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Research Article

Preparation and Application of Carboxymethyl Yam (*Dioscorea esculenta*) Starch

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Abstract. Yam (*Dioscorea esculenta*) starch was modified by carboxymethylation. The effect of reaction parameters, amount of sodium hydroxide (NaOH), amount of sodium monochloroacetate (SMCA), and reaction time on the degree of substitution (DS) of carboxymethyl yam starch (CMS), was studied using the Box–Behnken experimental design. Physicochemical and potency to be a tablet disintegrant of CMS were evaluated. CMS with DS in the range of 0.08–0.19 were obtained. The results from regression analysis indicated that the most important factor in controlling DS was the amount of NaOH followed by SMCA content and reaction time. However, high concentration of NaOH and SMCA lowered the DS. The optimal conditions to achieve the highest DS (0.19) were found to be at molar ratios of NaOH and SMCA to anhydroglucose unit of 1.80 and 2.35, respectively, and with the reaction time of 4.8 h. The swelling power and viscosity of CMS increased with an increase in the degree of modification. CMS showed satisfying tablet disintegrant properties. The tablets containing 1.0–4.0 % CMS disintegrated faster than 5 min. Hence carboxymethyl yam starch can be used as an excellent tablet disintegrant in low concentration.

KEY WORDS: carboxymethyl yam starch; *Dioscorea esculenta*; disintegrant; optimization; yam starch.

INTRODUCTION

Modified starches have played a major role in the food and pharmaceutical industries over the past few decades. They possess unique properties not found in natural starches, which are suitable for the development of new products. Examples are solubility in unheated water, specific changes in rheological profiles, lower gelatinization temperature, less retrogradation, and greater pH stability (1). Carboxymethyl starch is a popular chemically modified starch. It is prepared by a reaction of starch (St-OH) and sodium monochloroacetate (SMCA) ($ClCH_2COO^-Na^+$) in the presence of sodium hydroxide (NaOH) (2–5). This is a two-step reaction that proceeds as

$$St - OH + NaOH \quad \leftrightarrows \quad St - O^-Na^+ + H_2O \qquad (1)$$

$$St - O^-Na^+ + ClCH_2COO^-Na^+ \rightarrow St - O - CH_2COO^-Na^+ + NaCl \qquad (2)$$

An undesired side reaction of SMCA with NaOH can also occur as

$$NaOH + ClCH_2COO^-Na^+ \quad \rightarrow \quad H - O - CH_2COO^-Na^+NaCl \qquad (3)$$

The amount of substituted carboxymethyl group is indicated by the degree of substitution (DS). The DS is defined as the average number of substituents per anhydroglucose unit (AGU). Each AGU contains three hydroxyl groups (C_2, C_3, and C_6); therefore, the DS lies between zero and three. From the studies of Volkert et al. (6) and Heinze et al. (7), substitution in the order $C_2 > C_6 > C_3$ was indicated.

Many studies have discussed the influence of reaction medium, reagent concentrations, reaction temperature, and reaction time as main factors that determine the DS of carboxymethyl starch (6,8–9). Some of these studies determined the effects of reaction parameters on the carboxymethylation of starch by changing one variable while keeping the others constant. Such an approach gives no insight into the interactions between the process variables unless a large number of combinations are carried out. A statistical approach using experimental design combined with response surface methodology can be employed to simultaneously study the influence of multiple factors on interesting responses (2,10). A Box–Behnken design (11) or an orthogonal balanced incomplete block design is an economical design for an experiment, which has three or more factors. This design is capable of fitting the second order model, which is required for optimization studies. In the Box–

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Behnken design, there are only three levels per factor, and no experiment with all factors at their extreme values is included. The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of the multidimensional cube.

The yam (*Dioscorea* sp.) is a local plant in many countries, i.e., Nigeria, Jamaica, Brazil, China, and Thailand (12–15). The nutrient content of yam tuber has been reported (13,14). The main use of yam is as food, usually consumed boiled or roasted. Yam (*Dioscorea alata*) is a food crop of economic value in southern Brazil. The yam flour has been used in bread products and snacks (12). In addition to being used as food, it is believed that the mixture of yam flour, rice flour, and some nutrients is beneficial to infant health (15). In traditional Chinese medicine, yam (*Dioscorea opposita*) tuber has been used as an important invigorant. In Thailand, the most common yam species is *Dioscorea esculenta* (14). The application of starch from this yam to the food industry and pharmaceutics has not been reported. This study was designed to modify yam starch (*D. esculenta*) by carboxymethylation. The Box–Behnken experimental design was used to investigate the influence of reaction variables and their interaction on the preparation. The tablet disintegrant properties of the modified starch were evaluated. The physicochemical properties of the modified carboxymethyl yam starch (CMS) were also examined.

MATERIALS AND METHODS

Materials

Monochloroacetic acid (Fluka, Germany) was used as an etherifying agent. Dicalcium phosphate dihydrate granular (DCP, China) and magnesium stearate (Faci Asia Pacific Pte Ltd., China) were used as a filler and a lubricant, respectively. Yam starch (*D. esculenta*) and CMS were used as disintegrants.

Extraction of Yam Starch

Yam tubers were washed, peeled, and trimmed to remove defective parts. The tubers were then sliced, diced, and blended with distilled water in a food blender. The mixture was sieved through an 80-mesh screen, and the retained solid was exhaustively rinsed on the sieve with distilled water. The filtrate was allowed to stand overnight at 15°C, the precipitate was collected, and the supernatant was discarded. Resuspension and sedimentation operations were repeated until white starch was obtained. The starch was dried at 50°C for 6 h. Finally, the dried yam flour was ground and passed through a 100-mesh sieve. Yam starch was kept in a tight light-resistant container.

Preparation of CMS

In the standard preparation, the native yam starch (10.0 g) was suspended in 2-propanol (100 mL). An aqueous sodium hydroxide solution was added. The mixture was stirred at controlled temperature (30°C) for 10 min. Sodium monochloroacetate was added and stirring was continued up to the designated time. The pH of the mixture was adjusted to about 5.0 by addition of 50% glacial acetic acid. The carboxymethyl starch was filtered and washed with aqueous ethanol. The modified starch was dried at 50°C for 6 h. The dried carboxymethyl starch was passed through a 100-mesh sieve.

Experimental Design

A three-factor, three-level Box–Behnken design was used to study the effect of reaction parameters on carboxymethylation of yam starch. The selected process variables were the following: SMCA concentration (1.443, 2.150, and 2.867 mol/mol AGU), NaOH concentration (0.5, 1.0, and 1.5 mol/mol AGU), and reaction time (2, 4, and 6 h). The experimental design is shown in Table I. The experiments

Table I. Levels for the Main Factors in the Box–Behnken Design

	Coded values			Real values					
Run no.	A	B	C	*n*NaOH/*n*AGU (mol/mol)	*n*SMCA/*n*AGU (mol/mol)	Time (h)	DS	Swelling power	Viscosity
1	0	0	0	1.5	2.150	4	0.1844	95.39	148.07
2	0	−1	1	1.5	1.433	6	0.1676	65.03	127.60
3	1	1	0	2.0	2.867	4	0.1833	96.27	143.50
4	−1	1	0	1.0	2.867	4	0.1377	38.73	122.00
5	1	0	1	2.0	2.150	6	0.1810	93.69	142.50
6	1	0	−1	2.0	2.150	2	0.1591	65.87	123.00
7	1	−1	0	2.0	1.433	4	0.1784	78.61	134.23
8	0	0	0	1.5	2.150	4	0.1852	96.44	149.17
9	0	1	1	1.5	2.867	6	0.1934	109.31	228.33
10	−1	0	1	1.0	2.150	6	0.1304	35.29	114.13
11	0	1	−1	1.5	2.867	2	0.1506	61.91	122.83
12	0	−1	−1	1.5	1.433	2	0.0833	21.19	59.17
13	−1	−1	0	1.0	1.433	4	0.0970	23.46	106.60
14	−1	0	−1	1.0	2.150	2	0.0954	22.95	97.13
15	0	0	0	1.5	2.150	4	0.1889	98.38	158.50

contained 15 runs, including three center points, to evaluate the experimental errors. The levels of each variable were coded as −1, 0, and +1 for statistical analysis. The dependent variable was the DS of CMS. The polynomial equation generated by this experimental design (using Statgraphic plus 7.1, StatPoint Inc.) is as follows:

$$Y_i = b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_0 \quad (4)$$

where Y_i is the dependent variable, b_0 is the intercept, b_1 to b_{33} are the regression coefficients, and A, B, and C are the independent variables that were selected from the preliminary experiments.

Determination of the DS

The DS of CMS was determined in accordance with the method reported by Stojanovic et al. (16). The carboxymethyl groups in the CMS were first converted to an acid form with hydrochloric acid (HCl). The acidified starch was then recovered by precipitation with methanol, filtration, washing with methanol, and drying. Then, 0.2 M NaOH (20 mL) was added to a suspension of accurately weighed CMS in 30 mL of purified water. The mixture was transferred to a 100-mL volumetric flask and adjusted to the mark with purified water. The solution (25 mL) was transferred to an Erlenmeyer flask and titrated with 0.04 M HCl using phenolphthalein as the indicator. The titration was repeated three times, and the average value of HCl volume was used for the calculations. A blank was also titrated. The DS was calculated using followed equations:

$$\mathrm{DS} = \frac{162 \times n\mathrm{COOH}}{\mathrm{mds} - 58 \times n\mathrm{COOH}} \quad (5)$$

$$\mathrm{mds} = \frac{(1 - W_{\mathrm{water}})}{100} \times \mathrm{ms} \quad (6)$$

$$n\mathrm{COOH} = (V_{\mathrm{b}} - V) \times C_{\mathrm{HCl}} \times 4 \quad (7)$$

where 162 is the molar mass of AGU (in g/mol); nCOOH (in mol) is the amount of COOH; mds (in g) is the mass of dry sample; ms (in g) is sample mass; W_{water} (%) is water content; V_{b} (in mL) is the volume of HCl used for the titration of the blank; V (in mL) is the volume of HCl used for the titration of the sample; C_{HCl} (in mol/L) is the HCl concentration; and 4 is the ratio of the total solution volume (100 mL) and the volume taken for titration (25 mL).

Morphology

The morphology of yam starch granules was studied using a scanning electron microscope (Camscan MX2000, England).

IR Determination

Infrared (IR) spectra of native yam starch and CMS were obtained using a Fourier transform infrared (FTIR) spectrophotometer (Spectrum One, Perkin Elmer, England). Substitution was confirmed by the presence of carbonyl groups in the IR spectrum.

Viscosity

The viscosity of a 2% *w/v* starch suspension was determined using a viscometer (Brookfield, RVDV-II$^+$PRO, USA) with a spindle no. RV-02 and a speed of 200 rpm at 25°C.

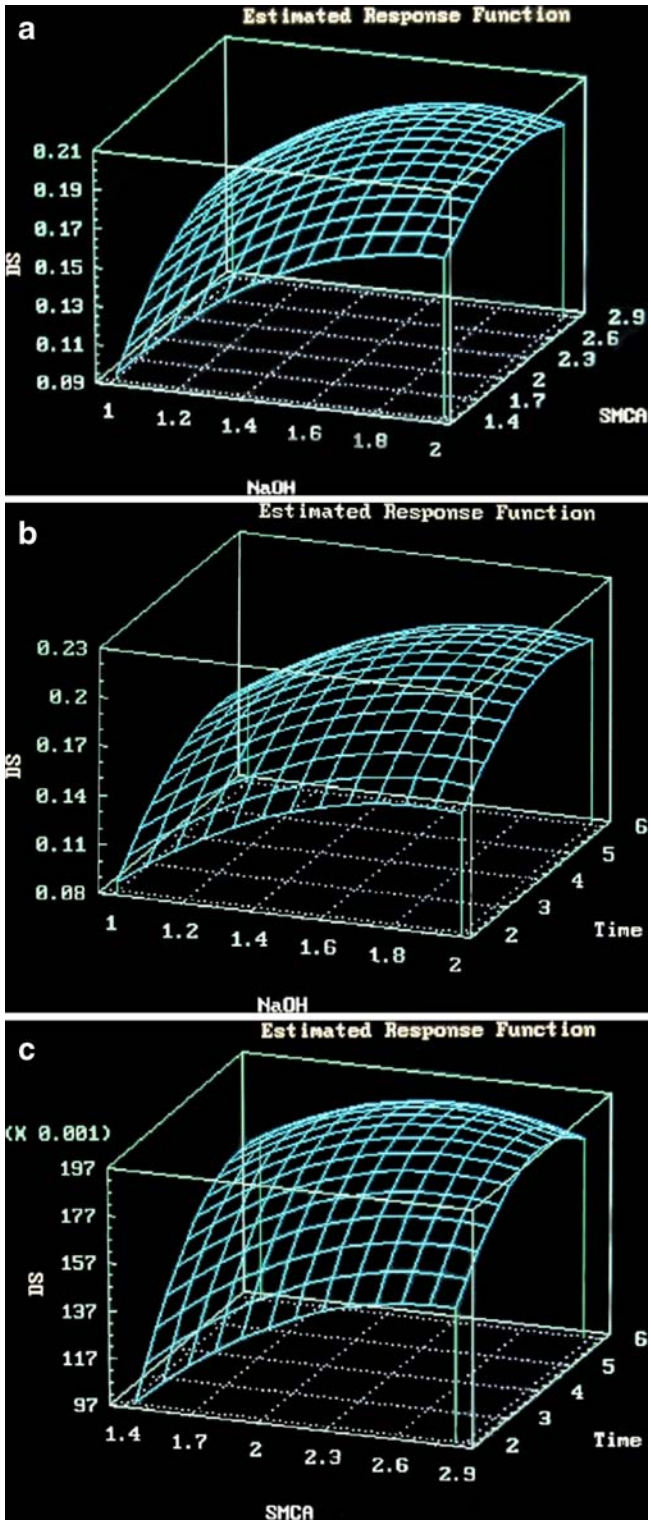

Fig. 1. **a** Effects of molar ratio of NaOH/AGU and SMCA/AGU on DS of CMS, **b** effects of time and molar ratio of NaOH/AGU on DS of CMS, **c** effects of time and molar ratio of SMCA/AGU on DS of CMS

Table II. Comparison of the Experimental Result and the Predicted Value at the Optimum Conditions

*n*NaOH/*n*AGU (mol/mol)	*n*SNCA/*n*AGU (mol/mol)	Time (h)	DS	
			Predicted	Experimental (SD)
1.80	2.35	4.8	0.2019	0.1966 (0.0056)

The readings of viscosity were taken after 30 s of rotation. All measurements were performed in triplicate.

Swelling Power

The swelling power (by weight) and solubility of starch were measured using a method modified from the one reported by Tester and Marison (17). Yam starch (0.2 g) was dispersed in water (20 mL). The suspension was heated to 85°C in a water bath for 30 min with vigorous shaking every 5 min. The starch gel was then centrifuged at 2,200 rpm for 15 min. The weight of sediment was used to calculate the swelling power. The solubility was obtained from the dried weight of dissolved starch in supernatant. The determination was done in triplicate. The swelling power was calculated as follow:

$$\text{Swelling power} = \frac{\text{Weight of sediment}}{(\text{Weight of dry starch} - \text{weight of dissolved starch})} \quad (8)$$

Tablet Disintegration

The efficiency of the optimized CMS as a tablet disintegrant was evaluated and compared with native yam starch. DCP tablets containing 0.5, 1, 2, 3, and 4 % *w*/*w* of starches were prepared. The blended powder was compressed into tablets using a hydraulic compressor (Riken Power, China) equipped with an 8-mm flat face punch. The tablets were compressed at controlled compression pressures ranging from 50 to 200 MPa. The tablet disintegration time was determined according to the US Pharmacopoeia method (18). Each reported value was the average reading of six tablets.

RESULTS AND DISCUSSION

Effect of Reaction Parameters on Carboxymethylation

The results for DS are shown in Table I, and the quadratic model from regression analysis was expressed as in the following equation:

$$\begin{aligned} \text{DS} = {} & 0.393A + 0.215166B + 0.077034C - 0.024965AB \\ & - 0.003275AC - 0.007235BC - 0.088633A^2 \\ & - 0.029B^2 - 0.005633C^2 - 0.588301 \end{aligned} \quad (9)$$

where A and B are the molar ratios of NaOH and SMCA to AGU, respectively, and C is the reaction time.

The results from the model analysis of variance indicated that the relationship between the DS and the variables was significant, with a p value less than 0.05 and a satisfied correlation coefficient of 0.95. Results from the regression analysis showed that the DS was primarily affected by the amounts of NaOH and SMCA, followed by the reaction time. The positive values of the regression coefficients indicated that the DS increased with increasing the amount of NaOH or the SMCA content or the time. The negative coefficients for the interaction terms suggested that the DS was decreased at certain levels of NaOH and SMCA contents and at certain times. These results indicated that there was an optimum value for the DS (Fig. 1).

From Eq. 1, the presence of NaOH in the reaction yields more reactive starch alkoxide, thus increasing the amount of NaOH in the reaction resulted in an increase of the DS. However, at high levels of NaOH, the side reaction of NaOH with SMCA (Eq. 3) became more significant and competed

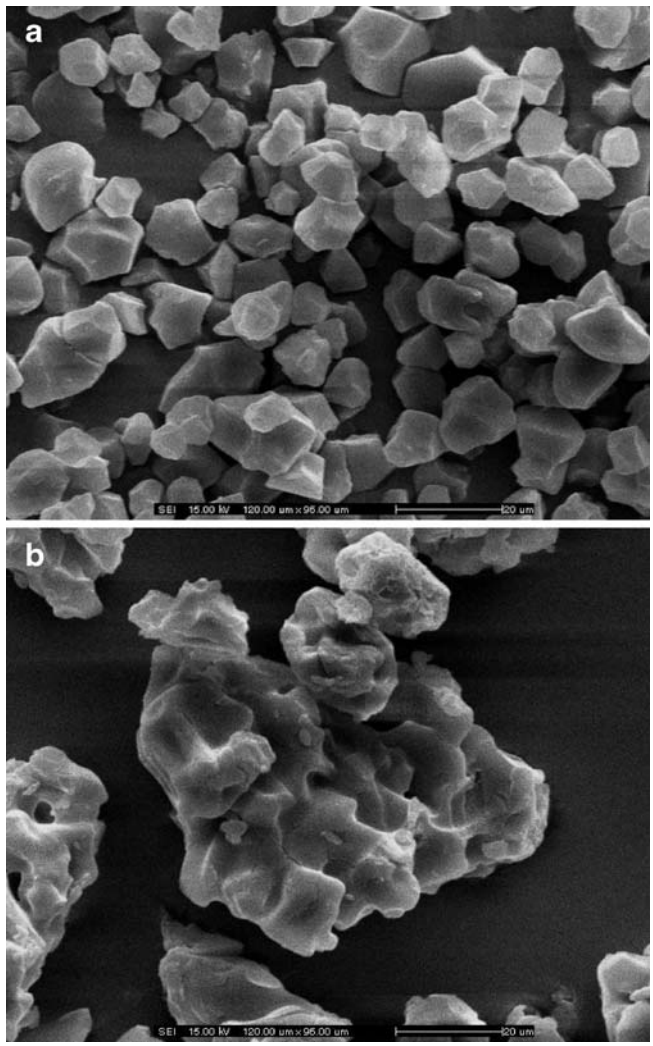

Fig. 2. Scanning electron micrograph of **a** native yam starch, **b** optimized carboxymethyl starch (scale bar=20 μm)

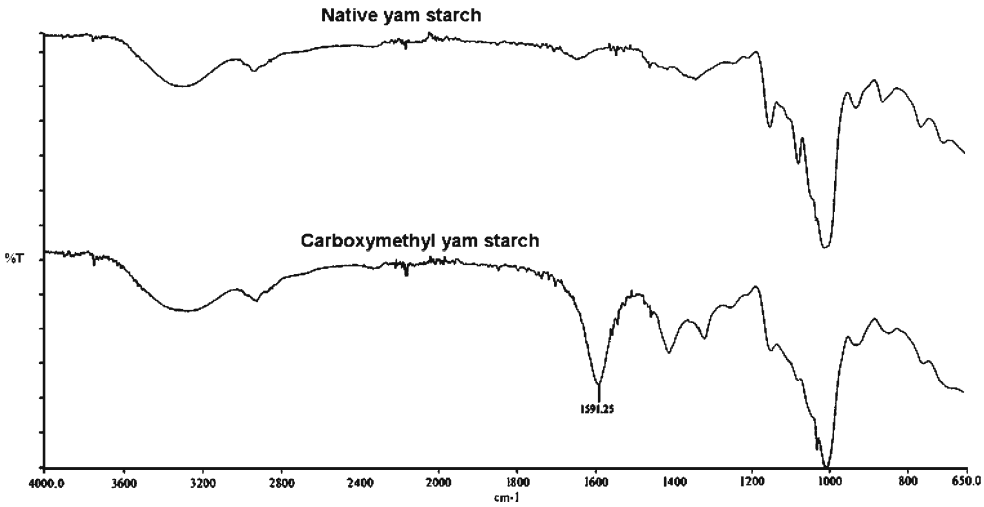

Fig. 3. IR spectrums of **a** native yam starch and **b** carboxymethyl yam starch

with the main reaction (Eq. 2). A decrease in the DS was observed in other studies (4,10,19).

SMCA had a positive effect on the DS, especially at high NaOH concentrations. At low levels of NaOH, the amount of activated alkoxide in starch molecules was limited. In the presence of a small amount of SMCA, the reaction was already saturated, thus increasing the amount of SMCA only slightly increased the DS value. As the concentration of NaOH increased, the effect of SMCA became prominent, the DS increased with increasing SMCA content (Fig. 1a). The analysis shown in Fig. 1b and c indicated that reaction time had little effect on the DS. However, the DS was slightly increased with increasing reaction time, until it reached the optimum value. After that, the DS was decreased. The optimum conditions are shown in Table II. The experimental values of DS were found to be very close to the predicted values.

Properties of CMS

Investigation of the starch granule surfaces by scanning electron microscopy (SEM) showed that the yam starch granule was polygonal with a smooth surface and particle size of approximately 2–20 μm. The SEM of optimized CMSs (Fig. 2b) showed partial eruption and coalescence of starch granules.

The presence of carboxyl groups was indicated by the presence of an absorption band at 1,591.25 cm^{-1} in the FTIR spectrum of CMS (shown in Fig. 3).

The swelling power and viscosity of CMS are shown in Table I. The swelling power and viscosity of CMS were higher than native starch. For CMS, the swelling power and viscosity increased with an increase in the DS. At higher degrees of substitution, more water can penetrate into the starch granules due to the hydrophilicity of the carboxymethyl groups (20), resulting in swelling of the starch granule.

Tablet Disintegrant

Tablets containing 0.5% *w/w* CMS, compressed at pressures higher than 50 MPa, did not disintegrate. The presence of less CMS possesses insufficient expansion power to break apart the tablet. The tablets containing 2.0% *w/w* CMS showed the fastest disintegration time. The disintegration times of tablets containing 3.0 and 4.0% *w/w* CMS were slightly retarded. This could be due to the formation of viscous gel mass of CMS after contact with water. Consequently, water hardly penetrated into the tablet, resulting in retardation of tablet disintegration (Fig. 4).

CONCLUSION

CMSs were prepared using the Box–Behnken experimental design. The results showed that the content of NaOH was found to be the most important factor to the DS of CMS, followed by the amount of SMCA and reaction time. These variables had positive correlations to the DS. The interaction between NaOH and SMCA possessed a negative influence on the DS. The optimum conditions to obtain CMS with DS of

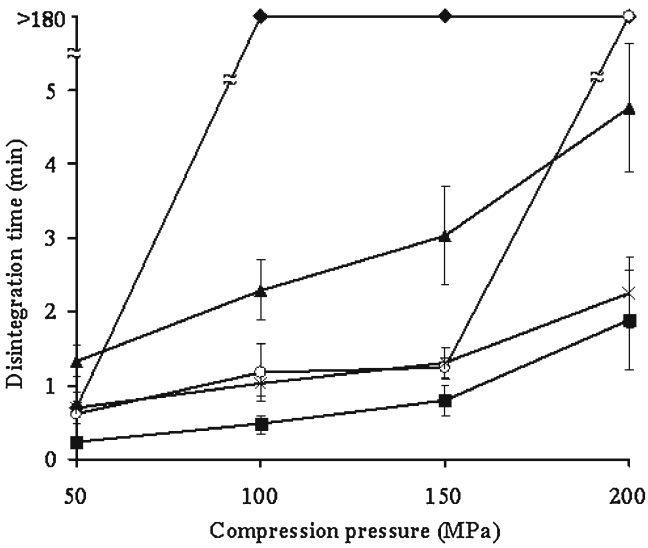

Fig. 4. Effect of various CMS concentrations on disintegration time of DCP tablet, *filled diamond* CMS 0.5% *w/w*; *empty circle* CMS 1.0% w/w; *filled square* CMS 2.0% *w/w*; *ex symbol* CMS 3.0% *w/w*; *filled triangle* CMS 4.0% *w/w*

0.2 were found to be molar ratios of NaOH and SMCA to AGU of 1.80 and 2.35, respectively, with reaction time of 4.8 h. The DS affected the properties of CMS. The swelling power and viscosity increased with increasing DS. The SEM photographs indicated that alkali treatment was responsible for the changed granular surface and structure of the modified starch. Tablets containing CMS concentrations of 1.0–4.0% disintegrated in less than 5 min. Thus, CMS can be used as a tablet disintegrant at low concentration. The use of CMS at high concentrations for tablet-controlled release has actively been investigated.

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