Chapter IV

Discussion and Conclusions

In this study, chitin was deacetylated to produce chitosans having different degree of deacetylation. Physicochemical and tablet-disintegrating properties of chitin and all chitosans were determined in order to compare their relative efficiency as tablet-disintegrants.

Chitin, a starting material, used in this experiment had been pulverized and sized through 60/200 mesh before it was deacetylated. Therefore, the particle size distribution was narrow. This meant one of the deacetylation factors was controlled. Another deacetylation factor controlled in this study was alkali reagent. It was reported in the literature review that a decrease in the alkali concentration during deacetylation, the longer time of reaction required so reduced molecular chains. It was proposed that an increase in the alkali concentration to saturation had little effect on degradation of molecular chains and viscosity of chitosan product. (Lusena and Rose, 1953) Consequently, concentration of 50% Sodium hydroxide was used in this experiment.

Variable factors in deacetylation processes were reaction time and temperature, and atmosphere condition (under normal or nitrogen atmosphere). Initially, method A was performed by using reaction temperature 110° C under air atmosphere. Chitosan products having different degree of deacetylation were obtained by varying reaction time. It was observed that reaction time longer than seven hours did not increase degree of deacetylation of chitosan product significantly. However, following the removal of the impurity by washing after

reaction time at seven hours, the degree of deacetylation could be increased when the reaction time was prolonged. By method A, degree of deacetylation of chitosans ranged from approximately 67-80 %.

By method B, the reaction occurred under nitrogen atmosphere. Degree of deacetylation of chitosans ranged from 67-75 %. In this case, chitosan having 80% deacetylation was not produced because the deacetylation process required washing step that may affect the amount of chemical constituents in the chitosan products. To avoid this error in deacetylation procedure, the chitosans in this study was produced up to approximately 75% deacetylation.

By method A and method B, it was observed that the rate of deacetylation under nitrogen atmosphere was faster than under air atmosphere. The reaction time required for producing chitosan having 67-68% deacetylation were 3 and 2.5 hours for air and nitrogen atmosphere, respectively, and at 71-72% were 3.5 and 3 hours. To produced 75%-deacetylation chitosan, the time required in both method A and B was equal at 7 hours. This indicated that, only the initial rate was different.

Although the reaction occurred under nitrogen atmosphere (method B) that was claimed to provide the chitosans of higher molecular weight, the chitosans obtained in this experiment had no significantly higher molecular weight than chitosans produced under air atmosphere (method A). It might be due to the high reaction temperature. Both method A and method B were carried out at 110°C leading to higher degradation of molecular chains. To reduce degradation, method C was performed at room temperature to produced high molecular weight chitosan.

Degree of deacetylation of each chitosan product was determined by colloidal titration. Infrared spectrometry was rapid method to observe the residual CONH groups in chitosan products. Chitosans from the same source having the same degree of deacetylation have identical infrared spectra. Thus, infrared spectrometry could be used to confirm the values attained from titration method. It was seen that deacetylation values from titration related with the IR spectra. The lower the 1650 cm^{-1} peak, the lower the amount of carbonyl groups and the higher the degree of deacetylation. This observation was exceptional for chitosan produced by method C, CTS60N. The IR spectrum of CTS60N was similar to of CTS3.5A and CTS3N that had degree of deacetylation approximately 71-72%. Surprisingly that, percentage of deacetylation of CTS60N determined by colloidal titration was very low at 58.51%. Perhaps, this because CTS60N had very high molecular weight and long chains packed densely leading to the hinderance of the hydrochloric acid to react with primary amine groups of chitosan during the formation of chitosan hydrochloride. To prove this hypothesis, chitosan hydrochloride solution of CTS60N and hydrochloric acid had been refluxed for ten minutes before chitosan hydrochloride precipitate was obtained. This step caused fragmentation of molecular chains. Therefore, hydrochloric acid easily reacted with primary amine in glucosamine units. Degree of deacetylation of CTS60N obtained by this method was higher to 74.13%. It was higher than expected, 71-72%. The excessive of 2% deacetylation may be due to the side reaction occurred while chitosan hydrochloride was formed in vigorous condition. (in strong acid and high temperature while it was refluxed) The possible reaction that yielded functional groups using Sodium hydroxide during titration could be performed in both two types of repeating units. The reaction included ester formation and protonation of side chains, CH_2OH; and hydrolysis of amide bonds in N-Acetyl-D-glucosamine repeating units leading to increase deacetylated units.

It was indicated that colloidal titration method proposed by Hayes (1978) could not use to determine the degree of deacetylation of very high molecular weight chitosans. In this study, the degree of deacetylation was expressed according to the method described by Hayes. However, IR spectrum of each chitosan was mainly used to classify chitosan products which had similar degree of deacetylation. The similar spectra obtained were of CTS3A and CTS2.5N (67-68%); CTS3.5A, CTS3N and CTS60N (71-72%); CTS7A and CTS7N (75-76%).

With the attempt to compare the molecular weight of chitosan products, mass spectrometry, differential thermal analysis (DTA), and viscosity determination were performed. The comparison of molecular weight by only viscosity determination was in agreement with the results of the previous studies. (Bough, Salter, Wu, and Perkins, 1978) Solution viscosity is a basic method to measure the size or extension in space of polymer molecules. Because of the simplicity of the method and the viscosity-molecular weight relationship, the viscosity determination is often used as a valuable tool to characterized the molecular weight of the polymers. (Flory, 1953)

The viscosity of the solution of chitosan produced by method A was reduced when the reaction time used in deacetylation process increased. Chitosans produced under the condition in method B exhibited the similar viscosity. This result was in agreement with the study by Wu and Bough (1978) that increasing time reduced molecular size. In addition, it was observed that the viscosity of chitosan solution produced by method A and method B at the same degree of deacetylation had no significant difference. The possible explanation was that at high deacetylation temperature (110° C), the amount of oxygen dissolving in Sodium hydroxide solution was almost zero. Therefore, purging

the reaction with nitrogen or not, the hydrolysis-degradation of molecular chains was still occurred. This degradation resulted from high reaction temperature, not from oxygen-catalyzed hydrolysis.

However, it was noticed that, at the same degree of deacetylation level, chitosans produced by method B (CTS2.5N and CTS3N) had higher viscosity than of by method A, (CTS3A and CTS3.5A). On the other hand, the viscosity of CTS7A was a slightly higher than of CTS7N and showed no difference. These results indicated that reaction time tended to play an important role on viscosity and molecular weight of chitosan products. At the same degree of deacetylation level, the chitosan produced by deacetylation using longer time had lower viscosity.

To produce chitosan having higher molecular weight, method C was performed at the room temperature. By using Sodium hydroxide as the reactor, the temperature of suspension was about 30° C. At low temperature, the reaction time required was prolonged and deacetylation should be carried out under nitrogen atmosphere to avoid oxygen-catalyzed hydrolysis. As it was expected, chitosan obtained, CTS60N, had the highest viscosity when compared with CTS3.5A and CTS3N, the chitosans at about 71-72 % deacetylation. This meant that CTS60N had the highest molecular weight.

The molecular-weight comparison interpreting from the electron impact spectra (EIS) was related with the viscosity values of chitosan solutions. However, there was an exception for CTS73A that showed higher molecular weight than of CTS7A according to EIS obtained. This was contrary to the results from viscosity determination, the molecular weight of CTS73A was less than of CTS7A. Another exception was also observed for CTS3N and CTS60N.

The molecular weight of CTS3N was higher than of CTS60N when using EIS, while the opposite result was shown from viscosity determination.

Another attempt was made to clarify the molecular weight of chitosans prepared from different deacetylation processes by using differential thermal analysis. It is expected that high molecular weight polymer may have DTA peak at high temperature. From the result, it could not be observed any relation between DTA peak temperature and reaction time used or other associated parameters. Thus, it was unable to use DTA as a method to compare molecular weight of chitosans.

Under electron photomicrographs, chitin and chitosan products possessed irregular shapes. It implied that chitin was deacetylated without changing of particle shapes. Chitosans obtained had similar particle shape as starting chitin. Particle size distribution of chitin and chitosans also seemed no difference. From these observations, it could be indicated that both particle size and shape were not the factors affecting the differences of disintegrating properties might be observed further.

True density of chitin and chitosans was about 0.33-0.34 g/cm^3. It was very relatively low density when compared with other disintegrants. The low density value of chitin and chitosans was undesired-property as it may cause segregation during mixing process. As a result, both chitin and chitosan should not be applied in direct compression technique. In wet granulation, segregation may be occurred while granule was mixed with extra-granular disintegrant (chitin/chitosan), if granule density was considerably higher than the density of disintegrant. However, irregular shape of chitin and chitosan may reduce this effect.

Hydration capacity was one of mechanisms of disintegrants studied in this experiment. The result indicated that degree of deacetylation of chitosans was not a factor affecting hydration capacity value. It was noticed that within method A or method B, hydration capacities of chitosans having difference in degree of deacetylation had no difference. A factor that may influence this parameter was molecular weight. It was indicated that CTS60N that showed the highest molecular weight among chitosans at 71-72% deacetylation level had the highest hydration capacity. As it was mentioned that, degree of deacetylation was not a factor affecting hydration capacity so that all chitosans having different degree of deacetylation could be compared. If higher molecular weight chitosan resulted in higher hydration capacity, The chitosans having molecular weight more than of CTS60N should have higher hydration capacity than of CTS60N too. But the hydration capacities obtained from CTS3A and CTS2.5N (chitosans having molecular weight more than of CTS60N) were lower than of CTS60N. Thus molecular weight of chitosan seemed not to be a factor influencing their hydration capacity.

In consideration of deacetylation factors (reaction time and temperature, and atmospheric condition) that affecting hydration capacity, it was probable to indicate that reaction temperature was a factor influencing hydration capacity. Both reaction time and exclusion of air were not influenced factors because chitosans prepared by method A and method B using various reaction times under air and nitrogen atmosphere seemed having no different hydration capacity. CTS60N produced by method C at low temperature had higher hydration capacity than chitosans deacetylated at 110° C. However, temperature should be studied further as a variable factor and larger sample size should be produced to obtain more reliable conclusion.

The swelling volume of chitin and chitosan obtained from this experiment was similar to their hydration capacity. This meant that chitin and chitosan can hold certain amount of water and swelled. Weight of water holding in the space of polymer molecules directly related to the swelling volume. Swelling capacity was a proper parameter employed to compare the ability of the polymer particles to swell when contacted with the medium, as the initial volumes of all polymers were different. It was found that the swelling capacities of all polymers were almost equal, except for CTS60N that had substantially higher than of the others. The possible explanation was the same as in the case of hydration capacity.

The result of rate of water uptake was clearly observed that the method of deacetylation influenced water uptake into the polymers. The profiles of water uptake (Figure 20) could distinguish into 4 groups that rate of water uptake decrease in the following order: chitin > chitosans produced by method A > chitosans produced by method B > chitosans produced by method C. However, in another view as in Figure 19, chitin, chitosans from method A and B did not have significantly difference in rate of water uptake and the volume of water uptake reached saturation within two minutes, whereas chitosan from methods C (CTS60N) had the slowest rate of water uptake and the highest saturation-volume of water uptake at the thirtieth minutes.

From hydration capacity, swelling capacity, and rate of water uptake data of all chitosan products, it was indicated that degree of deacetylation, and molecular weight were not factors influenced these properties that might be use to describe the mechanisms of disintegration. Reaction temperature, a deacetylation variable, was only possible factor affecting these properties.

With the attempt to distinguish the crystal structure of the polymers, x-ray diffraction was performed. There are three main crystal structures among chitins, α- , β- , and γ- chitins. Two of the crystal structure, α- , and β- chitins are already well known. (Minke, and Blackwell, 1989; Gardner and Blackwell, 1975) The x-ray diffraction patterns reported by Takai, Shimizu, and Hayashi (1989) are shown in Figure 33. The difference could be observed in the spectra of α-chitin (derived from crab shell and tendon) and β-chitin (derived from squid, *Loligo* pen). These included the higher intensity of the first peak and the shift of this peak to the left of β-chitin.

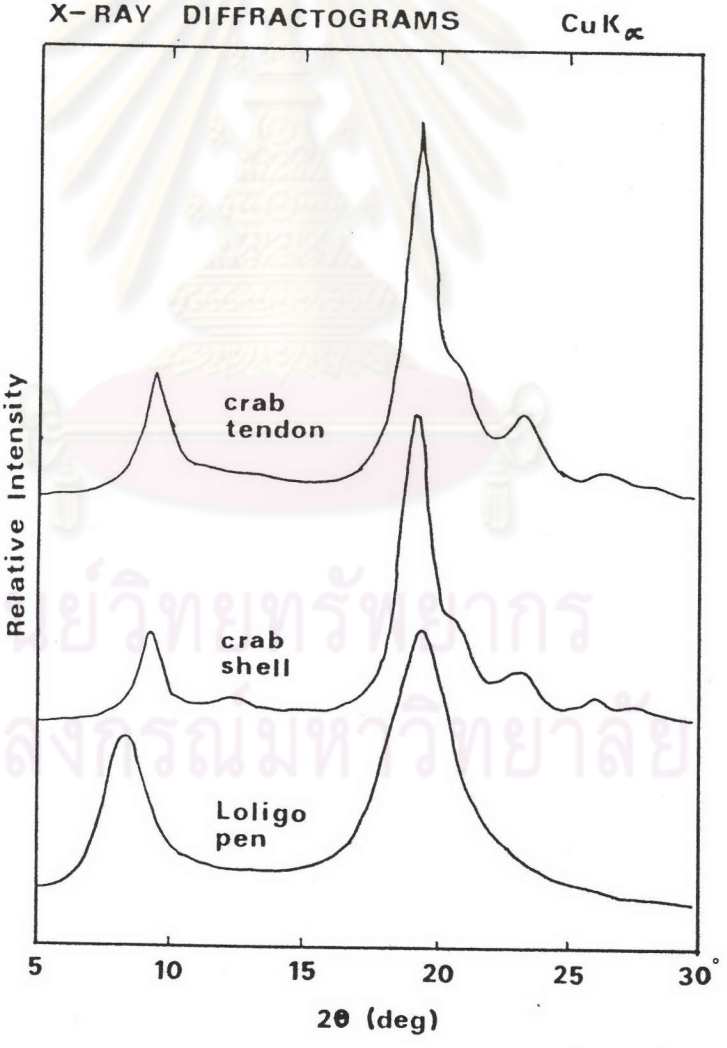

Figure 33 X-ray diffractograms of chitins from crab shell and tendon and *Loligo* pen.

The results obtained from this experiment had provided evidences to indicated that chitin and especially CTS60N had the same molecular conformation as β-chitin. Firstly, it was found from the x-ray diffractograms. As discussed above that there was two characteristics in the x-ray diffractogram of β-form: the higher of the first peak and the shift of this peak to the left. These was also observed in diffractograms of chitin and CTS60N. This conclusion was related to the results obtained from hydration capacity determination. Secondly, in β-chitin, the sheets are all arranged in a parallel manner and inter-molecular hydrogen bonding was absence. This results in the ease with which β-chitin can be swollen in water. (Backwell, Minke, and Gardner, 1978) Thus, the hydration capacity and swelling volume of chitin and CTS60N was significantly higher than of the other chitosans.

Chitin from crab shell was proposed that it is commonly found mainly in α-form. However, chitin used in this experiment may be a mixture of α- and β-form and the amount of β-form was more than usually found. It might be caused by the difference in production procedure of chitin. In the case of CTS60N, the first peak height was equal to the second peak. It was indicted that there was a large amount of β-form liked structure in CTS60N molecules.

All chitosans, except CTS60N, had molecular arrangement as α-form more than of chitin. This caused the reduction of their ability to absorb water or swell in water. The observation may be explained that the partial deacetylation, especially at high temperature, of β-chitin may result in the conversion to α-chitin. This behavior was found in the report by Kandaswamy (1978) as well.

The inter-molecular hydrogen bonding in the chitin from crab shell was shown in IR spectrum stated by Takai, Shimizu and Hayashi (1989) and depicted

in Figure 34. It was indicated that chitin used in this experiment had not only β-form but also had α-form liked structure.

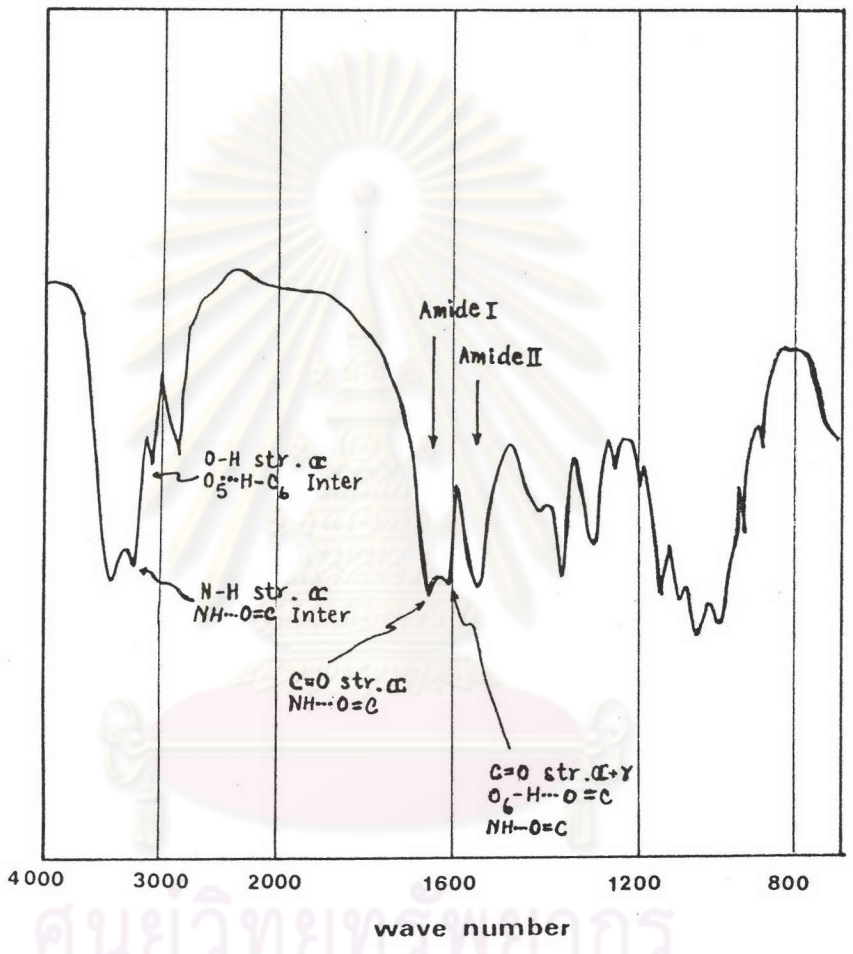

Figure 34 Infrared spectrum of chitin from crab shell that exhibited inter-molecular hydrogen bonding.

Moreover, by using base-line technique, the peak height ratio of the first to the second peak of chitosans produced under nitrogen atmosphere seemed to increase when the reaction time increased. The peak-height ratio of all polymers are given in Table 16. From this result, it was indicated that atmospheric condition during deacetylation may affect the arrangement of chitosan molecules. In addition, if chitosan was deacetylated under nitrogen atmosphere, the opportunity that β-form of the product was found was increase when the reaction time increased.

Table 16 Peak-height ratio of the first to the second peak from x-ray diffractograms of chitin and various chitosans.

polymer	Peak-height ratio
Chitin	0.58
CTS3A	0.45
CTS3.5A	0.48
CTS7A	0.53
CTS73A	0.47
CTS2.5N	0.46
CTS3N	0.59
CTS7N	0.72
CTS60N	1.23

To evaluate disintegrating properties of various polymers, paracetamol tablets were prepared. Pressure-hardness profiles of paracetamol tablets containing chitin/chitosans at any level of disintegrant seemed having no

difference. This meant that chitin and all chitosans used in this experiment affected no difference in hardness of paracetamol tablets. However, paracetamol tablets obtained from different batches had a little variation in hardness. As a result, to compare disintegration time of tablets containing various disintegrants at the same pressure and hardness, the data were presented as three-axis graphs.

When disintegration time was determined in deionized water, it was found that disintegration time of paracetamol tablets containing various disintegrants was nearly the same. This was found at 3, 5, and 10 % disintegrant level. An exception was found for tablets containing 3% CTS60N that had significantly lower disintegration time than the others at the same amount of disintegrant at 5000 lb. (Figure 29). In this case, it may be resulted from fragmentation of molecular chain at high compression pressure. (5000 lb.) The reason that this phenomenon occurred only in this case may be the absence of inter-molecular hydrogen bonding of CTS60N causing the ease of fragmentation. However, it was difficult to explain why this did not exhibited at 5 and 10 % disintegrant level.

The result that disintegration time of paracetamol tablets containing various disintegrants was no difference, could explain as follow. All disintegrants had similar hydration capacity, swelling capacity and rate of water uptake, except CTS60N. In addition, these parameters were evaluated using water as in disintegration test. In the case of CTS60N, it had higher hydration and swelling capacity but had slowest rate of water uptake than other chitosans.

Chitosan had the properties to dissolve and form gel in some acid. Hence, disintegration test of paracetamol tablets was performed in 0.1 N. HCl. Although disintegration time of tablets containing various disintegrants in water

was not significantly different, disintegration time in acid may be prolonged. The result obtained may be a useful application to sustained- release preparation.

Although the disintegration test in 0.1 N. HCl was not the official method, it should be suitable method to evaluate disintegration time of tablets containing chitin or chitosan. Because in general, the gastric contents are usually quite acid pH ~ 1. (Thompson, 1983)

Disintegration test of paracetamol tablets in 0.1 N. HCl was carried out only in the case of 5% disintegrant level. The result was clearly observed that degree of deacetylation of chitosan was a disintegration-time affecting factor. Disintegration time of tablets containing chitin in water and in 0.1 N. HCl were not different. In contrast, disintegration times of tablets containing all chitosans in acid were longer than in water. The higher the degree of deacetylation the longer the disintegration time. Moreover, it was found that disintegration time of tablets containing chitosan that had 75% or more deacetylation, was more than 30 minutes. Thus, degree of deacetylation of chitosan for using as vehicle in sustained-release preparation should be above 75%.

It could be noticed that the disintegration in 0.1 N. HCl of paracetamol tablets containing chitin and chitosan was different. The tablets containing chitin disintegrated into small flakes. After the disintegration test had been completed, the disintegrating medium remained clear. On the other hand, the tablets containing chitosan disintegrated by erosion of the tablets leading to the turbidity of the disintegrating medium. As these observations, it was indicated that the solubility of drug from the tablets containing chitosans seemed to be better than from tablets containing chitin. From these results, chitosans with low degree of deacetylation (CTS3A and CTS2.5N 67-68%, CTS3.5A and CTS3N 71-72%) should be better disintegrants in acid medium. Although low degree of

deacetylation chitosan slightly retarded the disintegration time, the dissolution of drug might be better. However, chitosans at about 75-80% could not be used as a disintegrant. Because they caused gel-formation leading to sustained-release of drug from the tablets. The rate of gel formation may depend on the degree of deacetylation of chitosan. However, chitosan of very high degree of deacetylation having very low molecular weight might have reversed effect on rate of drug release. It is possible to indicate that there is the optimum degree of deacetylation and molecular weight for chitosan to be sustained-released vehicle.

Chitosan having degree of deacetylation about 90% or more was not used in this experiment. Because the production of this chitosan require more procedures, time, and reagents causing very high expense. As this result, highly or fully deacetylated chitosan is not proper to use as disintegrant, whereas in the market, the price of the other disintegrants are so low. However, highly deacetylated chitosan may be a good disintegrant if disintegration time in 0.1 N. HCl of tablets containing it was not so longer. It was proposed by Machida, Nagai and Inouye (1989) that the release of prednisolone from the granules containing 93% deacetylation chitosan was considerably faster than those containing 60% deacetylation chitosan. Thus the enhancement of dissolution of highly deacetylated chitosan was better than of the lower.

Conclusions

The results emphasized that some deacetylation variables influenced the characteristics of chitosan products. Reaction temperature was an important variable affecting the molecular weight of chitosan obtained. An increase in the reaction temperature decreased molecular size. To produce high molecular weight chitosan, the reaction should be carried out at low temperature under

nitrogen atmosphere. In addition, deacetylation under air or nitrogen atmosphere at high reaction temperature produced chitosan having no significant difference in molecular weight. The degree of deacetylation of chitosan products increased when the reaction time increased. At the same time, the molecular weight decreased.

Viscosity determination was an easy and reliable method used to compare the molecular weight of chitosans in this experiment. By this method, the comparison obtained was in agreement with the results of the previous studies. (Bough, Salter, Wu, and Perkins, 1978)

The degree of deacetylation value was expressed according to the method described by Hayes (1978). However, infrared spectrometry was used to confirm and classify the chitosan products having similar degree of deacetylation.

The physicochemical properties that related to disintegrating properties of all chitosans obtained from method A and B had no significant difference. Only chitosan from method C (CTS60N) had properties superior to the others. These included the higher in hydration and swelling capacity of CTS60N. Although the hydration and swelling capacity of CTS60N was higher , its rate of water uptake was slower than chitosans from method A and B. However, disintegration time in water of paracetamol tablets containing 3, 5, or 10 % disintegrant level of various chitosans were not significant different. Chitin had higher hydration capacity, swelling volume and had faster rate of water uptake than from method A and B. But these superior properties of chitin did not showed the better disintegrating behavior.

The x-ray diffractograms clearly revealed that there was different in molecular arrangement of chitosan molecules. Deacetylation at high temperature may result in the conversion of β-form to α-from leading to the lower of hydration

capacity and swelling volume of chitosans from method A and B than of chitin. At low reaction temperature, the molecular rearrangement occurred resulting in the increasing of β-form. As this result, CTS60N had the highest hydration and swelling capacity. In the case of CTS60N, it was possible to indicate that the inter-molecular hydrogen bonding decreased while the intra-molecular hydrogen bonding considerably increased. An increase in intermolecular hydrogen bonding resulted in the difficulty of primary amine groups in CTS60N to react completely with hydrochloric acid during the determination of degree of deacetylation by colloidal titration, and also the slowest rate of water uptake of CTS60N was observed.

Atmospheric condition tended to be a factor affecting the properties of chitosan products. Deacetylation under air or nitrogen atmosphere may influence the difference in molecular arrangement of chitosan obtained. There were two reasons to support. Firstly, the difference in rate of water uptake of chitosans from method A and B could be observed. Secondly, the increasing of reaction time increased the peak-height ratio of the first to the second peak of x-ray diffractograms in the case of chitosans deacetylated under nitrogen atmosphere.

The disintegration time of paracetamol tablets containing chitosan in 0.1 N. HCl was longer than in water. In contrast, in the case of chitin there was no difference. The disintegration time increased when the degree of deacetylation of chitosan increased. At 75% or more deacetylation level, disintegration time was more than 30 minutes. The difference in disintegration time may be due to the difference in rate of gel-formation of chitosans having difference in degree of deacetylation.

The suitable chitosans in this experiment for using as disintegrants were CTS3A, CTS3.5A, CTS2.5N, and CTS3N. In the case of CTS60N, the high expense of production caused no practical use as a disintegrant. The prolong of disintegration time in the case of 75% or more degree of deacetylation chitosan was a useful information for development of chitosan as a vehicle in sustained-release dosage-forms. Chitosans produced by deacetylation under various conditions would have difference in physicochemical properties. The suitable condition could be searched for the production of chitosans. The conditions of deacetylation are needed to be strictly controlled to obtain the same quality of chitosan product having the properties as required for various applications.

On the basis of the information obtained under these investigations, many aspects of further studies were recommended.

1. Chitin should be deacetylated at various temperature under air and nitrogen atmosphere in order to study their physicochemical and disintegrating properties.

2. An examination of assessment of X-ray diffraction to evaluate the possible relation between reaction temperatures and molecular arrangement should be carried out. Moreover, aging and the rate of transformation of β-form to α-form should be investigated.

3. The various chitosan products should be investigated in order to find out the optimum degree of deacetylation for using as a vehicle in sustained-release preparation.

4. It is essential to study the effects of chitosan on the released-behavior of both acid and basic drugs, and soluble and insoluble drugs from pharmaceutical preparations.