CHAPTER III
EXPERIMENTAL

3.1 Chemicals

Biopolymer flake was purchased from Bio21 Co., Ltd., (Thailand). Its average molecular weight and a degree of deacetylation was about 200,000 g/mole (Mw) and 90%, respectively. To gain highly deacetylated biopolymer, the biopolymer was purified with sodium hydroxide solution (NaOH, commercial grade, Union Chemical 1986, Thailand) and sodium borohydride ($NaBH_4$, 97%, Labchem, Australia). The degree of deacetylation was charecterized by tritationandfourier transform infrared spectroscopy (FTIR). Hydrochloric (RCI Labscan, AR grade, Thailand) and sodium hydroxide (Merck, $\geq$ 99%) were used as solvents in a titration method. Sodium hydroxide (Merck, $\geq$ 99%) and acetic acid (RCI Labscan, AR grade, Thailand) were used as solvents for film preparation for analysis by Fourier transform infrared spectroscopy (FTIR). The purified biopolymer was dispersed into a mixture of isopropyl alcohol (RCI Labscan, AR grade, Thailand) and glacial acetic acid (RCI Labscan, AR grade). Piperazine-2-carboxylic acid dihydrochloride (Sigma-Aldrich Ltd., synthesis grade) was dissolved in deionized water prior to mixing and subsequently added to the solution of biopolymer to obtain the piperazine biopolymer.

3.2 Procedures

3.2.1 Purification of Biopolymer

The purchased biopolymer (100 g) and sodium borohydride (0.5 g) were mixed in 2 liters of 50% w/w sodium hydroxide solution. The mixture was deacetylated in an auto-clave for 1 h at 120 °C (Wan *et al.*, 2010). The deacetylated biopolymer was filtered and washed with deionized water until a neutral pH was reached. The purified biopolymer was dried at 80 °C overnight in a vacuum oven and later kept in a desiccator.

3.2.2 Determination of Degree of Deacetylation (%DD) of Biopolymer

3.2.2.1 Titration

According to an acid–base titration method (Avadi*et al.*, 2004), the purified biopolymer (0.05 g) was dissolved in 20 mL 0.10 N HCl in an erlenmeyer flask. The mixture was titrated pH-metrically by adding in a standardized solution of 0.1000 N NaOH solution while pH of the solution was measured. The titration was completed when the solution pH become constant. The two equivalent points in the titration graph corresponds to the excess of HCl and the protonated amino groups were plotted between pH of solution and the consumed volume of NaOH. The degree of deacetylation (% DD) was calculated based on equation (3.1) as follow.

$$DD = \frac{16.1(Y-X)f}{w} \tag{3.1}$$

Where X and Y are the consumed NaOH volume of the first and second equivalent points, respectively. f is the molarity of the NaOH solution and w is the initial purified biopolymer weight.

3.2.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were recorded on a FTIR spectrometer (Nicolet/Nexus 670 Model, Massachusetts, USA) in a transmission mode. A thin film was cast by dispersing 0.2 g purified biopolymer in 20 mL of 1%w/v of acetic acid solution to make 1% w/v of the polymeric solution upon stirring for 30 minutes. The polymeric solution (0.75 mL) was poured into a mold and dried in the oven at 80 °C overnight to obtain the thin film. 0.5 M sodium hydroxide solution was poured into the mold in order to remove the thin film which subsequently soaked in the same solution for 10 min. Finally, the obtained film was dried at 80 °C for 6 h. The FTIR measurement was carried out with 16 resolutions at 64 scans. Wave numbers used was from 400 $cm^{-1}$to 4000 cm^{-1}. Air was used as background.

3.2.3 Preparation of Modified Biopolymer

The purified biopolymer was modified by amine functionalization with piperazine-2-carboxylic acid. A mole ratio of biopolymer to piperazine-2-carboxylic acid was 1:1 and the ratio of the isopropyl alcohol, 1% w/v glacial acetic acid and water was 10:5:2. The purified biopolymer powder (0.1655 g) was dissolved in 26.21 mL of 1% w/v glacial acetic acid solution followed by adding in 52.42 mL of isopropyl alcohol. Piperazine-2-carboxlic acid dihydrochloride (0.1983 g) was dissolved in deionized water (10.48 mL). The piperazine-2-carboxlic acid solution was gradually added to the biopolymer solution while being kept in iced water. The mixture was stirred for 30 minutes, while it was cool. In addition, it was stirred for 1 hour at room temperature before being kept at 4 °C for 5 days to obtain brown precipitate. The precipitate was filtered and washed with solvent mixture of isopropyl alcohol and deionized water (5:1, v/v) to obtain the modified biopolymer (1A), which was later dried in the oven at 110 °C for 24 hours in order to remove the isopropyl alcohol and deionized water (Singh and Dutta, 2010).The filtrate was collected and analyzed by HPLC for determination of unreacted piperazine -2-carboxylic acid in the solution.

Similarly, the modified biopolymer was prepared by varying the mole of purified biopolymer to piperazine-2-carboxylic derivative ratios (1:2 for 2A and 1:5 for 3A) to find the most piperazine-2-carboxylic acid addition in the modified biopolymer as probable adsorbent for CO_2 adsorption.

3.2.3.1 Structural Characterization of Modified Biopolymer Using FTIR

The functional group of modified biopolymer sample was characterized by FTIR. A sample pellet was prepared by mixing the sample with dried potassium bromide with a ratio of 3:100 (w/w) in a mortar. The sample and KBr was thoroughly ground until mixed well. The mixture was then pelletized by hydraulic pressure. The IR spectrum was obtained with DTGS detector using 4 resolutions and 64 numbers of scan. A background spectrum was obtained with a pure KBr pellet.

3.2.3.2 Thermal stability of Modified Biopolymer

Thermogravimetric analysis in nitrogen atmosphere was done using a Simultaneous TG/DTA Thermal Analysis Instrument (Perkin Elmer, Pylis Diamond, Massachusetts, America). It was operated from ambient temperature to 750°C with a heating rate of 10°C/min. The temperatures of the decomposition onset, temperature at maximum process rate and percentage weight loss were evaluated from thermogravimetric (TG) and derivative thermogravimetric (DTG) curves. Simultaneously, the enthalpy changes (DTA curves) were also recorded.

3.2.3.3 Determination of Degree of Substitution of Modified Biopolymer (%DS)

The unreacted piperazine-2-carboxylic acid in the reaction solution before filtering with solvent mixture of isopropyl alcohol and deionized water was measured by high-performance liquid chromatography (HPLC) (Eichhorn*et al.*, 1997). HPLC separation was carried out on an Agilent 1100 series LC system consisting of a pump, a degasser, a diode-array detector, a column compartment, and ChemStation data management software. Chromatographic separation performs on Inertstil ODS-3 column, 250 ×4.6 mm, 5-μm particle size. The UV detector was set at 250 nm. Deionized water, acetonitrile and 250mM potassium dihydrogen phosphate (KH_2PO_4) at pH 2.3 were used as a mobile phase in the ratio of 50:30:20. The flow rate of mobile phase was always 0.5 mL/min.