CHAPTER IV
RESULTS AND DISCUSSION

4.1 Purification of Biopolymer and Characterization

4.1.1 Purification of Biopolymer

The biopolymer was purified by alkali treatment (50 %w/w NaOH) in order to obtain a higher degree of deacetylation (% DD). Sodium borohydride was used to suppress degradation of the biopolymer. The hydrolysis of biopolymer occurred to increase amine group in the structure (Aranaz, 2009).

4.1.1.1 pH-metrical Titration

The degree of deacetylation of the purified biopolymer was determined by pH-metrical titration. This method was done by adding excess amount of 0.1000 N hydrochloric acid solution to react with biopolymer. Hence, two forms of HCl exist in the system that are HCl bound to NH_2 group of biopolymer and free HCl in the solution. The pH-metrical titration curve of biopolymer solution with 0.1 N standardized solution of sodium hydroxide plotted between pH of solution and consumed volume of sodium hydroxide is shown in Figure 4.1.

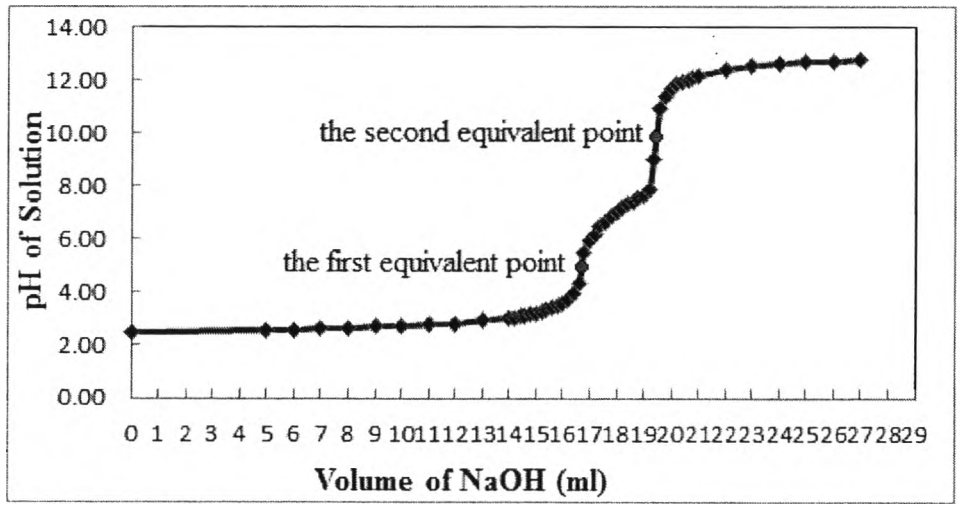

Figure 4.1 Titration curve of purified biopolymer.

The graph contains two equivalent points. At the initial stage before the first equivalent points, NaOH reacted with the excess of hydrochloric acid until depleted. Therefore, the first point corresponds to the neutralization of hydrochloric acid excess. After the first point, NaOH gradually displaced HCl bound to the primary amino groups of biopolymer hydrochloride. The titration reached the second equivalent point when bound HCl was completely reacted by NaOH. (Arbatskii *et al.*, 2006). The linear extrapolation to the adjacent portions of the titration curve was used for determination of the correct equivalence points. The degree of deacetylation of purified biopolymer identified by titration technique was 96.05 % with 0.066% standard deviation. The calculation was carried out according to the method proposed by Avadi *et al.* (2004).

4.1.1.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The FTIR spectra of the virgin biopolymer and the purified biopolymer are shown in Figure 4.2. This method was used to confirm the result obtained from the pH-metrical titration method.

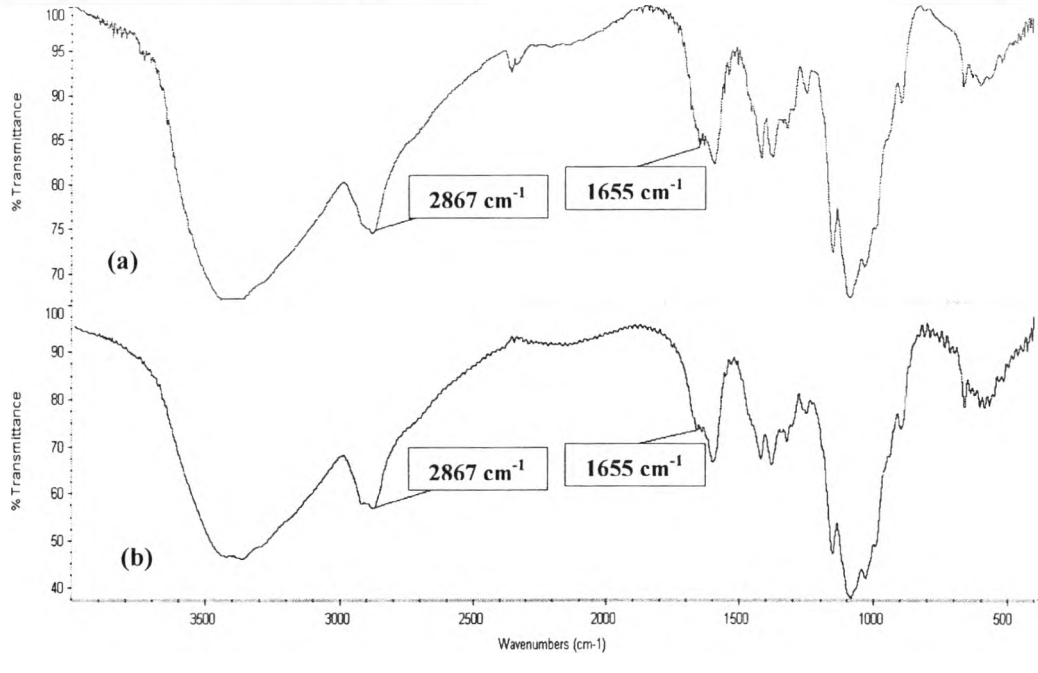

Figure 4.2 FTIR spectra of virgin biopolymer (a) and purified biopolymer (b).

The most noticeable difference was the decrease in the characteristic peak at 1655 cm^{-1} in the purified biopolymer representing amide I band (C=O) of remaining *N*-acetyl glucosamine units in its structure. Therefore, this peak was used to determine the remaining *N*-acetyl glucosamine units in highly deacetylated biopolymer expressed as degree of deacetylation. The peak ratio of absorbance of 1655 $cm^{-1}$and 2867 cm^{-1}(A_{1655}/A_{2867}) can be evaluated to give the degree of deacetylation of purified biopolymer as shown in the method proposed by Miya *et al.* (1980).The amide band (1655 cm^{-1}) and the C-H stretching band (2867 cm^{-1}) and the peak ratio of both bands (A_{1655}/A_{2867}) were calculated using equations (4.1), (4.2) and (4.3), respectively.

$$\log_{10}\left(\frac{AC}{AB}\right) = A_{C-H\ stretching,2867\ cm^{-1}} \quad (4.1)$$

$$\log_{10}\left(\frac{DF}{DE}\right) = A_{amide,1655\ cm^{-1}} \quad (4.2)$$

$$Absorbance\ ratio = \frac{A_{amide,1655\ cm^{-1}}}{A_{C-H\ stretching,2867\ cm^{-1}}} \quad (4.3)$$

Where AB and AC are relative intensities of C-H stretching band of purified biopolymer and DF and DE are relative intensities of amide I band, that their measurement are presented in Figure 4.3.

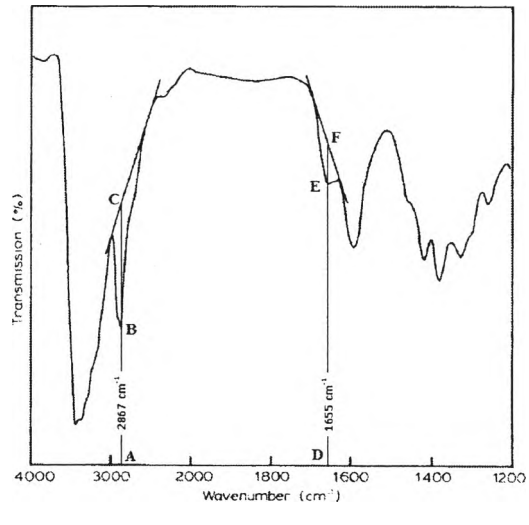

Figure 4.3 Baselines for determination of the peak absorbance and relative intensity of amide and CH-stretching. (Miya *et al.*, 1980).

Figure 4.4, which was obtained from Miya *et al.* (1980), shows a graph plotted between the peak ratio of absorbance of the band at 1655 cm^{-1} to that of the band at 2867 $cm^{-1}$and the degree of deacetylation. Based on Figure 4.4, the degree of deacetylation of purified biopolymer was found to be 96.80 % with 0.358 % standard deviation.

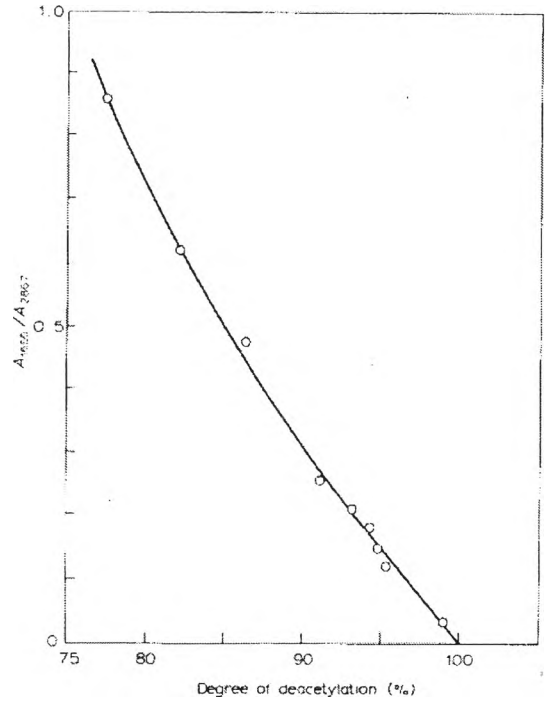

Figure 4.4 Peak ratio of absorbance of the band at 1655 $cm^{-1}$to that of the band at 2867 cm^{-1} (A_{1655}/A_{2867}) against the degree of deacetylation. (Miya *et al.*, 1980).

The results of the degree of deacetylation obtained from FTIR and the pH-metrical titration were very similar with the average of 96.43 % with 0.53% standard deviation.

4.2 Modification and Characterization of Biopolymer

4.2.1 Modification of Biopolymer

The main objective of this research was to chemically react NH_2 sites of biopolymer with piperazine for CO_2 adsorption. A concept of CO_2 adsorption using piperazine was proposed based on success of CO_2 absorption, that its rate constant of piperazine is higher than monoethanolamine (MEA) and other amines that mostly used in industry (Cullinane and Rochelle, 2006). To achieve the goal, the carboxylic groups in piperazine-2-carboxylic acid react with amine group of the glucosamine units in the purified biopolymer. The unreacted piperazine-2-carboxylic acid and unreacted biopolymer was removed by the mixture solution of isopropyl alcohol and deionized water. The reaction between purified biopolymer and piperazine-2-carboxylic acid is shown in Figure 4.5. The average yield of modified biopolymer analyzed was 42.50% w/w for **1A**, 76.84% for **2A** and 75.10% for **3A.**

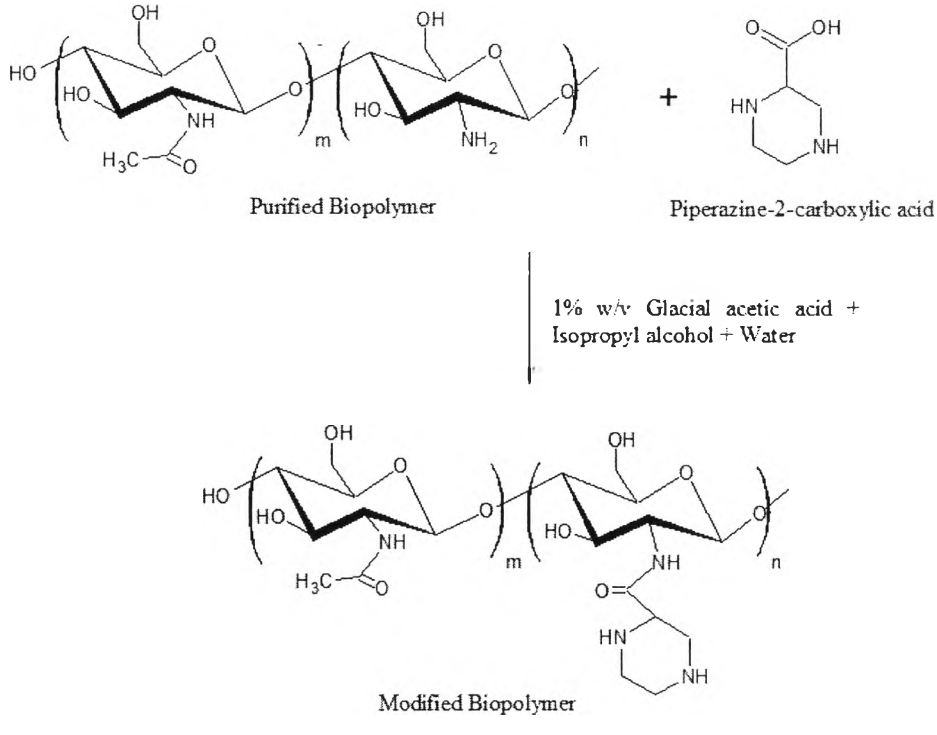

Figure 4.5 Reaction of purified biopolymer with piperazine-2-carboxylic acid.

4.2.2 Characterizations of Modified Biopolymer

4.2.2.1 Structural Characterization of Modified Biopolymer

The FTIR spectra of purified biopolymer, piperazine-2-carboxylic acid dihydrochloride and modified biopolymer are shown in Figures 4.5, 4.6 and 4.7, respectively. In Figure 4.5, the assigned peaks of purified biopolymer are (i) the peak at 3365 cm^{-1}corresponding to O-H stretching which overlaps with N-H stretching, (ii) the peak at 2867 cm^{-1} assigned to C-H stretching (Singh *et al.*, 2009), (iii) the peak at 1655 cm^{-1} corresponding to amide I (C-O stretching of remaining acetyl group) and the peak at 1598 cm^{-1}, given to peak overlap of free amino group of biopolymer and amide II (N-H stretching of remaining acetyl group) (Bhattarai *et al.*, 2006), (iv) the peak at 1419 $cm^{-1}$and 1384 cm^{-1} recognized as asymmetric C-H bending of CH_2 group (Singh *et al.*, 2009), (v) the peak at 1153 $cm^{-1}$and 1076 cm^{-1} representing the skeletal vibration involving the bridge C-O stretching (Singh and Dutta, 2010) and (vi) the peak at 895 cm^{-1}given to pyranose ring of the biopolymer. The assigned peaks of piperazine-2-carboxylic acid dihydrochloride are; (i) the peak at 3474 cm^{-1} corresponding to secondary amino heterocyclic amine stretching(John Coates, 2000), (ii) the peak at 3025 cm^{-1} and 2928 cm^{-1}recognized as C-H bending (iii) the peak at 1756 cm^{-1} corresponding to carbonyl group (C=O) of carboxylic groups (Singh and Dutta, 2010), (v) the peak at 1631 cm^{-1} corresponding to N-H bending of secondary amine, (vi) the peak at 1431 cm^{-1} and 1383 cm^{-1} representing C-O stretching of acid, and (vii) the peak at 1239 cm^{-1} and 1210 cm^{-1}recognized as C-O-H bending of acid. By comparing the FTIR spectra, the modified biopolymer (1A) shows an increase of amide I peak and amide II peak intensities, owing to N-H and C-O stretching, respectively. These peaks slightly shift from 1655 cm^{-1} to 1631 cm^{-1} and 1598 cm^{-1} to 1520 cm^{-1} for amide I and amide II, respectively (Qu *et al.*, 1999). Disappearing of the carbonyl group (C=O) of carboxylic groups in the piperazine-2-carboxylic acid was also observed. The spectrum has confirmed the formation of amide linkage between amine group of glucosamine in biopolymer and carboxylic group of piperazine-2-carboxylic acid. Moreover, the modified biopolymer shows the characteristic peak at 1154 cm^{-1} and 1093 cm^{-1} (skeletal vibration involving the bridge C-O stretching) and 894 cm^{-1} (pyranose ring of biopolymer (Singh and Dutta, 2010).

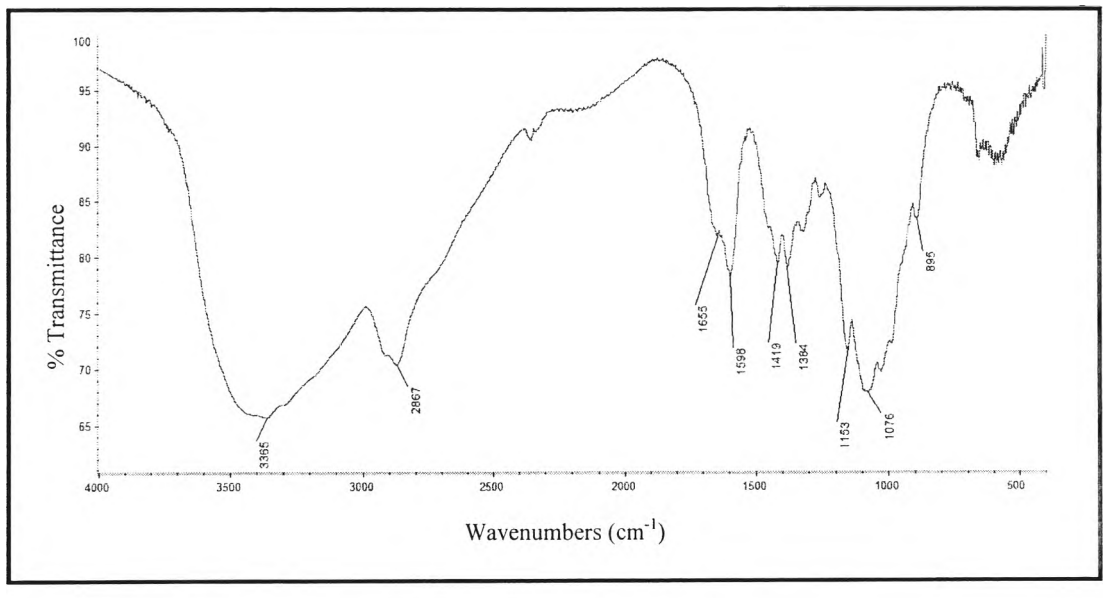

Figure 4.6 FT-IR spectrum of purified biopolymer (BP).

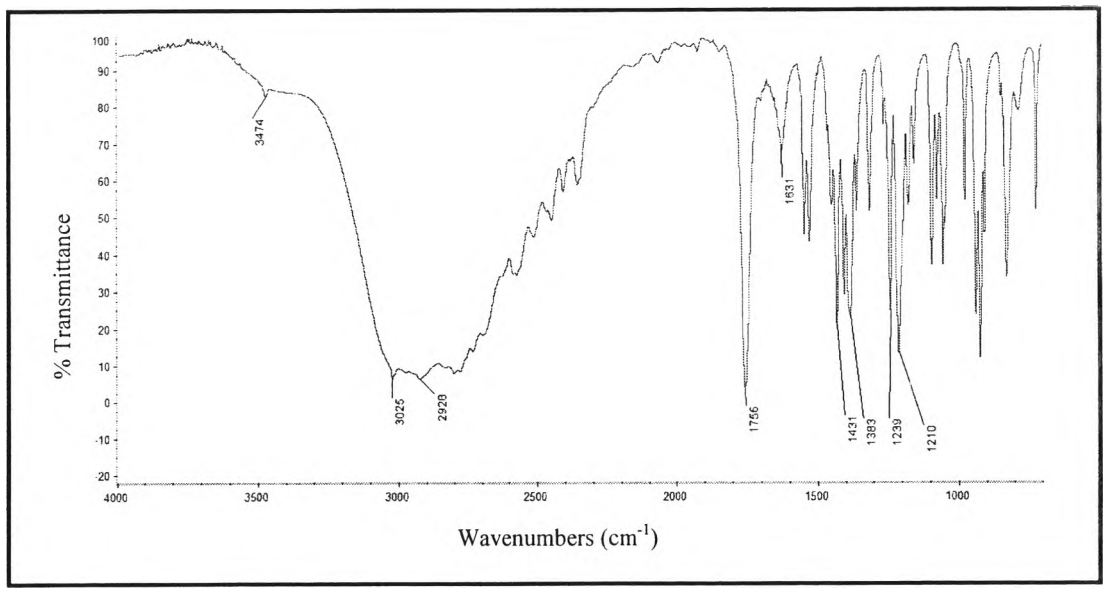

Figure 4.7 FT-IR spectrum of piperazine-2-carboxylic acid dihydrochloride.

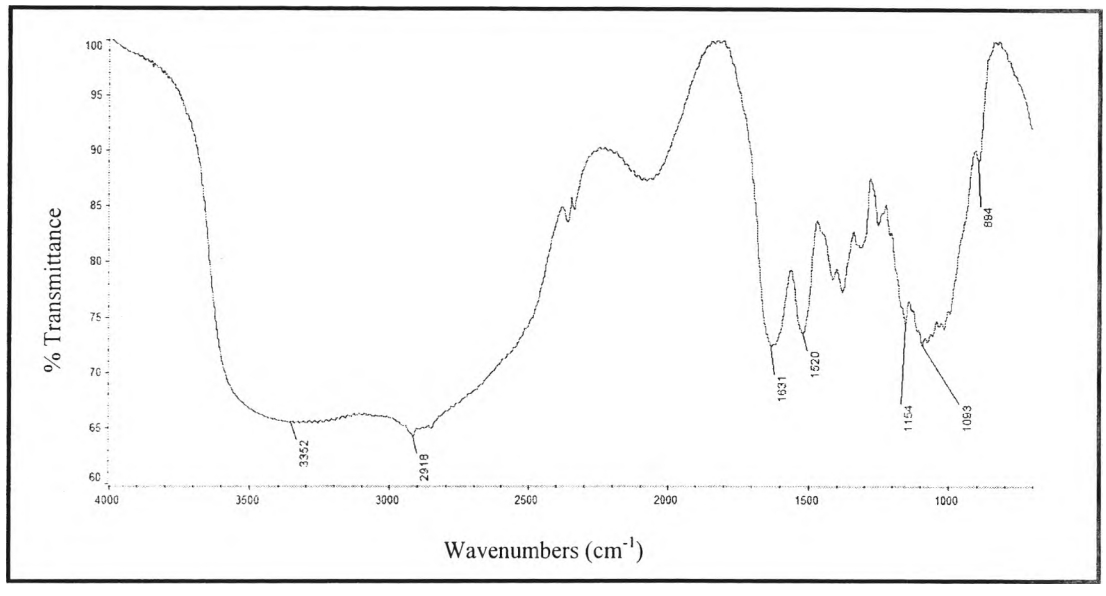

Figure 4.8 FT-IR spectrum of modified biopolymer (1A).

4.2.2.2 Determination of Degree of Substitution of Modified Biopolymer (DS)

In the modification of purified biopolymer, the effect of the ratio of piperazine derivative to biopolymer was studied by comparing the degree of substitution. The degree of substitution of each ratio was determined by peak area of unreacted piperazine-2-caboxylic acid in the filtered solution after the reaction of modification of biopolymer that was detected by high-performance liquid chromatography (HPLC). Figure 4.8 shows the chromatogram of 0.5 % w/v standard piperazine-2-carboxylic acid (0.0246 mol in 100 mL of standard solution). The retention time of the piperazine-2-carboxylic acid is 4.137 min. The calibration curve of various moles of piperazine-2-carboxylic acid is shown in Figure 4.9.

When the mole ratio of biopolymer to piperazine derivative was varied (1:1 for 1A, 1:2 for 2A and 1:5 for 3A), the retention times of residual piperazine-2-carboxylic acid in the filtered solution of 1A, 2A and 3A were 4.172 , 4.157 and 4.171 min as shown in Figures 4.10, 4.11 and 4.12, respectively. The average peak area of residual piperazine-2-carboxylic acid of1A, 2A and 3A was 29,231.50, 61,769.50 and 208,882.50, respectively. According to the calibration curve, it was found that the remaining of piperazine-2-carboxylic acid of 1A, 2A and 3A was 5.85×10^{-4}, 12.36×10^{-4} and 41.78×10^{-4} mole. Finally, the degree of

substitution which is the ratio of mole of reacted of piperazine-2-caboxylic acid and mole of glucosamine group of purified biopolymer was calculated. The degree of substitution of 1A, 2A and 3A was 39.80 %, 72.16% and 71.08%, which is summarized in Table 4.1. It can be seen that the degree of substitution in biopolymer was increased with increasing mole ratio of biopolymer to piperaine-2-carboxylic acid, since increase of piperazine derivative rose the opportunity of reaction between purified biopolymer and piperazine derivative. However, when the mole ratio of biopolymer to piperaine-2-carboxylic modified reached 1:5, the degree of substitution was not significantly different compared as mole ratio of 1:2. Therefore, the maximum addition ratio of piperazine-2-caboxylic acid is the ratio of 1:2 for modification of biopolymer. It shown that the 72.71 % DS of modified biopolymer would probably be the good adsorbent for CO_2 adsorption because of the high amine group in the structure which is reactive site in reaction between carbon dioxide and amine group.

Table 4.1 Degree of substitution of modified biopolymer

Mole ratio		Peak area at 4.1 min	Residual Piperazine-2-carboxylic acid ($\times 10^{-4}$mole)	Degree of substitution (%DS)
Biopolymer	Piperazine-2-carboxylic acid			
1	1	29231.50	5.85	39.80
1	2	61769.50	12.36	72.16
1	5	208882.50	41.78	71.08

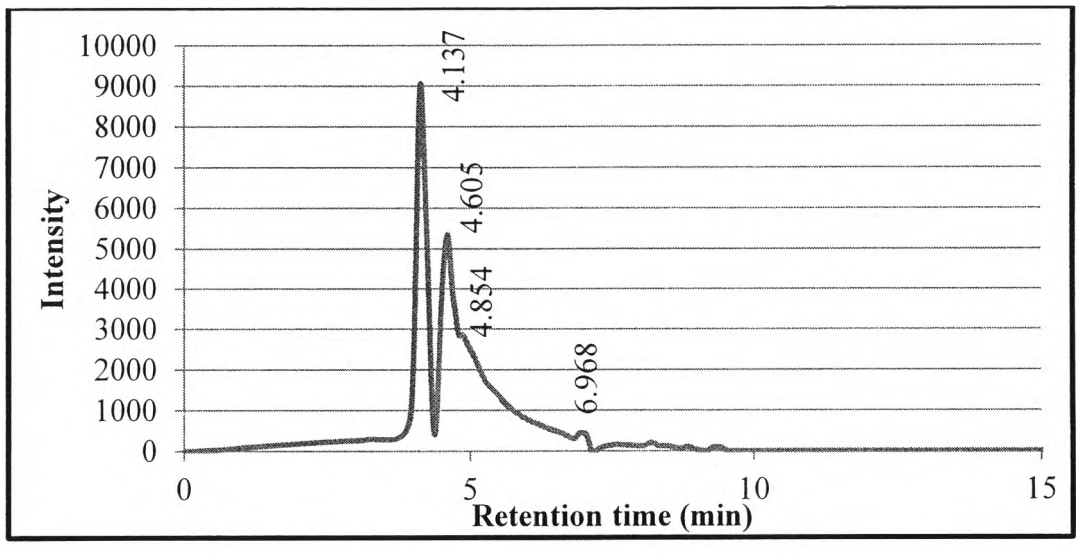

Figure 4.9 Chromatogram of 0.5 % w/v piperazine-2-carboxylic acid standard.

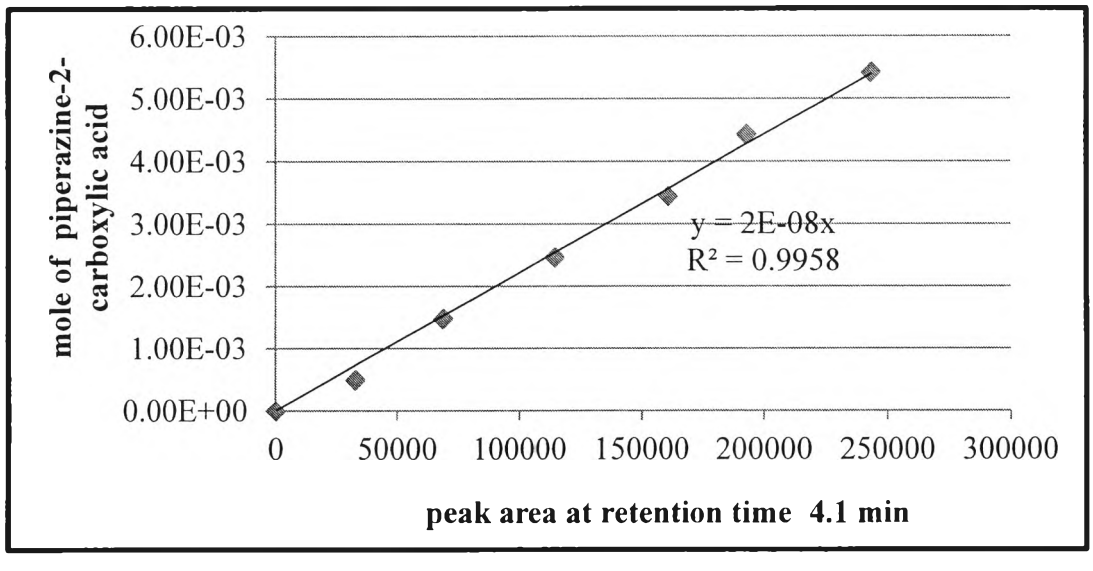

Figure 4.10 Calibration curve of piperazine-2-carboxylic acid at various moles.

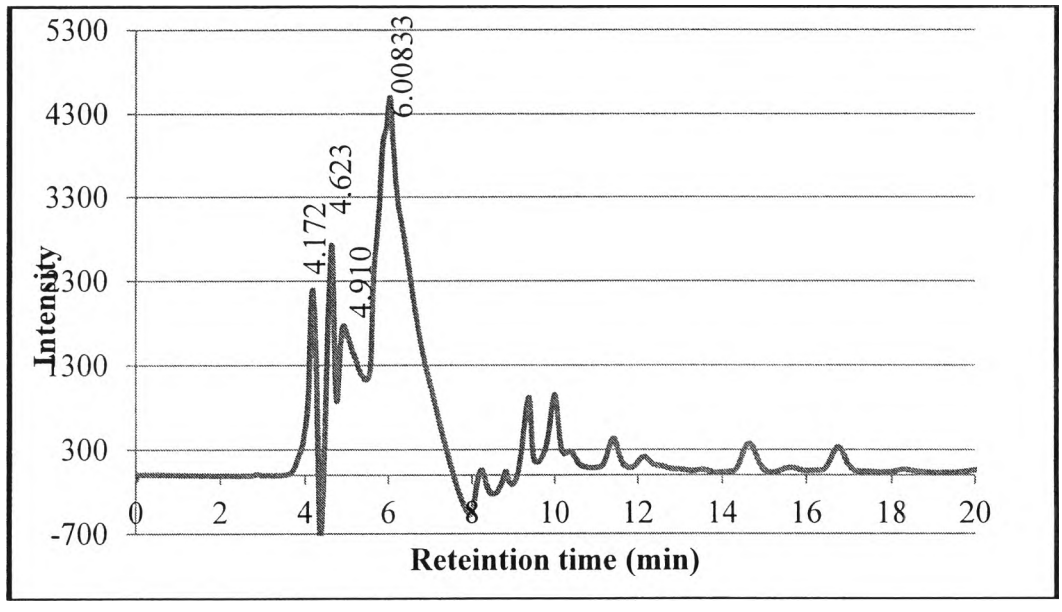

Figure 4.11 Chromatogram of filtered solution of modified biopolymer at ratio of 1:1 (1A).

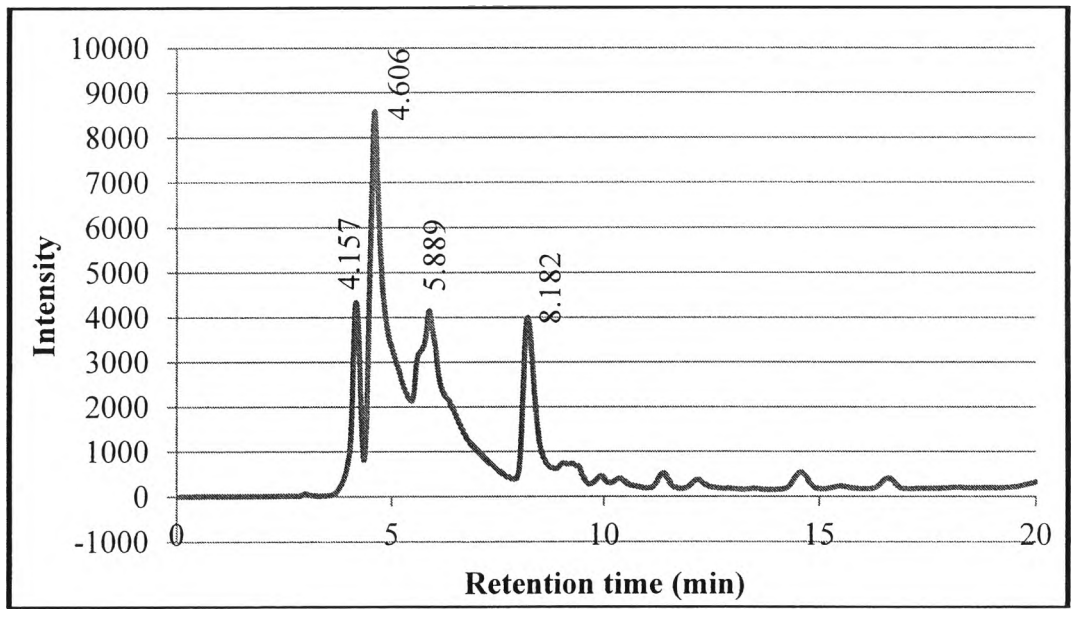

Figure 4.12 Chromatogram of filtered solution of modified biopolymer at ratio of 1:2 (2A).

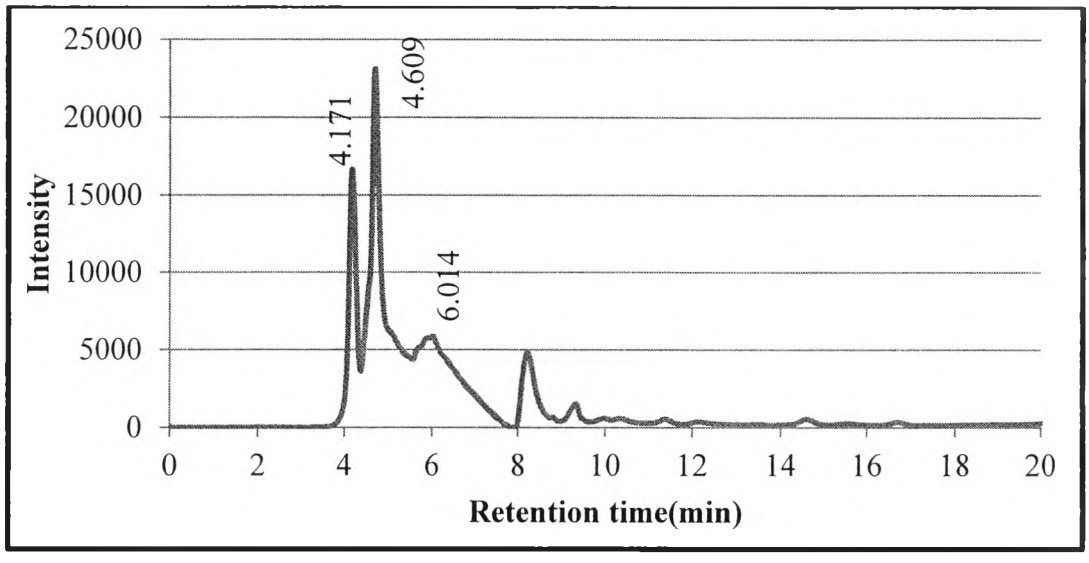

Figure 4.13 Chromatogram of filtered solution of modified biopolymer at ratio of 1:5 (3A).

4.2.1.3 Thermal Stability of Modified Biopolymer

Thermogravimetric measurements of the purified biopolymer, piperazine-2-carboxylic acid and modified biopolymer carried out from ambient temperature to 750 °C in the nitrogen atmosphere with a heating rate of 10°C/min for determination thermal stability of adsorbent. The TG curve is shown in Figure 4.13. First of all, purified biopolymer contains about 10.87% of adsorbed water from the air or in the inner polymer that is evaporated below 100 °C in the first stage. It indicated that this water physically adsorbed and/or weakly hydrogen-bonded to biopolymer molecules. In the second stage of purified biopolymer, a rapid weight loss about 57.37% occurred between 230- 400 °C (decomposition temperature at 278 °C) which causes by the depolymerisation of polymeric chains, decomposition of pyranose rings through dehydration and deamination and finally ring-opening reaction. The 30.27 % char residue is constant at least up to 750°C (López *et al.*, 2008: Zawadzki *et al.*, 2010). For piperazine-2-carboxylic acid, it showed only one step of decomposition. A mass loss of 93.8 % is associated with decomposition of organic functional group in a range of 200-350 °C and it remained 6.17% of char residue. Comparatively, the modified biopolymer shows a similar weight loss as the purified biopolymer at low temperature (below 100 °C) because of water

evaporation, but the predominant stage of thermal degradation appears at 190–300 °C that is lower than the purified biopolymer. The char residues of the modified biopolymers are similar to the purified biopolymer. Moreover, the decomposition temperatures in the second step of all ratios of modified biopolymers are similar which are 214.81°C for 1A, 212.05 °C for 2A and 214.69 °C for 3A, respectively. Hence modification of biopolymer with piperazine-2-carboxylic acid affects the thermal stability of biopolymer, which decreased the decomposition temperature of biopolymer because of the disruption of crystalline structure, especially through the loss of hydrogen bonding (Singh *et al.*, 2009: Singh and Dutta, 2010). The decomposition temperature of biopolymer modified with piperazine-2-carboxylic acid was thermally stable up to190 °C.

Table 4.2 Thermal parameters (characteristic temperatures and weight loss) obtained from thermogravimetric analysis

sample	Decomposition temperature(°C)		Weight loss (%)		Char Residue (%)
	1st step	2rd step	1st step	2rd step	
Purified biopolymer	36.90	277.99	10.87	57.27	30.27
Piperazine-2-carboxylic acid	242.25	-	93.798	-	6.17
Modifed biopolymer 1:1(**1A**)	63.00	214.81	8.29	57.26	34.05
Modifed biopolymer 1:2(**2A**)	49.71	212.05	10.30	59.21	29.98
Modifed biopolymer 1:5(**3A**)	46.52	214.69	5.64	63.05	30.74

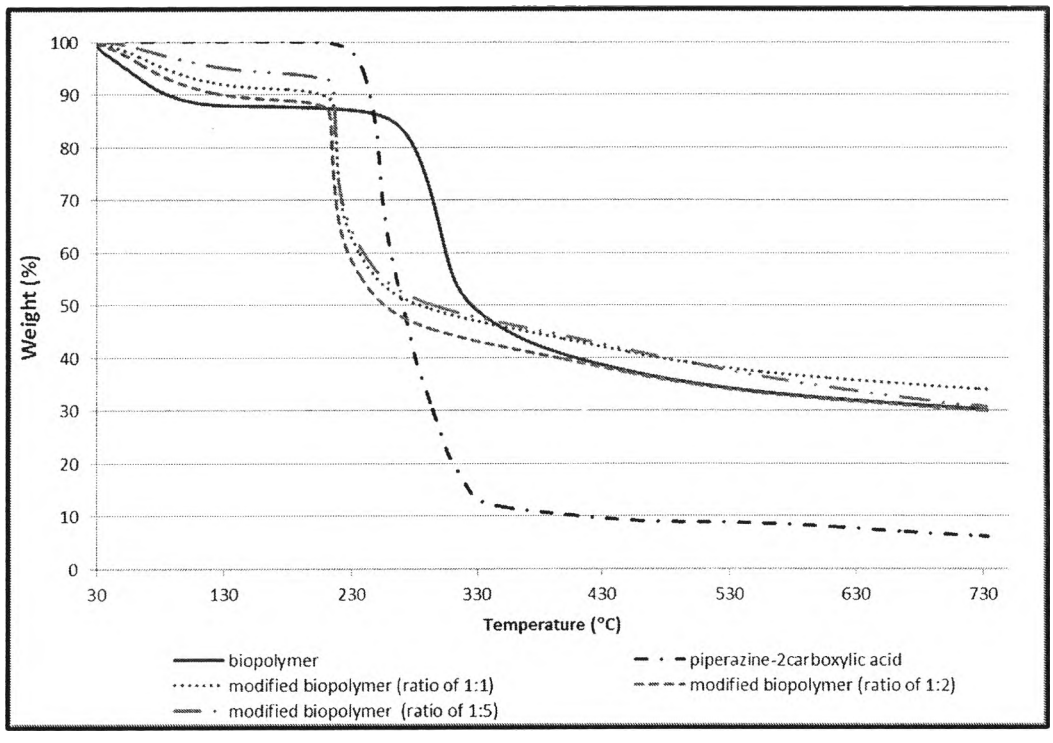

Figure 4.14 Thermograms of purified biopolymer, piperazine-2-carboxylic acid and modified biopolymer in the nitrogen atmosphere with a heating rate of 10°C/min.