CHAPTER III
EXPERIMENTAL

3.1 Materials

Biopolymer (M.W. 200 kDa) was supplied by Bio21 (Thailand). Biopolymer was purified with sodium hydroxide (NaOH, commercial grade, Union Chemical 1986, Thailand) and sodium borohydride ($NaBH_4$, 97%, Labchem, Australia). Hydrochlroric acid (AR grade, RCI Labscan, Thailand) and sodium hydroxide (NaOH, $\geq$ 99%, Merck) were used in titration method to find degree of purification of purified biopolymer. Acetic acid (AR grade, RCI Labscan) was used to dissolve biopolymer to make a solution and thin film for FTIR. Divinylbenzene (DVB, AR grade, Merck) was used as a monomer. Sorbitan monolaurate (Span 80), dodecylbenzenesulfonic acid (DDBSS) and cetyltrimethylammonium bromide (CTAB, $\geq$ 96.0 %) were supplied by Sigma – Aldrich. They were used as surfactants in the emulsion. Potasium persulphate ($K_2S_2O_8$, 99.0%, Merck), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, AR grade, J.T. Baker Chemicals, Holland) and distilled water were used as aqueous phase. Toluene (AR grade, RCI Labscan) used as a porogen in emulsion. Ethanol (AR grade, RCI Labscan) used to be a solvent in Soxhlet process.

3.2 Methodology

3.2.1 Purification of Biopolymer

100 g of biopolymer and 0.5000 g of $NaBH_4$ were immersed in 2 L of 50% w/w NaOH solution at 120 °C for 1 h in the autoclave and then washed with water until the washed water is neutralized. The purified biopolymer was dried at 80 °C overnight in a vacuum oven and kept in a desiccator.

3.2.1.1 Determination of Degree of Purification in Purified Biopolymer by Titration

0.0500 g of purified biopolymer was dissolved in 20.0 mL of 0.1000 N HCl and then titrated pH-metrically with a standardized solution of 0.1000 N NaOH solution to obtain the titration graph that is a plot of pH of solution against the consumed volume of NaOH.

3.2.1.2 Determination of Degree of Purification of Purified Biopolymer by Fourier Transform Infrared Spectroscopy (FT-IR)

The 1% w/v of the polymeric solution was prepared by dissolving 0.2000 g of purified biopolymer in 20 mL of 1% acetic acid solution. After stirring for 30 min, the 0.75 mL of the solution was taken and poured into a mould. The sample was dried at 80 ^{o}C overnight to obtain a thin film. The thin film was removed from the mould by pouring 0.5 M NaOH into the mould and soaking it for 10 min. The obtained film was washed with deionized water until pH of washed water was 7. Finally, the film was dried at 80 ^{o}C for 6 h to obtain the sampling film.

3.2.2 Preparation of PolyHIPE

Biopolymer was ground up to the smaller size by using Ball Mill (particle size less than 62 μm). High surface materials were prepared by first adding surfactants (Span 80 0.3611 g, DDBSS 0.0228 g and CTAB 0.0717 g) and biopolymer into a flask. 1 mL of DVB and 1 mL of Toluene were added into the flask and then the solution was magnetically stirred until the solution well mixed into the same phase (about 1 hr). The aqueous phase containing $K_2S_2O_8$ 0.0405 g as an initiator, $CaCl_2 \cdot 2H_2O$ 0.2089 g and distilled water 18 mL was mixed together and slowly dropwised to the stirring mixture. To obtain a highly viscous emulsion, the stirring rate (120 rpm) was kept constant for 1 h after adding at room temperature. After that the emulsion was transferred in the mold and placed in water bath at 70 ^{o}C for 24 h. The polymerized sample was removed from the mold and put into the cellulose thimble. After that it was extracted in a soxhlet apparatus with ethanol for 12 h to remove any impurities, and then dried to constant weight at 80 ^{o}C.

- Effect of Biopolymer

The amount of biopolymer in a solid form was loaded in emulsion. The size of biopolymer was sieved in a range of below 230 Mesh (≤ 62 microns). It was varied 0, 7, 11, 16 and 23 wt%, respectively. The wt% of biopolymer was related to weight of monomer (DVB).

Biopolymer in a solution form was also used to improve amount of loading and better distribution in polyHIPE. In this part, aqueous phase contained biopolymer solution was. Firstly, biopolymer was dissolved in 1%v/v of acetic acid and stirred until biopolymer completely dissolved. After that distilled water, $K_2S_2O_8$ and $CaCl_2 \cdot 2H_2O$ were added into biopolymer solution. The aqueous phase was slowly dropwised to oil phase. The next step was carried out the same as described in the previous experiment. The wt% of biopolymer related to weight of monomer (DVB) was varied, 30, 50, 70, 100, 120 and 150 wt%.

- Effect of Mixed Surfactant

The amounts of mixed surfactant were used to study. The total wt% of mixed surfactant were varied, 20, 23 and 25 which the amount of each surfactant used are presented in Table 3.1. However, the amount of aqueous phase was still the same.

Table 3.1 The amount of each surfactant at each condition

Mixed-surfactant			
Total (wt%)	SPAN80 (wt%)	DDBSS (wt%)	CTAB (wt%)
20	18	1.1429	0.857
23	20.7	1.3143	0.986
25	22.5	1.4286	1.071

3.2.3 Characterizations

3.2.3.1 Fourier Transform Infrared Spectrometer

FTIR spectrometer (Nicolet/Nexus 670 Model, Massachusetts, USA) was used to analyze the functional groups of the purified biopolymer (amide groups) to determine degree of deacetylation and samples were in the form of thin film casting. The another purpose of this equipment was used to analyze the functional group of amine in polyHIPE after adding biopolymer solution and samples were in form of sample pellet. It was obtained by mixing samples with potassium bromide (KBr) with ratio 3:100 w/w and then using the hydraulic pressure to pelletize the sample. The FTIR spectra were obtained with 16 resolutions, 64 numbers of scans in range of wave number from 400 to 4000 cm^{-1} and used air as a background.

3.2.3.2 Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) was used to analyze the morphology and porous features of polyHIPEs. This characterization was performed on Hitachi/S - 4800 Model (Tokyo, Japan). Samples were coated with platinum under vacuum before observation to make them electrically conductive.

3.2.3.3 Surface Area and Pore Size of PolyHIPE

Specific surface area and pore size distribution were characterized by nitrogen adsorption/desorption measurements with surface area analyzer (Quantachrome/Autosorb 1-MP Model, Florida, USA). Before analysis, the samples were dried in a vacuum oven overnight at 150 °C and degassed at 180 °C for 8 h in vacuum furnace. Surface area was calculated by using BET method.

3.2.3.4 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed to measure the thermal stability of the polyHIPEs. Thermogravimetric analyser (TGA/DSC Model, METTLER TOLEDO, Ohio, USA) was performed under nitrogen gas. Samples were cut into small pieces about 2-3 mg and loaded in alumina pans, then heated from 25°C to 600 °C at a heating rate of 10 °C/min.

3.2.3.5 CHNS Analyzer

The amount of added biopolymer in PolyHIPE was analyzed by CHNS analyzer (LECO - CHNSO Model, Michigan, USA) to determine the quantity of nitrogen (N) in structure of biopolymer. Sample (5 mg) was wrapped with thin foil before measuring and combusting at 950 °C.