# CHAPTER IV

FACTORS INFLUENCING PHYSICO-CHEMICAL PROPERTIES OF CARBOXYMETHYL TAPIOCA STARCH.
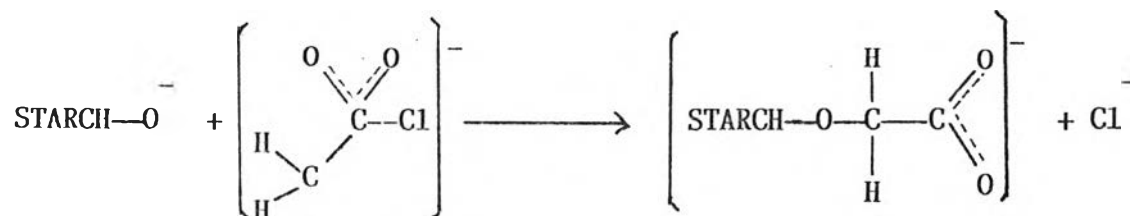
The production of water-soluble carboxymethyl starches in water miscible organic agent was established by Filbert (1952). Monochloroacetic acid or its alkali salts thereof was used as an etherifying agent. The finely divided starch containing the moisture usually present in the commercial product may be used directly. The alkali was introduced in the form of an aqueous solution. Good results could be obtained by an using alcohol of a lower aliphatic group. A reaction operated at the temperature in the range of 50 $^{\circ}$C to boiling point temperature of alcohol. He found that too low an alcohol/water ratio caused the formation of a lumpy mass which could not be conveniently purified by slurring with aqueous alcohol. As the molecular weight of the alcohol increased, the alcohol/water ratio for the process also had to be increased. They suggested the relationship between alcohol/water ratios and number of carbon atom in alcohol used was alcohol/water ratio = 1.75 $\{1+0.5\ (N-1)\}$ where N is the number of carbon atoms in the alcohol. in equation

The mechanism of the reaction of carboxymethyl substitution

Although the α-haloacetate can undergo a unimolecular displacement of the halogen, probably by way of an intermediate α-lactone, in the presence of a strong base the reaction mechanism is undoubtedly $S_N 2$.
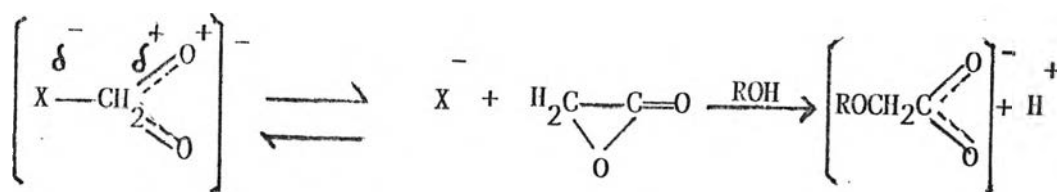
The etherification of starch with sodium chloroacetate in aqueous sodium hydroxide must be taken place according to the following equation (Roberts, 1965).

$$\text{STARCH---O}^{-} \quad + \quad \left[\begin{array}{c} O \diagdown \diagup O \\ C\text{---Cl} \\ H \diagdown C \\ H \end{array} \right]^{-} \quad \longrightarrow \quad \left[\begin{array}{c} H \\ | \\ \text{STARCH---O---C---C} \diagup O \\ | \diagdown O \\ H \end{array} \right]^{-} \quad + \quad \text{Cl}^{-}$$

The substituent groups of a partially substituted starch derivative are distributed among the three hydroxyl groups (C-2, C-3 and C-6) of anhydroglucose unit of starch molecule. The distribution is determined by the relative reactivity of hydroxyl groups and the nature of the substitution reaction. The carboxymethyl starches and cationic starches are predominantly attacked to the C-2 of anhydroglucose units. The remaining substituents are located mainly at the C-6 position with only negligible substitution at C-3 (Product information written by Swinkle).

Purpose of the study

The previous studies in chapter III, showed that carboxymethyl tapioca starch exhibited the best disintegrant characteristics. Hence, the objective of the study in this chapter is to investigate the factors influencing the physico-chemical properties of carboxymethyl tapioca starch. A factorial design study was used to evaluate these factors.

Materials and Methods

Materials

Tapioca starch   (Thai wah, CO., Ltd., Thailand)

Chloracetic acid   (Sigma Chemical CO., USA)

Methanol   (BDH Lab., England)

Sodium hydroxide (Eka Nobel, Sweden)

Acetic acid   (Riedel-de Hain, Germay)

Hydrochloric acid (BDH Lab., England)

Silver nitrate   (E Merck, Germany)

Ammonium thiocyanate (Fluka Chemical, Switzerland)

Sodium chloride   (E Merck, Germany)

Methods

1. Determination of physical property of tapioca starch

1.1 Moisture Content

The moisture content of starch was determined by the Ohaus moisture determination balance (model 6100H, serial No. 026733), the heater control knob was set to 6.5 watts with drying time of 15 minutes. The mean of three determinations was calculated.

1.2 Determination of destructible acidity(Radley, 1976).

The starch concentration of 5% were stirred to the suspension, and then its pH was determined electrometrically(see Appendix 30). The mean of three determination was calculated.

1.3 Determination of Extractable acidity

A sample was ground and 10.0 g of it was stirred in 100.00 ml of distilled water for 30 minutes, gravity filtered and titrated with 0.1 N NaOH against phenolhthalein indicator (Radley, 1976). The mean of three determinations was calculated.

1.4 Determination of the paste acidity

Ten grams of sample was suspended in 300.00 ml of water and the suspension was brought to boil on a hot plate, with stirring ocassionally, and allowed to boil for 10 minutes. The sample was titrated immediately with 0.1 N NaOH against phenolphthalein indicator (Radley,1976). The mean of three determinations was calculated.

1.5 Total ash determination

The total ash content was determined by modified USP method. 500 mg of sample was accurately weighed in a tarred crucible and incinerated in muffle furnace at 650 °C until free from carbon and then the weight of the ash was determined. The mean of three determinations was calculated.

1.6 Total fat determinations

The total fat was determined by fat extraction analyzer, Soxtec system HT, model 1043, Tecator, Sweden.

1.7 Determination of protein content.

The amount of protein content in a sample of starch was estimated by applying a factor to the nitrogen content as determined by Kjeldahl digestion method on Kjetec KD-02, Tecator, Sweden.

2. Factorial design study

It was reported by other workers that the reaction of carboxymethylation of starches was able to take place at room temperature if allowed to stand for one day. Elevating the temperature of the reaction mixture to reduce the prolonged reaction period resulted in the formation of a lumpy product difficult to purify (Filbert, 1952). A highly satisfactory results can be obtained by a more prolonged operating periods, 30-60 minutes, at the temperature as low as 50 °C. The reaction took place in the presence of sodium hydroxide and pH of the reaction was about 11.0.

To evaluate the effects of the factors influencing the carboxymethylation of tapioca starch, the factorial design with two-level and three factors was used to study (Armstrong and James, 1990). The factors were times (A), temperatures (B) and amounts of 50% NaOH used (C). The responses were degree of substitutions. The factorial study of carboxymethyl tapioca starch was shown as the following :

	Low level	High level
Factors : Times (A)	30 min.	60 min.
: Temperatures (B)	40 $^{\circ}$C	60 $^{\circ}$C
: 50 % NaOH (C)	10% w/w	16% w/w

Response : Degree of substitution

Levels : Low (−)

High (+)

Trials : $(2)^3$

Factors Combination	Times	Temperatures	Alkali
(1)	−	−	−
a	+	−	−
b	−	+	−
ab	+	+	−
c	−	−	+
ac	+	−	+
bc	−	+	+
abc	+	+	+

2.1 Preparation of carboxymethyl tapioca starch for factorial study.

Weighed 508.0 g of methanol and 17.5 g of monochloroacetic acid, mixed thoroughly, and heated to the temperature to be studied, then 218.0 g of finely divided tapioca starch were added with continuous mixing, followed by 50% NaOH aqueous solution in the amounts to be studied. The reaction was held at the temperature to be studied for various time intervals with maintenance of good agitation, and then neutralized with acetic acid. After removal of the mother liquor, the product was washed several times with 80% methanol until free of chloride and finally with 100% methanol. Dry the product in the hot air oven at 55° C overnight to evaporate the trace solvent. The dried product was screened through a 80 mesh-sieve and then kept in an air tight container.

3. Evaluation of physical properties.

3.1 Chemical evaluations.

3.1.1 Determination of degree of substitution of carboxymethyl starch by determination of carboxyl contents (Smith, 1967).

One gram of finely ground sample was weighed accurately, slurred in 25.00 ml of 2N HCl in 70% methanol. The sample was recovered by filtration and washed with 70% methanol (v/v) until washing are free of chloride as shown by silver nitrate test. The demineralized sample was transferred quantitatively to a beaker and dispersed in 300.00 ml of distilled water. The dispersion was heated in a steam bath and stirred continuously until starch gelatinized, heating was continued for about 15 minutes to insure complete gelatinization. The paste sample was titrated hot with 0.1 N NaOH solution to a phenolphthalein end point.

The unmodified original starch should be titrated to provide as a blank value. A percentage of carboxyl content and degree of substitution(DS) were calculated by :

Percent carboxyl = [(sample titer - blank titer) in ml x alkali normality x

0.045 x100]/sample weight in gram.

$$DS = \frac{(220)}{100 \times 45} \times percent\ carboxyl\ content$$

3.1.2   Determination of degree of substitutions of carboxymethyl starch using modified USP method (USPXXII).

Transfer about one gram of sample, accurately weighed, to a glass-stopped, 500-ml conical flask, add 300 ml of sodium chloride solution (1 in 10), then add 25.00 ml of 0.1 N NaOH. Insert the stopper, and allow to stand for five minutes with intermittent shaking.   Add   five drops of m-cresol purple TS and add 15.00 ml of 0.1 N HCl from burette.   Insert in the stopper and shake. If the solution is purple, add 0.1 N HCl in 1-ml portion until solution becomes yellow, shaking after each addition. Titrate with 0.1NaOH to purple end-point. Calculate the net number of meq (M) of the base required to neutralized 1.0 g of carboxymethyl starch on dried basis. Determine the percentage of residue on ignition(C) of sample as the total ash determination method. Calculate the degree of acid carboxymethyl substitution (A) by:

$$A = \frac{1150\ M}{(7120 - 412\ M - 80C)}$$

Calculate the degree of sodium carboxymethyl substitution (S) by

$$S \ = \ \frac{(162+58A)}{(7102-80C)}$$

The degree of substitution is the sum of A+S

3.1.3 Determinations of sodium chloride contents

The sodium chloride contents were conducted using USP method (USP XXII).

Weighed accurately about 1.0 g of sample, transferred to a conical flask. Add 20.00 ml of 80 % alcohol, stirred for 10 minutes and filtered. Repeat the extraction until chloride has been completely extracted as shown by a test with silver nitrate TS. Evaporate the combined filtrate and dry the residue at 105 °C to constant weight. Transferred 150.0 mg of residue to 200 ml volumetric flask with the aid of water. Add 5.00 ml of nitric acid and 40.00 ml of 0.1 N $AgNO_3$, mix and dilute with water to volume. Allow to stand in the dark for 30 minutes and then filter. To 100.00 ml of filtrate, add 5.00 ml of ferric ammonium sulfate TS and titrate with 0.1 N ammonium thiocyanate. Perform a blank determination, and calculate the sodium chloride content. Each ml of 0.1 N $AgNO_3$ equivalent to 5.844 mg of NaCl in 100.00 ml and to 11.66 mg of NaCl in amount of sodium starch glycolate weight

3.2 Physical property evaluations

3.2.1 Water uptakeThe amount of water uptake of modified tapioca starch was determined by modified Nogami's apparatus (Nogami, et al., 1969). The apparatus is demonstrated in Figure 14 and Figure 15.
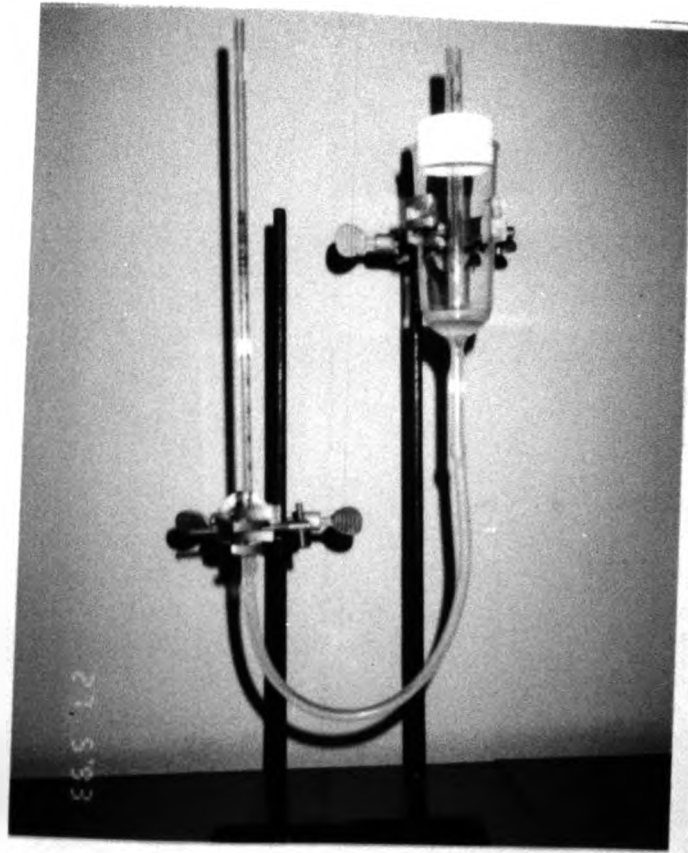
Figure 14 Apparatus for Determination of Water
Uptake of Disintegrant Powders.

Figure 15 Apparatus for Determination of Water Uptake
of Tablets.

A sample of 100.00 mg of each starch or modified starch was placed in the sample tube and the volume of powder and liquid taken up into the sample were observed simultaneously at pre-determined time intervals. A distilled water was used as test media. The experiment was performed at a constant temperature of $37 \pm 1^{\circ}C$. The mean of three determinations was calculated.

3.2.2 Bulk swelling

During the water uptake measurement, the swelling of starch and modified starch powders were studied. At the constant volume of water uptake of sample the bulk swelling was determined. The bulk swelling of sample (V_b) was estimated as a percentage of original volume by following the equation:

$$V_b = [(h_t - h_0)100]/h_0$$

where h_0 is the original height of powder bed and h_t is the height of powder bed at time "t" (Rudnic, et al.,1982). The mean of three determinations was calculated.

3.2.3 Sedimentation Volume.

The sedimentation volume was determined by using cylindrical method. Five grams of sample was suspended in distilled water and transferred to a 100-ml graduated cylinder with the aid of water. Then the volume was adjusted to 100 ml with water. The cylinder was stoppered and inverted for three times. It was allowed to stand at 37 °C for 24 hours and the sediments was measured. The sedimentation volume was calculated in ml per gram. The result was the mean of two determinations.

3.2.4 Hydration capacity

Method of hydration capacity measurement was modified from the procedure described by Kornblum and Stoopak (1973). A sample of 0.1 g was transferred to 10 ml centrifuge tube and tarred. Ten milliliters of distilled water was added. The tube was stoppered and shaked vigorously. It was allowed to stand for 10 minutes and then was centrifuged at 2535 gravities for 15 minutes (Labofuge 1, France). The supernatan' was decanted and the tube was inverted to drain. The tube contained wet sediment waw accurately weighed. Hydration capacity could be calculated as follows :

Hydration capacity = $\dfrac{(Wt.\ of\ tube\ +\ sediment) - (Wt.\ of\ tube)}{Samle\ weight\ on\ dry\ basis}$

3.2.5 Cold water soluble fraction

Cold water soluble fraction was determined from the dry matter content of the clear upper layer of a 1% aqueous dispersion at 37 °C after centrifugation at 2350 rpm for 15 minutes (Labofuge 1, France).

3.2.6 Viscosity

The viscosity was determined in one percent aqueous dispersion using cone and plate viscometer with cone No. CP41 and a speed of 100 rpm (Brookfield Engineering Laboratories Inc., USA).

3.2.7 Moisture determination.

Ten gram of each sample was accurately weighed on a pan of Ohaus moisture determination balance. It was dried at dial setting 6.5 watts for 15 minutes until constant weight was attained. The percentage of moisture loss was calculated.

3.2.8 pH determination.

The starch sample concentration of 5% were stirred to suspension, and the pH of the suspension was determined electrometrically.

3.2.9 Bulk density and tapped density.

Bulk density was performed by pouring 3.0 g of smple into 10-ml graduated cylinder and the volume was read in ml.

Tapped density was performed by dropping the graduated cylinder onto a glass surface from a height of 2 centimeters for 100 times. The resulted volume was determined.

Results and Discussion

1. Physico-chemical specification of native tapioca starch

The results of determination of the specification of tapioca starch used for modification are shown in Table 6.

Table 6 Specification of Experimental Tapioca Starch

Properties	Results
Moisture content (%)	12.96 (0.15)
Destructible acidity (pH)	4.73 (0.02)
Extractable acidity (meq.)	0.02 (0.00)
Paste acidity (meq.)	0.07 (0.01)
Total ash (%)	0.15 (0.01)
Total fat (%)	Trace
Protein content (% nitrogen)	0.16 (0.06)
Viscosity (centipoise)	1.35 (0.00)

Standard Deviations are in parentheses.

2. Factorial design studies

2.1 Determination of degree of substitutions.

Degree of substitution of carboxymethyl tapioca starch are demonstrated in Table 7

Table 7 Degree of Substitutions (DS) of Carboxymethyl Tapioca Starch (CMTS).

Types	Degree of Substitutions (DS)	
	Carboxyl method	USP method
(1)	0.0028 (0.0005)	0.0029 (0.0007)
a	0.0112 (0.0001)	0.0182 (0.0007)
b	0.0353 (0.0006)	0.0278 (0.0015)
ab	0.0646 (0.0003)	0.0595 (0.0013)
c	0.0319 (0.0011)	0.0333 (0.0011)
ac	0.0416 (0.0013)	0.0437 (0.0035)
bc	0.1188 (0.0079)	0.1021 (0.0014)
abc	0.1797 (0.0003)	0.1888 (0.0023)

Standard deviations are in parentheses.

Table 7 indicated that both methods of DS determination of CMTS gave similar results.

Figure 16 exhibited that the factors : time, temperature and amount of alkali were essential for the reaction of substitution. The reaction occurred in the presence of alkali. When the amount of alkali (50% NaOH) was below 10% w/w, the reaction was not readily. While a large amount of alkali used caused a formation of gel which could not be facilitated the reaction. The reaction take place at room temperature for one day. When the temperture was elevated to accelerate the rate of reaction, the product was more soluble in cold water (Filbert, 1952). If the reaction temperature was above $60\,^{o}C$, the starch gelatinized to form a lumpy mass which could not be readily purified in the final step. The pasting temperature of tapioca starch is $60\text{-}65\,^{o}C$.
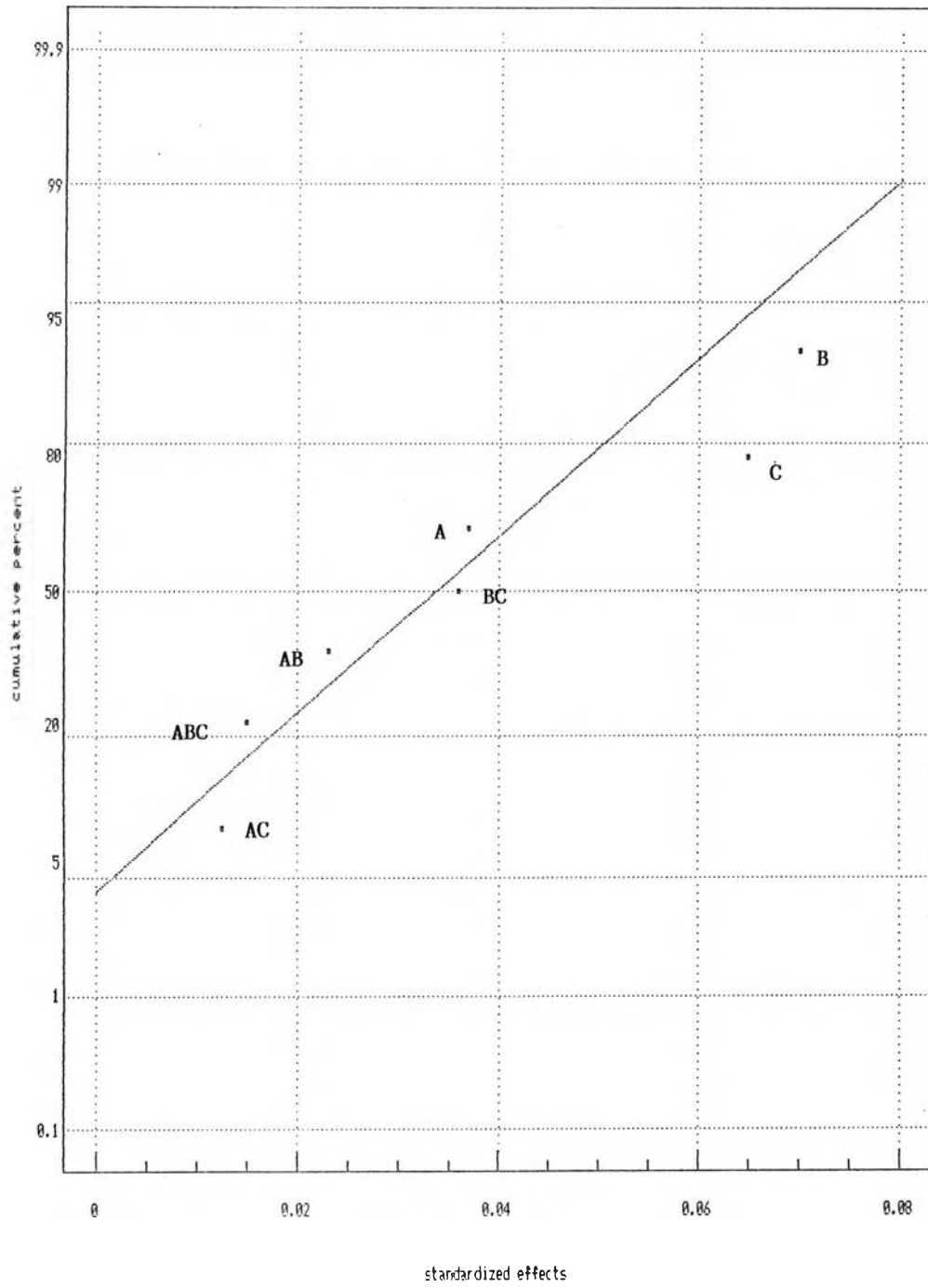
Normal Probability Plot for DS

Figure 16. Normal Probability Plot of the Estimated Effect for Degree of Substitution.

2.2 Sodium chloride contents.

The sodium chloride contents of CMTS was a byproduct of substitution reaction. The sodium chloride may affect efficiency of disintegrant. The presence of sodium chloride in carboxymethyl starch reduced both viscosity and swelling capacity (Bolhius, et al.,1984).

The soduim chloride contents in CMTS were shown of Table 8. The percent of soduim chloride contents of prepared CMTS were more closely resembled.

Table 8   Sodium Chloride Contents of CMTS

Types	% NaCl Contents
(1)	0.0064
a	0.0064
b	0.0058
ab	0.0054
c	0.0067
ac	0.0057
bc	0.0051
abc	0.0080

2.3 Water uptake

Since the purpose of modification of tapioca starch was to make a super disintegrant, the factors influencing the process of CMTS production which affected disintegrating properties have been studied. The important requirements imposed on tablet disintegrants are : high swellability, low solubility, low tendency to gelling and great ease of hydration.

Lowenthal (1972) suggested that the disintegrants that absorb about 20% water and are insoluble in water are said to be good disintegrant. Water uptake has been an important mechanism of tablet disintegrant. It is the first step of a sequence of tablet disintegration.

Water uptake of CMTS in factorial disign study were shown in table 9

Table 9 Water Uptake of CMTS.

Types	ml
(1)	0.45 (0.01)
a	0.53 (0.02)
b	0.91 (0.02)
ab	1.47 (0.03)
c	1.15 (0.00)
ac	0.74 (0.01)
bc	0.98 (0.00)
abc	0.31 (0.00)

Standard deviation are in parentheses.

It was found that increased in the DS (Table 7) increased water uptake of CMTS powder (Table 9). However, samples bc and abc showed low values of water uptake; it was due to they absorbed water and produced viscous gel barrier resulted decreasing in quantity of water uptake at the surface of powder bed.

2.4 Bulk swelling

One of the most widely accepted mechanism of tablet disintegration is swelling (Kanig and Rudnic, 1984). Bulk swelling of CMTS in factorial design study were demonstrated in Figure 17. It was shown that the bulk swelling incressed when the DS increased. The bulk swelling values of bc and abc were low, in spite of the high DS of bc and abc, the reason for this phenomena was that their solubility processes produced viscous gel barriers to retard the water uptake. In fact, the bulk swelling took place when the bulk contacted with water and absorbed enough water to swell.

2.5 Sedimentation volume.

The sedimentation volumes of CMTS in water were shown in Figure 18. The sedimentation volumes of ab, bc and abc in factorial design study were maximum since CMTS were soluble and form clear gels. The bc and abc formed semi-solid gels while the ab formed liquefied gel. The soluble gel formation occures because the carboxymethyl substitution made the starch more hydrophilic as carboxymethyl group is preferred to from hydrogen bond with water causing starch absorbed water and swelled and dissolved to form clear gel.
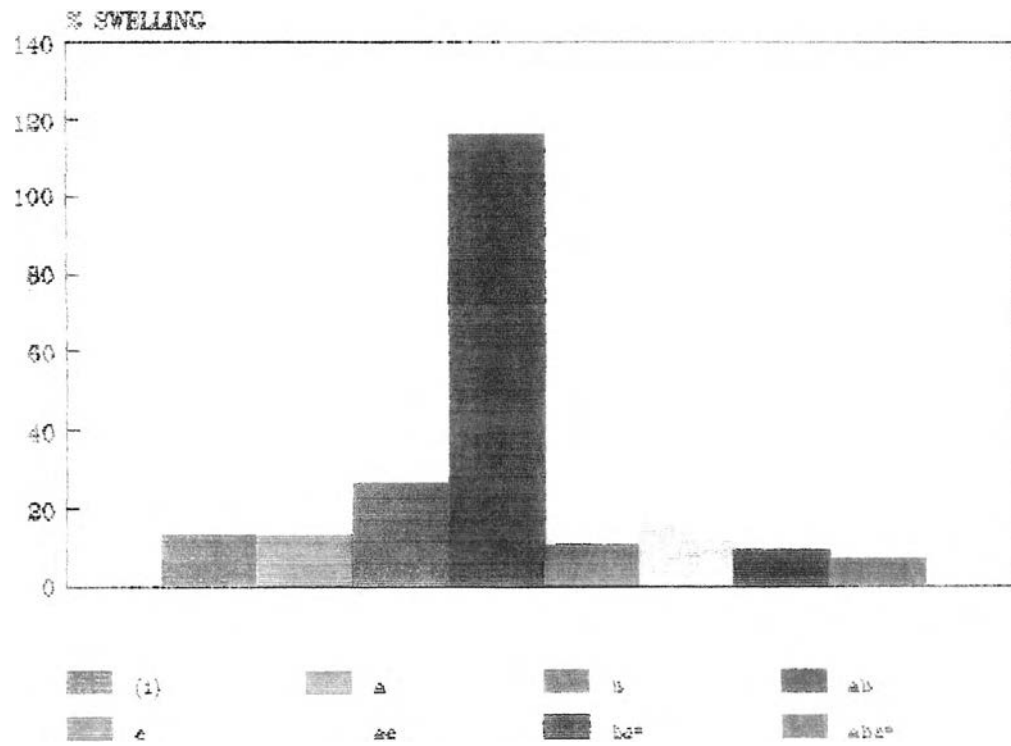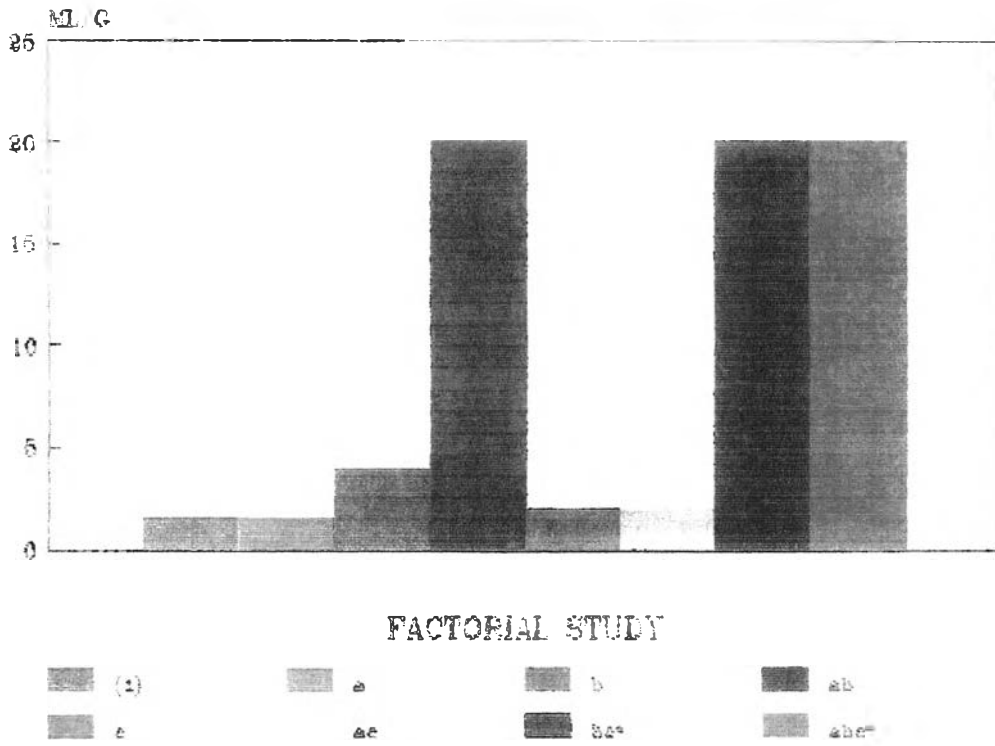
Figure 17 Bulk Swelling of CMTS.

Figure 18 Sedimentation Volumes of CMTS in Water.

The sedimentation volumes of CMTS were corresponding to DS of CMTS which was previously shown in Table7. Increases in DS increased the sedimentation volume. CMTS was soluble when its DS was at least 0.06.

The sedimentation volumes of CMTS in 0.1N HCl were illustrated in Figure 19. Sedimentation volume of CMTS at DS of 0.06 was low which differed from the result in water. This could be attributed to the mechanism of welling of carboxymethyl starch by mutual repulsion of anions caused the polymer expanded when contact to water. At the low DS, the carboxymethyl anions were interfered by cation from 0.1 N HCl . In the other hand at high DS (0.1 or above), the amounts of carboxymethyl anions were enough to overcome the effect of cation from medium, therefore the sedimentation volumes of the bc and abc were high with gel formation.

2.6 Hydration capacity

The hydration capacity of CMTS were presented in Figure 20. The good disintegrant should be great ease to hydration. The results of hydration capacity were corresponding to sedimentation volume of CMTS in water and also degree of substitutions. In addition, increases in DS increased the hydration capacities. The hydration capacity of abc was lower than ab and bc because abc was more soluble and partially dissolved out during the experiment was performed hence, the lower hydration capacity was achieved.
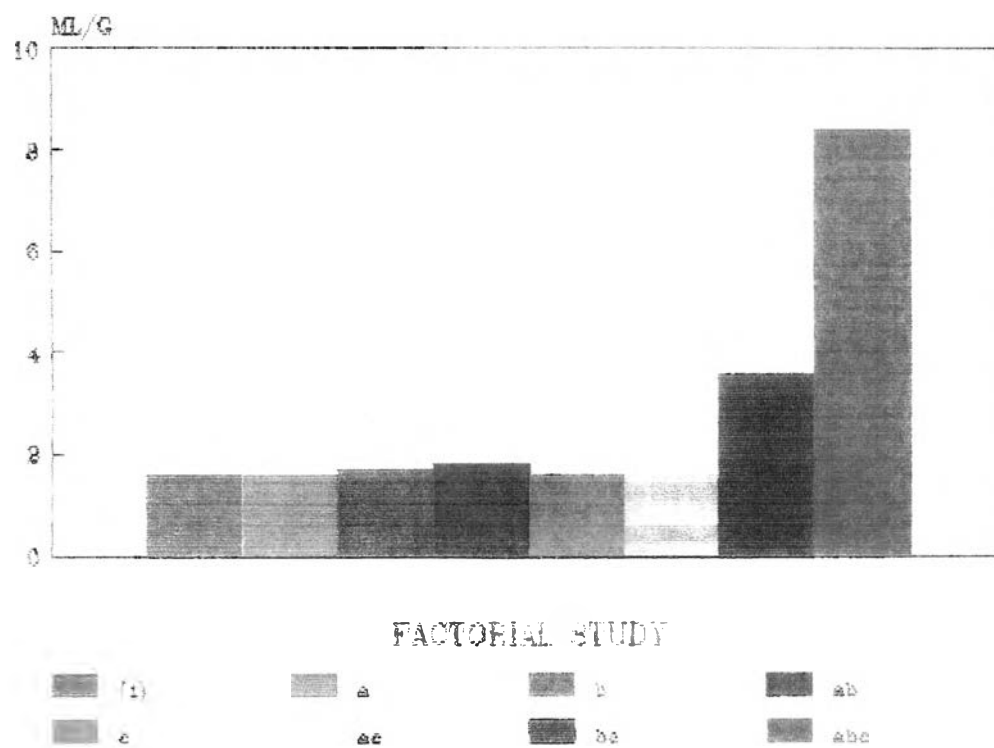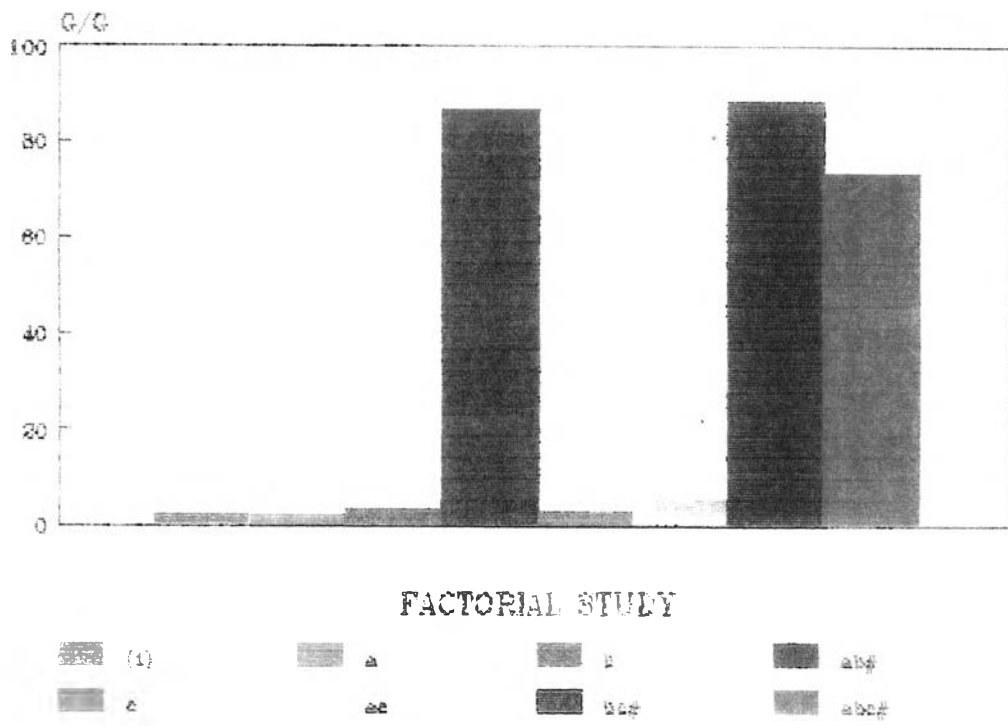
Figure 19 Sedimentation Volumes of CMTS in 0.1 N HCl.

Figure 20 Hydration Capacity of CMTS.

2.7 Cold water soluble

The cold water soluble of CMTS were illustrated in Figure 21. The cold water soluble of CMTS were corresponding to degree of substitution. Increases in DS of CMTS increased the cold water soluble due to increases in carboxymethyl anion which facilitated formation of hydrogen bond with water.

2.8 Viscosity

The viscosity of CMTS were performed using 1% aqueous suspension and the results of determination were shown in Table 10. The viscosity values were parallel to hydration capacity of CMTS. The more hydration capacity, the more viscosity values. The viscosity of ab, bc and abc were higher than the others as to they swelled and partially soluble in medium produced a viscous disperse system.
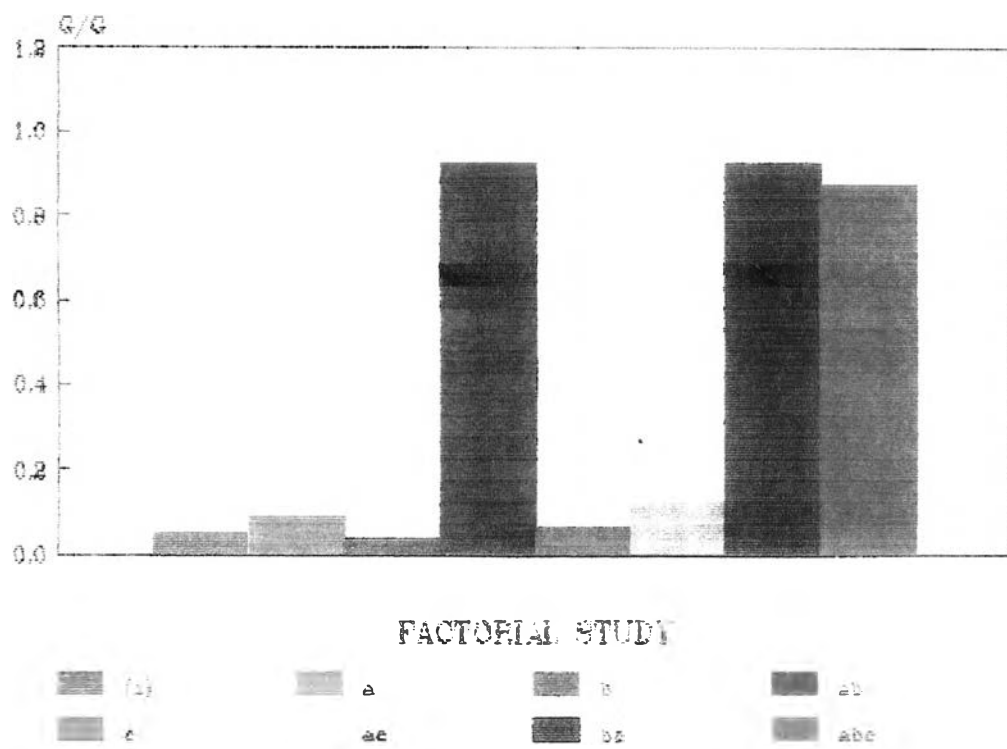
Figure 21 Cold Water Soluble Fractions of CMTS.

Table 10 Viscosity of CMTS.

Types	Viscosity (cps.)
(1)	1.3517 (0.00)
a	1.3517 (0.00)
b	1.4131 (0.08)
ab	523.3125 (1.52)
c	3.0720 (0.01)
ac	2.0582 (0.04)
bc	54.1900 (0.16)
abc	49.7049 (0.09)

Standard deviations are in parentheses.

2.9 The moisture content, pH, bulk density, tapped density were presented in table 11.

The moisture content of all CMTS derivatives are closely resembled. So are their pH values, bulk densities and tapped densities.

Table 11 Some Physical Properties of CMTS,

Types	Moisture (%)	pH	Bulk density g/ml	Tapped density g/ml
(1)	12.45 (0.07)	4.70 (0.00)	0.5133(0.00)	0.7333(0.00)
a	11.30 (0.14)	4.70 (0.00)	0.4911(0.00)	0.7250(0.00)
b	10.25 (0.21)	4.15 (0.07)	0.4956(0.00)	0.7222(0.01)
ab	9.15 (0.07)	4.10 (0.00)	0.4194(0.00)	0.7506(0.01)
c	11.60 (0.14)	4.25 (0.07)	0.5592(0.00)	0.7947(0.00)
ac	9.05 (0.07)	4.10 (0.00)	0.5041(0.00)	0.7562(0.01)
bc	10.20 (0.28)	3.95 (0.07)	0.4941(0.01)	0.7701(0.00)
abc	8.50 (0.00)	4.00 (0.00)	0.4350(0.01)	0.6619(0.00)

Standard deviatiens are in parentheses.

Conclusions

Factors which influenced on carboxymethylation of tapioca starch also affected physico-chemical properties of prepared carboxymethyl tapioca starch. There were linear relationships among the sedimentation volume, hydration capacity, cold water soluble and degree of substitution. This study emphasized on the disintegrant properties of prepared carboxymethyl tapioca starch such as water uptake and swelling, which were the main mechanisms of disintegration of tablets containing starch as disintegrant. From the factorial design studies reaction time and temperature were the important factors affected both water uptake and swelling of carboxymethyl tapioca starch. Consequently, the production of various degree of substitution of carboxymethyl tapioca starches to achieve appropriated disintegrant properties could be performed by varying reaction time and temperature.

However, the point of order about disintegrant properties of carboxymethyl tapioca starch was considered. The most important problem of using carboxymethyl tapioca starch as tablet disintegrant was its solubility produced viscous gel barrier around the granules and tablet which retarded tablet disintegration. Therefore, a further modification of carboxymethyl tapioca starch is necessary. To overcome this problem, decreasing the viscous gel barrier was performed by reducing its solubility via crosslinking with suitable crosslinking agent.