CHAPTER IV

PRERARATION AND CHARACTERIZATION OF HEXANOYL CHITOSAN

4.1 ABSTRACT

Hexanoyl chitosan (H-chitosan) was synthesized to obtain the organic solvent soluble chitosan derivatives. H-chitosan was synthesized directly by repeating reacted chitosan with hexanoyl chloride in the mixture of anhydrous pyridine and chloroform to obtain products with various degrees of hexanoylation [Degree of substitution = 2.06, 3.78, 3.91 and 3.92 for the number of repeated reaction = 1, 2, 3 and 4, respectively]. The chemical structures of hexanoyl chitosan were characterized by FT-IR, ^{1}H-NMR, and elemental analysis. The obtained H-chitosan exhibited much improvement in the solubility in organic solvents such as chloroform, dichloromethane and tetrahydrofuran. Thermal analysis results indicated lower thermal stability of H-chitosan than that of chitosan. WAXD indicated that lower packing of chitosan main chains in H-chitosan. The thermal stability and crystallinity of H-chitosan were lower than those of chitosan due to the loss of intra- and inter-molecular hydrogen bonds and large substituents group resulting in poor packing of H-chitosan main chains.

4.2 INTRODUCTION

Chitin is a high-molecular weight linear polymer of *N*-acetyl-*D*-glucosamine and is the second most abundant natural polymers. It occurs in nature as the fibrous component of the exoskeleton of insects, crustaceans, and invertebrates and also in cell wall of fungi. Chitin has excellent properties such as biocompatibility, biodegradability, non-toxicity and so on (Muzzaerelli, 1977). However, intractability of chitin such as poor solubility, reactivity, and difficult processability has limited its utilization as a polymeric material. Chitosan is the *N*-deacetylated derivative of chitin, though this *N*-deacetylation is almost never complete (Robert, 1992). A sharp nomenclature border has not been defined between chitin and chitosan based on the degree of *N*-deacetylation.

Efficient procedures for the preparations of organic soluble chitosan derivatives have been established on the basic of chemical modifications. Solubility of chitosan derivatives in organic solvents in essential requirement for effecting fine molecular design leading to novel types of functional materials. The removal of the two hydrogen atoms of amino groups of chitosan and introduction of some hydrophobic nature by chemical modification cause change the chitosan's inherent crystalline structure and polarity. There are several researches that have been emphasized on chemical modification of the structure of chitosan.

Nishimura *et al.* (1991) prepared *N*-phathaloyl chitosan by the reaction of chitosan with phthalic anhydride in *N*,*N*-dimethylformamide (DMF) at 130°C. The modified chitosan obtained exhibited much improved solubility in common organic solvent such as DMF, *N*, *N*-dimethylacetamide, dimethyl sulfoxide, and pyridine. Yalpani and Hall (1988) indicated that the attachment of carbohydrate to the 2-amino functions of chitosan transforms linear polymer into branched-chain polymers, which were soluble in both aqueous and organic solvents. This conversion can be achieved by reductive alkylation using sodium cyanoborohydride and any aldehyde or keto sugar, by Shift base formation, or by amidation reactions using carboxylic acid or lactone derivatives. These procedures facilitate the chitosan exhibited a number of useful and uncommon properties in terms of their solution characteristics.

A simple and improved method of preparing highly soluble chitosan (half *N*-acetylated chitosan) was developed using a chitosan samples of low molecular weight, and the solubility of the half *N*-acetylated chitosan in water and organic solvents was investigated. To reduce the molecular weight, chitosan was treated with $NaBO_3$ under the condition that chitosan was homogeneously dissolved in aqueous acetic acid. Chitosan was *N*-acetylated with acetic anhydride under the condition that chitosan was homogeneously dissolved in aqueous acetic acid again. The results indicated that half *N*-acetylated chitosan had increased water solubility with decreasing molecular weight and good solubility in aqueous dimethylacetamide and dimethylsulfoxide (Kubota *et al.*, 2000).

N-acyl chitosan had high susceptibility to lysozyme and showed more blood compatible properties than *N*-acetyl chitosan, in particular, *N*-hexanoyl chitosan was the most blood compatible (Lee *et al*, 1995). Novel *N*-acylchitosan fibers were obtained by treatment the filament surface of chitosan fiber with a series of carboxylic anhydrides in methanol at room temperature. Their filament tenacity and elongation values were little influenced by the *N*-acylation (Hirano *et al.*, 1998). Zong *et al.* (2000) synthesized three kinds of acylated chitosans by reacting chitosan with hexanoyl, decanoyl and lauroyl chlorides. In contrast to the chitosan, all of acylated chitosans showed excellent solubility in common organic solvents such as halogenated hydrocarbons and aromatic solvents, but poor solubility in polar solvents. Thin transparent films could be obtained by casting their solutions in chloroform. The white chitosan film was rigid and tough; the films of the acylated chitosans were softer and became even more sticky and elastic at room temperature with increasing chain length of the acyl substituents. Among various acylated chitosans, hexanoyl chitosan (H-chitosan) was found to be anti-thrombogenic and resistant to hydrolysis by lysozome (Lee *et al.* 1995; Hirano and Noishiki 1985). As a result, H-chitosan is a very interesting derivative of chitosan to be used in biomedical applications.

This work is investigated on chemical modification of chitosan to prepare an organic solvent derivative of chitosan, H-chitosan. H-chitosan was prepared by repeating reacted chitosan with hexanoyl chloride. Chemical structure and properties

of the product were characterized. The solubility of the H-chitosan was also investigated.

4.3 EXPERIMENT

Materials and Sample Preparation

Shrimp shell was kindly supplied by Surapol Food Co., Ltd. Sodium hydroxide solution 50% (w/w) was kindly supplied by KPT Coorporation, Thailand. Hexanoyl chloride was purchased from Fluka Co., Ltd. Methanol was purchased from Labscan Co., Ltd. Pyridine and chloroform obtained from Aldrich Co., Ltd. were distilled and dried over molecular sieve prior to use. The other chemicals were analytical grade and were used without further purification.

Chitin was prepared by acid and alkali treatment. Briefly, shrimp shells were cleaned and dried before grinding into smaller pieces. Demineralization was performed by immersing shrimp shells in 1 N HCl solution for 2 day with occasional stirring. The demineralized product was neutralized by washing with deionizing water and protein removal was performed in 4% (w/w) of NaOH solution by boiling at 80-90°C for 4 h. The deproteinized portion was washed with deionized water until neutral. Chitin obtained was dried at 60°C for 24 h.

Chitosan was obtained from deacetylation of α-chitin by using α-chitin flasks in 50% (w/w) NaOH solution. $NaBH_4$ 0.5% (w/w) was added based on the weight of chitin to prevent depolymerization. The mixture was heated in an autoclave at 110°C for 1 h. The deacetylated product was washed thoroughly with deionized water until neutral. The resulting chitosan flakes were dried in an oven at 60°C for 24 h. Chitosan powder was sieved using Restch Seived Machine type Vibro and the portion with the size of 70-75 μm was collected.

Hexanoyl Chitosan Preparation

H-chitosan was synthesized by reacting chitosan with hexanoyl chloride in a mixture of anhydrous pyridine and chloroform, as shown in Figure 4.1. The hexanoylation of chitosan was thoroughly described in an earlier work by Zong *et*

al., 2002. Chitosan (3.20, 19.13 mmol) was soaked in pyridine for one week, and filtered off before further soaking in a mixture of pyridine (90 ml) and chloroform (45 ml) for one day. The mixture was cooled to -10°C in an ice-salt bath, and hexanoyl chloride (21.18 g, 160.67 mmol) dissolved in chloroform (15 ml) was added dropwise in 2 h. The mixture was then stirred for 2 h at room temperature and further refluxed for 6 h. at 98°C. A heterogeneous aggregation of the product was observed in the mixture. The resultant mixture was poured into methanol (300 ml), and the precipitated product was filtered off. The product was dissolved again in chloroform, then precipitated by pouring into methanol, filtered off, extracted in a Soxhlet extractor with methanol for 8 h, and dried in vacuum oven at 40°C for 24 h. The sticky yellowish product was obtained.

The drying acylated derivatives of chitosan, fresh pyridine, and chloroform were placed in a flask in the amount described above. This procedure was repeated several times in order to vary the degree of substitution of resultant hexanoyl chitosan.

Instruments

FT-IR spectroscopic analysis was conducted using Bruker Instrument (EQUINOX55) with a resolution of 4 cm^{-1}. The solid samples were prepared by mixing 1% of sample with dried KBr, while the liquid samples were analyzed using Zn-Se window cell and the film samples were prepared with the thickness of 10-20 μm and attached to the sample holder.

^{1}H-NMR spectrum was recorded by using FT-NMR 500 MHz. spectrometer (JEOL, JNM-A500). Hexanoyl chitosan was dissolved in $CDCl_3$ and used tetramethylsilane (TMS) as reference for chemical shift measurement.

Elemental analysis results were obtained from CHNS/O analyzer (Perkin Elmer PE2400 Series II: option CHN) with combustion temperature at 950°C. The sample (1-2 mg) was filled in tin foil and analyzed under air with oxygen as a combustion gas (flow rate of 20 ml/min) and He as a carrier gas (flow rate of 200 ml/min).

Thermal properties were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) mode. TGA thermograms were performed

using Perkin Elmer instrument: TGA7 analyzer while DSC thermograms were conducted on Mettlor DSC 822e/400 analyzer at a heating rate of 10°C/min under nitrogen atmosphere. Aluminum pans were used in DSC analysis with sample size of 5 – 10 mg, while platinum pan was used in TGA with sample size of 10 – 20 mg.

Crystallinity of products was characterized using Rigaku X-ray Diffractometer at scanning speed of 5 degree/sec using CuKα as a source and CuKβ as a filter. The working ranges were over the 2θ range of 2 to 40°.

4.4 RESULTS AND DISCUSSIONS

Characterization of Chitin, Chitosan, and H-chitosan

α-Chitin was prepared from shrimp cells. Chitosan was prepared by deacetylation of α-chitin in concentrated alkali solution. The degrees of deacetylation of α-chitin and chitosan obtained form FT-IR measurement were 10.71 and 91.00%, respectively (Figure 4.2) (Sabnis, 1997). The molecular weights of chitin and chitosan were determined by viscometric method. The viscosity-average molecular weight of chitin and chitosan were 8.21×10^5 and 3.72×10^5, respectively.

Hexanoyl chitosan (H-chitosan) was synthesized and its chemical structure was characterized by FT-IR, ^{1}H-NMR and elemental analysis.

The FT-IR spectra of chitosan and various hexanoyl chitosans that were synthesized by repeatedly doing the hexanoylation reaction for one (H1-chitosan), two (H2-chitosan), three (H3-chitosan) and four (H4-chitosan) times are shown in Figure 4.3. The characteristic absorption at 3000~4000 cm^{-1} (OH, NH_2) in the FT-IR spectrum of chitosan were absent in H1-, H2-, H3-, and H4-chitosan after first, second, third and forth repeated reaction. The spectra of hexanoyl chitosan also showed new characteristic absorption at 1717 cm^{-1} (C=O of $N(COR)_2$), 1749 cm^{-1} (C=O of OCOR), 2958 cm^{-1}, 2932 cm^{-1} (ν_{as} CH_2), 2873 cm^{-1} (ν_s CH_2), 1458 cm^{-1} (δ CH_2), and 1171 cm^{-1} (twisting vibration of CH_2). These characteristic absorption peaks of hexanoyl chitosan were stronger and shaper as the number of the repeated reaction were increased.

The characteristic absorption peaks of chitosan which were observed at 3000~4000 cm^{-1} (OH, NH_2) were absent in the FT-IR spectra of H-chitosan, indicating that hexanoylation reaction occurred at the hydroxyl groups of chitosan. The FT-IR analysis suggested that hexanoyl groups substituted into hydroxyl and amino groups on the monosaccharide units of chitosan.

^{1}H-NMR was used to confirm the chemical structure of H-chitosan. ^{1}H-NMR spectra of H-chitosan in $CDCl_3$ (Figure 4.4) show signals at 5.6 (H1), 5.2 (H3), 4.2 (H4), 3.4~3.6 (H6, H5), and 2.6 (H2) ppm due to the protons of the polysaccharide ring and the signals at 2.4 ($-CO-CH_2-$), 1.3-1.6 ($-CH_2-$) and 0.9 ($-CH_3$) ppm are assigned to the peaks of hexanoyl chains. The degrees of substitution of H4-chitosan determined from ^{1}H-NMR was 2.91.

From FT-IR and ^{1}H-NMR analysis, the results indicated that H-chitosan was obtained after the chemical modification of chitosan by hexanoylation reaction.

The results of elemental analysis of H1-, H2-, H3- and H4-chitosan are listed in the Table 4.1. Experimental results were compared with the calculated values and the degree of substitution was determined based on the C/N ratio of the product. The degrees of substitution of H1-, H2-, H3- and H4-chitosan were 2.06, 3.78, 3.91, and 3.92, respectively

Characteristic Properties of H-chitosan

In contrast to chitosan, all of the H-chitosans showed excellent solubility in common organic solvents (Table 4.2) such as chloroform, dichloromethane and tetrahydrofuran, but poor solubility in polar solvent.

DSC thermograms of the chitosan and hexanoyl chitosans are shown in Figure 4.5. The chitosan shows a broad endothermic peak around 120°C while H1-Chitosan shows smaller endothermic peak at the same position but the peak absent when the degree of substitution of hexanoyl groups increased. The exothermic of H1-, H2-, H3- and H4-chitosan at position close to 240°C correspond to their thermal decomposition.

For thermogravimetric thermogram of hexanoyl chitosans, H1-chitosan shows one T_d but H2-, H3- and H4-chitosan shows two T_{ds}. Figure 4.6 shows two T_{ds} of H-chitosan at 255-270°C and 315-330°C which correspond to the T_d of

hexanoyl groups and glysidic linkage of chitosan, respectively. The decomposition behavior of H-chitosan is more different far from chitosan when the degree of substitution increased. Zong *et al.* (2000) investigated thermal properties of chitosan with a degree of deacetylation of 90%, H-chitosan, decanoyl chitosan (D-chitosan), and lauroyl chitosan (L-chitosan) by DSC analysis. It was found that the exothermic peaks of chitosan, H-, D-, and L-chitosan observed at 298°C, 225°C, 246°C, and 255°C, respectively, were their thermal decomposition temperatures. Grant *et al.* (1990) also reported that chitosan with degree of deacetylation of 86.4% and its lauroyl derivative had the exothermic decomposition peaks at 322°C and 214°C, respectively. These evidences suggested that the thermal stability of H-chitosan decreased when the hexanoyl groups were introduced to chitosan backbone. The hexanoyl chitosan is stable below 255°C. Introduction of lateral substituents or flexible units into the polysaccharide structure should disrupt the crystalline structure of chitosan, especially through the loss of the hydrogen bonding.

WAXD patterns of the H-chitosans were measured to compare with the crystalline structure of chitosan (Figure 4.7). H-chitosan shows a strong diffraction peak at diffraction angles (2θ) of 2-6° together with a broad peak at 2θ of 20°. When degree of substitution of hexanoyl groups increased, the peak at 20° became broader and the peak at 2-6° was stronger. This suggested that the packing of hexanoyl side chains formed a new type of ordering structure. The loss of 10° reflection indicated that crystallinity of chitosan was decreased from the loss of hydrogen bonds due to the substitution of hexanoyl groups. Similar results have been reported by Zong *et al.* (2000), who synthesized and characterized the structure of acylated chitosans which were hexanoyl, decanoyl, and lauroyl chitosan. They found that the *d*-spacing of diffraction angles (2θ) of 2-6° of each chitosan derivative increased as the number of carbon atoms of acyl subsistent increased. As a result, the side chains were packed with each other forming a layered structure with the main chains extended.

4.5 CONCLUSION

Hexanoyl chitosans was synthesized by reacting chitosan with hexanoyl chlorides to obtain products with various degrees of hexanoylation [Degree of substitution = 2.06, 3.78, 3.91 and 3.92 while the number of repeated reaction = 1, 2, 3 and 4, respectively]. The chemical and crystalline structures of hexanoyl chitosan were determined. With increasing degree of substitution, solubility in organic solvent increased. The thermal stability and crystallinity of H-chitosan were lower than those of chitosan due to the loss of intra- and inter-molecular hydrogen bonds and large substituents group resulting in poor packing of H-chitosan main chains.

4.6 ACKNOWLEDGMENTS

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Table 4.1 Elemental analysis and the degree of substitution of hexanoyl chitosan

Derivatives	C%	H%	N%	Degree of substitution
Calculated values	64.89	9.17	2.56	4.00
H1-chitosan	52.55	8.57	4.02	2.06
H2-chitosan	66.09	9.41	2.76	3.78
H3-chitosan	66.84	10.24	2.74	3.91
H4-chitosan	66.48	10.01	2.68	3.92

Table 4.2 Solubility of chitosan and H-chitosans

Sample	Solubility				
	$CHCl_3$	CH_2Cl_2	THF	DMAc	DMSO
Chitosan	–	–	–	–	–
H1-chitosan	±	±	±	±	±
H2-chitosan	+	+	+	–	–
H3-chitosan	+	+	+	–	–
H4-chitosan	+	+	+	–	–

Note: + dissolvable ± swelling or partially dissolvable – undissolvable

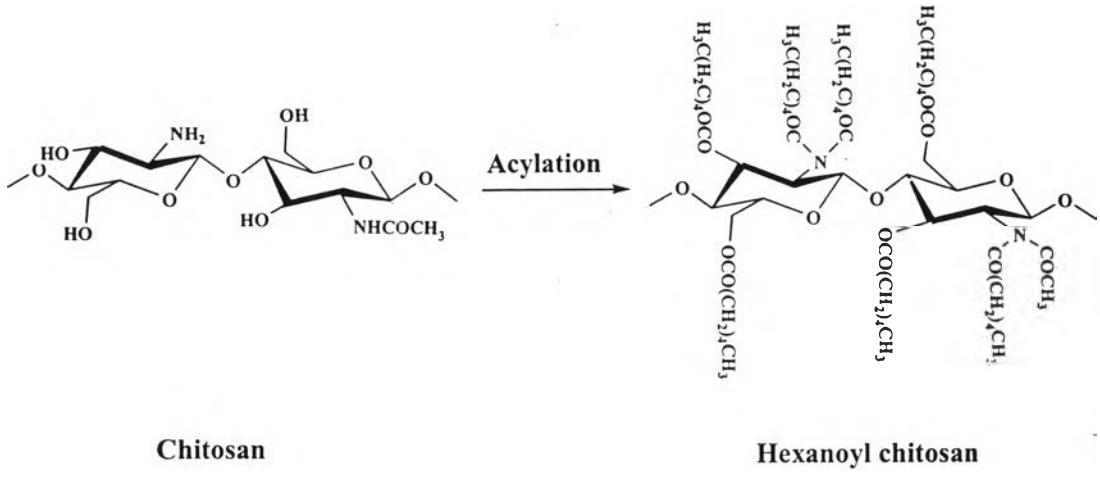

Figure 4.1 Synthesis reaction of hexanoyl-chitosan.

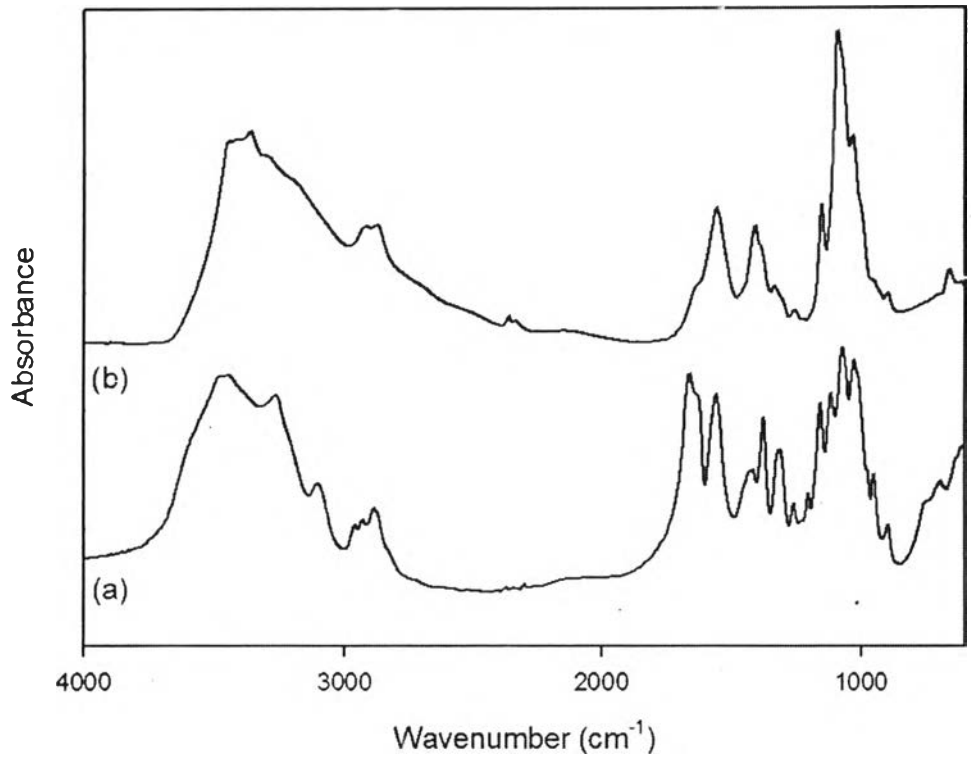

Figure 4.2 FT-IR spectra of (a) α-chitin, and (b) chitosan.

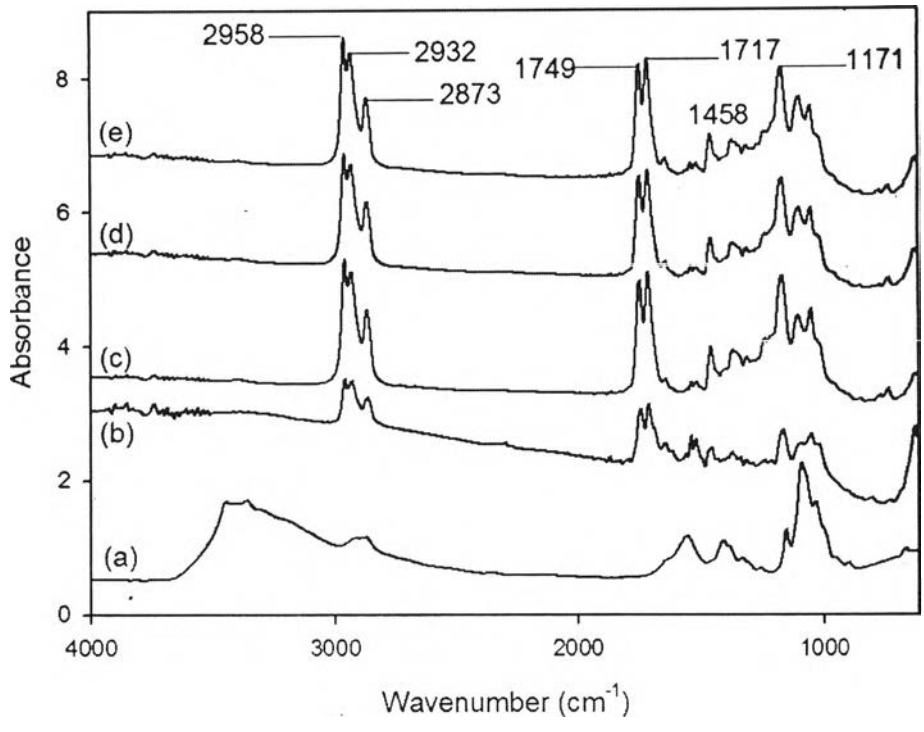

Figure 4.3 FT-IR spectra of (a) chitosan, (b) H1-chitosan, (c) H2-chitosan, (d) H3-chitosan and (e) H4-chitosan.

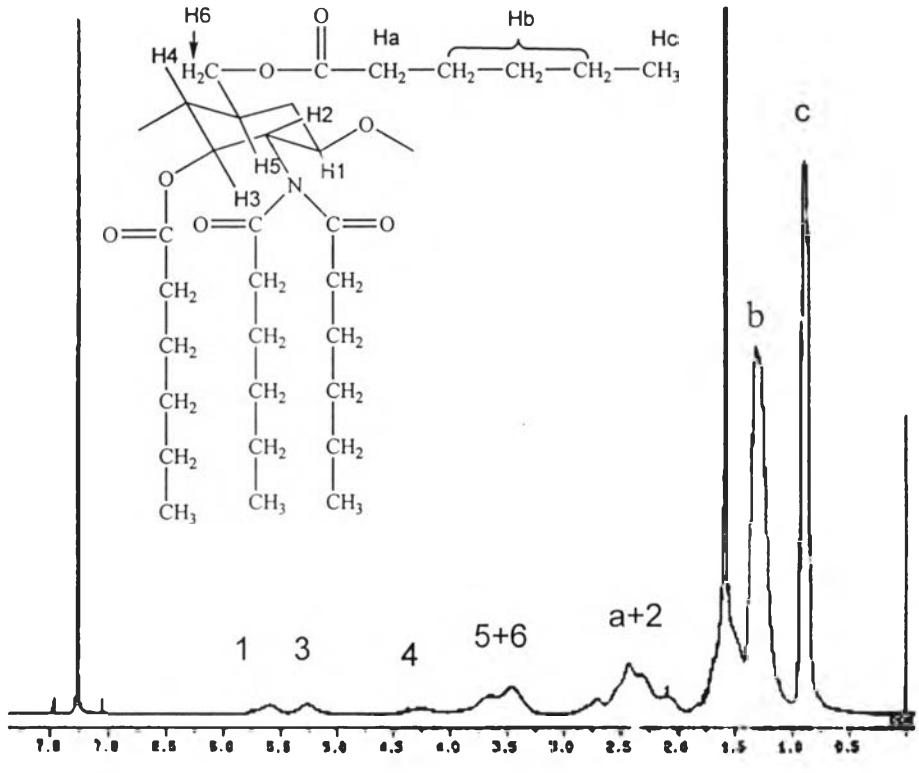

Figure 4.4 ^{1}H-NMR spectrum of H4-chitosan in $CDCl_3$.

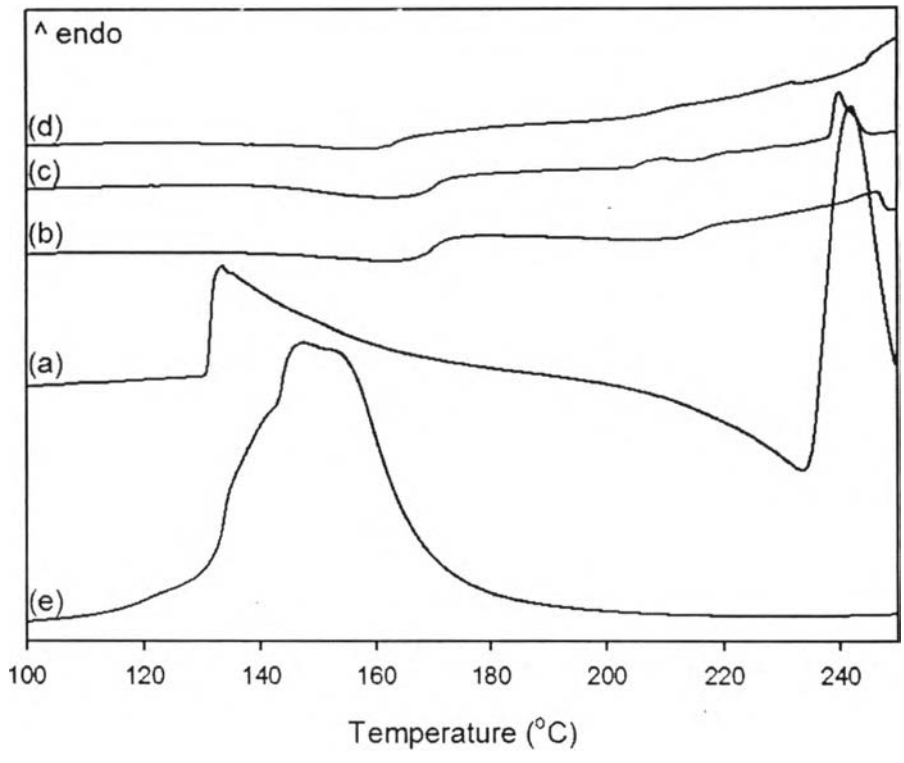

Figure 4.5 DSC thermograms of (a) H1-chitosan, (b) H2-chitosan, (c) H3-chitosan, (d) H4-chitosan and (e) chitosan.

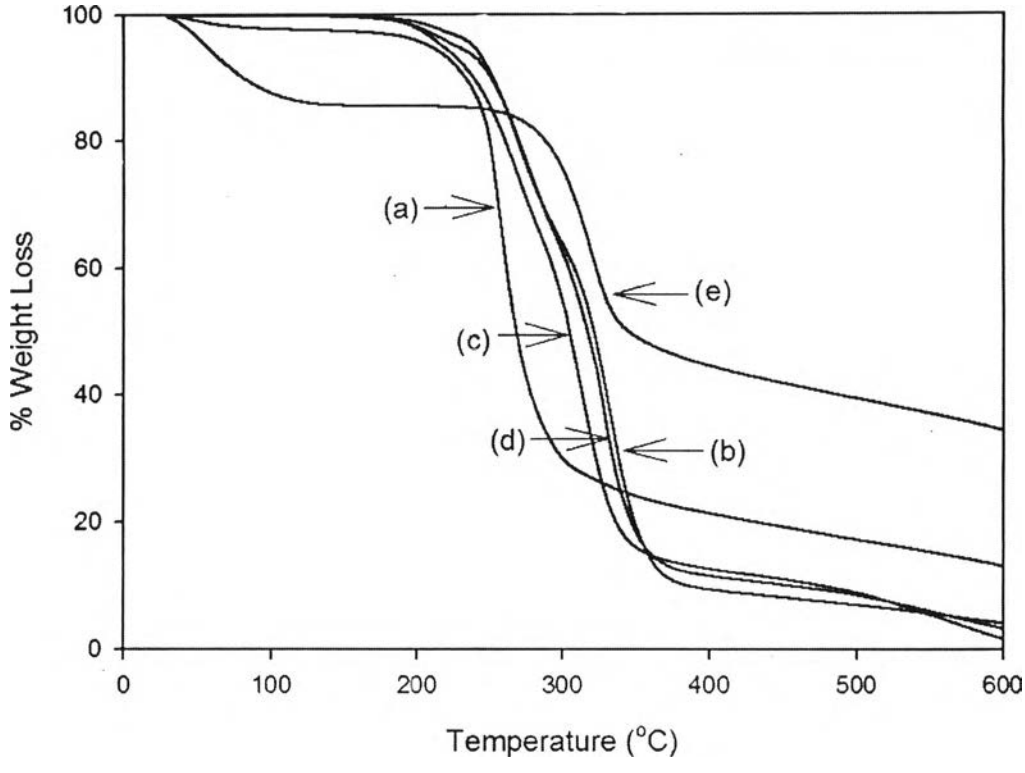

Figure 4.6 TGA thermograms of (a) H1-chitosan, (b) H2-chitosan, (c) H3-chitosan, (d) H4-chitosan and (e) chitosan.

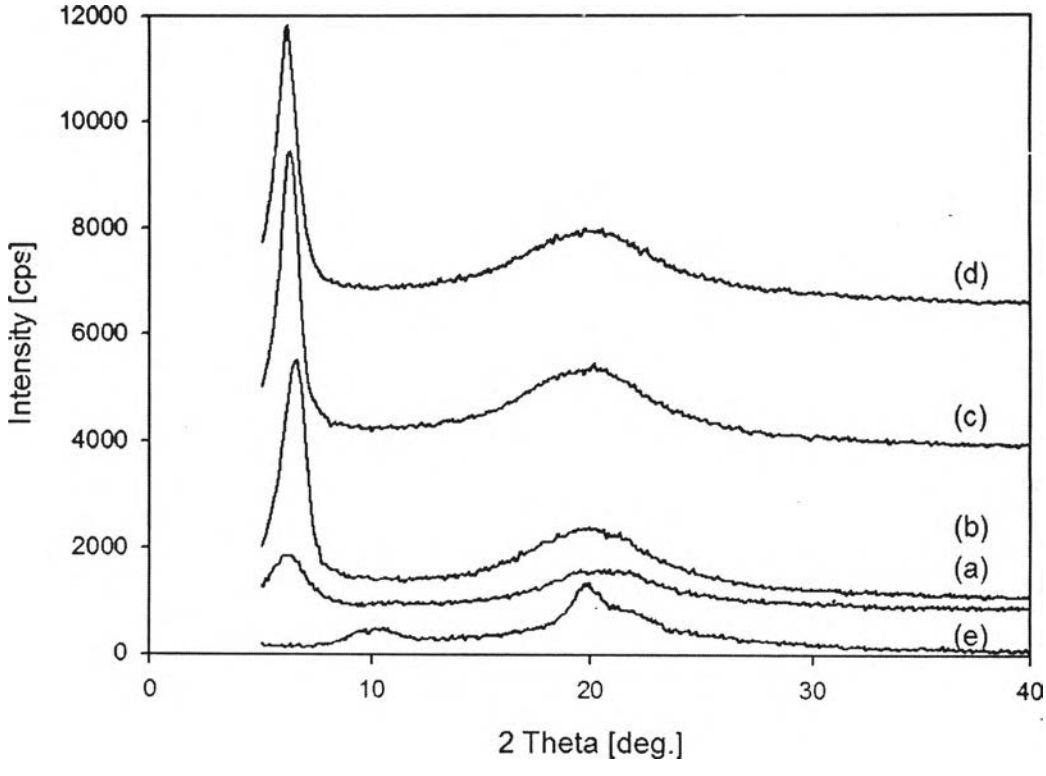

Figure 4.7 WAXD patterns of (a) H1-chitosan, (b) H2-chitosan, (c) H3-chitosan, (d) H4-chitosan and (e) chitosan.