

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary Study on Hydroxypropylation of Tapioca Starch

Hydroxypropylation of tapioca starch was scarcely reported. In the earlier work, Buachamras (26) studied the hydroxypropylation of tapioca starch. When the reaction condition was carried out with sodium sulfate 15%, sodium hydroxide 1.5% propylene oxide 9% based on the dry starch weight at 40°C for 12 hours, the hydroxypropylated tapioca starch was obtained with degree of substitution of 0.0927.

In this study, the hydroxypropylation of tapioca starch was primarily carried out using the same condition as Buachamras reported. Three repetitions of this reaction were performed. The degree of substitution was determined by ¹H-NMR technique as presented in Table 4.1 and Figure 4.1.

Table 4.1 Hydroxypropylation of tapioca starch at the primary condition.

Entry no.	Degree of substitution (DS)	Average degree of substitution
1	0.1013	0.1006
2	0.1007	
3	0.0999	

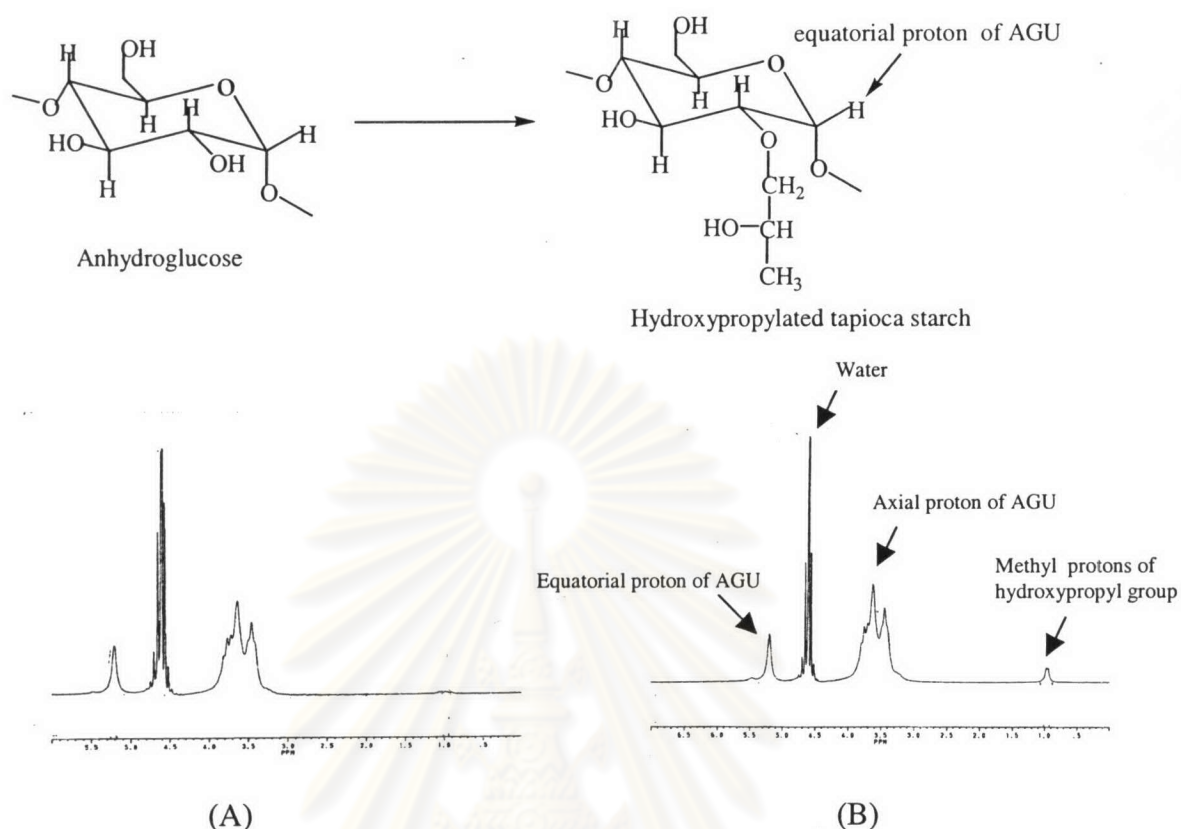


Figure 4.1 The ¹H-NMR spectra of native tapioca starch (A) and hydroxypropylated tapioca starch with DS 0.0999 (B)

Figure 4.1 exhibits the ¹H-NMR spectrum of native tapioca starch and hydroxypropylated tapioca starch. The degree of substitution was calculated from the ratio of the peak areas of methyl protons of hydroxypropyl group on starch to the equatorial proton of anhydroglucose unit of starch as described in Appendix B. The average DS obtained was 0.1006 which was higher than the one reported by Buachamras (26). This was probably due to the different method of DS determination. The ¹H-NMR technique was applied in this study but the colorimetric method was used by Buachamras. However, it has already been proved that ¹H-NMR technique gave more accurate result (21, 22). It is noteworthy to mention that gelatinization in entry no.3 was observed but only little gel appeared while gel was not formed in entry no.1 and no.2. This observation was correlated with the slightly lower DS in entry no.3 than no.1 and no.2.

4.1.1 The Pasting Property of Hydroxypropylated Tapioca Starch

The most important of all industrial tests used to characterize starches is the paste viscosity. Different types of starches will have different pasting properties. Liu (17) reported that hydroxypropylated starches generally have markedly altered pasting properties, compared with their parent starches. Pal *et al.* (19) also reported that the pasting property of corn starch was higher as compared with that of amaranth due to the waxy nature of amaranth starch. The similar observation was reported by several other researchers who worked on other types of starch (7, 13). The pasting properties of native tapioca starch and hydroxypropylated tapioca starch (DS= 0.0999) are shown in Figure 4.2.

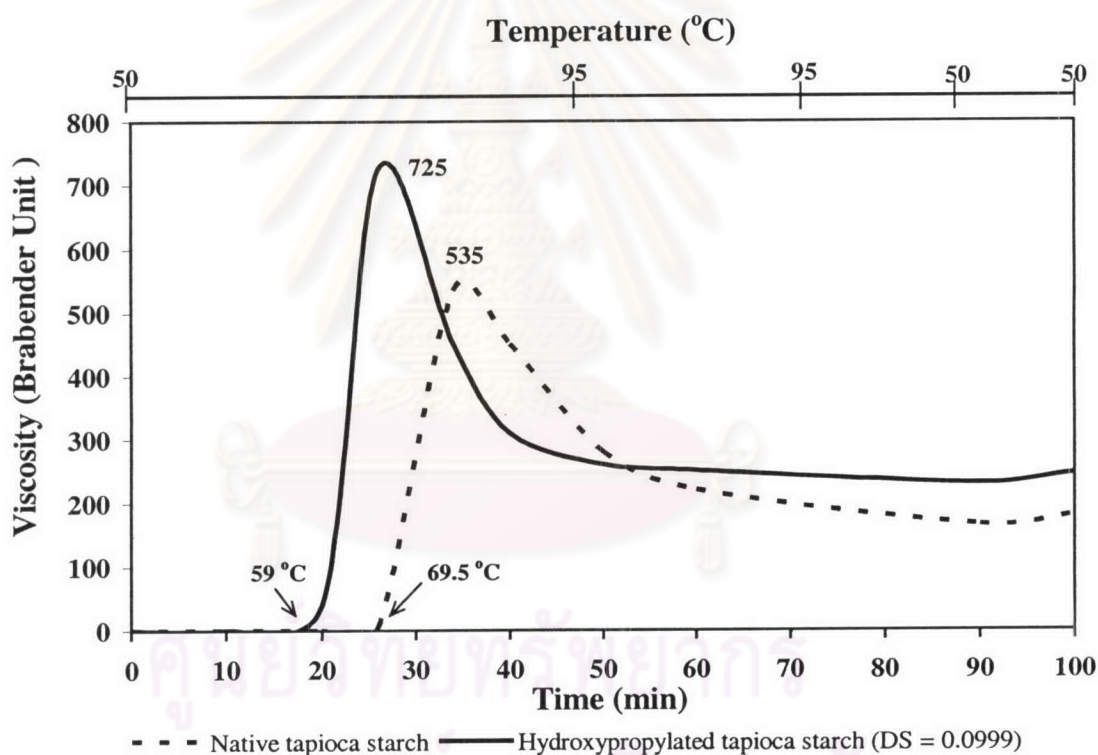


Figure 4.2 Pasting properties of native starch and hydroxypropylated tapioca starch

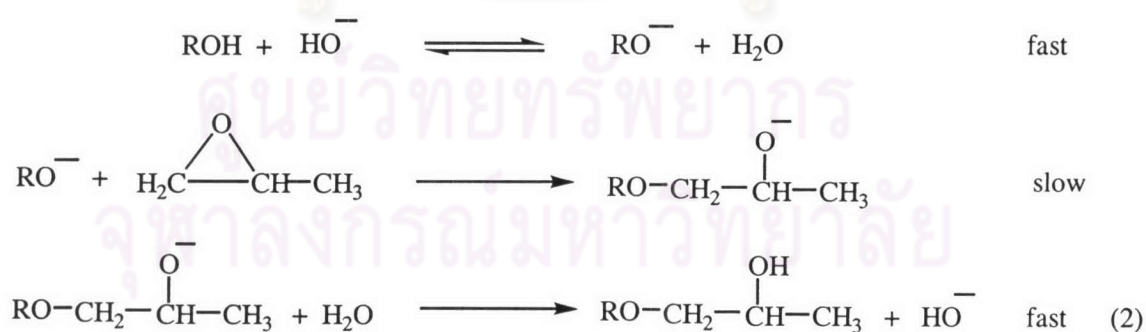
Figure 4.2 shows the pasting property of native tapioca starch comparing with hydroxypropylated tapioca starch. It obviously indicated that the hydroxypropylated tapioca starch has gelatinization temperature of 59°C and peak viscosity of 725 BU which are lower and higher than those of native tapioca starch 69.5°C and 535 BU, respectively. Accordingly, the hydroxypropylation can improve the pasting property of tapioca starch.

4.2 The Formation of Hydroxypropylated Tapioca Starch

It has been known that hydroxypropylated starch can be synthesized by reacting starch with propylene oxide under alkaline condition. Starch dissolved in water behaves as a weak polyacid. Sodium hydroxide added to a solution of starch thus reacts with the hydroxyl group of the starch as follows:



The amount of RO^- formed depends on the acidity constant of the hydroxyl groups of the starch molecule. In a mixture of such an alkaline starch solution, propylene oxide can react with both the hydroxyl groups of starch and water. The reactions are summarized in the following reaction scheme:



The reactions proceed according to $\text{S}_{\text{N}}2$ reaction mechanism. The main reaction parameters which influence the hydroxypropylation process are reaction time, temperature, the concentration of sodium hydroxide, sodium sulfate and propylene oxide concentration. Different types of starches will respond to the chemical modification differently. Pal *et al.* (16) found that the reaction condition to yield the

same degree of substitution in hydroxypropylation differed from one starch type to another. Wang (23) also reported that the degree of substitution (DS) of hydroxypropylated starch at the same condition was differing from one starch type to the others. Bae and Lim (30) studied the hydroxypropylation of normal and high amylose corn starch. It was found that the degree of substitution of high amylose corn starch was higher as compared to that of normal corn starch. Therefore, it can be concluded that the degree of substitution correlated to the amount of amylose. With higher amylose, the DS of hydroxypropylated starch will be higher. Since amylose content of tapioca starch was lower than other starches, such as corn and potato starch, the highest degree of substitution and reaction efficiency of hydroxypropylated tapioca starch is thus expected to be lower.

The appropriate reaction temperature for hydroxypropylation of starch depends on the gelatinization temperature of each type of starch. It is generally in the range of 37- 50°C. At temperature below 37°C, reactions proceed very slowly. Above 50°C, the risk of swelling or pasting the starch granules, unless high levels of salt are used is concern (7). Native tapioca starch has the gelatinization temperature between 59-70°C (7,13). Thus, hydroxypropylation of tapioca starch must be handled at the temperature lower than 59°C to prevent the occurrence of gelatinized starch. Accordingly the variation of all parameters affecting on the hydroxypropylation of tapioca starch was performed except the reaction temperature which was kept constant at 40°C.

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4.3 Parameters Affecting on Reaction Efficiency

The study on the parameters affecting on reaction efficiency was performed by using tapioca starch 40 g in 100 ml of water in all reactions. The reaction temperature and the stirring speed were kept constant at 40°C and 400 rpm, respectively.

4.3.1 Effect of Sodium Sulfate

The hydroxypropylation of starch commonly takes place in aqueous solution. The gelatinization can normally occur due to the interaction between starch and water. When the solution is heated, it will lose hydrogen bond and short molecules of amylose starts to dissolve but amylopectin swells up. This problem had on degree of substitution and reaction efficiency. To avoid the gelatinization, it is necessary to add gelatinization inhibitor in the condition. Sodium sulfate is the most common gelatinization inhibitor and was used in this research.

In this section, the concentration of sodium sulfate was studied by varying from 0 to 25% (w/w) based on the dry starch weight, while sodium hydroxide and propylene oxide were kept constant at 1% and 8% (v/w) based on the dry starch weight, respectively. In addition, all the reactions were allowed to run for 24 hours. After the reaction was stopped, the product was filtered and thoroughly washed with water. Then it was dried and its $^1\text{H-NMR}$ spectrum was recorded. The reaction at each condition was repeated three times. Then portions of each sample were subjected to $^1\text{H-NMR}$ recording. The results are shown in Table 4.2 and Figure 4.3.

Table 4.2 Effect of the concentration of sodium sulfate on degree of substitution and reaction efficiency

Sodium sulfate (% by dry starch weight)	Degree of substitution (DS)	Reaction efficiency (%) (RE)
0*	0.0358	-
10	0.0806 ± 0.0068	36.08
15	0.0827 ± 0.0083	37.02
20	0.1004 ± 0.0078	44.94
25	0.1095 ± 0.0050	49.02

Note; * gelatinization of starch was observed.

In the absence of sodium sulfate, gel formation occurred after 10 hours of reaction. Though it was difficult to work up the reaction, the DS of the resulted hydroxypropylated tapioca starch was determined to be 0.0358. At 10% (w/w) sodium sulfate, the degree of substitution and reaction efficiency was nearly the same as 15% (w/w) sodium sulfate. With higher sodium sulfate content, higher DS and RE were obtained as shown in Table 4.2. It indicated that salt had an effect on degree of substitution and reaction efficiency. That is sodium salt can prevent the starch from swelling or pasting by dissolubility in water.

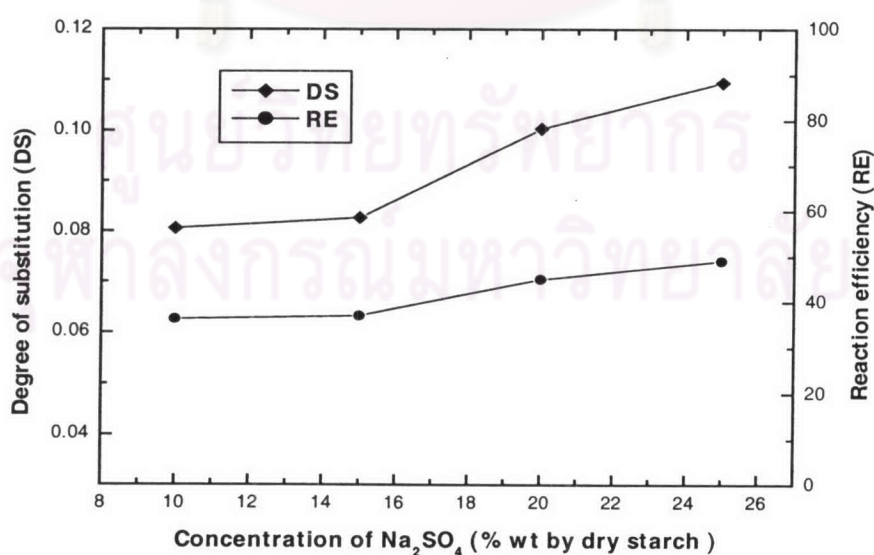


Figure 4.3 Dependence of DS and RE on the concentration of sodium sulfate

A better parameter to optimize the reaction condition is the reaction efficiency which can be calculated from the DS as described in Appendix C. The reaction efficiency determination was thus included. The reaction efficiency indicated that the percentage of the hydroxypropyl group upon starch. The effect of sodium sulfate on the reaction efficiency was shown in Table 4.2 and Figure 4.3. When the amount of sodium sulfate increased, higher the reaction efficiency was obtained. At 25% sodium sulfate, the reaction efficiency was 49.02%, this means that 40% of propylene oxide had been used in too much. Though higher sodium sulfate concentration would give higher reaction efficiency, but not large increment in the reaction efficiency would be obtained while too much salt was left in the solution which had to be removed later. Therefore, 10% sodium sulfate would be used for the other experiments. Sodium sulfate had interacted with water more readily than approached the starch molecules. Thus the gelatinization could not occur. Similar results had been reported by other research groups (20, 28).

4.3.2 Effect of Sodium Hydroxide

The reactions in this experiment were carried out with sodium sulfate 10%, propylene oxide 8% (v/w) based on the dry starch weight at 40°C for 24 hours. The effect of sodium hydroxide was studied by varying in range of 0.5 to 2.2% (v/w) based on the dry starch weight. The results are presented in Table 4.3 and Figure 4.4.

Table 4.3 Effect of the concentration of sodium hydroxide on degree of substitution and reaction efficiency

Sodium hydroxide (% by dry starch weight)	Degree of substitution (DS)	Reaction efficiency (%) (RE)
0.5	0.0503 ± 0.0065	22.50
1.0	0.0806 ± 0.0068	36.08
1.5	0.0975 ± 0.0037	43.64
2.0	0.1025 ± 0.0047	45.88
2.2	gel	gel

The values of the degree of substitution and reaction efficiency increase more rapidly at early reaction with the increasing concentration of sodium hydroxide. The increase in degree of substitution of hydroxypropylated tapioca starch was because of the catalytic effect of sodium hydroxide on the hydroxypropylation reaction (Scheme 2.1). The more sodium hydroxide was added, the more starch nucleophile was formed. The higher DS and RE thus were obtained in shorter reaction time. Since hydroxypropyl group was hydrophilic, hydroxypropylated starch exhibited better dispersibility in cold water than native starch. However, gelatinization would easily occur due to the presence of a large number of hydroxypropyl groups. This explanation was corresponded to the report by Ragheb (29). He observed that the gelatinization of starch depended mainly on the concentration of sodium hydroxide on the paste and its rheological characteristics of pastes depended on both the temperature of treatment and on the concentration of sodium hydroxide. The maximum DS and RE are 0.1025 and 45.88, respectively by using 2.0% sodium hydroxide. With 2.2% sodium hydroxide, the starch slurry formed very thick slurry which caused the difficulty for stirring. At this sodium hydroxide concentration, the hydroxypropylation was presumably induced to occur rapidly. Hence, more hydroxypropyl groups in starch molecule were developed in a short time.

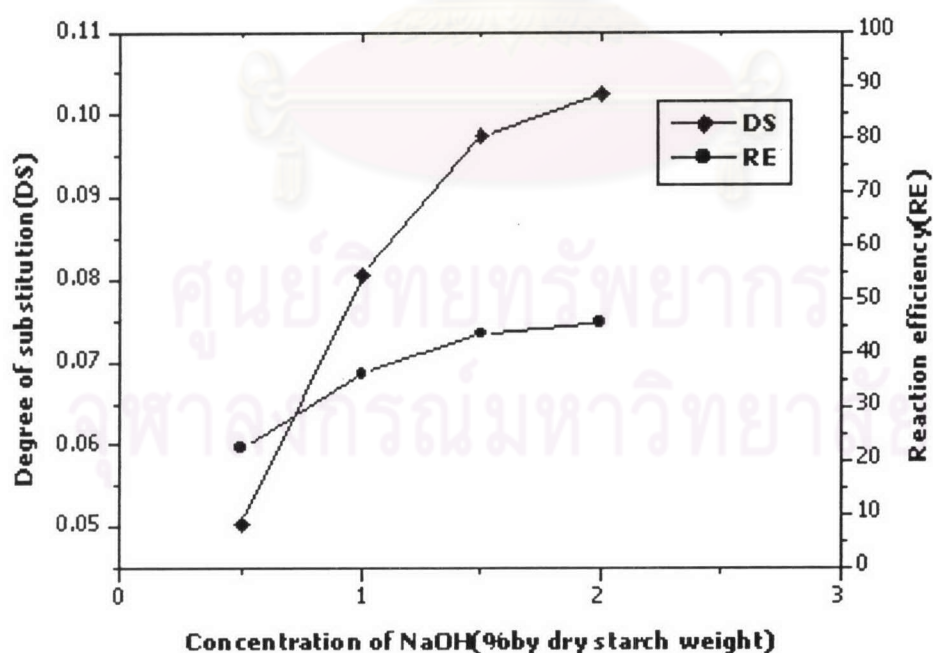


Figure 4.4 Dependence of DS and RE on the concentration of sodium hydroxide

Consequently, it indicated that the sodium hydroxide concentration had influenced on the reaction efficiency and degree of substitution of hydroxypropylated

starch. Thus, the using amount of sodium hydroxide was limited. If the sodium hydroxide is present in large amounts, the gelatinization could occur. Also, the DS and RE may be decreased. Therefore, 1% sodium hydroxide would be used for the other experiments to prevent the starch from swelling or pasting.

4.3.3 Effect of Propylene Oxide

The propylene oxide was another vital parameter for the hydroxypropylation of starch. Therefore, the effect of propylene oxide was studied by varying from 5 to 20% (v/w) based on the dry starch weight, while sodium hydroxide and sodium sulfate were kept constant at 1% and 10% based on the dry starch weight, respectively. In addition, all reactions were allowed to run for 24 hours. The resulted examination was taken as shown in Table 4.4 and Figure 4.5.

Table 4.4 Effect of propylene oxide concentration on degree of substitution and reaction efficiency

Propylene oxide (% by dry starch weight)	Degree of substitution (DS)	Reaction efficiency (%) (RE)
5	0.0443 ± 0.0057	31.70
8	0.0806 ± 0.0068	36.08
12	0.1365 ± 0.0054	40.70
14	0.1668 ± 0.0084	42.66
20	0.2565 ± 0.0085	45.92

From Table 4.4 it obviously indicated that the DS and RE were increased with the increasing concentration of propylene oxide. The explanation was that the great amount of propylene oxide would allow more possibility for starch-O⁻ to react with leading to higher substitution. The same observation had been reported by other researchers who worked on the different types of starch (16, 23).

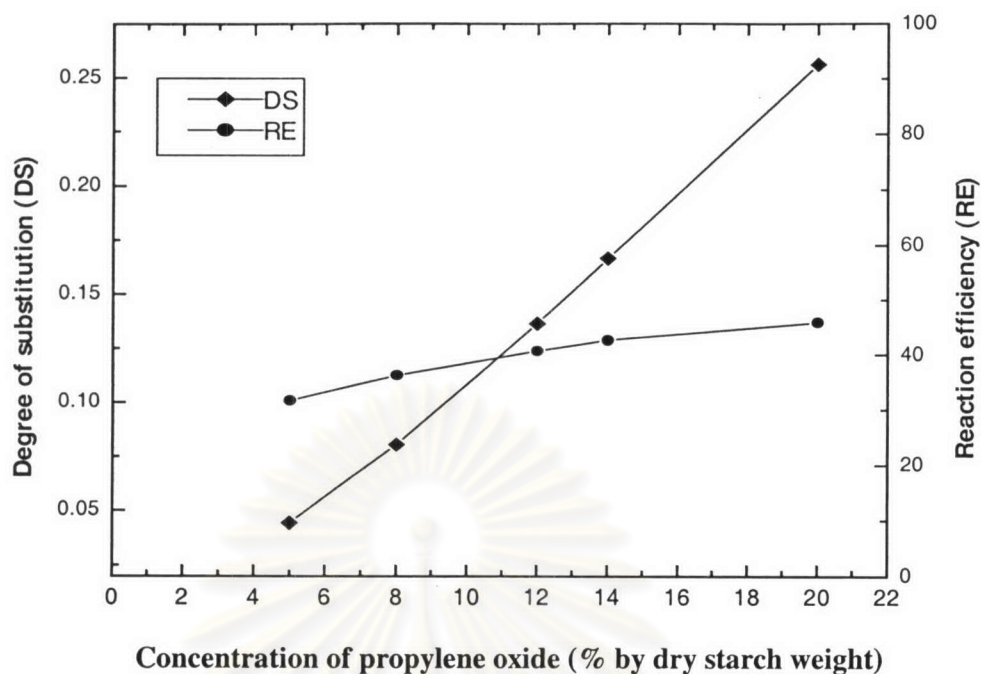


Figure 4.5 Dependence of DS and RE on the concentration of propylene oxide

The values of the DS and RE increase almost linearly with the concentration of propylene oxide. At 20% propylene oxide, the gelatinization starch was observed. This was due to the presence of more hydroxypropyl groups in starch. When they were introduced into starch granules, the internal bond holding the granule together was weakened. Then the hydration of starch granule occurred easily even at low temperature. On the other hand, it can be said that the hydroxypropylated starch was lower gelatinization temperature. Thus the gelatinization would easily occur. The degree of substitution and reaction efficiency would be decreased.

Consequently, the more concentration of propylene oxide was added, the gelatinization could be occurred. Therefore, the presence of propylene oxide was much or less depended on other severe conditions in each reaction such as temperature, sodium hydroxide and sodium sulfate.

4.3.4 Effect of Reaction Time

To increase the degree of substitution and reaction efficiency, high concentration of sodium sulfate, sodium hydroxide and propylene oxide were used. Thus, to avoid the gelatinization of modified starch, the shorter reaction time was thus attempted. In this section, the effect of reaction time was studied by varying from 3 to 24 hours, while sodium hydroxide, propylene oxide and sodium sulfate were kept constant at 1%, 14% and 10% based on the dry starch weight, respectively. The results are presented in Table 4.5 and Figure 4.6.

Table 4.5 Effect of reaction time on degree of substitution and reaction efficiency.

Reaction time (hrs.)	Degree of substitution (DS)	Reaction efficiency (%) (RE)
3	0.0825 ± 0.0045	21.10
6	0.1398 ± 0.0066	35.75
14	0.2318 ± 0.0047	59.28
18	0.2565 ± 0.0074	65.60
21	0.2693 ± 0.0044	68.87
24	0.2725 ± 0.0043	69.70

From Table 4.5 and Figure 4.6, it showed that the degree of substitution and reaction efficiency of hydroxypropylated tapioca starch increased significantly at early reaction. Greater contact time with continuous agitation led to efficient absorption or diffusion of propylene oxide into starch granules, which increased the chances of collisions between the starch nucleophile and propylene oxide, which induced a higher degree of substitution and reaction efficiency. However, after 18 hours increasing of degree of substitution no significant change was observed. It implied that 18 hours reaction accomplished almost complete hydroxypropylation. The similar observation had been reported by several other researchers who worked on other types of starch (16, 24).

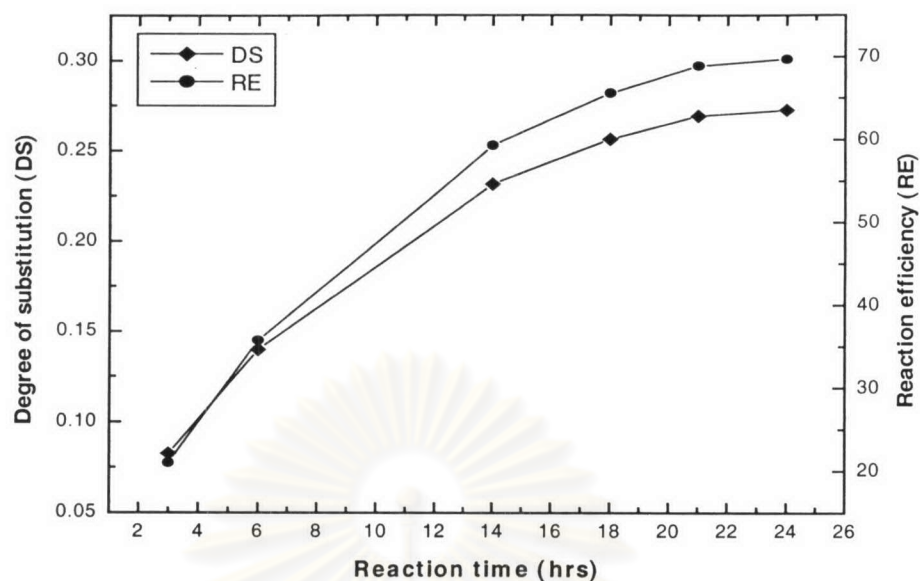


Figure 4.6 Dependence of DS and RE on the reaction time

Different types of starches will thus respond to the complete hydroxypropylation differently. Pal (16) reported that reaction time parameter had an effect on hydroxypropylation of corn and amaranth starch. It was found that optimized time of hydroxypropylation in corn starch and amaranth starch were 8 and 12 hrs, respectively. This is due to each starch type having the ratio of amylose and amylopectin differently. High amylose content, the optimum reaction time are shorter. The optimum reaction time for hydroxypropylation in tapioca starch had completed within 24 hours.

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4.4 Experimental Factorial Design for Starch Hydroxypropylation in Water

Though the hydroxypropylation of tapioca starch in water has been studied by Buachamras (26), the highest degree of substitution and reaction efficiency of 0.0927 and 36.9%, respectively were reported. This means that 63% of propylene oxide was wasted. To improve reaction efficiency, the optimum condition of this reaction must be investigated.

4.4.1 Hydroxypropylation of Tapioca Starch According to 3^k Factorial Design.

In general, the reaction temperature in range of 37-50°C. Buachamras (26) studied the effect of temperature on degree of substitution of hydroxypropyl group by varying from 30 to 50°C. It was found that at 50°C, the gelatinization was observed. The highest DS of hydroxypropylated tapioca starch was obtained and no gel formation appeared during the reaction when the reaction condition was carried out at 40-45°C. At 40°C, the highest degree of substitution was nearly the same as 45°C. It can be concluded that the optimum temperature for hydroxypropylation of tapioca starch is 40°C. In section 4.3.4, the reaction time was studied by varying from 3 to 24 hours. It was found that, the degree of substitution and reaction efficiency were increased with the reaction time but appropriated constant at 24 hours. It implied that 24 hours reaction accomplished complete hydroxypropylation. The next experiments, the reaction temperature and reaction time were kept constant at 40°C and 24 hours, respectively.

To determine the optimum condition for hydroxypropylation of starch, 3^k factorial design was used (Appendix A). In this study, k is defined as three variables of the reaction, *i.e.* the concentration of sodium hydroxide, sodium sulfate and propylene oxide. Each variable was assigned into three levels, *i.e.* sodium hydroxide 1.0, 1.5 and 2.0%, sodium sulfate 10, 15 and 20% and propylene oxide 8, 14 and 20% based on the dry starch weight. Accordingly, 3³ or 27 experiments of tapioca starch hydroxypropylation in water were carried out at 40°C for 24 hours. The reaction at each condition was repeated three times. The DS and RE were determined by ¹H-NMR technique as shown in Appendices B and C, respectively. The results are illustrated in Table 4.6.

Table 4.6 Hydroxypropylation of tapioca starch in water.

Experiment No.	NaOH (% v/w)	PO (% v/w)	Na ₂ SO ₄ (% v/w)	DS	RE (%)
1	1.0	8	10	0.0806 ± 0.0068	36.08
2	1.0	14	10	0.1668 ± 0.0084	42.66
3	1.0	20	10	0.2565 ± 0.0085	45.92
4	1.5	8	10	0.0975 ± 0.0037	43.64
5	1.5	14	10	0.2093 ± 0.0034	53.53
6*	1.5	20	10	-	-
7	2.0	8	10	0.1025 ± 0.0047	45.88
8*	2.0	14	10	-	-
9*	2.0	20	10	-	-
10	1.0	8	15	0.0827 ± 0.0083	37.02
11	1.0	14	15	0.1871 ± 0.0033	47.85
12	1.0	20	15	0.2598 ± 0.0040	46.51
13	1.5	8	15	0.1062 ± 0.0026	47.54
14	1.5	14	15	0.2112 ± 0.0068	54.02
15*	1.5	20	15	-	-
16	2.0	8	15	0.1175 ± 0.0059	52.60
17*	2.0	14	15	-	-
18*	2.0	20	15	-	-
19	1.0	8	20	0.1004 ± 0.0078	44.94
20	1.0	14	20	0.1906 ± 0.0050	48.75
21	1.0	20	20	0.2614 ± 0.0037	46.80
22	1.5	8	20	0.1144 ± 0.0031	51.21
23	1.5	14	20	0.2260 ± 0.0014	57.80
24*	1.5	20	20	-	-
25	2.0	8	20	0.1212 ± 0.0015	54.25
26	2.0	14	20	0.2725 ± 0.0043	69.70
27*	2.0	20	20	-	-

Note; * gelatinization of starch was observed.

In the case that gelatinization had occurred during the reaction, degree of substitution would not be determined and the reaction efficiency could thus not be obtained.

4.4.2 Degree of Substitution of Hydroxypropylated Tapioca Starch as Determined by $^1\text{H-NMR}$ spectroscopy

It had been reported that DS of hydroxypropylated tapioca starch can be determined correct by $^1\text{H-NMR}$ spectroscopic technique (22). The $^1\text{H-NMR}$ spectrum of each product was obtained using deuterium oxide (D_2O) as a solvent. Figure 4.7 illustrates $^1\text{H-NMR}$ spectrum of native tapioca and hydroxypropylated tapioca starch with various DS.

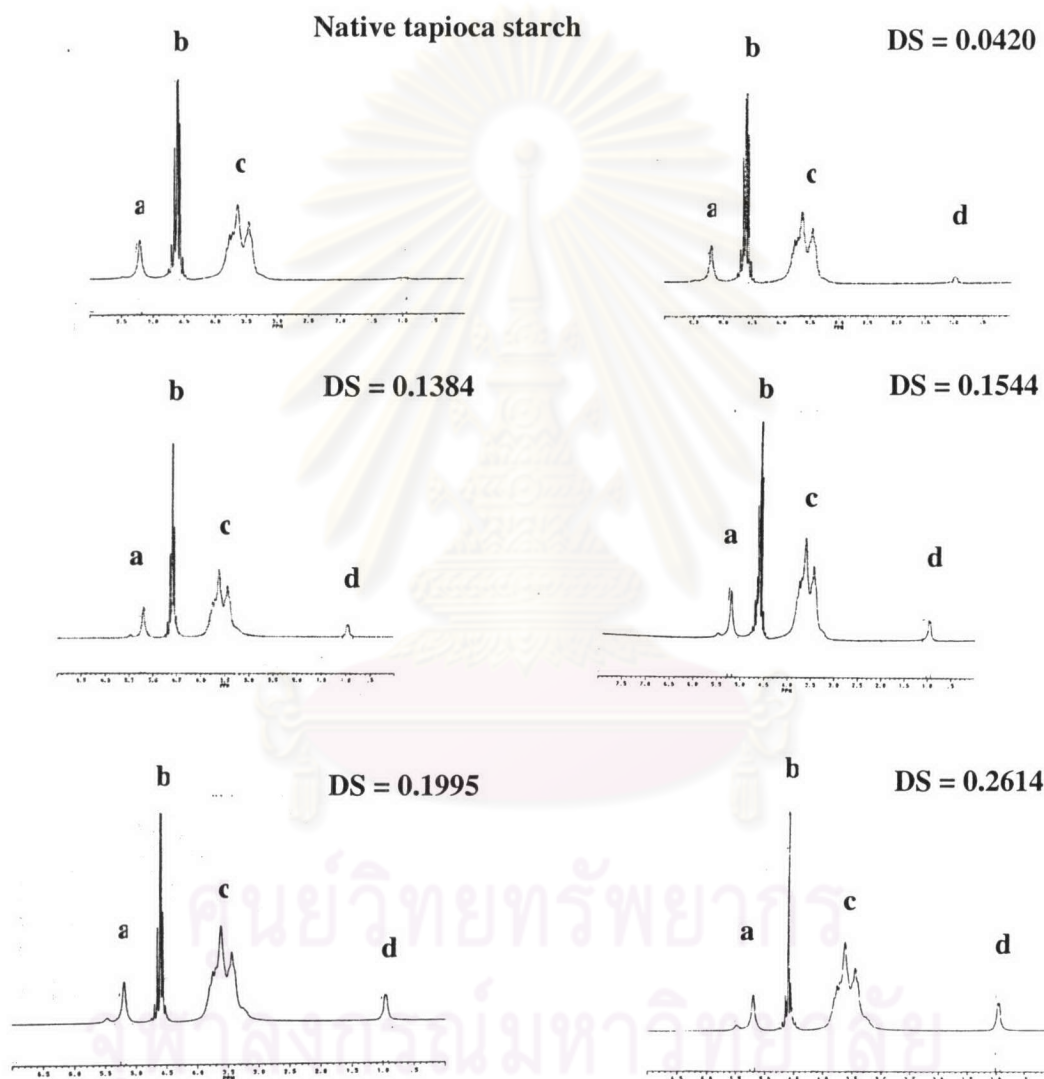


Figure 4.7 The $^1\text{H-NMR}$ spectrum of native and hydroxypropylated tapioca starch with various DS

- a) Equatorial proton of anhydroglucose unit
- b) Water
- c) Axial protons of anhydroglucose unit
- d) Methyl protons of hydroxypropyl group on starch

The $^1\text{H-NMR}$ spectrum of native tapioca starch shows the signal of axial proton of the anhydroglucose units, water and equatorial proton of the anhydroglucose unit appeared at $\delta = 3.3\text{-}4.1$, $4.5\text{-}4.8$ and 5.3 respectively. In case of the hydroxypropylated tapioca starch, the additional doublet at $\delta = 1.1$ ppm appeared in the spectrum. This attributed to methyl protons of hydroxypropyl group on starch. The DS was calculated using the integrated intensities of the signal at $\delta = 1.1$ ppm from methyl proton in hydroxypropyl group and the integrated intensities of the signal at $\delta = 5.3$ ppm from the equatorial proton of the anhydroglucose unit. The more hydroxypropyl group is, the higher integration the signal at $\delta = 1.1$ ppm in relative with the singlet proton at $\delta = 5.3$ ppm. Therefore, the DS was increased.



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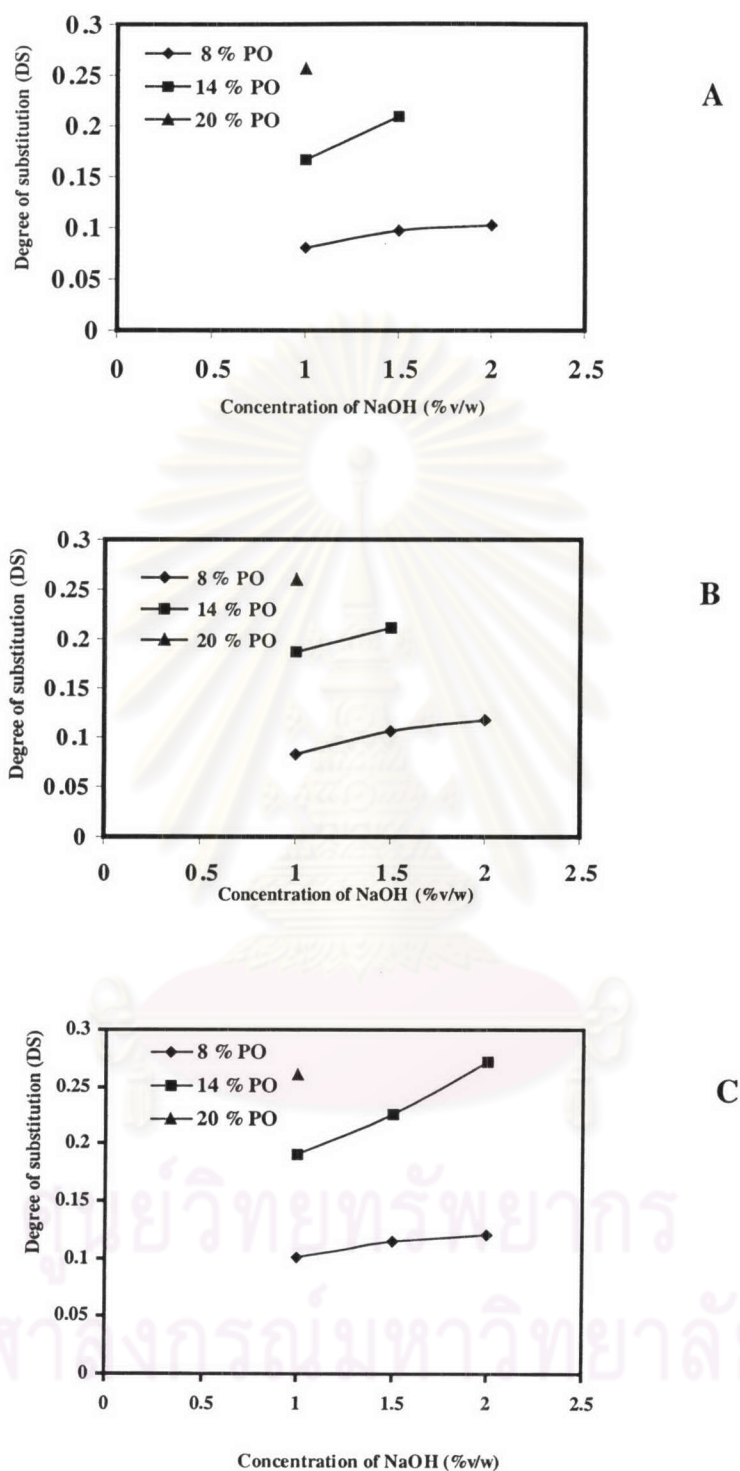


Figure 4.8 Degree of substitution of hydroxypropylated tapioca starch depending on sodium hydroxide and propylene oxide at certain sodium sulfate concentrations A) 10% w/w, B) 15% w/w and C) 20% w/w based on the dry starch weight.

From Figure 4.8 (A, B and C), the concentration of sodium sulfate was fixed at 10%, 15% and 20% based on the dry starch weight, respectively. The concentration of sodium hydroxide and propylene oxide were varied in the range of 1 to 2% and 8 to 20 %v/w based on the dry starch weight, respectively. In Figure 4.8(A, B), it obviously indicated that when the concentration of propylene oxide increased, the DS was higher. The reaction with 20% propylene oxide gave the DS higher than the concentration of propylene oxide 14% and 8% in a sequence. This is probably due to the greater availability of propylene oxide at higher concentrations in the proximity of starch granules (16). The sodium hydroxide concentration had effect on DS. It appeared that the DS was little increased when the concentration of sodium hydroxide was increased. The concentration of sodium hydroxide and propylene oxide were used correlating to the DS. With 14% and 20% propylene oxide, the high concentration of sodium hydroxide will permit, 1.5% and 1%, respectively. The gelatinization could not occur during the reaction. Addition of sodium hydroxide to the starch dispersions also introduces small ions to the system, affecting the polyelectrolyte properties of the polymer. Hydroxyl groups may promote charge screening of the junction zones on the starch chains, disrupting the hydrogen bonding which causes the formation of a gel. This would weaken the starch network and produce the more fluid-like gels. The DS would be decreased. In case of the result of Figure 4.8 (C) and Figure 4.8 (A, B) were differed. At 8% and 14% of propylene oxide concentration and the concentration of sodium sulfate was fixed at 20% w/w by dry starch weight, all for reactions with no gel formation appeared during the reaction when the concentration of sodium hydroxide was in the range of 1 to 2% v/w based on the dry starch weight were used. This can be due to the higher concentration of sodium sulfate. This could be explained that the salt dissolved in water and had interacted with water by surrounded sodium ion and sulfate ion. As the result, the water molecule could not approach the starch molecule. Thus the gelatinization could not occur. With 20% propylene oxide, the concentration of sodium hydroxide should be not higher than 1% v/w based on the dry starch weight due to the occurrence of gelatinization starch during the reaction. This can be concluded that each parameter such as sodium hydroxide, sodium sulfate and propylene oxide had an effect on the degree of substitution of hydroxypropylation of starch. Other reports also described the similar observation on the hydroxypropylation of various parameters. (16, 20, 26). The reaction efficiency was considered as

compared with the DS. It was also found that the RE was depended on these parameters. The effect of these parameters on the RE is shown in Figure 4.9.

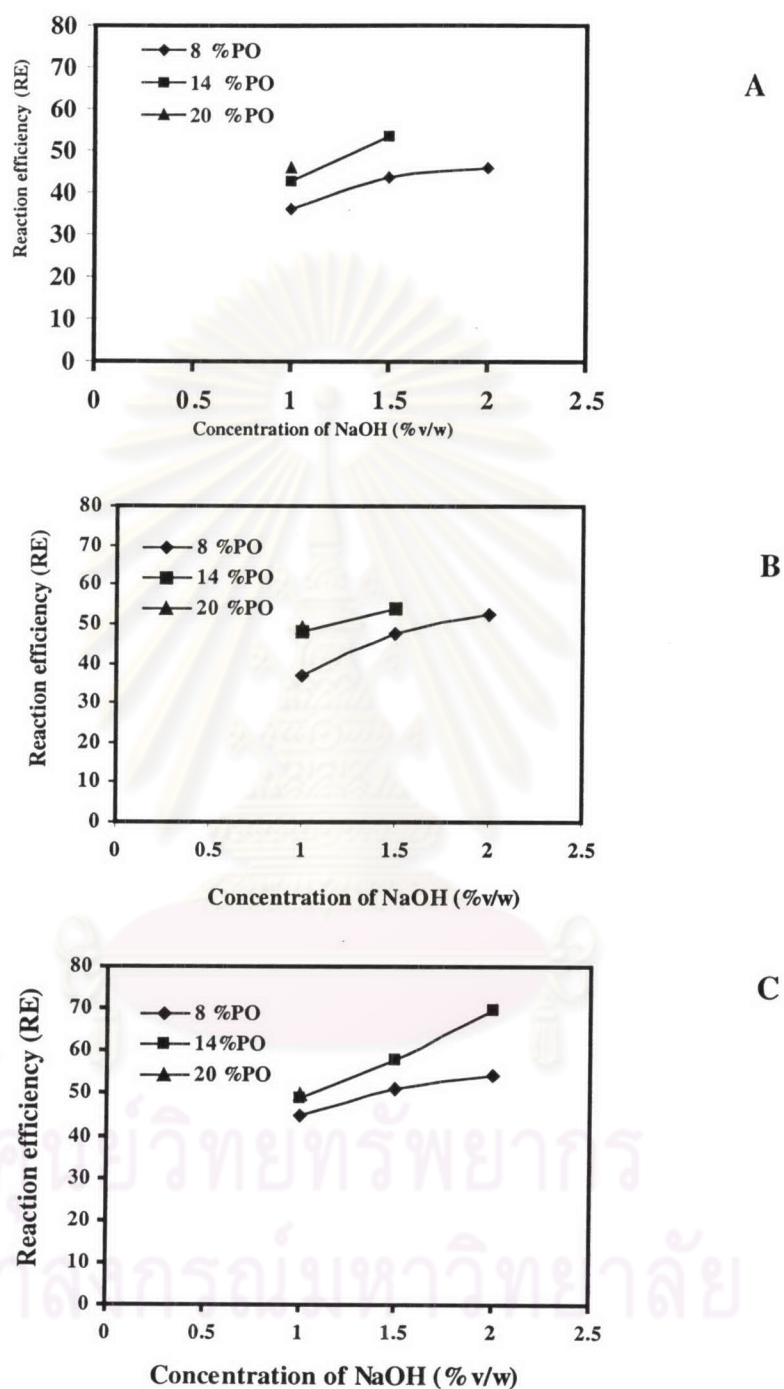


Figure 4.9 Reaction efficiency of hydroxypropylated tapioca starch depending on sodium hydroxide and propylene oxide at certain sodium sulfate concentration A) 10% w/w B) 15%w/w and C) 20% w/w based on the dry starch weight.

Figure 4.9 presents the effect of sodium hydroxide, sodium sulfate and propylene oxide concentration had on the RE of hydroxypropylation tapioca starch. It was obviously found that the RE was higher when the concentration of sodium hydroxide, sodium sulfate and propylene oxide were increased. However limitation of each parameters was needed for preparing the high RE. With higher propylene oxide, higher RE was obtained. At 14% propylene oxide, the RE was nearly the same as 20% propylene oxide by using 1% sodium hydroxide. This can be explained that at 20% propylene oxide, the more hydroxypropyl groups were, the more starch slurry formed very thick slurry. Thus, the RE obtained was decreased. That means the amount of sodium sulfate was not high enough to prevent the occurrence of gelatinized starch. These results give different the trend of DS since the DS is defined as the number of substituted hydroxypropyl groups upon the starch. While the RE is defined as the percentage of propylene oxide upon starch as compared with starting propylene oxide. During the reaction, the propylene oxide can react with both starch nucleophile and sodium hydroxide to form both hydroxypropylated starch and by-product. Therefore, the RE was decreased. At high concentration of sodium sulfate, the gelatinization could decreasingly occur. Thus, the RE would be increased. It indicated that salt could reduce granule swelling. The highest DS and RE obtained were 0.2725 and 69.7%, respectively for hydroxypropylated tapioca starch in water.

4.5 Experimental Factorial Design for Starch Hydroxypropylation in Aqueous Alcohol

Another means to avoid the gelatinization is the preparation of hydroxypropylated starch in aqueous alcohol medium such as ethanol, isopropanol and methanol *etc.* The hydroxypropylated starch had little or no tendency to swell in these media (20, 22). Ethanol was chosen, as medium for hydroxypropylation of tapioca starch due to its less hazardous than other alcohols. In this research the hydroxypropylated tapioca starch was synthesized in aqueous ethanol by using 3^3 factorial designs, the same method as the reaction in water, but the variables are the concentration of sodium hydroxide, propylene oxide and the percentage of ethanol in water. In this case, no sodium sulfate was added since ethanol could not swell the starch molecule. All the reactions were carried out at 40°C for 24 hours. The DS and RE were determined

by $^1\text{H-NMR}$ technique as shown in Appendix B and Appendix C, respectively. The results were presented in Table 4.7

Table 4.7 Hydroxypropylation of tapioca starch in aqueous ethanol.

Experiment No.	NaOH (% v/w)	PO (% v/w)	Ethanol:H ₂ O	DS	RE (%)
28	1.0	8	30:70	0.0426 ± 0.0029	19.07
29	1.0	14	30:70	0.1013 ± 0.0022	25.91
30	1.0	20	30:70	0.1482 ± 0.0048	26.53
31	1.5	8	30:70	0.0667 ± 0.0015	29.86
32	1.5	14	30:70	0.1289 ± 0.0021	32.97
33*	1.5	20	30:70	-	-
34	2.0	8	30:70	0.0694 ± 0.0053	31.07
35*	2.0	14	30:70	-	-
36*	2.0	20	30:70	-	-
37	1.0	8	50:50	0.0397 ± 0.0063	17.77
38	1.0	14	50:50	0.0842 ± 0.0013	21.53
39	1.0	20	50:50	0.1156 ± 0.0044	20.69
40	1.5	8	50:50	0.0532 ± 0.0067	23.81
41	1.5	14	50:50	0.0975 ± 0.0052	24.94
42	1.5	20	50:50	0.1568 ± 0.0038	28.39
43	2.0	8	50:50	0.0580 ± 0.0084	25.96
44	2.0	14	50:50	0.1203 ± 0.0054	30.77
45	2.0	20	50:50	0.1899 ± 0.0034	34.00
46	1.0	8	70:30	0.0374 ± 0.0027	16.74
47	1.0	14	70:30	0.0566 ± 0.0035	14.48
48	1.0	20	70:30	0.0935 ± 0.0052	16.74
49	1.5	8	70:30	0.0422 ± 0.0062	18.89
50	1.5	14	70:30	0.0773 ± 0.0022	19.77
51	1.5	20	70:30	0.1323 ± 0.0087	23.68
52	2.0	8	70:30	0.0510 ± 0.0039	22.83
53	2.0	14	70:30	0.1037 ± 0.0036	26.52
54	2.0	20	70:30	0.1603 ± 0.0044	28.70

Note: * gelatinization starch was observed

From Table 4.7 it obviously indicated most experiments were no gel formation appeared during the reactions. It indicated that ethanol can be prevented gelatinization of starch but the DS and RE were lower. Other reports described the similar observation on the hydroxypropylation of different types of starch (24, 30). These observations explained that the structure of ethanol was larger and more hindrances than water molecules. As the result, water molecule could not approach the starch molecule. Thus the gelatinization could not occur. Hence, the DS and RE were lower than the results from the hydroxypropylation of tapioca starch in water. It is not fully understood. Possible reasons advanced for this are sodium hydroxide and propylene oxide exhibited lower dispersibility in ethanol solution than water. Thus sodium hydroxide and propylene oxide were difficulty penetrated to react with starch granule. Therefore, the DS and RE was decreased. The reaction in 50% ethanol in water provided higher DS and RE, 0.1899 and 34% yield, respectively when propylene oxide 20% and sodium hydroxide 2% based on the dry starch weight were used.

4.6 High Degree of Substitution of Hydroxypropylated Tapioca Starch

Propylene oxide is a versatile chemical intermediate and its boiling point was low (bp. = 34.23°C). Since the reaction was operated at room temperature which was around its boiling temperature, propylene oxide could easily evaporate during the addition period. To keep propylene oxide cool, the dropping funnel with a water jacket was used for adding propylene oxide dropwise into the starch solution. The reaction condition for this experiment was the same as experiment No. 26 in Table 4.6. The reaction was repeated three times. The degree of substitution was determined at least three times for each sample. Then the average value of degree of substitution and reaction efficiency were taken as shown in Table 4.8.

Table 4.8 High degree of substitution of hydroxypropylated tapioca starch in water

Reaction No.	Degree of substitution	Average degree of substitution	Average Reaction efficiency (%)
1	0.2847	0.2797	71.50
2	0.2747		
3	0.2796		

From Table 4.8 it was found that the degree of all three trials was very close to each other. The average degree of substitution of all three trials was 0.2797. Accordingly, the reaction efficiency was 71.5% yield. The degree of substitution and the reaction efficiency were higher than those reported earlier were little increased as compared the best condition of the reaction in water that gave highest degree of substitution. Although, the DS and RE were not greatly higher but it improved that this equipment design can be increase DS and RE. It can be concluded that besides reactant concentrations and container for propylene oxide are parameter influencing the DS and RE. The highest reaction efficiency obtained was 71.5%. The amount of propylene oxide only lost about 30%. The hydroxypropylated tapioca starch having DS greater than 0.25 was not found. It indicated that this research could be developed the reaction condition that provided the highest degree of substitution and reaction efficiency, 0.2797 and 71.5%, respectively. Figure 4.10 illustrates $^1\text{H-NMR}$ spectrum of hydroxypropylated starch with highest degree of substitution, 0.2797 as compared with native tapioca starch.

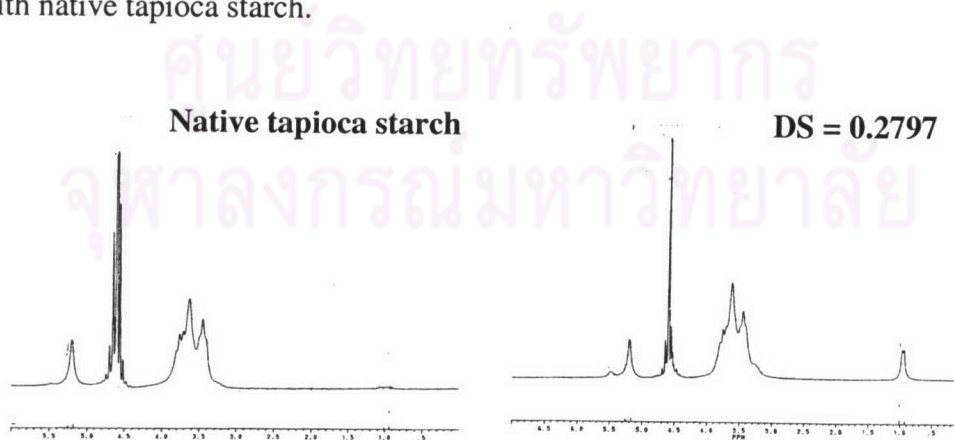


Figure 4.10 The $^1\text{H-NMR}$ spectrum of native tapioca starch and high degree of substitution (DS = 0.2797).

The signal in the $^1\text{H-NMR}$ spectrum of high degree of substitution is a new signal appeared at $\delta = 1.1$ ppm. This signal did not appear in the $^1\text{H-NMR}$ spectrum of native tapioca starch. The signal at $\delta = 1.1$ attributed to methyl protons in the hydroxypropyl groups on starch. The increase of degree of substitution, also the peak intensity of this signal was higher. The peak intensity depends on the hydroxypropyl group on the starch.

4.7 Examination of Physical Properties of Hydroxypropylated Tapioca Starch

4.7.1 Gelatinization Temperature

When a starch is heated with water the granules lose their birefringence, then as the temperature rises they swell to several times their original size, the viscosity begins to increase and the appearance changes from that of a cloudy suspension to a translucent paste. These changes occur in temperature ranges, which are the characteristic of the type of starch and the nature and extent of its modification. This makes observations of the temperatures at which they occur of value in the differentiation and identification of starch (7, 13). The pasting property of starch is shown in Figure 4.11.

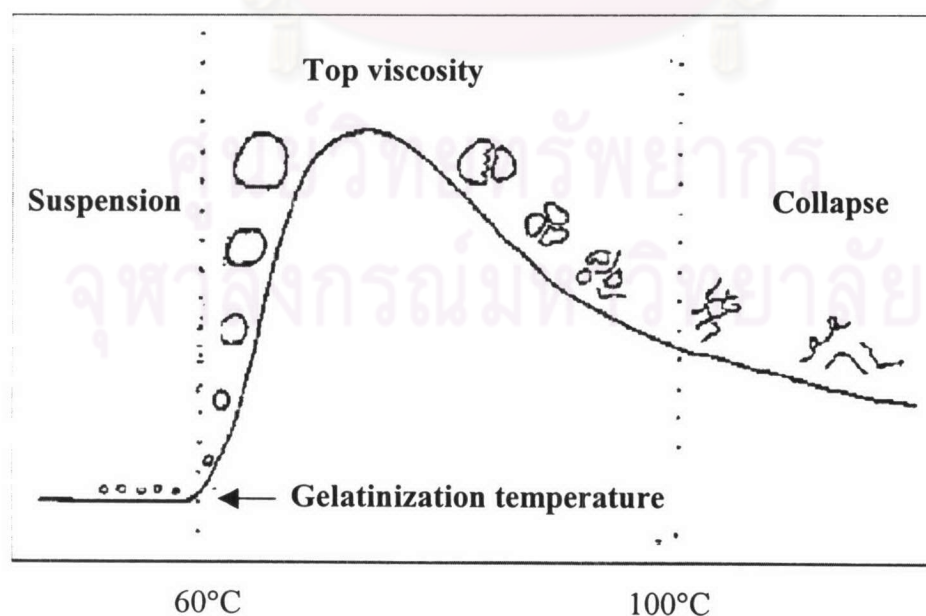


Figure 4.11 The pasting property of starch

The paste curves can be explained that when a starch is heated in hot water, the starch molecules vibrate more vigorously, breaking intermolecular bonds and allowing their hydrogen bonding sites to engage more water molecules. The granule size and viscosity begins to increase. Continued heating in the presence of abundant water results in a complete loss of crystallinity. The point at which birefringence first disappears is regarded as gelatinization point or gelatinization temperature. Highly swollen granules and peak viscosity, with continued heating, the hydrogen bonds holding the granule together rupture, the swollen granules collapse and disintegrate, and the viscosity drops (7, 13).

In general, the gelatinization of native tapioca starch ranges from 59-70 °C depending on the size of granule and the composition of amylose and amylopectin. Different starches exhibit different gelatinization temperature. The gelatinization range refers to the temperature range over which all the starch granules are fully swollen. The gelatinization of starch was observed by increased viscosity of the starch slurry. As the starch undergoes hydroxypropyl substitution, the gelatinization temperature is decreased. Therefore, another indirect method for relatively determining degree of substitution of hydroxypropylated tapioca starch is the starch-gelatinization temperature drop. The Brabender Viscoamylograph is a convenient instrument to use for determining the decrease of gelatinization temperature (7).

Table 4.9 Effect of substitution on gelatinization temperature of hydroxypropylated tapioca starch

Samples	Gelatinization temperature (°C)	Peak viscosity (BU)	Viscosity at 95°C (BU)	Viscosity after holding at 95°C for 30 min (BU)	Viscosity after cooling to 50°C (BU)
Native tapioca	69.5	535	265	230	265
DS = 0.0426	63.5	600	340	265	320
DS = 0.0806	59.5	720	400	330	420
DS = 0.1384	57.8	730	480	375	460
DS = 0.2797	54.5	660	320	295	380

Table 4.9 showed the Brabender characteristics of the hydroxypropylated tapioca starches having degree of substitution of 0.0426, 0.0806, 0.1384 and 0.2797 compared with native tapioca starch. It was observed that the gelatinization temperature of hydroxypropylated was lower and the peak viscosity was higher. Hydroxypropylated tapioca starch with DS 0.0426, 0.0806, 0.1384 and 0.2797, the gelatinization temperature was lowered 63.5, 59.5, 57.8 and 54.5, respectively. This can be easily explained by the fact that the hydroxypropyl group is hydrophilic in nature, which facilitates the penetration of water molecules inside the starch granule by weakening the internal bond holding the granule together. Then it increases the ease of hydration of the starch granule. From these results it can be concluded that the hydroxypropylation process was more effective for the starches with high swelling power. The similar observation was reported by other researchers (17, 19, 24).

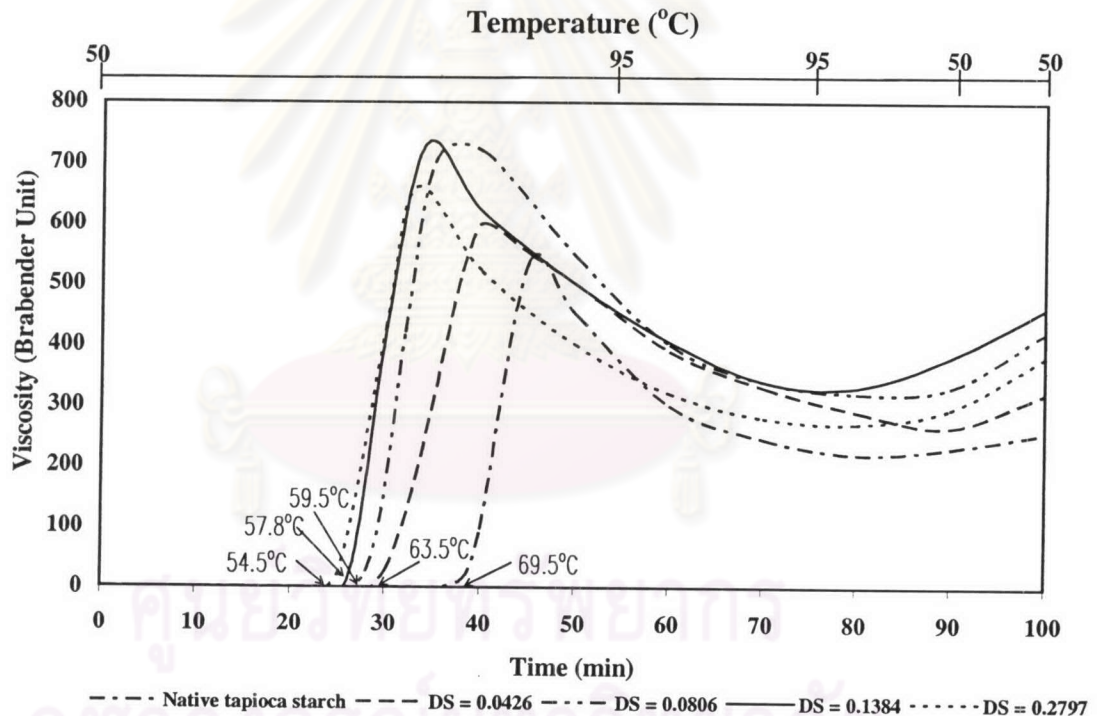


Figure 4.12 Brabender Viscograph of tapioca starch and hydroxypropylated tapioca starch with various DS (DS = 0.0426, 0.0806, 0.1384 and 0.2797).

From Table 4.9 and Figure 4.12, it also indicated that the gel from hydroxypropylated starch was highly viscous as compared to native tapioca starch. This was probably depended on the hydroxypropyl group in modified starch. The hydroxypropyl group caused loosening of the network and allowing additional water to enter starch granule. Thus the increase in hydration volume resulted in higher viscosity. In this case, cohesive forces in the starch granular structure still remained after hydroxypropylated groups slightly occurred. But hydroxypropylated tapioca starch of high degree of substitution 0.2797 showed remarkable decrease in peak viscosity as compared to the other hydroxypropylated starches probably caused by very weak cohesive force in the granular structure because the amount of hydroxypropyl groups was higher than previous reactions. The similar observation had been reported by Pal (19).

As heating was continued to 95°C, the starch granules tended to rupture, collapsed and fragment released the smaller molecules of the starch chain. The viscosity was dropped. When the gelatinized starch was cooled to 50°C, the viscous tended to rise, caused by molecular association from amylose chains. It formed a network that increased the viscosity (16).

Consequently, the gelatinization temperature of hydroxypropylated tapioca starch was dropped. The peak viscosity increased because of hydrophilic nature of hydroxypropyl groups. Therefore, the Brabender Viscoamylograph was the one of all methods to use for determining degree of substitution of hydroxypropylated tapioca starch.

4.7.2 Moisture

The moisture content of a starch is of economic importance as purchaser does not wish to pay for an excessive amount of water and also the storage properties of the starch will be dependent upon moisture content. A high moisture content will permit the growth of moulds and other microorganisms and will affect its free-flowing properties by balling. The normal moisture content varies from starch to starch. For tapioca starch, the moisture content is range from 10-13% (13).

The moisture of hydroxypropylated tapioca starches was measured by Sartorius Moisture Analyzer. Both hydroxypropylated tapioca starch obtained from both reaction in water and aqueous alcohol contained moisture 10-14% by dry starch weight (Appendix I and Appendix J). Therefore, the hydroxypropyl groups incorporated into tapioca starch did not increase moisture absorption, which was about the same as the native tapioca starch.

4.7.3 Granule Morphology of the Hydroxypropylated Starch

In general, the granules of tapioca starch are range from 5 μm to 35 μm . In this study, the native tapioca starch has the granules in the range of 5-15 μm as shown in Figure 4.13.

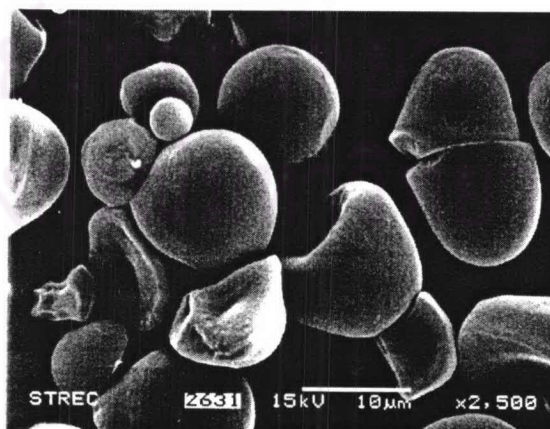


Figure 4.13 Scanning electron micrograph of native tapioca starch

The granules appear round with a flat surface on one side containing a conical pit which extends to a well-defined eccentric hilum. Some granules are very nearly circular. The surfaces appeared to be smooth. Figure 4.14 illustrates the granule morphology of hydroxypropylated tapioca starch having degree of substitution of 0.0426, 0.0999, 0.1384 and 0.2797.

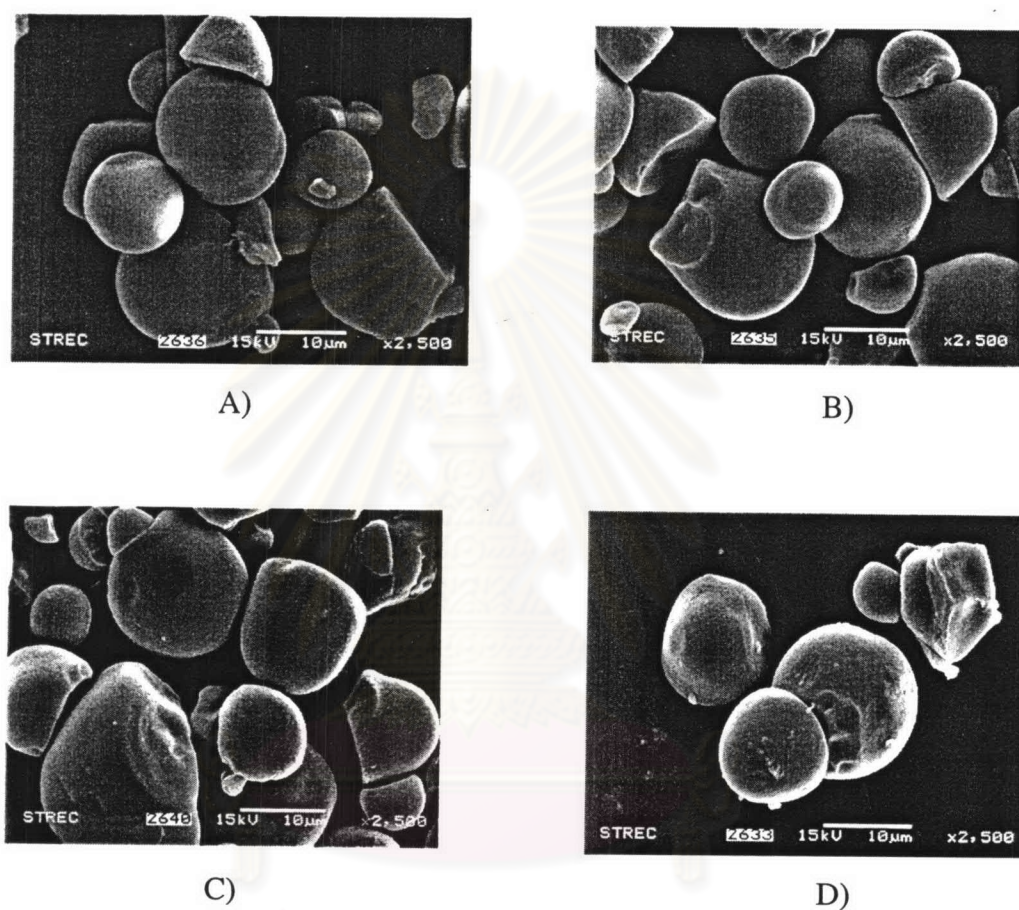


Figure 4.14 Scanning electron micrographs of hydroxypropylated tapioca starch:

A) DS = 0.0426

B) DS = 0.0999

C) DS = 0.1384

D) DS = 0.2797

Although different conditions of hydroxypropylation were used, it was obvious that these did not affect the size, shape and the granule surface of the starch. That means the starch granules were not damaged by hydroxypropylation under these conditions.