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Preparation and physicochemical properties of sodium carboxymethyl mungbean starches

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Abstract

Fifteen sodium carboxymethyl mungbean starches (SCMMSs or MMSs) were prepared from native starch through a substitution reaction using monochloroacetic acid, using different modification conditions and solvents. Physicochemical properties, including the determination of degree of substitution, solubility studies, SEM and X-ray diffraction analyses, and rheological studies under normal and freeze-thaw conditions were investigated. The degree of substitution (DS) of the prepared MMSs ranged from 0.06-0.66. Nine modified mungbean starches were freely soluble in unheated water while the other six were partially soluble or only swelled. The pH of water-soluble MMSs were between 9.0–10.5. Scanning electron microscopy (SEM) pictures showed that the particles of all of the soluble modified starches were mostly ruptured, with the exception of MMS-M-04 and MMS-E-04 for which the starch granules remained rounded. X-ray diffraction results concurred with the SEM findings, showing that all the rounded-particle MMSs retained their crystallinity while those with destroyed surface SEMs showed loss of crystallinity. Ethanol (92.4%) appeared to be the most effective solvent in the preparation but the condition that yielded modified starches with the most viscous paste was methanol (100%) at 70 °C for 60 min. The potential applications of these modified starches in food and pharmaceutical industries are discussed.

Keywords: Modified starch; Mungbean; Scanning electron microscopy; X-ray diffraction; Rheology; Viscosity

1. Introduction

Modified starches (MSs) have played a major role in the food and pharmaceutical industry in the past few decades. They possess some unique properties, not found in natural starches, which are suitable for the development of new products. Examples are the solubility in unheated water, specific changes in rheological profiles, lower gelatinization temperature, less retrogradation, pH stability, etc. Water-soluble MS such as sodium carboxymethyl starch (SCMMS or MMS), an ester starch derivative prepared by a reaction between native starch and chloroacetic acid in an alkaline condition, yields paste with smoother texture, greater flexibility and strength than those of pregelatinized starches (Mishra, Jain & Agrawal, 1990). MMS is generally safe and is used in the food industry as an ingredient in many types of sauces and instant foods (Chen & Jane, 1994). It is also officially listed in the United States Pharmacopeia (USP) and the British Pharmacopoeia (BP). The expansion of the markets and the R&D for new products in both food and pharmaceutical industries are the main driving force to the development of new modified starches.

Mungbean (*Vigna radiata* L. Wilczek, Papilionaceae), a native plant of southeast Asia, is now widely grown in Africa, South America and Australia as well as in the US. Known to be a rich source of protein (21–28%), the seeds also contain a high amount of starch which is mainly amylose (30–45%) (Hoover et al., 1997; Jung, Shin & Choi, 1991; Kasemsuwan, Bailey & Jane, 1998). When heated, the starch solution becomes a 'transparent' gel, a unique characteristic of mungbean starch, while remaining resilient with strong gel-strength. The viscosity of the gel in hot water is comparable to that of cassava starch but with higher shear resistance than other starches (Takahashi, Kitahara & Kainuma, 1981). The physicochemical and rheological

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Table 1
Degree of substitution (DS) and water solubility of MMSs prepared using four different alcoholic solvents and under different conditions

MMS #	Solvent	T (°C)	Time of reaction (min)	DS	Solubility in water	pH
M-01	92.4% MeOH	50	20	0.0590	×	
M-02	100% MeOH	60	60	0.1953	×	
M-03	100% MeOH	60	120	0.1725	×	
M-04	100% MeOH	70	60	0.3597	✓	9.0
P-01	1-Propanol	50	60	0.3096	✓	9.1
P-02	1-Propanol	50	120	0.5192	✓	9.6
P-03	1-propanol	81	120	0.5277	×	
I-01	2-Propanol	50	60	0.3443	✓	9.2
I-02	2-propanol	81	30	0.5585	✓	9.7
I-03	2-propanol	81	60	0.5526	×	
I-04	2-Propanol	50	120	0.4374	×	
E-01	92.4% EtOH	50	22	0.2011	✓	9.0
E-02	92.4% EtOH	50	30	0.3696	✓	9.4
E-03	92.4% EtOH	50	40	0.5271	✓	9.8
E-04	92.4% EtOH	81	15	0.6551	✓	10.5

properties, as well as structural characterizations of mungbean starch have been reported (Hoover et al., 1997; Ohwada, Ishibashi, Hiranaka & Yamamoto, 2003). In Thailand, mungbean is the most cultivated legume crop (Srinives et al., 2001) and mungbean starch is used predominantly in the food industry for the preparation of glass noodles or mungbean vermicelli and a popular Thai dessert called ‘Sah-lim’. However, the application of mungbean starch in the pharmaceutical industry has not been much investigated. A work by Sinchaipanid (1989) suggested that pregelatinized and crosslinked mungbean starch had potential as a tablet disintegrant. The preparation of other types of modified mungbean starch has not been reported.

The objectives of this work were to prepare sodium carboxymethyl mungbean starches with different degree of substitution and to evaluate their physicochemical properties as potential materials for food and pharmaceutical industries.

2. Experimental

2.1. Materials and modified starch preparation

Mungbean starch was from Sitthinan Company Ltd (Bangkok, Thailand). All chemicals used in the preparation and analysis of modified starches were AR grade or equivalent. Double-distilled commercial grade methanol was used in the washing of the final products, with the exception of the final wash in which the AR grade methanol was used.

MMSs were prepared by a substitution reaction modified using the method described by Filbert (1952). The reaction utilized monochloroacetic acid (MCA) as a reagent under alkaline condition, with various types of solvent and reaction conditions to yield 15 different MMSs (Tables 1 and 2). First, MCA was dissolved in one of the four low-molecular weight, water-miscible alcohols (methanol, ethanol, 1-propanol, or 2-propanol). Then, while stirring, native starch powder was added into the solution followed by the solution of sodium hydroxide. The mixture was heated, if necessary, to the required temperature and was maintained at that temperature for the required amount of time, with continuous stirring. At the end, the reaction was stopped by neutralization with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 80% methanol and a final wash with 100% methanol. Following an oven-drying at 50 °C for 6 h, the modified starch obtained was passed through sieve no. 80.

2.2. Determination of degree of substitution

The degree of substitution (DS) of each MMS was determined by the USP XXIII method described for Croscarmellose sodium, which included two steps—titration and residue on ignition. The DS can be calculated

Table 2
Viscosity of 1% w/v solution of water-soluble MMSs under normal (room temperature) and freeze-thaw (FT) conditions (eight cycles of a 2 days storage at 8 °C and 2 days storage at 45°) and the thixotropic values

Samples	1% Viscosity–normal (mPa s)	1% Viscosity–FT (mPa s)	Thixotropic value (mPa s)
MMS-M-04	149.7±1.5	59.3±0.6	8795±458
MMS-P-01	31.3±0.6	26.0±2.0	−1137±51
MMS-P-02	29.7±0.6	25.7±1.5	−448±143
MMS-I-01	37.3±0.6	29.7±1.5	−1599±38
MMS-I-02	32.7±0.6	26.0±1.0	−505±90
MMS-E-01	31.3±0.6	27.7±1.5	−1557±16
MMS-E-02	22.0±0.0	17.7±0.6	−102±28
MMS-E-03	31.7±0.6	25.0±1.7	−273±70
MMS-E-04	26.0±2.3	22.7±0.6	369±121

using the following equation;

$$\mathrm{DS} = A + S \tag{1}$$

when A is the degree of substitution of carboxymethyl acid and S is degree of substitution of sodium carboxymethyl. A and S can be calculated using the information from the titration and ignition steps;

$$A = \frac{1150M}{(7120 - 412M - 80C)} \tag{2}$$

$$S = \frac{(162 + 58A)C}{(7102 - 80C)} \tag{3}$$

when M is the mEq of base required for in the titration to end point. C is the percentage of ash remained after ignition. The reported DS values are means of three determinations.

2.3. Solubility and pH of solution

The solubility of MMSs in unheated water was tested by adding 0.1 g of MMS in to 10 mL of water (1% w/v), mixed thoroughly and observed the solubilization and/or swelling of MMSs. The pH of water-soluble MMS solutions (1%) was determined directly using a Waterproof pHScan WP 2 (Eutech Instruments, USA)

2.4. IR determination

IR spectra were obtained using a Nicolet 510 FT-IR (Nicolet Instrument Corp., USA) using KBr disc technique. The substitution reaction was confirmed by the presence of a carbonyl group in the IR spectrum.

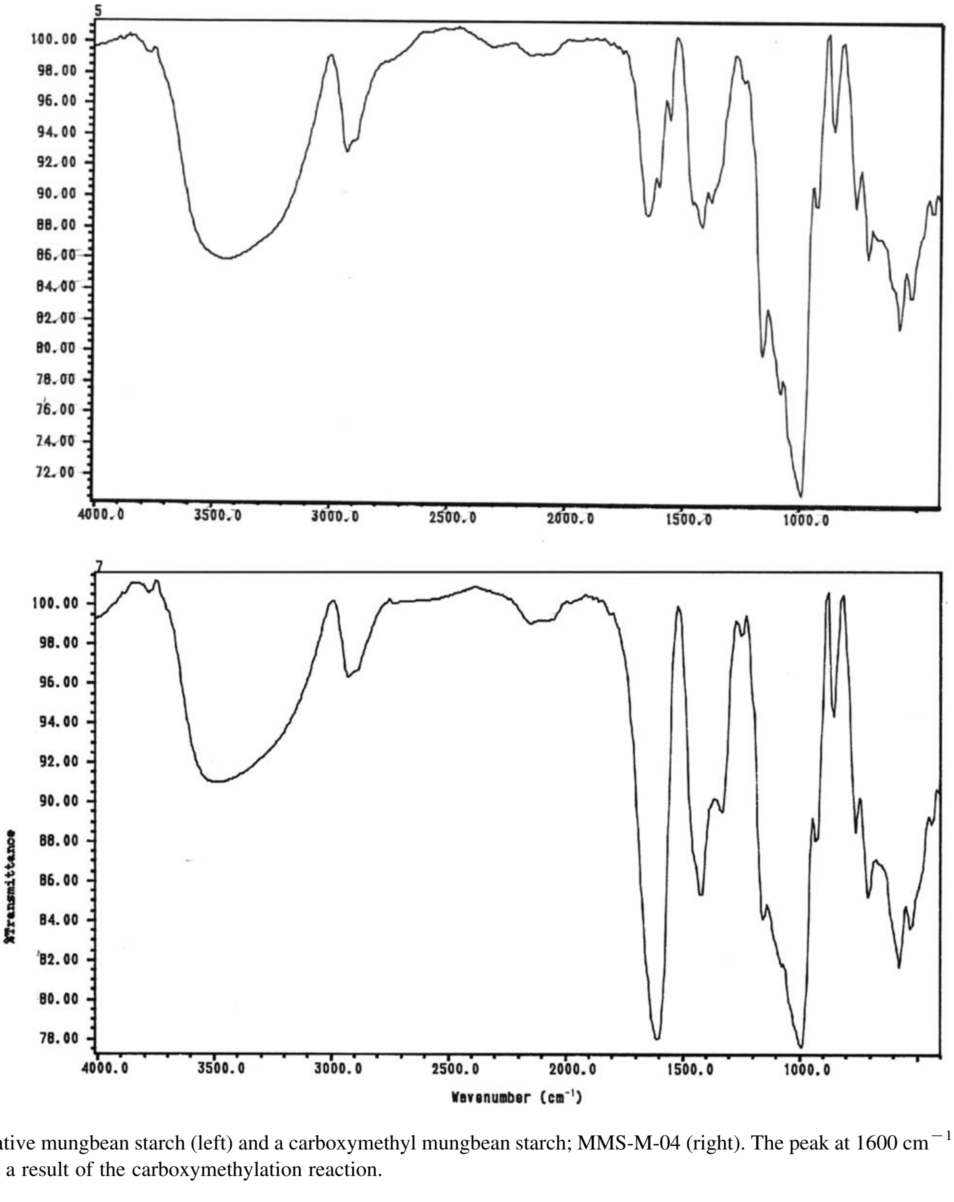

Fig. 1. IR Spectra of native mungbean starch (left) and a carboxymethyl mungbean starch; MMS-M-04 (right). The peak at 1600 cm^{-1} indicates the presence of a carbonyl group as a result of the carboxymethylation reaction.

2.5. Paste clarity and freeze-thaw stability

A 1% w/v paste (0.2 g in 20 mL distilled water) was prepared for each MMS. The clarity of pastes were determined by placing 2.5 mL in a disposable cuvette and measured the absorption on a spectrophotometer at 650 nm against a water blank. Freeze-thaw stability of MMS pastes was assessed by subjecting the pastes to ten (10) freeze-thaw cycles (cold storage at −20 °C for 18 h, then thawed to 28 °C for 6 h). At the end of each freeze-thaw cycle, the absorption of the paste was measured at 650 nm against a water blank. A 1% w/v paste of native starch, prepared with the aid of heating, was used as a control.

2.6. SEM analysis

The scanning electron microscopy (SEM) experiments to analyze the granule surface, shape and size were conducted using a Phillip XL 30 ESEM (FEI company, Hillsboro, OR, USA) equipped with a large field detector. The acceleration voltage was 10–20 kV under low vacuum mode (0.7–0.8 torr).

2.7. X-ray diffraction

X-ray diffraction patterns of native and modified starches were recorded in the reflection mode on a Siemens D-500 X-ray diffractometer. Diffractograms were registered at Bragg angle $(2\theta)=5$–40° at a scan rate of 5 °/min.

2.8. Viscosity

Apparent viscosity was measured using a Brookfield R/S-CPS rheometer (Bob-and-Cup format) in suspensions of 1.0 g MMS in 100 mL water (1% w/v). The samples were prepared by dispersing MMS powder in the stirring water, mixed thoroughly, and the suspensions were allowed to stand overnight for complete swelling. Each sample was divided into two portions; one portion was directly subjected to viscosity measurement at room temperature (normal condition), the other portion was processed through an accelerated stability test before viscosity measurement. The test was performed by subjecting the samples through eight (8) freeze and thaw (FT) cycles, in which each cycle consisted of 2 days storage at 8 °C and another 2 days storage at 45°. The measurement procedure was (1) an increase of rotation speed from 0 to 1000 rpm in 1 min, (2) held at 1000 rpm for 1 min, and (3) a decrease of rotation speed from 1000 to 0 rpm in 1 min. All measurements were performed in triplicate, at a controlled temperature of 25 ± 1 °C. The data were analyzed with a Brookfield Rheo 2000 software. Viscosity was expressed in mPa s.

2.9. Statistical analysis

All tests were performed at least in triplicate. The statistical significant tests were performed using Duncan's multiple range test at 95% confidence level ($P<0.05$).

3. Results and discussion

Modified mungbean starches (MMSs) were prepared as described by Filbert (1952) with some modifications of the conditions. The degree of substitution (DS) values of the obtained MMSs were from 0.06–0.66. A carbonyl peak at 1600–1700–cm^{-1} on the IR spectrum confirmed the introduction of a $\text{–CH}_2\text{COO}^-$ group into the starch molecule (Fig. 1). The intensity of the peak for each MMS was in agreement with the DS value. For all solvents, an increase in temperature or time of reaction resulted in an increase in the DS value. The use of 1-propanol or 2-propanol as solvent generally produced MMSs with high DS (0.31–0.56), while methanol yielded MMSs with lower DS (0.06–0.36). However, the higher DS did not render a better solubility as the result showed that the DS of seven water-soluble MMSs was between 0.20–0.52. MMSs with DS lower than 0.20 or higher than 0.52 were insoluble or only partially soluble, with the exception of ethanolic solvent in which two MMSs with DS 0.53 (MMS-E-04) and 0.66 (MMS-E-02) were water-soluble. Of the fifteen MMSs, nine were completely soluble—including all four MMSs preparing in 92.4% ethanol, while the other six MMSs were not or only partially soluble in water. The pH of 1% solution of water-soluble MMSs, ranged from 9.0–10.5, showed good correlation with the degree of substitution (Table 1).

The clarity of the 1% water-soluble MMS pastes, determined by the absorption at 650 nm, was significantly higher than that of the native starch paste (0.01–0.15 vs. 1.40). The stability of MMS pastes after ten (10) freeze-thaw cycles was evidence, as the absorbance of most MMS pastes remained unchanged or only slightly increased throughout the observed period. In contrast, the native starch paste showed lack of freeze-thaw stability as the turbidity of the paste increased gradually within 2–3 days to reach a maximum value ($A_{650}=2.90$), at which point a phase separation was observed.

Investigation of granule surface by scanning electron microscopy (SEM) showed that the native mungbean starch granules were predominantly elliptical, with a smooth surface, visible thickness and wide range of size distribution (4–16 μm in width and 8–30 in length). SEM of MMS granules can be divided into two groups. The first group showed granule that were elongated elliptical with rough surface together with some small debris, while granules of the other group were of similar appearance to those of the native starch, but with more size uniformity (Fig. 2). The granules of the first group were observed in all water-soluble

MMSs, while those of the other group were characteristic of the MMSs, which were not soluble in water. The exception was noted in case of the water-soluble MMS-M-04 and MMS-E-04, where the shape observed in SEM images remained similar to that of the native starch but with more uniformed size distribution (10–25 μm) and slightly smoother surface.

The X-ray diffractogram of native mungbean starch showed strong reflections at 15, 17 and 23° of diffraction angle 2θ. These values are in agreement with those reported

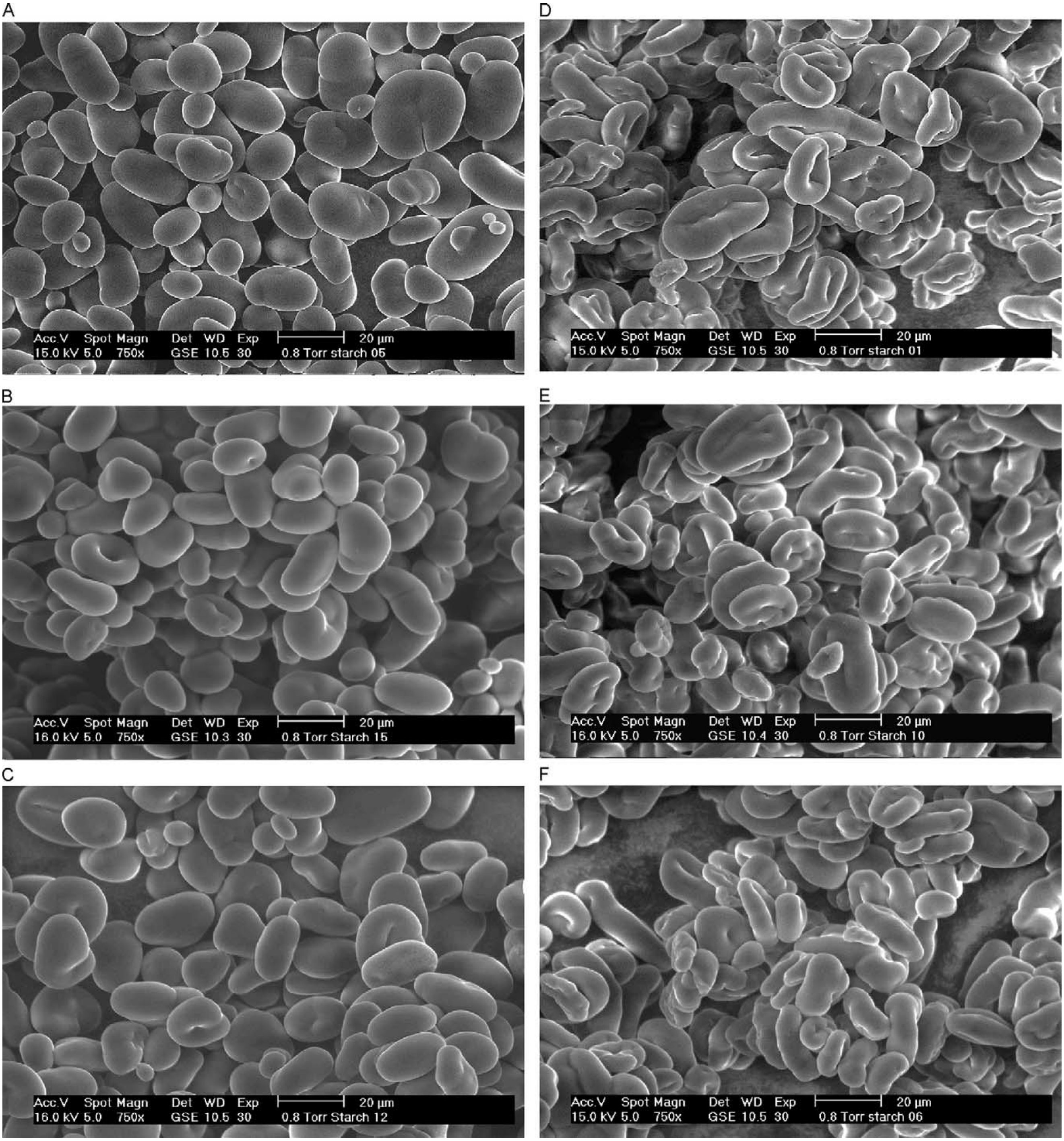

Fig. 2. SEM images of Native and Modified Mungbean starches. (A) MMS-M-01; (B) MMS-P-03; (C) MMS-I-04; (D) MMS-E-01; (E) MMS-P-01; (F) MMS-I-01; (G) MMS-E-04; (H) MMS-M-04; (I) Native starch. A–C are partially-soluble in water. D–F are water soluble, with marked change in granule properties. G and H are water soluble, with no significant change in granule properties and similar to native starch (I).

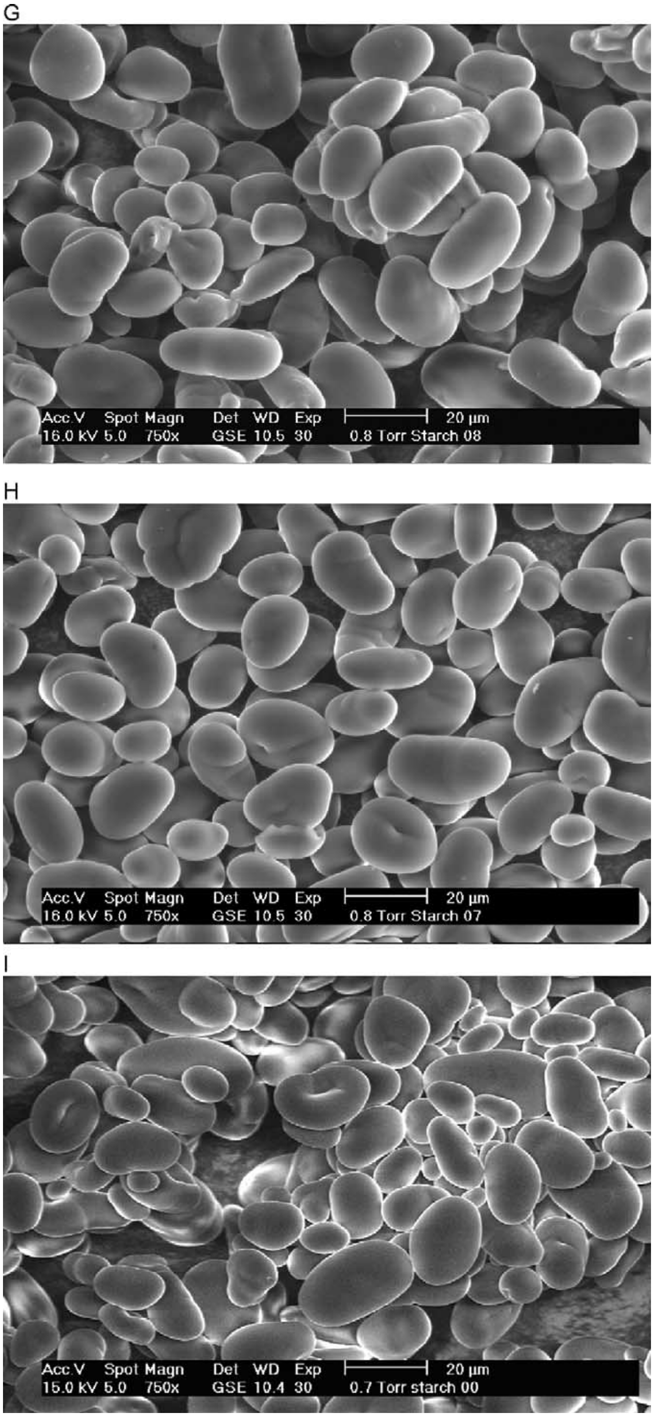

Fig. 2 (*continued*)

by Ohwada and co-workers and indicates that mungbean starch, like other cereal starches, have an A-type crystal pattern (Ohwada et al., 2003; Koo, Park, Jo, Kim, Hur & Baik, 2005), although the presence of a few small peaks at 22–24° of diffraction angle 2θ could also be interpreted as a C-type pattern as suggested by Hoover et al. (1997). Similar to the SEM results, two types of X-ray diffractogram of MMSs can be observed. The first group showed similar pattern of X-ray diffraction to that of the native starch, with a slight decrease in peak intensities, while the other group showed losses of all visible reflections previously seen in the diffractogram of the native starch (Fig. 3). Since, the reflections observed in the X-ray diffractogram indicated the presence of crystalline starch molecules, the disappearance of these reflections afterwards was an indication of a decrease in crystallinity within the starch molecules.

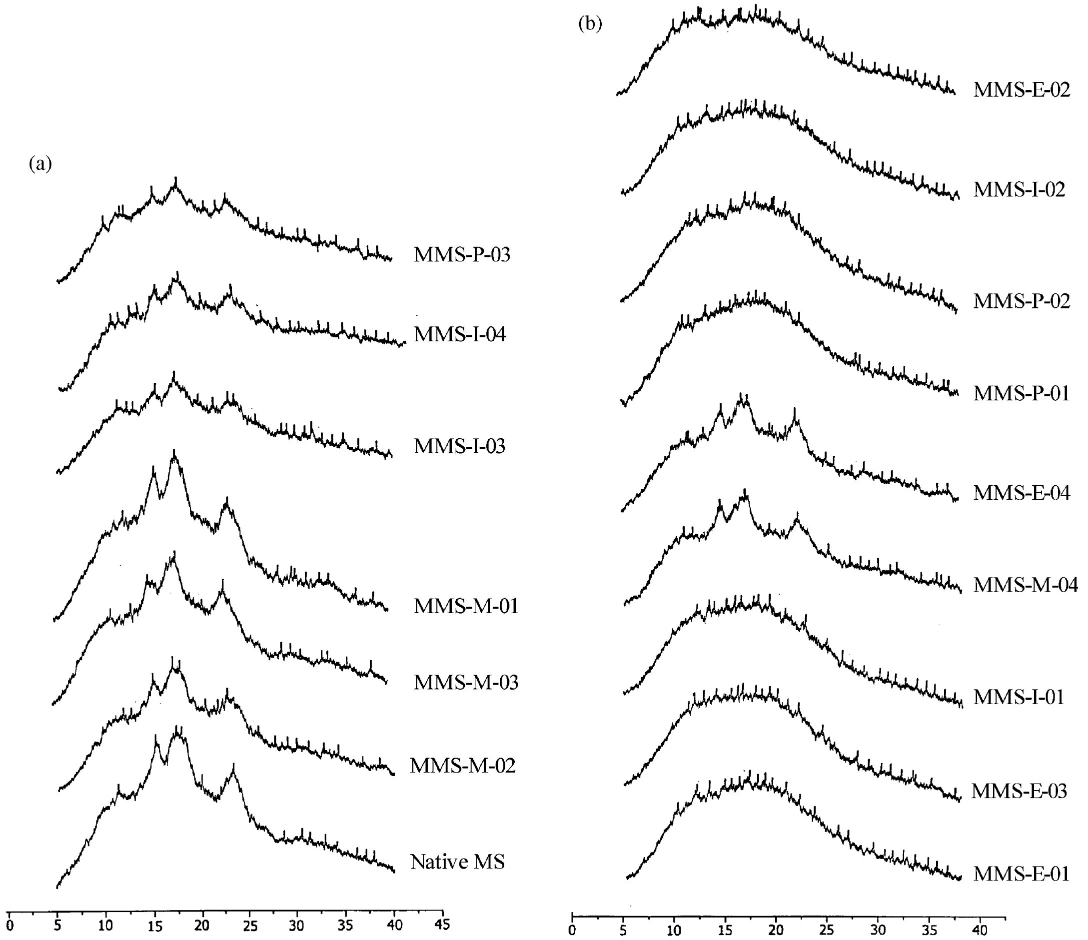

Fig. 3. X-ray diffractograms of native and modified mungbean starches. (a) native and water-insoluble MMSs; (b) water-soluble MMSs.

From the SEM and X-ray results, a correlation could be drawn between the SEM images and the X-ray diffraction pattern such that the loss of crystalline structures inside the granules (indicated by the 'halo pattern') resulted in rough surface granules observed on the SEM images. Further correlation with water solubility of each MMS showed that, with the exception of MMS-M-04 and MMS-E-04, MMSs with rough surface granule caused by the loss of crystallinity were completely water-soluble, while MMSs with SEM and X-ray pattern similar to that of the native starch were only partially soluble in water. The results suggested that the loss of crystallinity could be due to the rupture of starch granules, which in the presence of water together with heat treatment caused the breakage of chemical bonds in starch molecules. This is especially the case when 92.4% ethanol was used as a solvent and although at the temperature below gelling temperature, the starch granules become 'pregelatinized'. A study on crystalline properties of starch reported that pregelatinized starches, which are water-soluble, lost the crystalline structures in the starch granules (Lui, Ruan, Wang & Wu, 2003). However, pregelatinized starch does not yield high viscosity gel, which is also observed in this study with most of the water-soluble MMSs. In case of MMS-M-04 and MMS-E-04, SEM and X-ray results indicated that most of the granules were modified mainly by carboxymethylation, with very little pregelatinization taken place.

The exception of MMS-M-04 and MMS-E-04, which showed no significant change in SEM and X-ray characteristics from those of a native starch but are both water-soluble, is of interest and should be noted. The conditions employed in the preparation of these two MMSs allowed the carboxymethylation to take place with no or little pregelatinization. In the case of MMS-M-04, this can be explained by the effect of methanolic solvent, which impeded the pregelatinization of starch granules by increasing their gelation temperature (Gerlsma, 1970; Hizukuri and Takeda, 1978). As for MMS-E-04, the explanation is less obvious, but it is plausible that a short time of reaction (15 min) plays an important role in limiting the pregelatinization. A 1% w/v solution of MMS-M-04 yielded a viscosity of 149.7 ± 1.5 mPa s, a 3–5 times higher than that of other MMS solutions (Table 2), which indicated that the ability to dissolve in water and form a thick gel was primarily due to the carboxymethyl modification. Freeze-thaw cycles resulted in a decrease of viscosity, especially MMS-M-04, partly due to the loss of water molecules out of the polymer paste. Phase separation, however, was not observed. The rheograms of water-soluble MMSs showed a

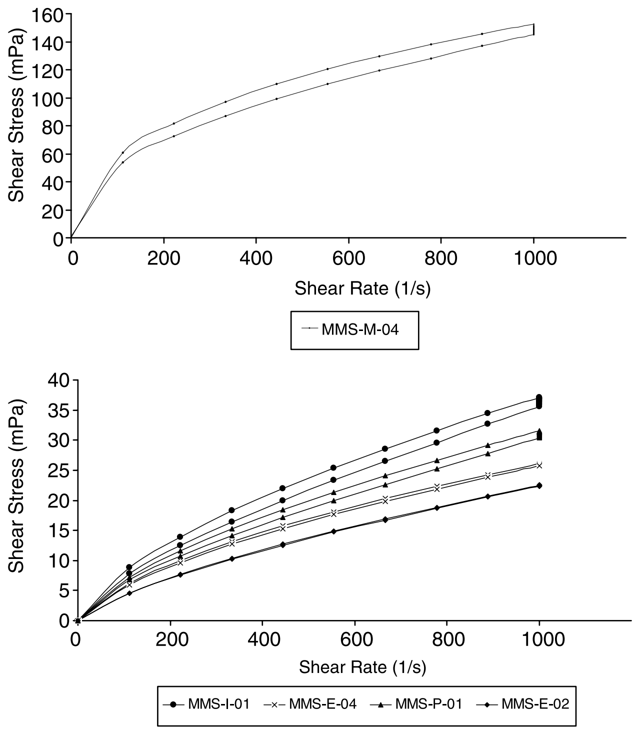

Fig. 4. Rheograms of selected water-soluble MMSs at 1% w/v concentration. (A) MMS-M-04. (B) MMS-I-01, MMS-E-04, MMS-P-01, MMS-E-02.

pseudoplastic-type rheology, with the formation of a hysteresis loop between the upcurve and the downcurve (Fig. 4). At 1% concentration, MMS-M-04 and MMS-E-04 showed thixotropic behavior (i.e. positive thixotropic value), while other MMSs were rheopexy (i.e. negative thixotropic value) (Table 2). These flow behaviors are important information for pharmaceutical applications of MMSs. For example, the high-viscosity MMS-M-04, which also shows a large area of hysteresis loop (Fig. 4(A)), is suitable for use as a suspending agent or a gelling agent (hydrogel). Those with lower viscosity and smaller loop area such as MMS-I-01 and MMS-P-01 (Fig. 4(B)), can be used as film-forming agent. MMS-E-04 and MMS-E-02, which are relatively non-viscous and show little hysteresis loop can be employed as binder in tablet formulation. In addition, MMSs that are partially soluble but have a high swelling capacity in water can be employed as a disintegrant.

4. Conclusion

Sodium carboxymethyl mungbean starches with various degrees of substitution, prepared using different solvents and reaction conditions, can be categorized into two groups—freely soluble and partially soluble in water. Changes in physicochemical properties from native starch were observed and have resulted in different and unique characteristics of each MMSs. This, together with the solubility in water and altered rheological behaviors, allows the broadening use of MMSs in many applications, including the use as pharmaceutical excipients. An appropriate MMS can be selected for a specific purpose of use. The application of these MMSs, based on their physicochemical properties, as pharmaceutical excipients are currently under investigation in our laboratory.

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