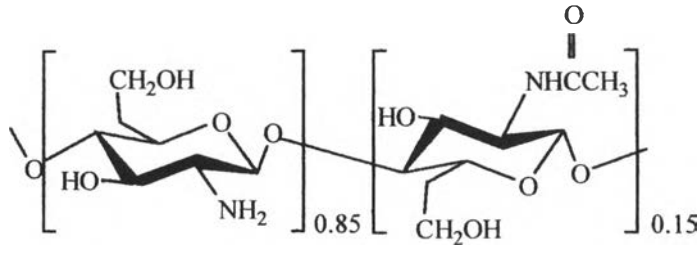
CHAPTER IV
RESULTS AND DISSCUSSION

4.1 Chitosan

Chitosan used as the starting material was prepared by deacetylation of chitin. The degree of deacetylation of chitosan obtained was approximately 85%. The chemical structure of chitosan was shown in Scheme 4.1.



Scheme 4.1 Chemical structure of chitosan starting material.

Generally, chitosan has higher amount of amino groups than acetamide groups. However, The degree of deacetylation of chitosan is dependent on the deacetylated condition especially NaOH concentration as shown in Figure 4.1.

The structure of chitosan obtained was confirmed by FT-IR spectrum shown in Figure 4.2 and the absorption of characteristic bands of the chitosan are summarized in Table 4.1.

Table 4.1 FT-IR characteristic absorption band of chitosan.

Wavenumber (cm^{-1})	Assignment
1659	C=O stretching
1561	NH deformation
1317	CN band and CH_2 wagging
1080 and 1031	C-O stretching vibration

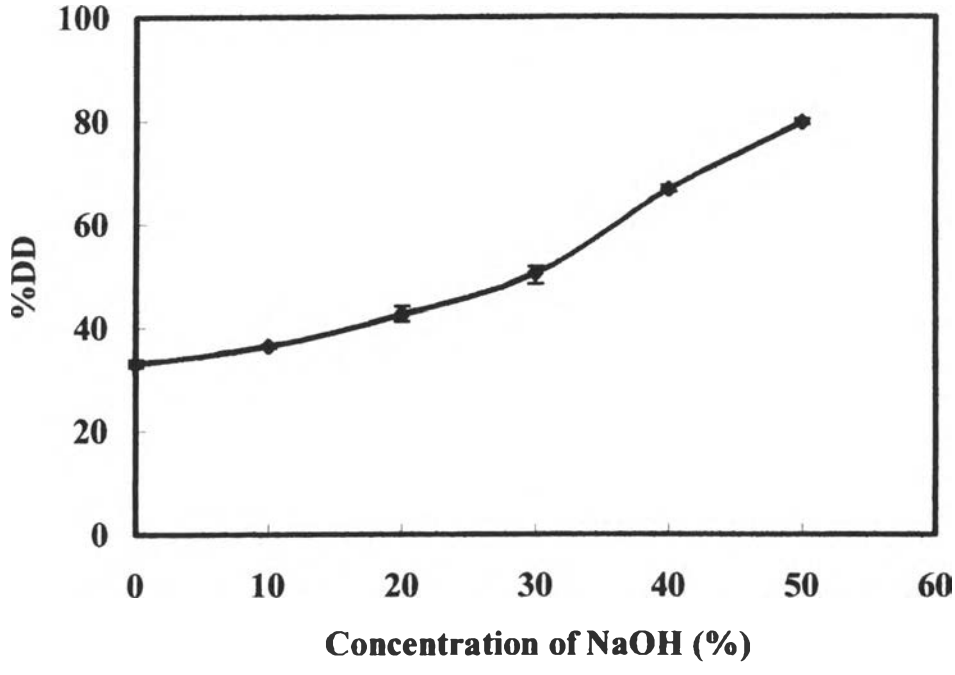


Figure 4.1 Effect of NaOH concentration on degree of deacetylation of chitosan.

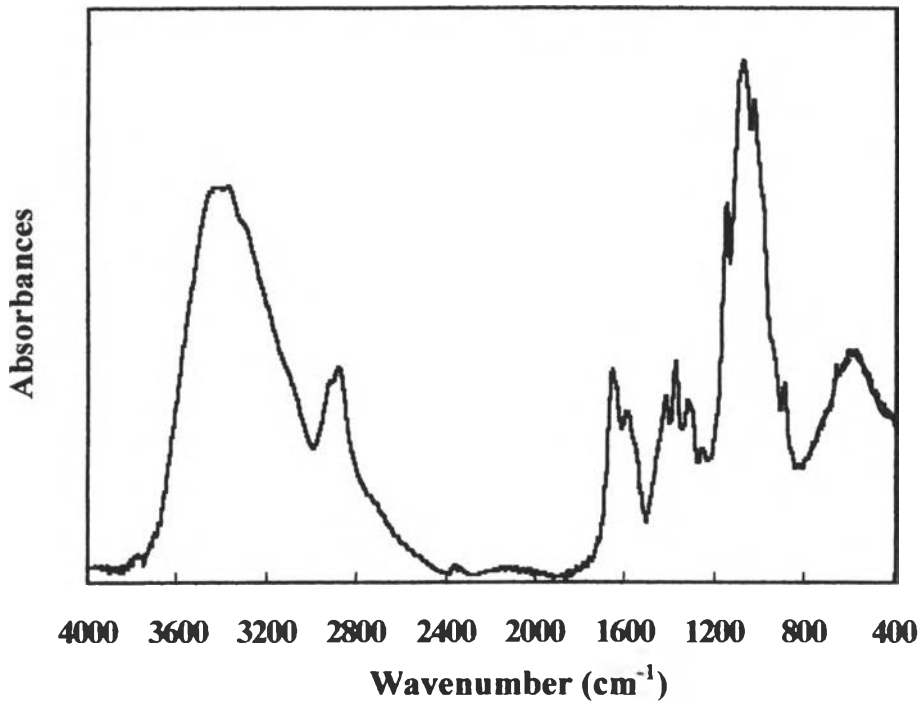


Figure 4.2 FT-IR spectrum of chitosan.

Following the method used by Lee (1978), the intrinsic viscosity of chitosan was found to be 10.86. This value was obtained from the interception of the plot between η_{sp}/c and $\ln(\eta_{rel}/c)$ against concentration of chitosan solution (see Figure 4.3). Using Mark-Houwink equation where "a" is 0.71 and "K" is 8.93×10^{-4} (Lee, 1978), the viscosity-average molecular weight of chitosan obtained was approximately 5.67×10^{5} g/mol.

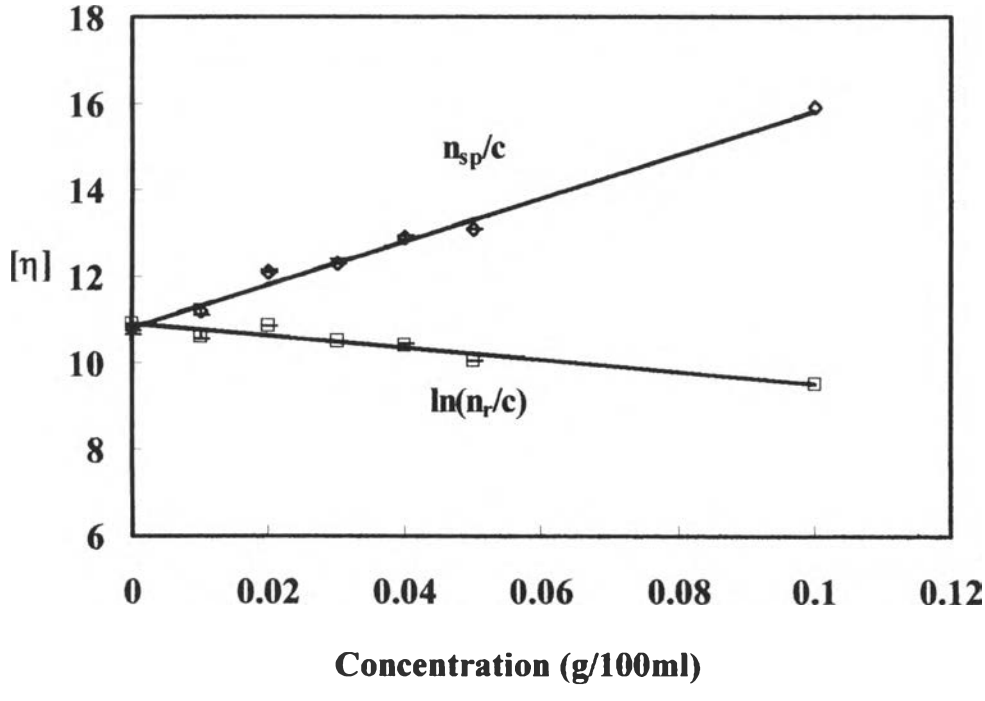


Figure 4.3 η_{sp}/c and ln (η_r/c) against concentration of chitosan solution.

4.2 Preparation of Hexanoyl Chitosan (Method I)

4.2.1 Characterization

Hexanoyl chitosans were synthesized directly in heterogeneous system by reacting chitosan with hexanoyl chloride in pyridine and chloroform solvent mixture. In this reaction, all amino, hydroxyl and acetamide groups of

chitosan were substituted with hexanoyl group after the reaction was repeated for 3 times. The products were characterized by FT-IR and NMR.

The characteristic absorptions at 3000~4000 cm^{-1} (OH, NH_2) in the FT-IR spectrum of chitosan (Figure 4.4) were reduced in hexanoyl-1 (H-1) after the first reaction and absent in the spectra of hexanoyl-2 and-3 chitosan (H-2 and H-3) after 2^{nd} and 3^{rd} repeated reaction. The spectra of hexanoyl chitosans also showed peaks at 1716 cm^{-1} (C=O of $N(COR)_2$), 1747 cm^{-1} (C=O of OCOR) and 2958, 2832, 2872, 1416 and 1182 cm^{-1}(CH_2). These characteristic peaks of hexanoyl chitosan were stronger and sharper as the number of repeated reaction increased.

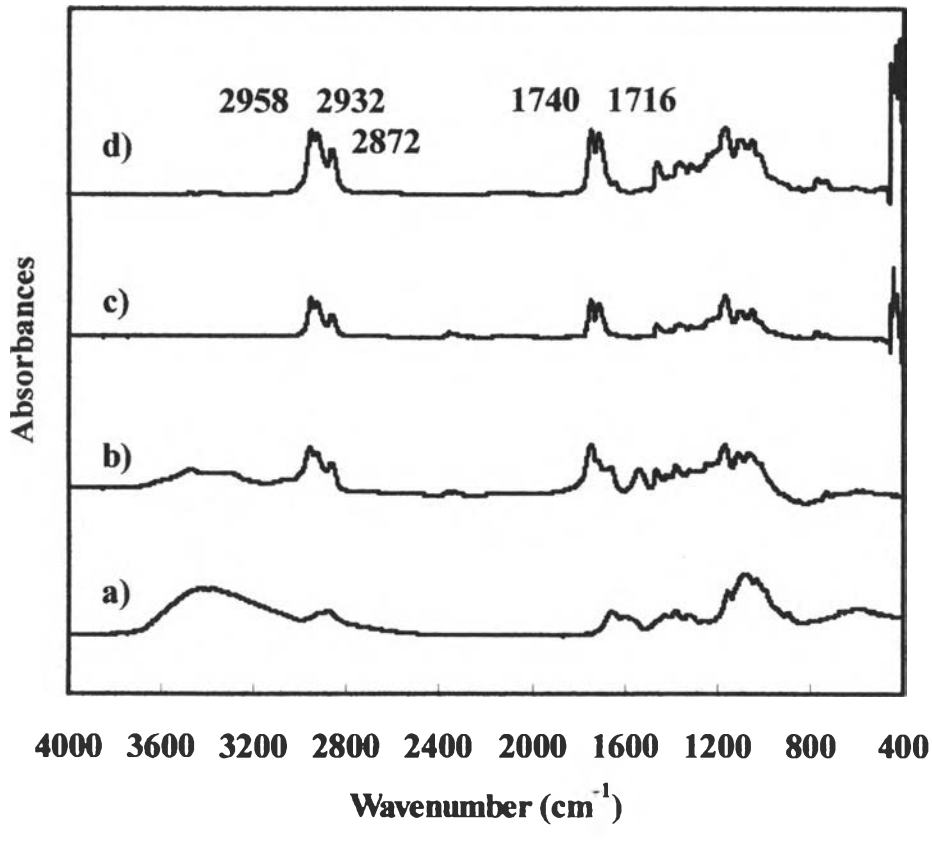


Figure 4.4 FT-IR spectra of a) chitosan, b) H-1 chitosan, c) H-2 chitosan, and d) H-3 chitosan.

Solid state ^{13}C-NMR spectrum of H-1 chitosan was shown in Figure 4.5. The signals observed at 55(C2+C2'), 63(C6+C6'), 73-86(C4+C4', C5', C3+C3', C5), 100(C1) and 104(C1') ppm were attributed to the carbon in the polysaccharide structure. The signals of β-ε carbons of hexanoyl groups were observed at 14(a), 23(b), 32(c) and 25(d) ppm as singlet signals while α-CH_2 were broad peak at 28-43 ppm corresponding to the hexanoyl groups in different environment (no obvious peaks were observed because of low degree of substitution). The signals observed between 165-185 ppm were assigned to the carbonyl carbons refered to the bonds between hexanoyl groups and chitosan.

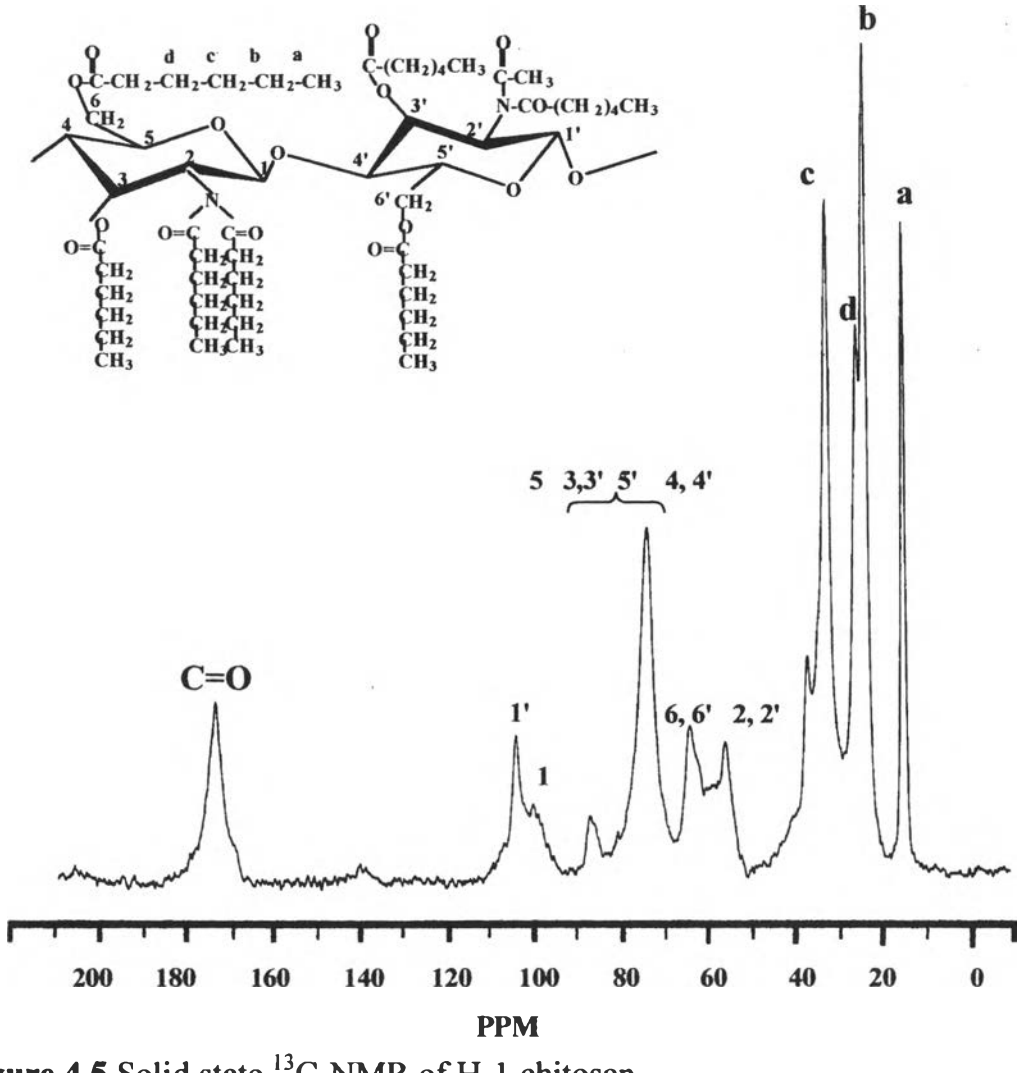


Figure 4.5 Solid state ^{13}C-NMR of H-1 chitosan.

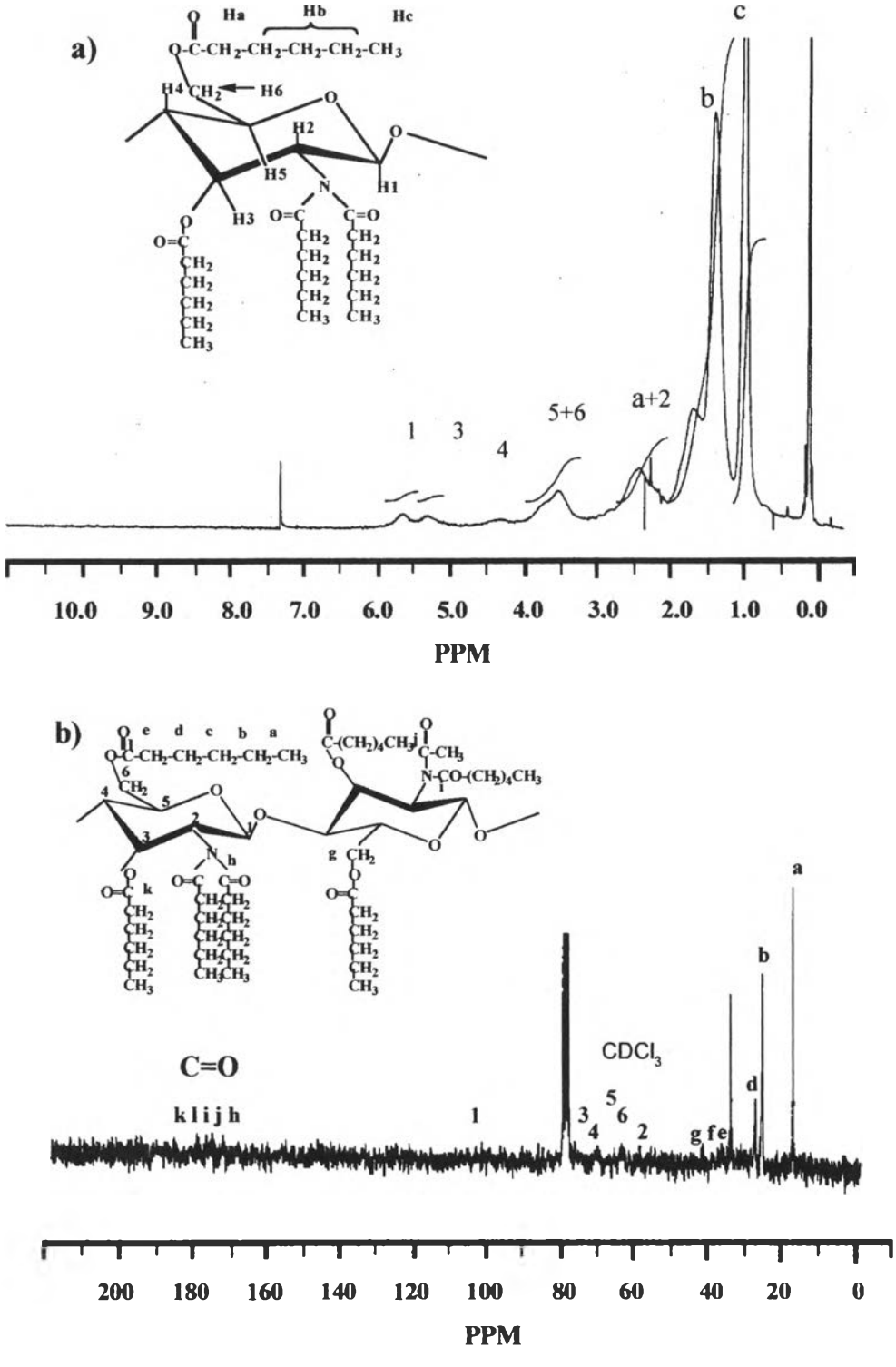


Figure 4.6 a) 300 MHz ^{1}H- NMR spectrum of H-2 chitosan, in $CDCl_3$, at 25°C, b) 300 MHz ^{13}C-NMR spectrum of H-2 chitosan, in $CDCl_3$, at 25°C.

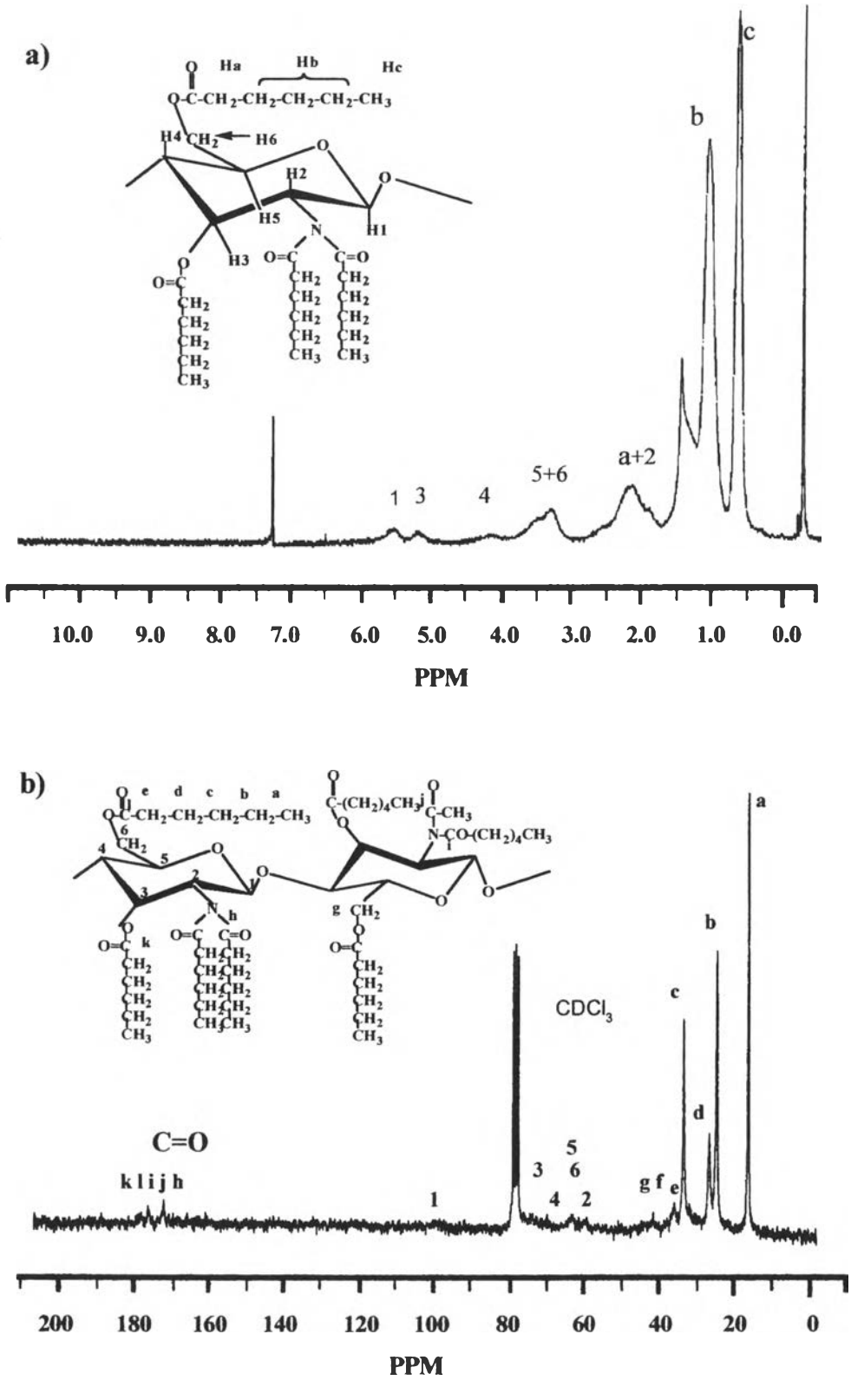


Figure 4.7 a) 300 MHz ^{1}H- NMR spectrum of H-3 chitosan, in $CDCl_3$, at 25°C, b) 300 MHz ^{13}C-NMR spectrum of H-3 chitosan, in $CDCl_3$, at 25°C.

The ^{1}H- NMR spectra of H-2 and H-3 chitosan in $CDCl_3$ (Figure 4.6 (a) and 4.7 (a)) display signals at 2.75-5.6 ppm which are peaks found in pyranose ring and the signals at 2.45 (-CO-CH_2-), 1.3~1.65 (-CH_2-) and 0.9 (-CH_3) ppm which are the characteristic peaks of the hexanoyl chains. Figure 4.6 (b) and 4.7 (b) showed ^{13}C-NMR spectra of H-2 and H-3 chitosan in $CDCl_3$. The signals observed at 58(C2), 62(C4), 69(C3) and 99(C1) ppm were attributed to the carbon in the pyranose ring. The signals of β-ε carbons of hexanoyl groups were observed at 14(a), 23(b), 32(c) and 25(d) ppm as singlet signals while α-CH_2 are multiplet signal (7 peaks) at around 27-43 ppm corresponding to the hexanoyl groups in different environment. The signals observed between 169-179 ppm were assigned to the carbonyl carbons. These results were consistent with the ^{13}C-NMR spectrum of fully hexanoylated chitosan [Zong *et al.*, 2000].

The results of elemental analysis of all derivatives are listed in Table 4.2. The experimental results were compared with the calculated values and the degrees of subsitution were determined based on the C/N ratio of the products. The degree of substitution was caculated per each repeating unit assuming H-3 chitosan was fully hexanoylated. The degrees of substitution of H-1, H-2 and H-3 chitosan were 2.76, 3.77 and 3.85, respectively. H-3 chitosan displayed higher amount of carbon compared to the value calculated from complete hexanoylation. These results indicated that all functional groups of H-3 chitosan were fully substituted with hexanoyl groups. The appearance of H-3 was much stickier than H-2 and H-1 chitosan, respectively. This may due to the chain scission of chitosan occured after the reaction was repeated resulting in lower molecular weight polymer chains and also the loss of intra- and inter-molecular hydrogen bonds from the introduction of hexanoyl substituents.

Table 4.2 Elemental analysis and degree of substitution of chitosan derivatives.

Derivatives	Calcd.			Found			Degree of substitution
	C%	H%	N%	C%	H%	N%	
Method I							
H-1 chitosan	63.25	8.96	3.02	60.71	8.99	2.90	2.76
H-2 chitosan	65.69	9.26	2.30	65.97	9.48	2.31	3.77
H-3 chitosan (fully hexanoyl chitosan)	65.83	9.27	2.26	66.02	8.85	2.23	3.85
Method II							
Ph-chitosan	54.06	5.55	5.67	54.06	4.14	3.20	0.60
H-Ph-chitosan	64.00	7.94	2.77	64.55	7.08	2.49	2.65
H-P chitosan	49.85	7.61	2.78	49.85	6.92	2.15	2.65

4.2.2 Thermal Stability

Thermal stability of H-l, H-2 and H-3 chitosan are shown in Table 4.3. H-1, H-2 and H-3 chitosan showed an initial weight loss at around 231± 1°C, 233±1°C and 232±1°C, respectively. This could be due to the degradation of hexanoyl groups and second decomposition observed at around 320°C was caused by the decomposition of glycosidic linkage of chitosan. These results suggest that the introduction of hexanoyl group onto chitosan decrease the thermal stability of chitosan. This could be due to the destruction

of crystalline structure of chitosan especially through the loss of intra- and inter-molecular hydrogen bonds of the chitosan structure.

Table 4.3 TGA results of chitosan and its derivatives.

Derivatives	**Degradation temperature (°C)**		
	1st decomposition	2nd decomposition	3rd decomposition
Method I			
Chitosan	-	-	311±1
H-1 chitosan	-	231±1	320±1
H-2 chitosan	-	233±1	293±1
H-3 chitosan	-	232±1	319±1
Method II			
Ph-chitosan	190±1	-	340±1
H-Ph-chitosan	185±1	256±1	345±1
H-P chitosan	174±1	235±1	293±1

4.2.3 Crystallinity

Figure 4.8 shows the WAXD patterns of all hexanoyl chitosans with different degree of hexanoylation compared with chitosan. Chitosan has two distint crystal forms I and II which showed the strongest reflection at 2θ = 11.4° and 20.1°, respectively [Samuels, 1981]. The hexanoyl chitosans showed a broad reflection at around 20° together with the strong reflection at

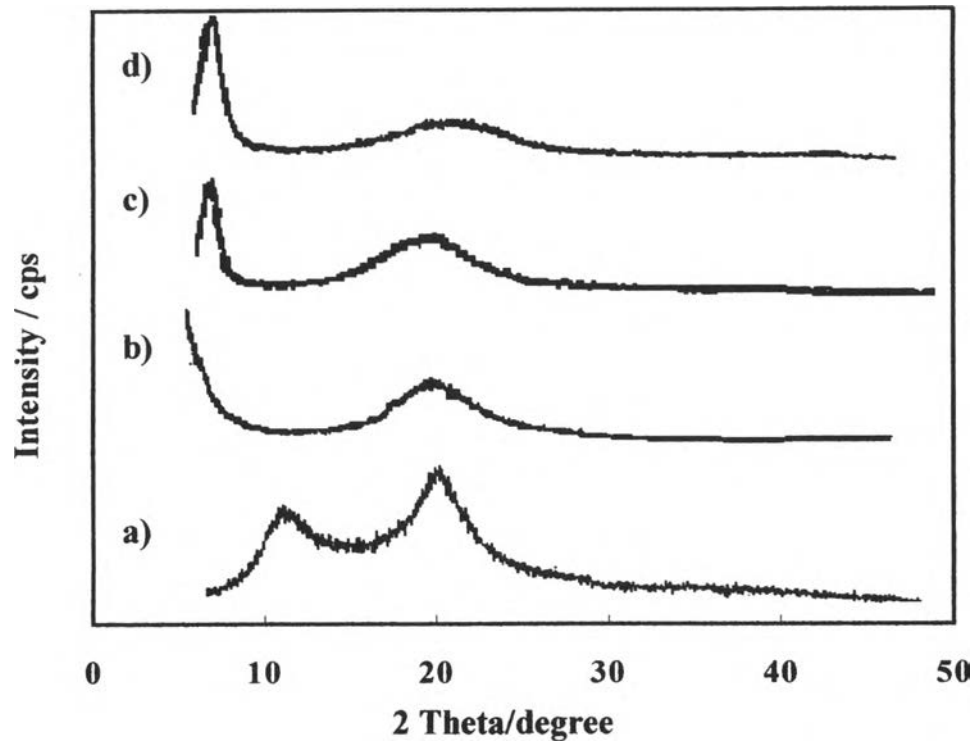


Figure 4.8 WAXD patterns of a) chitosan, b) H-1 chitosan, c) H-2 chitosan, and d) H-3 chitosan.

around 2-6°. The loss of 11.4° reflection indicated that crystallinity of chitosan was decreased from the loss of hydrogen bonds. The latter reflection at 20° suggested a new type of ordering structure that may caused by the packing of hexanoyl side chains. The peak at 20° is broader with increasing the degree of substitution of hexanoyl groups while the peak at 2-6° is stronger. A lower degree of ordered packing of chitosan main chains was due to the bulky side chains having rotational freedom and prevent the occurrence of well-defined reflection of main chains.

Table 4.4 Solubility of chitosan and its derivatives.

Derivatives	Solubility									
	$CHCl_3$	CH_2Cl_2	Xylene	$C_6H_5CH_3$	THF	Dioxane	Pyridine	DMF	DMAc	DMSO
Method I										
Chitosan	–	–	–	–	–	–	–	–	–	–
H-1 chitosan	±	±	±	±	±	±	±	±	±	±
H-2 chitosan	+	+	+	+	+	±	+	–	–	–
H-3 chitosan	+	+	+	+	+	+	+	–	–	–
Method II										
Ph-chitosan	–	–	–	–	–	–	+	+	+	+
H-Ph-chitosan	±	±	±	±	±	±	+	±	±	±
H-P chitosan	–	–	–	–	–	–	±	±	±	+

Note : + dissolvable ± swelling or partially dissolvable – undissolvable

4.2.4 Solubility

All hexanoyl chitosans showed much improve in solubility with increasing degree of substitution (see Table 4.4). H-1 chitosan swelled in most organic solvents while H-2 and H-3 chitosan dissolved easily in halogenated hydrocarbons and aromatic solvents such as chloroform, methylene chloride, toluene and xylene, but gave poor solubility in polar solvents like DMSO. Thin transparent films could be obtained by casting their solutions in toluene. While the chitosan film is rigid and tough, the films of the hexanoyl chitosans were softer and became sticky and elastic at room temperature.

4.3 Preparation of Hexanoyl Chitosan with Free Amino Groups (Method II)

4.3.1 Characterization

4.3.1.1 *Phthalimido Chitosan (Ph-Chitosan)*

Phthaloyl group was chosen as the most suitable protective group for the amino group of chitosan. The reaction proceeds without any side reaction with the primary and secondary hydroxyl groups at C_3 and C_6 positions. The characteristic absorptions of the substituted phthalimido groups at 1776 and 1714 cm^{-1} were also observed in the FT-IR spectrum of the protected chitosan [Nishimura *et al.*, 1991] as shown in figure 4.9 (b).

The chemical structure of Ph-chitosan was also confirmed by ^{1}H- and ^{13}C-NMR spectra shown in figure 4.10 (a) and (b), respectively. From the ^{1}H-NMR spectrum, peak at 1.8 ppm could be assigned to the acetyl methyl group. The peaks of hydrogen of pyranose ring at around 3-5.5 ppm and benzene ring in phthalimido group at around 7-8 ppm were observed in this

spectrum. From the ^{13}C-NMR spectrum, the peaks of carbon from acetyl methyl and pyranose rings of chitosan were observed at 20 (single) and 55-110 (multiplets) ppm, respectively. While the characteristic peaks at around 122-140 and 168 ppm were assigned to carbon from benzene ring and carbonyl of phthalimido group. The result from NMR corresponded to the result from FT-IR indicated N-phthalimido chitosan were succesfully synthesized.

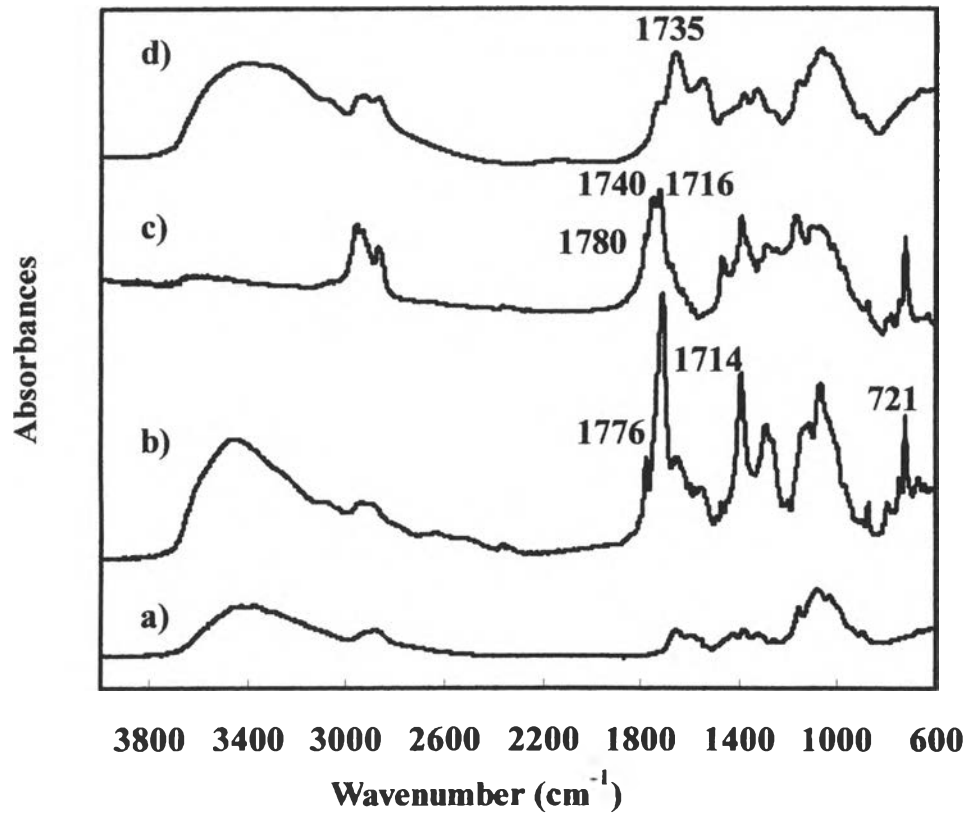


Figure 4.9 FT-IR spectra of a) chitosan, b) Ph-chitosan, c) H-Ph-chitosan, and d) H-P chitosan.

4.3.1.2 *Hexanoyl-N-Phthalimido Chitosan (H-Ph-Chitosan)*

H-Ph-chitosan was prepared by hexanoylation of Ph-chitosan. In this reaction the remaining amino, hydroxyl and acetamide groups of chitosan were substituted with hexanoyl groups after the

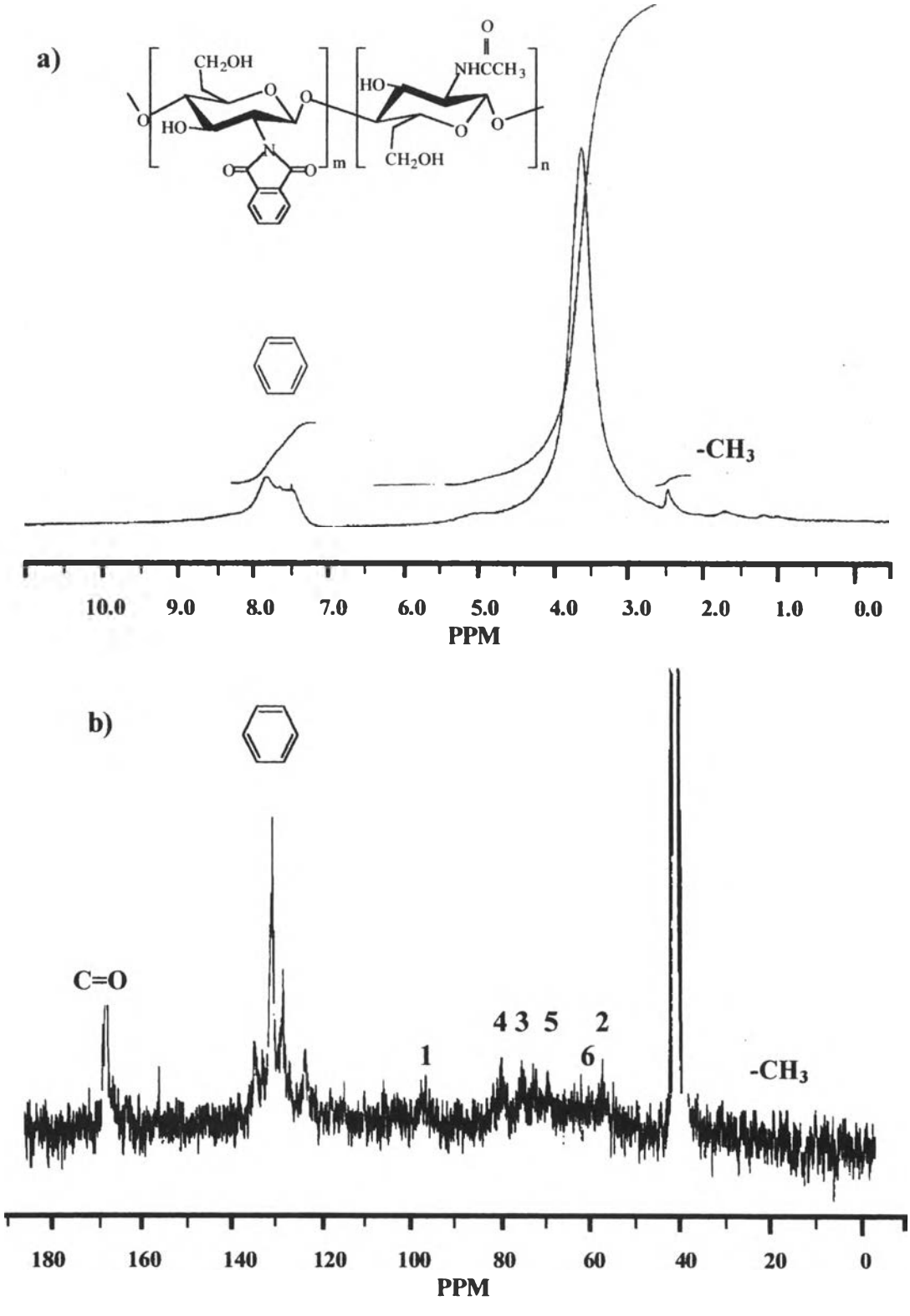


Figure 4.10 a) 300 MHz ^{1}H- NMR spectrum of Ph-chitosan, in DMSO-d6, at 25°C, b) 300 MHz ^{13}C-NMR spectrum of Ph-chitosan, in DMSO-d6, at 25°C.

hexanoylation was repeated twice. It is interesting to note that solubility behaviour of Ph-chitosan and H-Ph-chitosan has been greatly improved in pyridine. As a result, the hexanoylation was prepared sucessfully in a more simple reaction since there is no need for soaking Ph-chitosan and H-Ph-chitosan in pyridine prior to the reaction for a week. This might be due to the loss of hydrogen bonds when dissolve in pyridine.

FT-IR spectrum of H-Ph-chitosan shows both characteristic peaks of phthalimido and hexanoyl groups (see Figure 4.9 (c)). The peaks of carbonyl groups observed at 1716 cm^{-1} were consistent with the amide (C=O of $N(COR)_2$) of hexanoyl superimposed with phthalamide structure. The peaks at 1740 cm^{-1} and 1780 cm^{-1} were assigned to the carbonyl of hexanoyl (substituted on -OH groups) and phthalimido groups, respectively. The peaks at 2932, 2872, 1468 and 1164 cm^{-1} were due to $-CH_2$ of hexanoyl groups comfirmed the success of reaction.

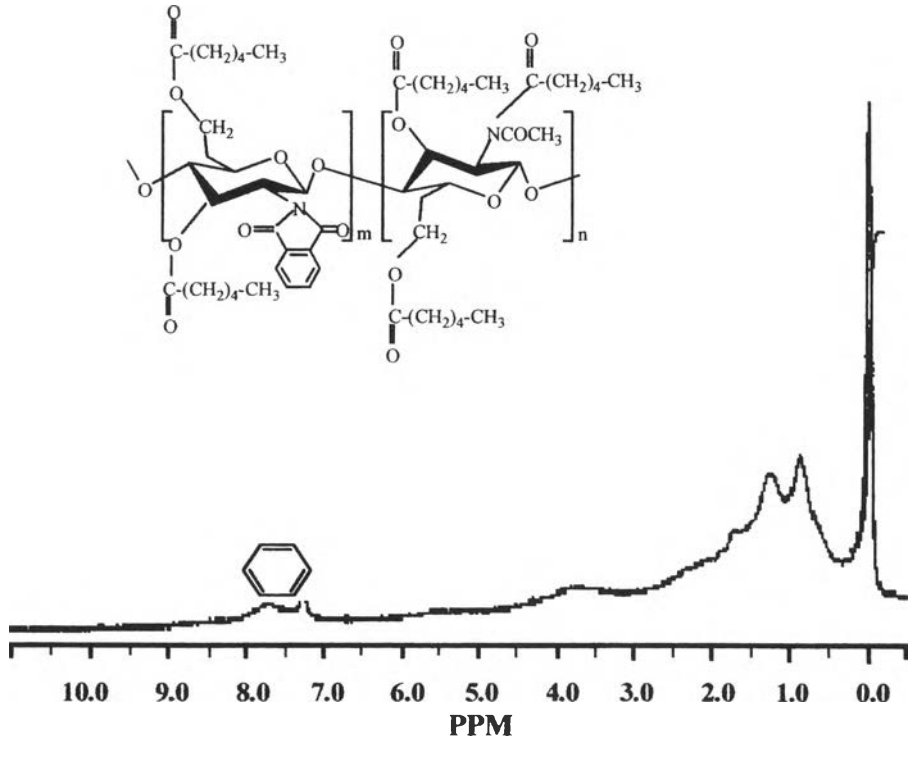


Figure 4.11 300 MHz ^{1}H-NMR spectrum of H-Ph-chitosan, in $CDCl_3$, at 25°C.

^{1}H-NMR spectrum of H-Ph-chitosan in $CDCl_3$ was shown in Figure 4.11. Both characteristic peaks of phthalimido and hexanoyl groups were observed from the spectrum of this derivative as displayed at around 7-8 ppm and 0.9-2.45 ppm, respectively.

4.3.1.3 *Hexanoyl Chitosan (H-P Chitosan)*
(obtained after removal of protecting group)

The phthalimido group was removed from H-Ph-chitosan by treating with hydrazine monohydrate. However, the removal of some hexanoyl groups was also observed in this reaction. After removal of protective groups, the present of free amino groups was confirmed by FT-IR spectrum (see Figure 4.9 (d)). The absorption of amino groups was observed at around 3300-3500 cm^{-1} (N-H stretching) and 1640,1520 cm^{-1} (N-H vibration). Hexanoyl groups substituted on both hydroxyl groups of chitosan were still remained in the structure observed from the peak of carbonyl of ester shown at 1735 cm^{-1} [Xu *et al.*, 1996]. Figure 4.12 shows the ^{1}H of H-P chitosan in duterated DMSO-d6, respectively. The ^{1}H-NMR spectrum of H-P chitosan with free amino groups displayed signal at 2.3-5.6 ppm which are peaks found in polysaccharide ring and the signals at 2.45 ($-CO-CH_2-$), 1.2~1.65 ($-CH_2-$) and 0.85 ($-CH_3$) ppm which are the characteristic peaks of the hexanoyl chains. The characteristic peak of benzene was also observed at 7.4-7.9 ppm implied that small amount of phthalimido groups remained in this product.

The elemental analysis results indicated that the degree of substitution of Ph-chitosan was 0.60 calculated based on the carbon percentage (Table 4.2). The fully substitution of hexanoyl groups on all hydroxyl, acetamide and the amino groups left from protection was confirmed in these results with degree of substitution of 2.65 for H-Ph-chitosan. By assuming no hexanoyl group was removed in the reaction used for removing

the protective group, the degree of sustitution of H-P chitosan obtained from elemental analysis also was 2.65.

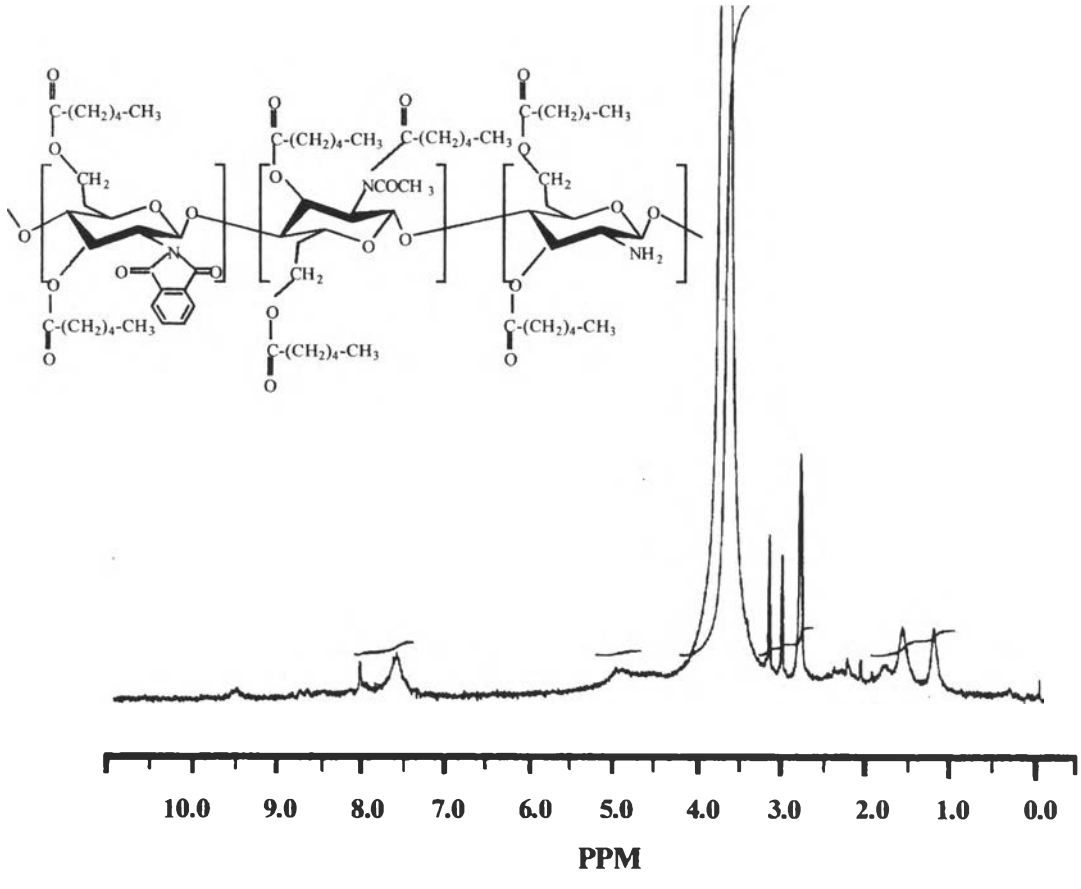


Figure 4.12 300 MHz ^{1}H- NMR spectrum of H-P chitosan, in DMSO-d6, at 25°C.

4.3.2 Thermal Stability

While chitosan starting material decomposed at 311±1°C (Table 4.3) due to decomposition of glucoside linkage between pyranose ring of chitosan, Ph-chitosan showed weight loss at 190±1°C revealed the degradation of phthalimido group. TGA result of H-Ph-chitosan showed two main decomposition processes due to the substituent groups. The first decomposition at 185±1°C could be assigned to the decomposition of

phthalimido group while another decomposition at 256±1°C revealed the degradation of hexanoyl group. After removing the protective groups from H-Ph-chitosan, the thermal stability of resulting hexanoyl chitosan was changed. Similar to H-Ph-chitosan, H-P chitosan showed the peaks at 174±1°C and 235±1°C due to the degradation of phthalimido group left in the structure and hexanoyl group, respectively. The results indicated that the introduction of phthalimido and hexanoyl groups to structure of chitosan would decrease the thermal stability of chitosan.

4.3.3 Crystallinity

Figure 4.13 shows the WAXD patterns of original chitosan compared to the other derivatives obtained by protection method. It was found that the peak assigned to the crystallinity of chitosan at 20° was broader after phthaloylation. The Ph-chitosan showed a broad peak at 22.32°. This could imply the decrease of crystallinity, which might be due to decreased in ability of chain packing from the introduction of bulky phthalimido groups. Similar to Ph-chitosan, H-Ph-chitosan showed the broad peak at 20.62° and another peak at 6.76°. This second peak can be reffered to the new ordering structure caused by the hexanoyl side chains. The final H-P chitosan obtained also showed the same WAXD pattern with H-Ph-chitosan. This results indicated that the introduction of bulky group and/or any side chains caused decrease in ability of main chain packing resulted in the destruction of crystalline structure of chitosan and prevent hydrogen bonds to take place.

4.3.4 Solubility

In contrast to chitosan, all derivatives showed an improved solubility in common organic solvents (see Table 4.4). The use of phthaloyl as a

protective group for the primary amino groups in chitosan gave a derivative with a much improved solubility in organic solvents such as dimethylformamide(DMF), dimethylacetamide(DMAc), dimethylsulfoxide (DMSO) and pyridine. Therefore, the further selective modification of primary and secondary hydroxyl groups can be performed in a more homogeneous system. The solubility of Ph-chitosan in organic solvents could be due to the bulkiness of substituted phthaloyl groups and the removal of two hydrogen atoms from the amino groups thus preventing intra- and inter-molecular hydrogen bonds between the polymer chains to occur.

H-Ph-chitosan was found to swell in most organic solvents but dissolved easily in pyridine due to the presence of both bulky phthaloyl groups and long acyl chains of hexanoyl groups that introduced both polar and non polar characteristics to the polymer.

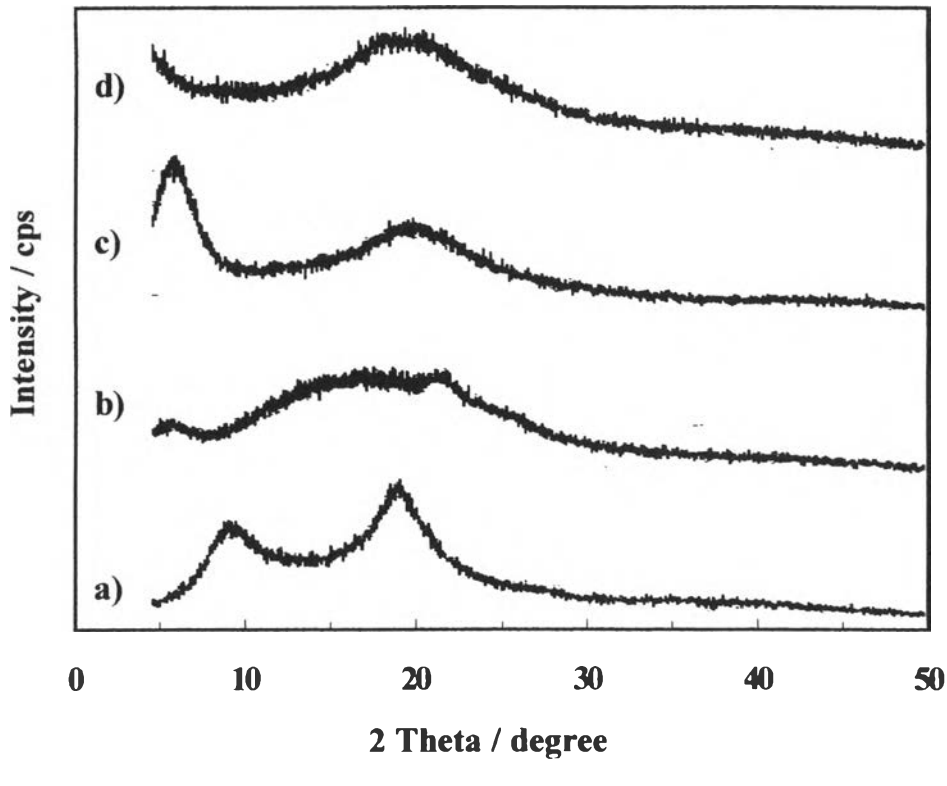


Figure 4.13 WAXD patterns of a) chitosan, b) Ph-chitosan, c) H-Ph-chitosan, and d) H-P chitosan.

H-P chitosan dissolved only in DMSO and swelled in DMF, DMAc and pyridine. The decrease in solubility of this derivative after removal of protective groups was due to the loss of phthalimido and some hexanoyl groups in this reaction and possibility of intra- and inter-molecular hydrogen bonding from the presence of free amino groups.

4.4 Comparison of Hexanoyl Chitosan (obtained from different methods)

4.4.1 Structure and Properties

In the first method of modification, H-1, H-2 and H-3 chitosan showed the substitution on all reactive sites of chitosan ($-NH_2$, OH and $-NHCOCH_3$). The high extent of hexanoylation was obtained especially through the amino group that was very reactive. With the protection technique, most of amino groups, that was protected, can be retrived after hexanoylation. Although some hexanoyl groups was also removed in the reaction for removing the protective groups, H-P chitosan obtained in this method still had great extent of hexanoylation on hydroxyl groups of chitosan.

Comparing the thermal stability of all hexanoylated chitosans, H-P chitosan with free amino groups (obtained from method II) was less stable than the hexanoyl chitosans obtained from direct hexanoylation (method I). This could be due to the phthalimido group left in this derivative.

All hexanoylated chitosans showed the same WAXD patterns. A reflection at 2-6° was stronger while a reflection around 20° was broader with the increment of hexanoyl groups of hexanoyl chitosans. As the results, H-P chitosan with free amino group displayed weaker reflection at 2-6° than H-1, H-2 and H-3 chitosan, respectively according to their degree of substitution of hexanoyl groups.

Hexanoyl chitosans obtained from different methods of modification gave distinct solubility. H-1 chitosan just swelled in most organic solvents. H-2 and H-3 chitosan dissolved easily in most halogenated hydrocarbons and aromatic solvents, but gave poor solubility in polar solvents while H-P chitosan dissolved easily in polar solvent like DMSO.

4.4.2 Metal Adsorption Ability

Metal adsorption of all hexanoyl chitosans were shown and compared to chitosan with different degree of deacetylation (see Figure 4.14). The relationship between degree of deacetylation of chitosan and metal adsorption ability was studied. The increase in the absorbed Cu^{2+} ions content leveled off after 6 hours of exposure of chitosan films, indicating the attainment of adsorption equilibrium. Chitosan showed much better Cu^{2+} ions adsorption compared with chitin in the same condition. The chitosan film with 92%DD showed the maximum sorption ability for Cu^{2+} ions. The order of Cu^{2+} ions adsorption by chitosan film increased with increasing %DD due to the increment of free amino groups in chitosan structure. The amino group of chitosan has been suggested as the active site for metal ions coordination. Kurita *et al.* (1979) also demonstrated a positive correlation between amino groups and the adsorption rate of copper ions. H-3 chitosan, fully hexanoylated derivative, showed almost no adsorption similar to chitin while metal adsorption ability of H-2 chitosan (4.19%) was better than H-3 chitosan (1.43%) but still very low compared to unmodified chitosan. H-1 and H-P chitosan displayed higher metal adsorption than H-2 and H-3 chitosan due to less substitution of hexanoyl group resulting in more free amino groups. Although H-P chitosan had lower degree of hexanoylation, it still had lower metal adsorption ability (17.36%) than H-1 chitosan (35.60%). This might be

due to some free amino groups of H-P chitosan was bound with the phthalimido group left in this derivative.

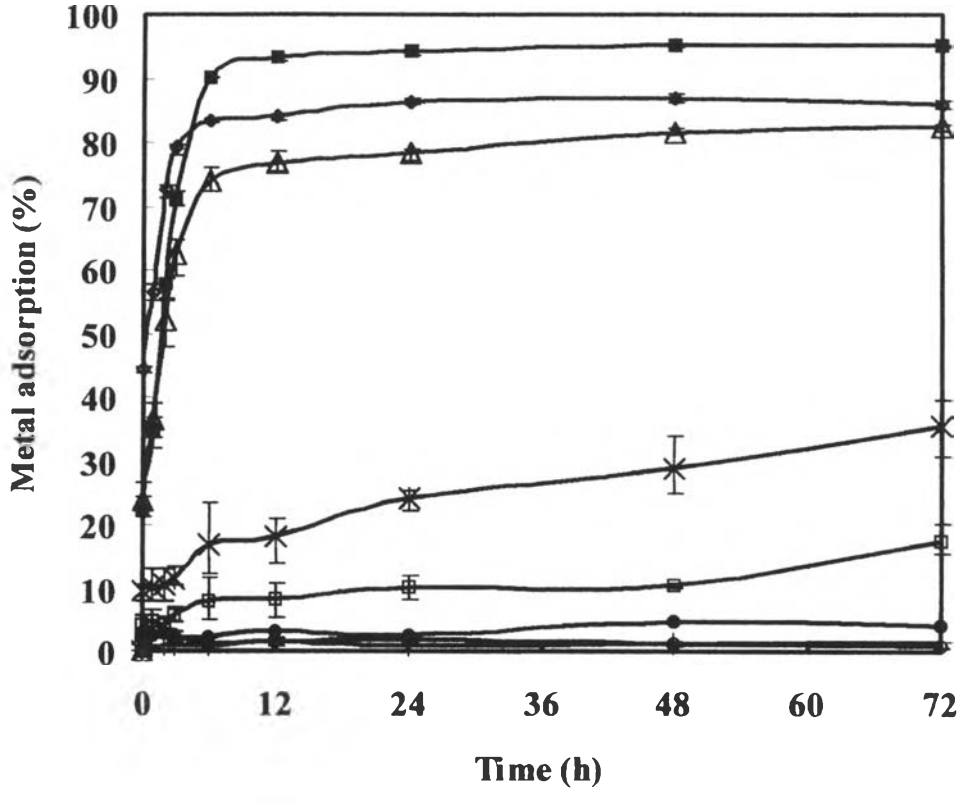


Figure 4.14 Cu^{2+} ions adsorption ability of chitosan and hexanoyl chitosan —■— 92%DD, —◆— 85%DD, —▲— 70%DD, —●— chitin, —✱— H-1, —●— H-2, —+— H-3, —□— H-P.