

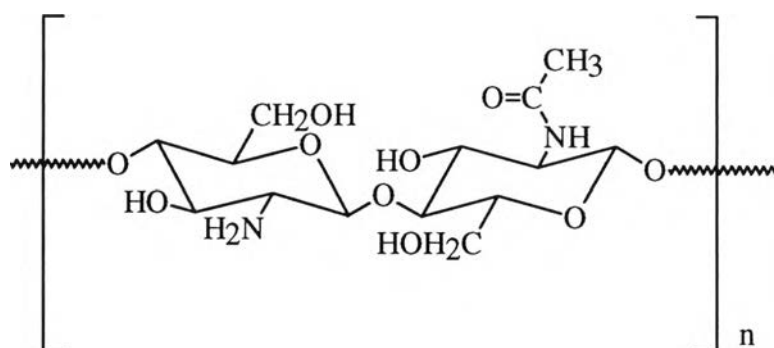
## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Analysis of Chitosan and Chitosan Acetate

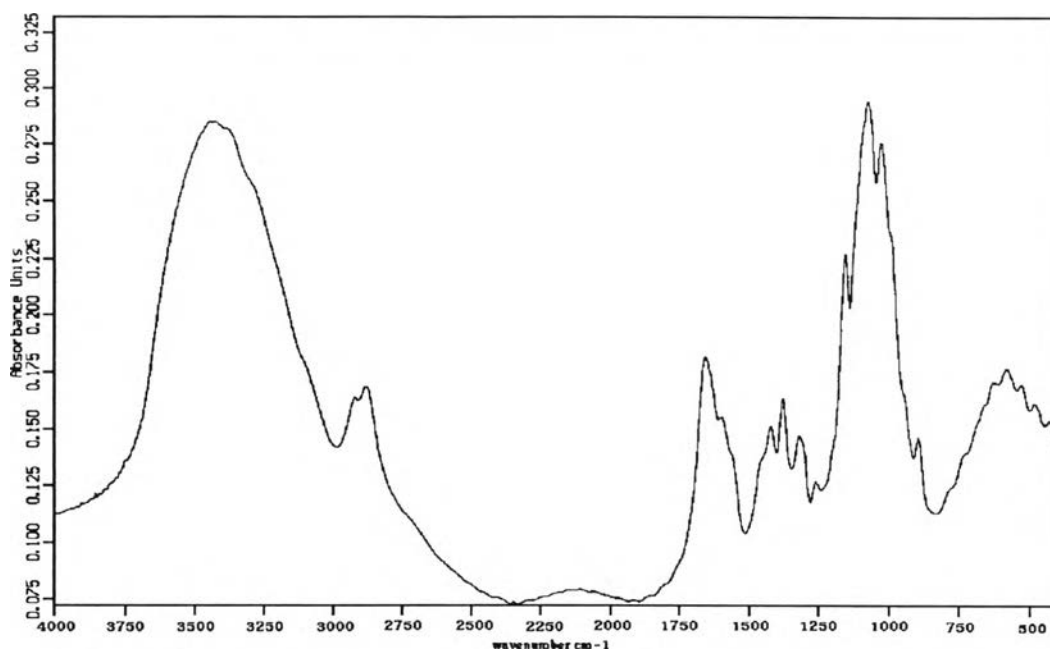
##### 4.1.1 Chitosan

Chitosan starting material was provided by Prof. Suwalee Chankrachang. The deacetylation was carried out by 50% NaOH at 40°C to obtain a degree of deacetylation of approximately 0.85 as determined by HPLC.



**Figure 4.1** Chemical structure of chitin-chitosan copolymer.

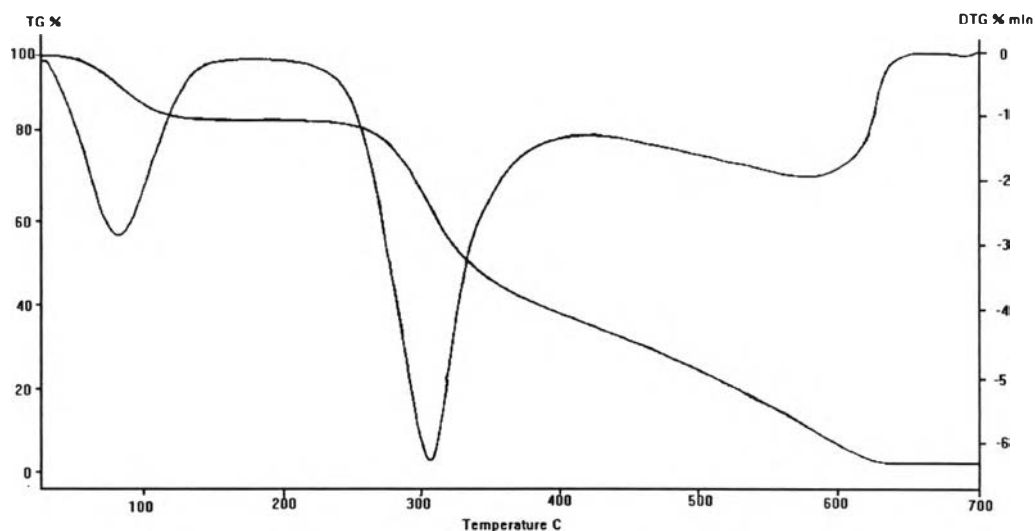
FTIR spectrum of chitosan is showed in Figure 4.2.



**Figure 4.2** FTIR spectrum of chitosan.

The FTIR spectrum shows characteristic peaks as follows, FTIR (KBr,  $\text{cm}^{-1}$ ) : 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 1656 ( $\text{NHC=O}$ ), 1075 (pyranose ring)

TGA and DTA diagrams of chitosan are shown in Figure 4.3. TGA shows the weight loss first at  $82.4^\circ\text{C}$  followed the second peak by  $306.4^\circ\text{C}$ , which can also be seen as two endothermic peaks in DTA. The former peak reveals the loss of water or moisture in chitosan material. The latter peak at  $306.4^\circ\text{C}$  shows the degradation of chitosan which may be due to the bonds breaking between pyranose rings.

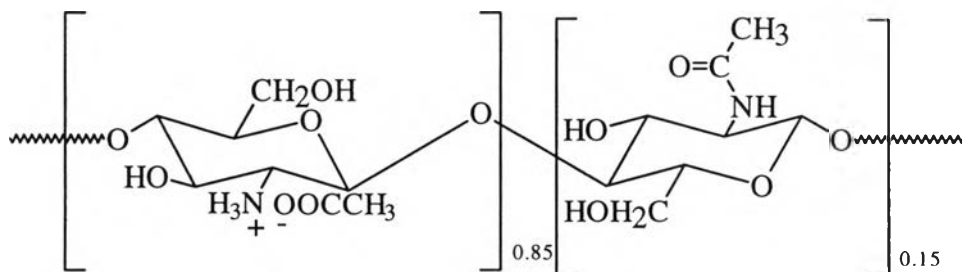


**Figure 4.3** Thermal stability of chitosan.

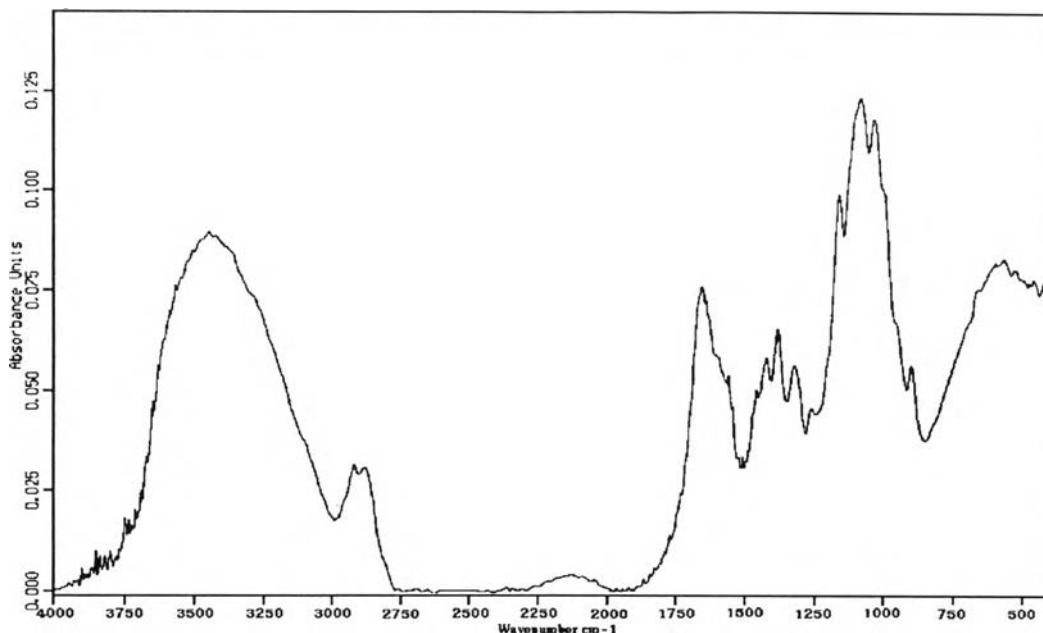
#### 4.1.2 Chitosan Acetate

Chitosan acetate was prepared to protect amino groups to be inert to the reaction and controlled the structure of chitosan derivative in which the reaction can be occurred at C-6 position selectively.

Chitosan acetate was carried out by reprecipitating the solution of chitosan in acetone. The obtained product was a white fibrous material.



**Figure 4.4** Chemical structure of chitosan acetate (Horton,1973).



**Figure 4.5** FTIR spectrum of chitosan acetate.

FTIR (KBr,  $\text{cm}^{-1}$ ): 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 3480-3380 (amine salt), 1654 ( $\text{NHC=O}$ ), 1076 (pyranose ring). Chitosan acetate shows the FTIR spectrum which is similar to that of chitosan. Characteristic peaks of chitosan and chitosan acetate are the same while the amine salt peaks overlap with amino and hydroxyl peak.

EA : Calculation for  $(\text{C}_8\text{H}_{15}\text{NO}_6)_{0.85}(\text{C}_8\text{H}_{13}\text{NO}_5)_{0.15}$

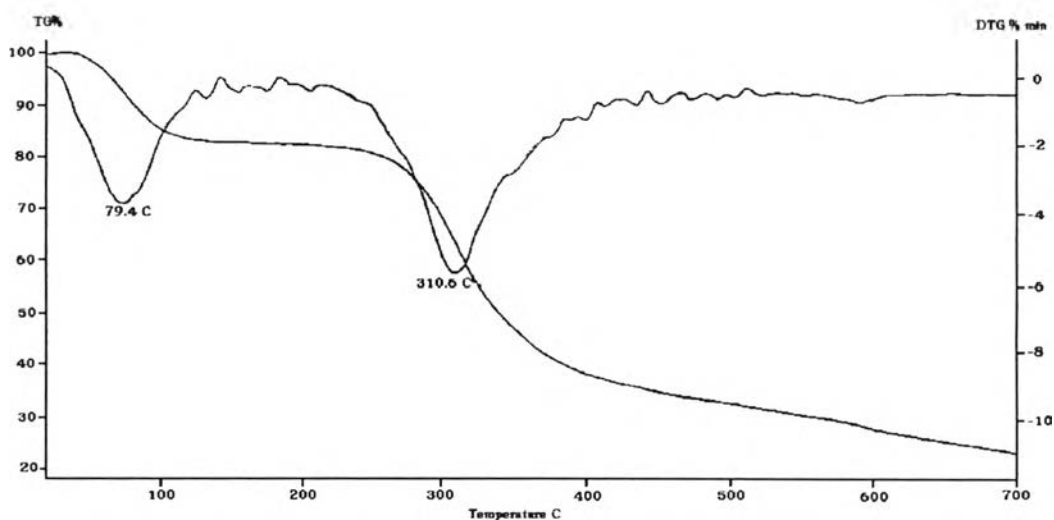
C, 43.98; H, 6.73; N, 6.41; O, 42.88

Found C, 38.92; H, 7.41; N, 6.58; O, 47.09.

This may be the impurities in chitosan. In the preparation step, chitosan flakes have to be ground by alumina balls in ball mill system. Alumina may be contaminated as small particles with chitosan. However, the present study focuses on the chemical modification and the quantitative analysis can be achieved by using N value as an internal standard.

Thermal analysis shows weight loss at  $79.4^\circ\text{C}$  which reveals the loss of water or moisture in chitosan acetate. The following temperature

at 310.6°C because bonds between pyranose ring are broken similar to chitosan meaning that the salt formation is not influenced to the thermal property.



**Figure 4.6** Thermal properties of chitosan acetate.

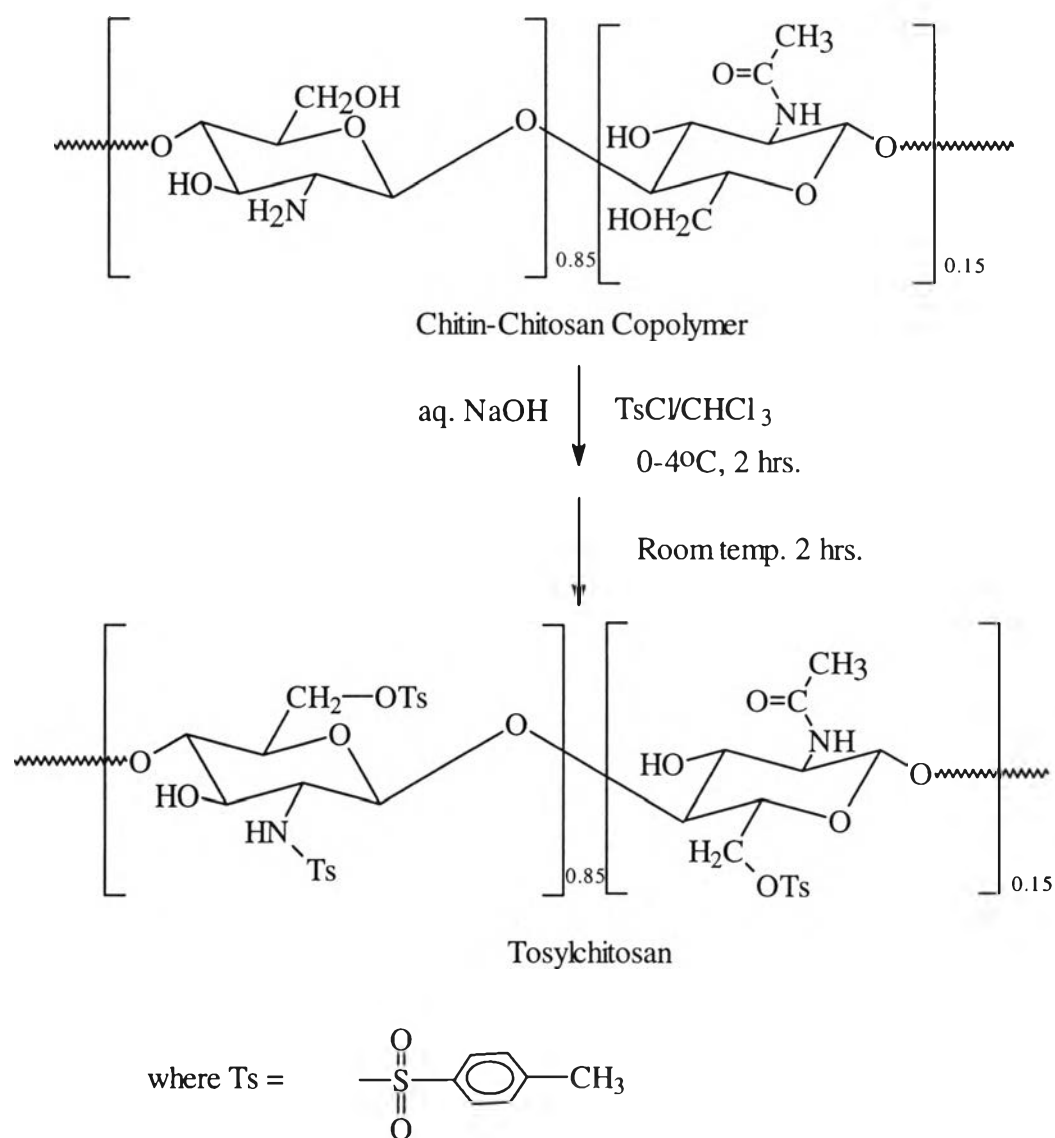
## 4.2 Preparation and Characterization of Tosylated Chitosan and Tosylated Chitosan Acetate

### 4.2.1 Tosylated Chitosan

Recently, the tosylation of chitin has been studied for using as a precursor for facile chemical modification. For example, The modification of tosylchitin to iodochitin for facile copolymerize with styrene monomer. In this article, the chitin derivative, chitosan, was studied as a reactive precursor for acylation.

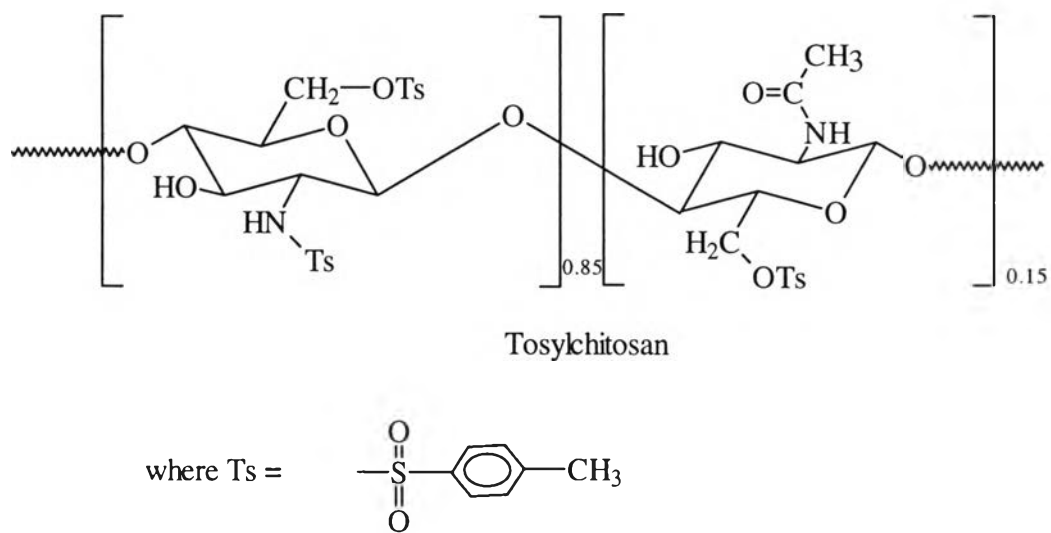
Tosylation was performed on alkaline chitosan by applying the technique of interfacial condensation between an aqueous alkali chitosan solution and a chloroform solution of tosylchloride. The reaction had to

performed in mild condition to induce high reproducibility and substitution (Figure 4.7).

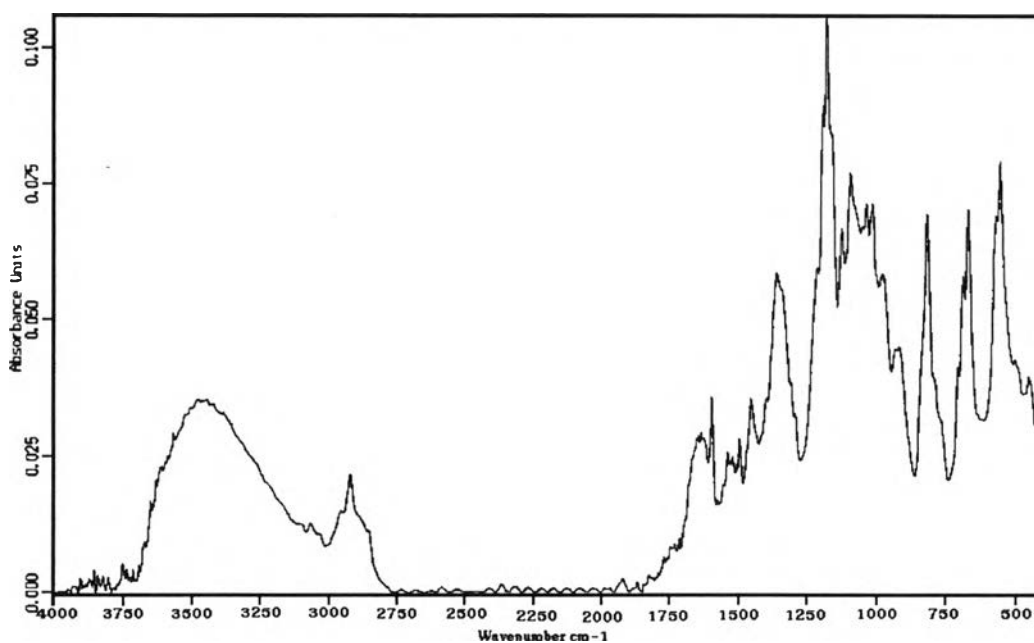


**Figure 4.7.** Schematic synthesis of tosylated chitosan.

The structure of the obtained tosylated chitosan is shown in Figure 4.8.



**Figure 4.8.** Chemical structure of tosylated chitosan.



**Figure 4.9** FTIR spectrum of tosylated chitosan.

FTIR (KBr,  $\text{cm}^{-1}$ ) : 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 1636 ( $\text{NHC=O}$ ), 1075 (pyranose ring), 1177 ( $\text{S=O}$ ) 1598 and 815 (*p*-phenylene).

In the case of tosyl chloride concentration for an equivalent mole, elemental analysis was obtained as follows.

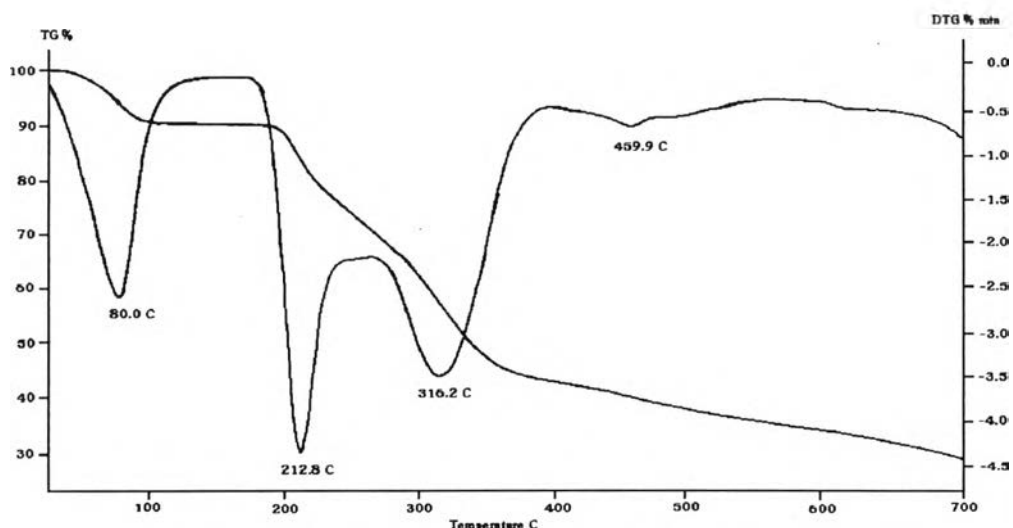
EA : Calculation for  $(C_{20}H_{23}NS_2O_8)_{0.85}(C_{15}H_{19}NSO_7)_{0.15}$

C, 51.08; H, 4.95; N, 3.10; S, 13.09; O, 27.78.

Found C, 44.91; H, 5.67; N, 3.01; S, 12.11; O, 34.30.

Elemental Analysis reveals that there is significant sulfur content. In the tosylation step, the sulfonyl group can appear at both C-6 ( $CH_2OH$ ) and C-2 ( $NH_2$ ). However, according to the structure (Figure 4.8), the nitrogen content is constant, thus it can be used as an internal standard.

TGA and DTG diagrams of tosylated chitosan are shown in Figure 4.10. TGA shows the weight loss at  $82.4^\circ C$  which is associated with the loss of water or moisture. The latter peak at  $212.8^\circ C$  reveals the loss of tosyl groups and the following peak at  $306.4^\circ C$  is due to the breaking of bonds between pyranose rings.



**Figure 4.10** Thermal degradation of tosylated chitosan.

The reaction was severely dependent on the stirring efficiency and temperature control because this reaction is a rapid reaction at the



interface, thus, vigorous stirring at 0°C in the initial stage was also important. When the reaction mixture was held to room temperature, the reaction temperature rose to 40°C owing to the exothermic reaction, which led to the problem of poor reproducibility and substitution. To avoid this problem, the reaction was cooled to around 0°C.

In the case of chitosan, the amino group at C-2 shows higher reactivity than hydroxyl group at C-6 position. A high concentration of NaOH was added in order to get rid of HCl which is a by product from the reaction. Thus it can be inferred that during tosylation, alkaline hydrolysis of acetamide groups was occurred while acetyl migration progressed. The degree of deacetylation will be higher than the starting chitosan.

The degree of tosylation was determined by S/N ratio.

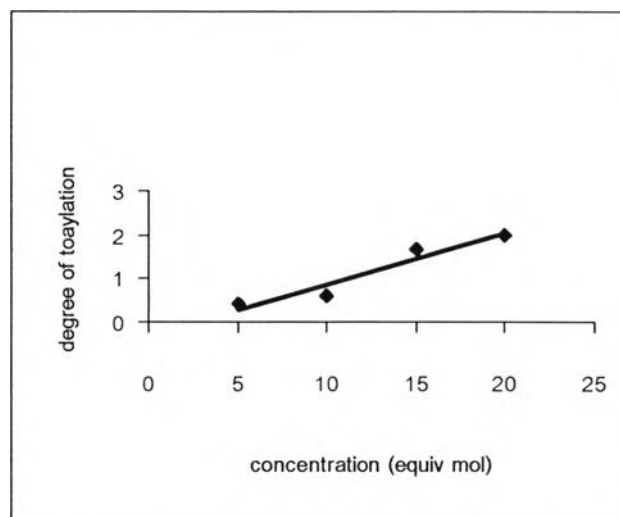
$$X = \frac{S/32}{N/14}$$

In order to evaluate the reaction efficiency, tosyl chloride was varied from 5-20 equivalent mole. Kurita et al. concluded that tosylchitin could be successfully prepared with the tosyl chloride concentration of 15 fold excess. Here, tosylation of chitosan was studied as summarized by varying tosyl chloride concentration in Table 4.1.

**Table 4.1** Summary of Elemental Analysis Data and Concentration

Concentration (equiv mol)	S	N	S/N	Degree of tosylation
5	5.31	5.87	0.90	0.40
10	7.60	5.37	1.42	0.62
15	11.97	3.13	3.82	1.67
20	12.02	2.65	4.54	1.99

The relationship between degree of tosylation and the tosyl chloride concentration is shown in Figure 4.11.



**Figure 4.11** Relationship between degree of tosylation and tosylchloride concentration (equivalent mole to pyranose ring).

It should be noted that, when the tosyl chloride concentration increases, degree of tosylation increases.

The linear relation is  $Y = 0.1164X - 0.285$

$$R^2 = 0.9317$$

where  $X$  = the concentration of tosylchloride

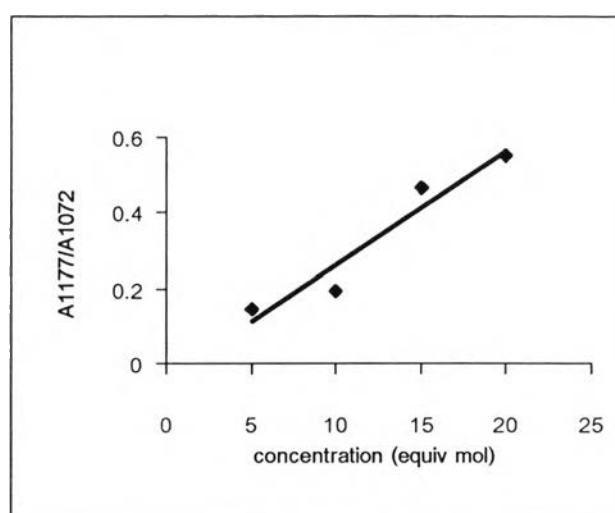
and  $Y$  = the degree of tosylation

According to FTIR, tosylgroup ( $1177 \text{ cm}^{-1}$ ) and pyranose ring ( $1072 \text{ cm}^{-1}$ ) can be applied for quantitative study. The ratio of tosyl group and pyranose ring is related to the amount of tosyl group substituted in the chain. Tosyl chloride concentration was found to be related linearly to the amount of substituted tosyl group as studied by quantitative FTIR as summarized in Table 4.2.

**Table 4.2** Summary of Quantitative FTIR and Concentration

Concentration (equiv mol)	$A_{1177}$	$A_{1072}$	$A_{1177}/A_{1072}$
5	0.793638	5.5183	0.1438
10	0.665257	3.41892	0.1946
15	4.62411	9.97383	0.4636
20	1.3085	2.3703	0.5520

The relationship between  $A_{1177}/A_{1072}$  and the concentration is also linear and exhibits the same trend as the relationship between degree of tosylation and concentration ( Figure 4.12).



**Figure 4.12** Relationship between  $A_{1177}/A_{1072}$  and tosylchloride concentration (equivalent mole to pyranose ring).

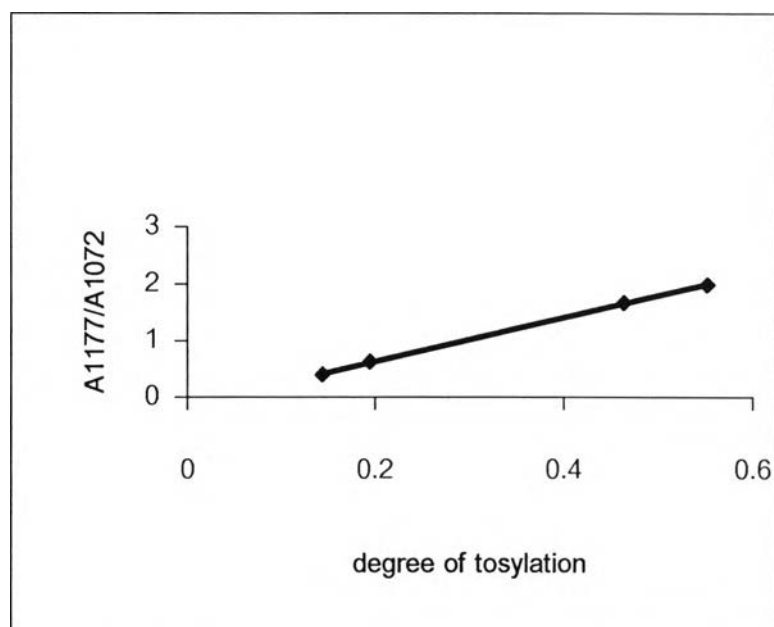
The linear equation is  $Y = 0.0299X - 0.03$

$$R^2 = 0.9307$$

where  $X$  = the concentration of tosylchloride

and  $Y$  = the value of  $A_{1177}/A_{1072}$

Degree of tosylation was determined by EA and the substituted amount of tosyl group are plotted as a calibration curve. Here, S/N ratio and  $A_{1177}/A_{1072}$  is found to be linear, as shown in Figure 4.13.



**Figure 4.13** Calibration curve to determine the degree of tosylation.

From this calibration line, The degree of tosylation can be calculated by the following equation.

$$Y = 3.894X - 0.1481$$

$$R^2 = 0.9997$$

where  $X$  = degree of tosylation which calculated from S/N ratio

$$S/N = 32X/14$$

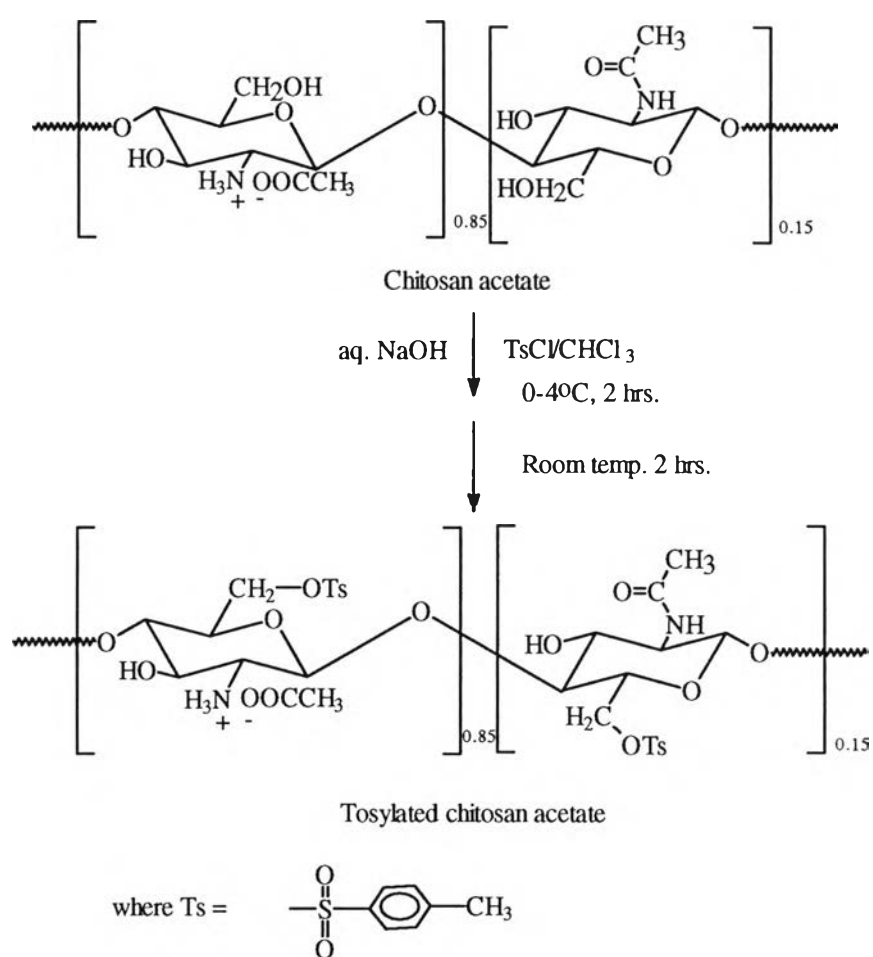
and  $Y = A_{1177}/A_{1072}$

Thus, by this quantitative FTIR, the degree of tosylation can be evaluated according to the calibration curve.

#### 4.2.2 Tosylated Chitosan Acetate

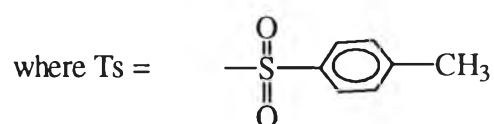
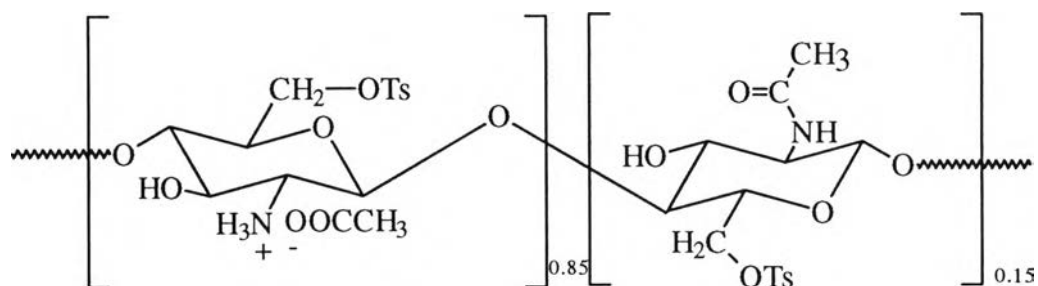
Chitosan was prepared for selective tosylation at C-6 position.

Similarly, tosylation of chitosan acetate was also performed by applying the technique of interfacial condensation between an aqueous alkaline chitosan acetate solution and a chloroform solution of tosylchloride (Figure 4.14).

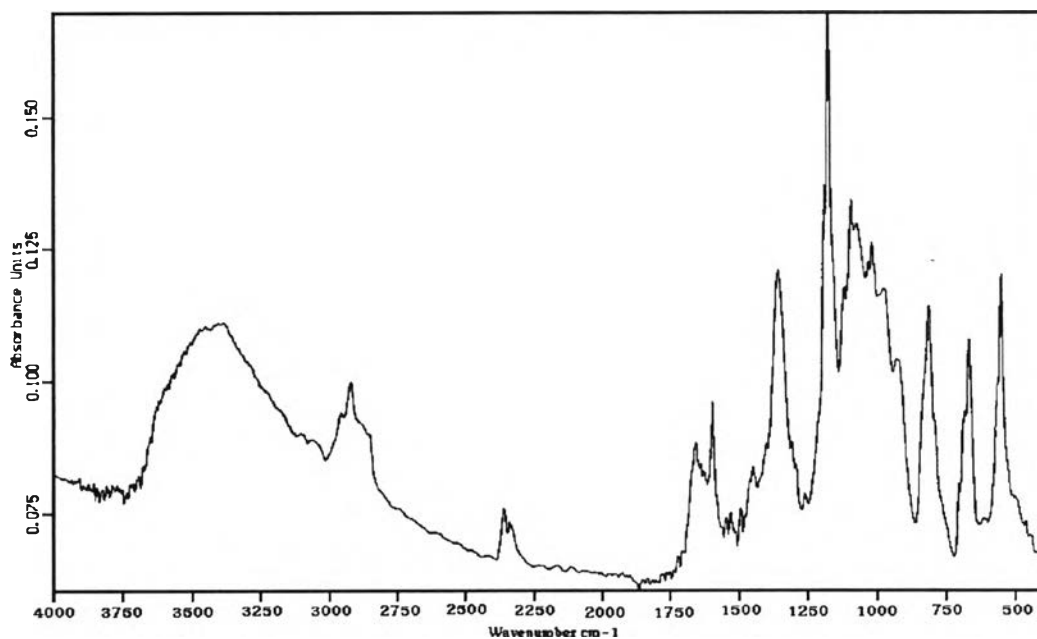


**Figure 4.14** Schematic synthesis of tosylated chitosan acetate.

The structure of tosylated chitosan acetate was studied by FTIR spectroscopy.



**Figure 4.15** Chemical structure of tosylated chitosan acetate.



**Figure 4.16** FTIR spectrum of tosylated chitosan acetate.

FTIR (KBr,  $\text{cm}^{-1}$ ) : 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 3480-3380 (amine salt), 1636 ( $\text{NHC=O}$ ), 1074 (pyranose ring), 1176 ( $\text{S=O}$ ) 1598 and 814 (*p*-phenylene).

In the case of tosyl chloride concentration 15 equivalent mole, elemental analysis is shown as follows.

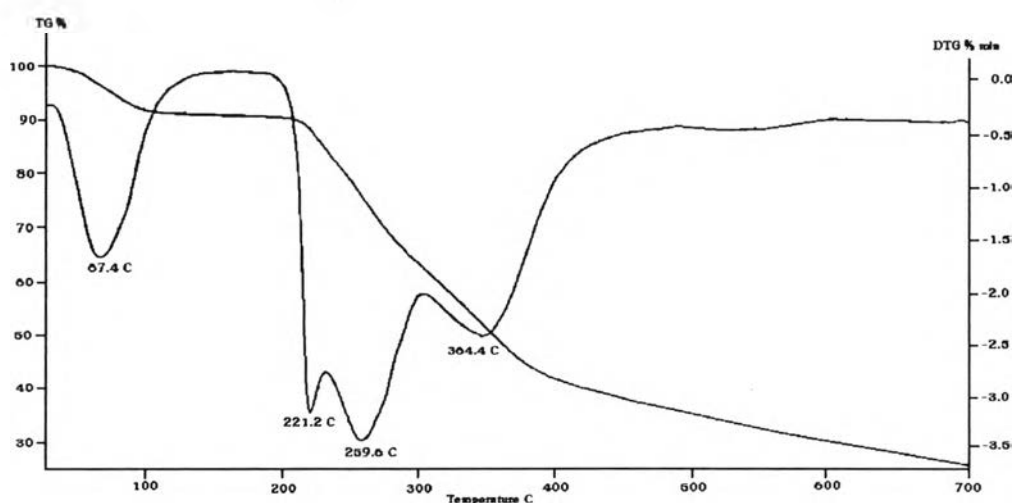
EA : Calculation for  $(C_{15}H_{19}NO_7S)_{0.85}(C_{15}H_{21}NO_8S)_{0.15}$

C, 50.04; H, 5.37; N, 3.89; S, 8.90; O, 31.80. (Assuming degree of tosylation equal to 1)

Found C, 43.58; H, 5.87; N, 5.76; S, 5.36; O, 39.43.

The obtained product was found to show less sulfur content than the calculated value, which is referred to the degree of tosylation equal to 1. According to the elemental analysis value, the obtained product has degree of tosylation equal to 0.41. This means the reaction can be achieved as a partial tosylation at 15 equivalent mole tosyl chloride concentration.

TGA and DTG diagrams of tosylated chitosan acetate are shown in Figure 4.17. TGA shows the first weight loss at 67.4°C which may be due to the loss of water or moisture. The second peak at 221.2°C refers to the loss of the tosyl group. The third weight loss at 259.6°C shown amine salt degradation. The last at 306.4°C is due to bonds between pyranose ring are broken.



**Figure 4.17** Thermal stability of tosylated chitosan acetate.

The thermal stability of tosylated chitosan acetate was studied and compared with that of chitosan, chitosan acetate and tosylated chitosan acetate. As shown in Figure 4.3 and 4.6, chitosan and chitosan acetate give degradation points close to one another. On the other hand, tosylated chitosan acetate gives 4 points of degradation, i.e. the loss of water, tosyl group, amine salt and the degradation of tosylated chitosan acetate (Figure 4.17), compared to tosylated chitosan which gives only 3 points, i.e. the loss of water, tosyl group and tosylated chitosan degradation (Figure 4.10).

#### 4.3 Preparation and Characterization of Acylation of Tosylated Chitosan and Tosylated Chitosan Acetate.

The condition of acylation of chitosan and chitosan acetate were studied. It is found that tosylated chitosan was not successful under heterogenous conditions in chloroform and N, N-dimethylacetamide. Some solvents were facilitated such as N, N-dimethylformamide. To find out the optimal condition for synthesis of stearylated chitosan and stearylated chitosan acetate, the condition for acylation of tosylated chitosan or tosylated chitosan acetate with acetic acid at 80°C in N, N-dimethylformamide was studied. The results are summarized in Table 4.3.

**Table 4.3** Reaction Condition of Acylation

Condition	40°C	60°C	80°C
CHCl <sub>3</sub>	U	U	U
DMAc	U	M	M
DMF	U	S	S

U = Unsatisfactory

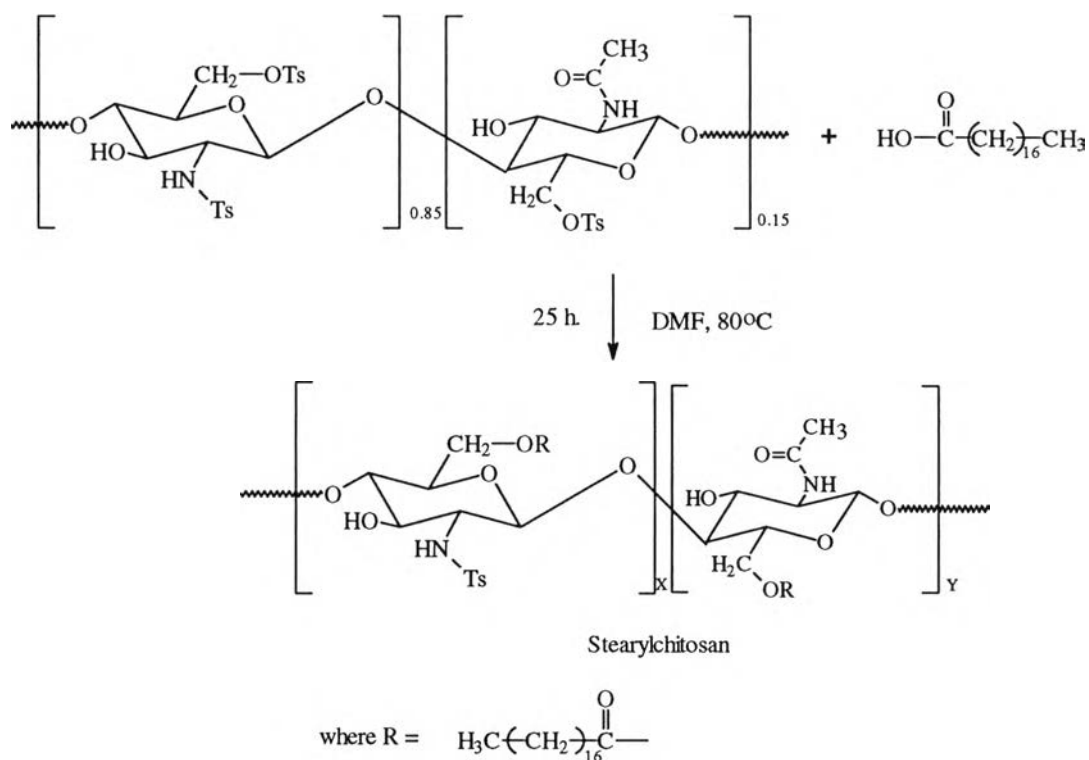
M = Medium

S = Satisfactory



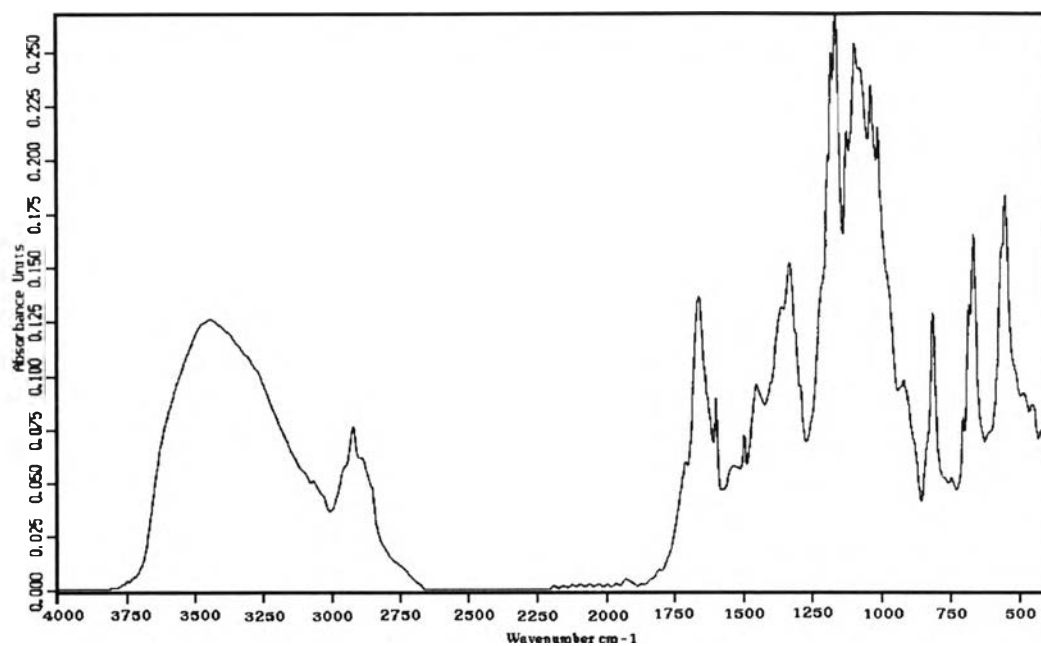
### 4.3.1 Stearylated Chitosan

The mixture of tosylated chitosan and 10 equivalent mole to pyranore rings of stearic acid in N,N-dimethylformamide was performed heterogenous phase at 80 °C for 25 hours.

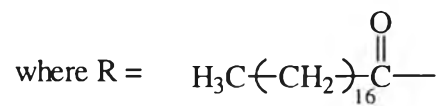
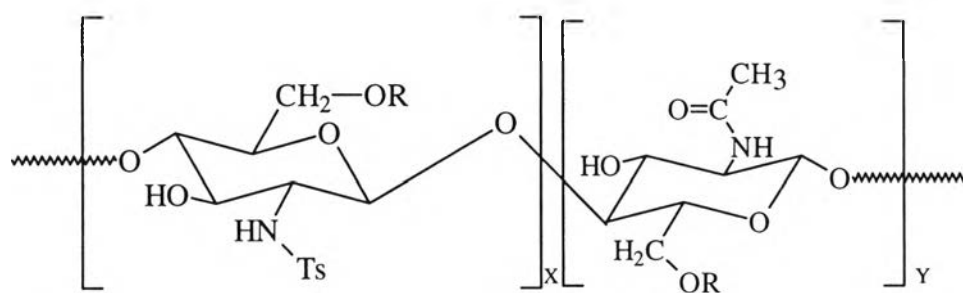


**Figure 4.18** Schematic diagram of the acylation of tosylated chitosan.

The stearylated chitosan were obtained as brown powdery materials. The structure of stearylated chitosan was characterized by FTIR spectroscopy.



**Figure 4.19** FTIR spectrum of stearylated chitosan.



**Figure 4.20** Chemical structure of stearylated chitosan.

FTIR (KBr,  $\text{cm}^{-1}$ ) : 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 1661 ( $\text{C}=\text{O}$ ), 1075 (pyranose ring), 1177 ( $\text{S}=\text{O}$ ) 1598 and 815 (*p*-phenylene), 2922 (C-H).

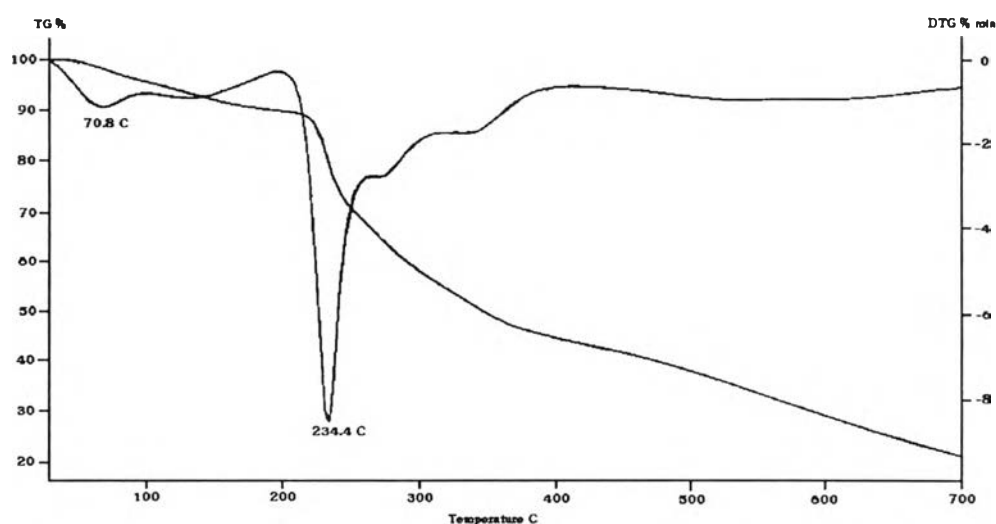
EA : Calculation for  $(\text{C}_{31}\text{H}_{51}\text{NSO}_7)_{0.85}(\text{C}_{26}\text{H}_{47}\text{NO}_6)_{0.15}$

C, 64.00; H, 8.89; N, 2.47; S, 4.80; O, 19.84.

Found for  $(\text{C}_{31}\text{H}_{51}\text{NSO}_7)_a(\text{C}_{26}\text{H}_{47}\text{NO}_6)_b(\text{C}_{15}\text{H}_{19}\text{NO}_7\text{S})(\text{C}_{15}\text{H}_{21}\text{NO}_8\text{S})_d$

C, 44.81; H, 5.50; N, 5.98; S, 7.73; O, 36.38.

TGA and DTG diagrams of stearylated chitosan are shown in Figure 4.21. There are two regions of mass loss. The first mass loss at  $82.4^\circ\text{C}$  corresponds to the loss of water or moisture. The second mass loss at  $234.4^\circ\text{C}$  refers to decomposition of stearylated chitosan, which is lower than chitosan perhaps because of stearic hindrance. This may be due to the cleavage of bonds between pyranose ring.



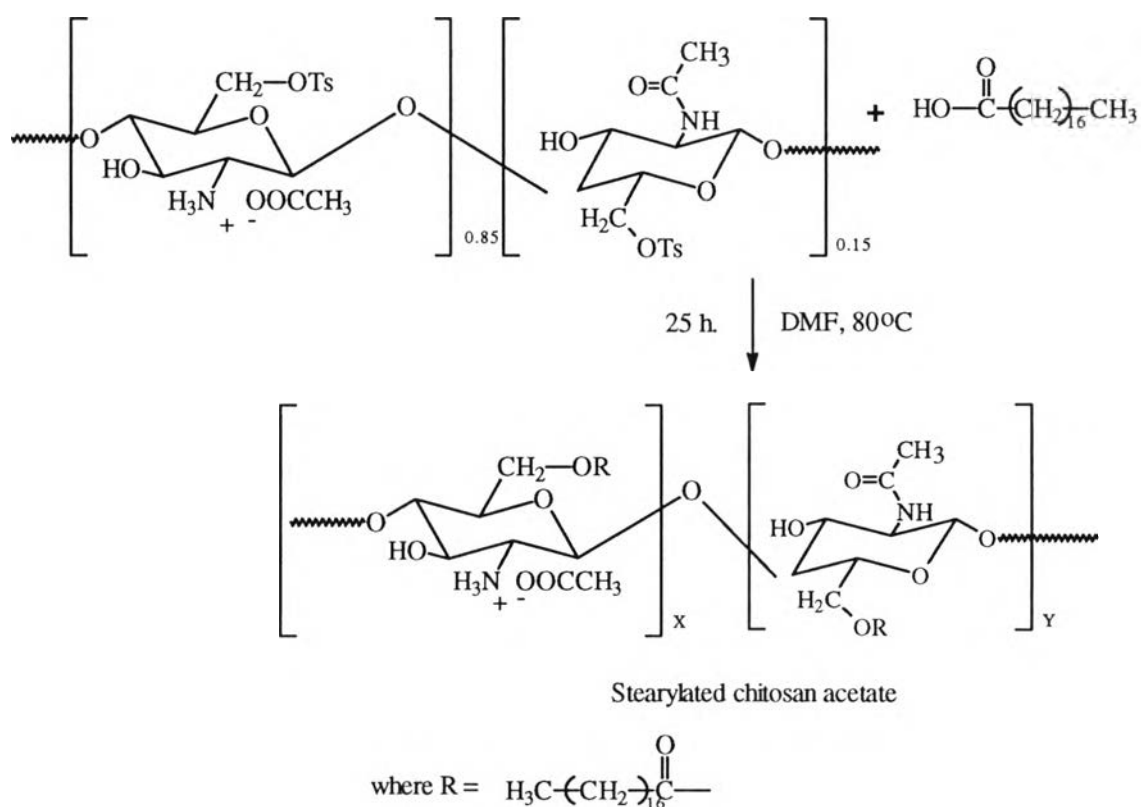
**Figure 4.21** Thermal properties of stearylated chitosan.

The degree of stearyl substitution on chitosan can be determined by elemental analysis. Tosylated chitosan gives S/N 4.02, which can be refer

to the total degree of tosylation of 1.76 ( $4.02 \times 14/32$ ). Stearylchitosan gives S/N 1.39 which reveals the remaining degree of tosylation to be 0.61 ( $1.39 \times 14/32$ ). The decreasing of degree of tosylation to be 0.61 indicated that some of tosyl groups were eliminated and substituted by stearyl groups. The degree of stearyl substitution is equal to 1.15 ( $1.76 - 0.61 = 1.15$ ).

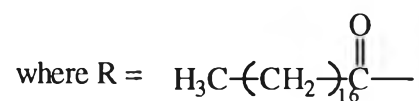
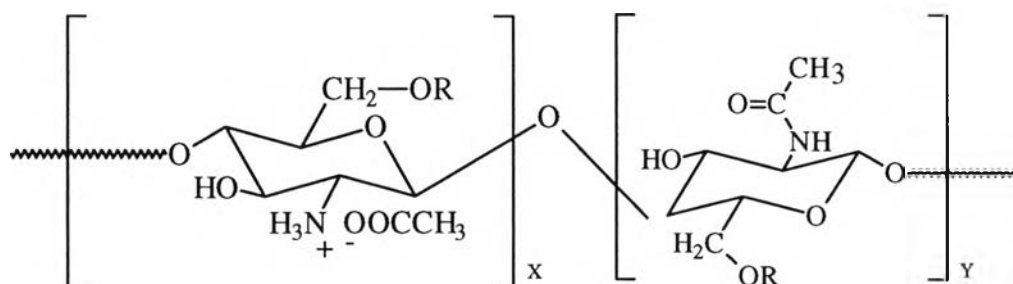
#### 4.3.2 Stearylated Chitosan Acetate

Similar to stearylated chitosan, a mixture of tosylated chitosan acetate and 10 equivalent mole of stearic acid in N, N-dimethylformamide were performed in a heterogeneous system.

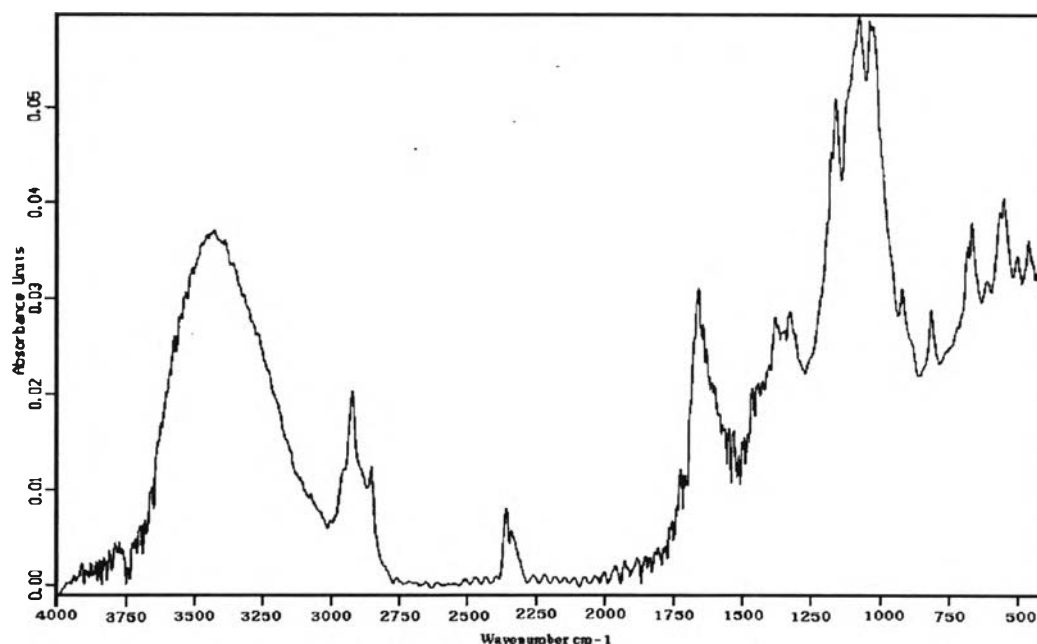


**Figure 4.22** Schematic diagram of the acylation of tosylated chitosan acetate.

The stearylated chitosan acetate was obtained as a brown powder. The structure of stearylated chitosan acetate was investigated by FTIR spectroscopy.



**Figure 4.23** Chemical structure of stearylated chitosan acetate.



**Figure 4.24** FTIR spectrum of stearylated chitosan acetate.

FTIR (KBr,  $\text{cm}^{-1}$ ) : 3500-3250 (OH), 3530-3400 ( $\text{NH}_2$ ), 3480-3380 (amine salt), 1658 (C=O), 1072 (pyranose ring), 1175 (S=O) 813 (*p*-phenylene).

EA : Calculation for  $(\text{C}_{26}\text{H}_{49}\text{NO}_7)_{0.85}(\text{C}_{26}\text{H}_{47}\text{NO}_6)_{0.15}$

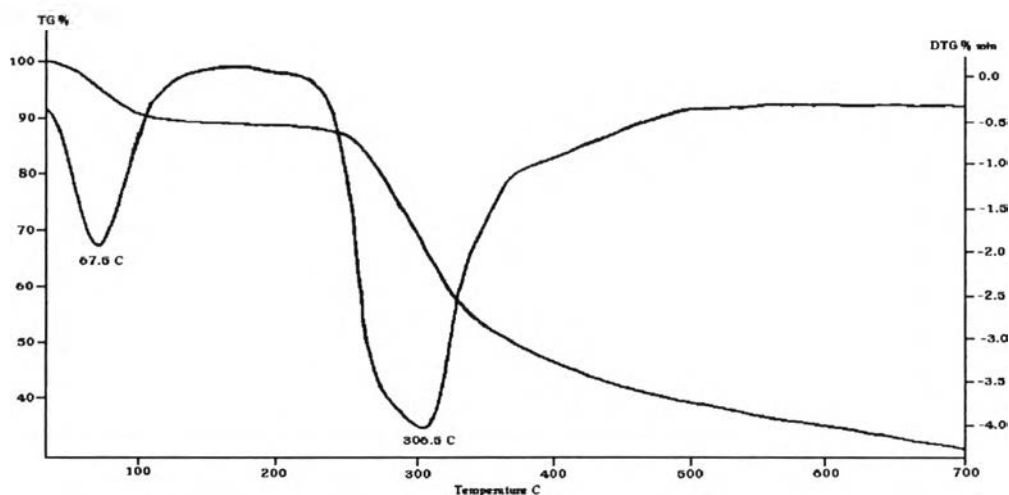
C, 64.42; H, 10.06; N, 2.89; O, 22.63.

Found for  $(\text{C}_{26}\text{H}_{49}\text{NO}_7)_a(\text{C}_{26}\text{H}_{47}\text{NO}_6)_b(\text{C}_{15}\text{H}_{19}\text{NO}_7\text{S})_c(\text{C}_{15}\text{H}_{21}\text{NO}_8\text{S})_d$

C, 41.90; H, 6.84; N, 6.93; S, 2.56; O, 41.77.

The degree of stearyl substitution onto chitosan acetate was determined by elemental analysis. Tosylated chitosan acetate gives S/N = 0.93, so the total degree of tosylation is 0.41 ( $0.93 \times 14/32$ ). Stearylated chitosan acetate gives S/N = 0.37 referring to the remaining degree of tosylation to be 0.16 ( $0.37 \times 14/32$ ). The decrease in degree of tosylation to 0.16 indicates that some tosyl groups are eliminated and substituted by stearyl groups. The degree of stearyl substitution is equal to 0.25 ( $0.41 - 0.16$ ).

TGA and DTG diagram shows two points of mass loss. The first mass loss at  $67.5^\circ\text{C}$  corresponds to the loss of water or moisture. The second mass loss at  $306.5^\circ\text{C}$  may be due to the cleavage bonds between pyranose ring as shown in Figure 4.25.



**Figure 4.25** Thermal stability of stearylated chitosan acetate.