6.25	3.75	2.50	1.0
Biomer/50PPG	50	6.00	3.00	3.00	1.0
Biomer + ESO					
Biomer/10ESO	10	6.00	5.40	0.60	1.0
Biomer/20ESO	20	6.75	5.40	1.35	1.0
Biomer/30ESO	30	6.50	4.55	1.95	1.0
Biomer/40ESO	40	6.75	4.05	2.70	1.0
Biomer/50ESO	50	7.50	3.75	3.75	1.0
f-PHB1 + PPG					
Pure f-PHB1	0	6.00	6.00	-	1.0
f-PHB1/10PPG	10	6.5	5.85	0.65	1.0
f-PHB1/20PPG	20	5.75	4.60	1.15	1.0
f-PHB1/30PPG	30	6.50	4.55	1.95	1.0
f-PHB1/40PPG	40	6.00	3.80	2.40	1.0
f-PHB1/50PPG	50	6.00	3.00	3.00	1.0

Table 4.3 The concentration and composition of polymer/modifying agent system (cont.)

System	% by weight of modifying agent	Concentration (g/ 30 ml of chloroform)	Composition (g)		Gap of the film applicator (mm)
			Polymer	Modifying agent	
f-PHB2 + ESO					
Pure f-PHB2	0	6.00	6.00	-	1.0
f-PHB2/10ESO	10	6.50	5.85	0.65	1.0
f-PHB2/20ESO	20	6.75	5.40	1.35	1.0
f-PHB2/30ESO	30	7.00	4.90	2.10	1.0
f-PHB2/40ESO	40	6.75	4.05	2.70	1.0
f-PHB2/50ESO	50	7.00	3.50	3.50	1.0

4.4 Tensile Properties Testing

Specimen preparation and condition of measurement

Film of PHB/modifying agent blend was cut into strip-shaped specimens. The strip is 10 mm in width, 10 cm in length and 0.12-0.15 mm in thickness. The specimens were kept for 10 days from the chloroform evaporation was completely finished to reach the complete crystallization [Bibers *et al.*, 1999]. The data of mechanical properties which varied with specimen storage time were summarized in Appendix B.

Maximum tensile strength, %elongation at break, the stress-strain curve and modulus of elasticity of the PHB/modifying agent blending system were measured using a "LLOYD 2000R" universal testing machine (at Polymer Engineering Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University). The test conditions follow ASTM D882. The determination of tensile properties was done at constant crosshead speed of 2mm/min with a 1000 N load cell. At least five specimens were used for each blend composition. The results of maximum tensile

strength, %elongation at break and modulus of elasticity were reported with the error bar of 95% confidence. The details of error analysis were presented in Appendix C.

4.5 Morphology

Specimen preparation and condition of measurement

Morphology of the PHB/modifying agent system was observed by using a scanning electron microscope JOEL JSM-5410LV at the Scientific and Technological Research Equipment Center, Chulalongkorn University. Since this technique requires good samples for electron conducting, it is necessary to provide conduction to specimens by coating with a thin metal layer (i.e. gold was used in this work). The coated specimens were kept in dry place before experiment. SEM was operated at 15 kV. This is considered to be a suitable condition since too high energy can burn the samples.