Effect of Dual Hydroxypropyl-Carboxymethyl Modification on the Physicochemical Properties of Mung bean Starch

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ABSTRACT

Modified mung bean starches were prepared by dual carboxymethylation and hydroxypropylation reactions. The reactions were used to react with native mung bean starch step by step to yield carboxymethyl mung bean starch (CMMS), hydroxypropyl-carboxymethyl mung bean starch (HPCMMS) and carboxymethyl-hydroxypropyl mung bean starch (CMHPMS). The modified starches were investigated for physicochemical properties. The degree of substitution (DS) of modified starches was 0.25, 0.27 and 0.31 for CMMS, HPCMMS and CMHPMS, respectively. The molar substitution (MS) was 0.05 and trace for HPCMMS and CMHPMS, respectively. Scanning electron microscope and X-ray diffraction showed that the reactions did not alter starch granules and crystal structure. IR experiments confirmed the substitution of carboxymethyl groups in modified starches. All of modified starches were water-soluble, 1% w/v of starch solutions yielded viscosity of 507.13, 301.63 and 255.61 at a shear rate 100 s^{-1} for CMMS, HPCMMS and CMHPMS, respectively. The 3% w/v solutions yielded 1075.17, 823.80 and 514.88 at the same shear rate. The water uptake investigations of modified starches showed that CMMS took up water less than the others. CMMS, however, showed the highest viscosity. Based on the high viscosity and good water uptake ability, CMMS and HPCMMS are considered hydrophilic polymers which can potentially be used as a controlled-release agent for pharmaceutical preparations.

Keywords: Mung bean; Modified starch; Carboxymethylation; Hydroxypropylation; Physicochemical property; Controlled-release agent

INTRODUCTION

Starch has long been used in pharmaceutical industry as diluent, disintegrant and binder for tablet formulation. The use of native starch, however, is limited due to its inherent characteristics such as insolubility in water, gel retrogradation and inconsistent viscosity. Thus, native starch is normally modified to improve properties that make it suitable for using in dosage forms. Modifications can cause changes in many physical characteristics such as the size, surface, crystallinity and solubility of granules, as well as certain pharmaceutical characteristics such as swelling or gel-forming abilities. Carboxymethyl starch (CMS) and hydroxypropyl starch (HPS) are modified starches obtained via etherification reactions which introduce carboxymethyl and hydroxypropyl groups, respectively, into the chains of native starch. CMS is soluble in water, forms gel with less retrogradation compared to native starch (Tijsen et al., 2001a; Tijsen et al., 2001b; Kittipongpatana et al., 2006a), and exhibits potential uses as pharmaceutical excipients (Mulhbacher et al., 2001; Kittipongpatana et al., 2006b; Kittipongpatana et al., 2006c; Nabais et al., 2007; Brouillet et al., 2008; Kittipongpatana et al., 2009). HPS is hydrophobic modified starch with low gelatinization temperature and increased water-holding capacity (Choi and Kerr, 2003) compared to native starch. HPS also exhibits freeze-thaw stability (Lawal et al., 2008; Ratnayake and Jackson, 2008) and has been used in many types of food products (Richardson et al., 2000).

The substitution of both groups on the starch molecules could yield a modified starch with combined or even unique properties. Since both reactions are etherification and require alkaline conditions, therefore, it is possible to carry out the reactions sequentially. Depending on the order of the reaction, either carboxymethyl-hydroxypropyl starch (CMHPS) or hydroxypropyl-carboxymethyl starch (HPCMS) can be prepared. This study reports the preparation of dual-modified mung bean starches, using carboxymethylation and hydroxypropylation (CMHPMS or HPCMMS). The physicochemical and functional properties of the modified starches are evaluated in comparison with carboxymethyl mung bean starch (CMMS). Potential uses of dual-modified starches as pharmaceutical excipients are also investigated.

MATERIALS AND METHODS

Materials

Mung bean starch (100%) was supplied by Sitthinan Company Ltd. (Bangkok, Thailand). Chloroacetic acid (CAA) and propylene oxide (PO) were products of Fluka (Germany). Sodium hydroxide and sodium sulfate were supplied by Merck (Germany). Analytical grade chemicals or equivalent were used in modified starch process and analysis of the starch. Double-distilled commercial grade methanol was used for washing modified starch after chemical reactions and the last washing was done with AR grade methanol.

Preparation of carboxymethyl mung bean starch (CMMS)

CMMSs were prepared using the method previously reported (Kittipongpatana et al., 2006a). CAA was used as a carboxymethylating reagent under alkaline condition. In brief, mung bean starch was added into the stirring mixture of methanol and CAA. After an addition of 50% w/w sodium hydroxide solution, the mixture was heated to 70°C and maintained for 1 h with continuous stirring. The reaction was neutralized with glacial acetic acid. The mixture was washed several times with 85% methanol until no salt was detected and finally washed with AR grade methanol. The modified starch was then dried in a hot-air oven at 45°C for 24 h.

Preparation of hydroxypropyl-carboxymethyl mung bean starch (HPCMMS)

Native mung bean starch was first modified by a hydroxypropylation, followed by a carboxymethylation reaction. Hydroxypropylation was carried out using the method of Pal et al., (2000) with slight modification. In brief, sodium sulphate was dissolved in 0.1% w/v sodium hydroxide. Mung bean starch was then added into the solution and the mixture was continuously stirred for 10 min. PO was added and the reaction was kept stirring at 40°C for 24 h. After stopping the reactions by neutralizing with 2M HCl, the mixture was washed three times with distilled water, followed by a wash with AR grade methanol. Carboxymethylation was then carried out as described in the preparation of CMMS.

Preparation of carboxymethyl-hydroxypropyl mung bean starch (CMHPMS)

Carboxymethylation was performed as described in the preparation of CMMS, followed by hydroxypropylation. After carrying out a carboxymethylation using the method described in CMMS, the reaction mixture was cooled down to 40°C. PO was then added and the reaction was kept at 40°C for 24 h with continuous stirring. After stopping the reactions by neutralization with glacial acetic acid, the mixture was washed with 85% MeOH until no salt was detected and finally washed with AR grade methanol. The modified starch was then dried in a hot air oven at 45°C for 24 h.

Table 1. Amounts of chemicals and conditions for mung bean starch modification.

Type of Modified Starch	Carboxymethylation			Hydroxypropylation		
	CAA (g)	T (°C)	Time (min)	PO (ml)	T (°C)	Time (h)
CMMS	40	70	60	-	-	-
HPCMMS	40	70	60	16.6*	40	24
CMHPMS	40	70	60	16.6*	40	24

*10% of medium used

Determination of degree of substitution (DS)

The degree of substitution of modified starches was determined using the method of Croscarmellose sodium in the USP 30, which consisted of two steps, i.e., titration and residue on ignition. The degree of substitution (*DS*) is the sum of A+S.

When A is the degree of acid carboxymethyl substitution and S is the degree of sodium carboxymethyl substitution. A and S are calculated using the information from the titration and ignition steps:

$A = 1150M/(7102 - 412\mathrm{M} - 80C)$

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

When M is the mEq of base required for in the titration to end point. *C* is the percentage of ash remained after ignition.

Determination of molar substitution (MS)

The hydroxypropyl substitution of modified starch was determined by the method described in Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1997), using ninhydrin reagent (3% solution of 1,2,3-triketohydrindene crystals in 5% sodium bisulfite solution) as an indicator. Then, the absorption of the solution was measured at 590 nm. The hydroxypropyl group substitution was calculated as:

Hydroxypropyl groups (%) = C x 0.7763 x 10 x F / W

C = amount of propylene glycol in the sample solution read from calibration curve (µg/ml)

F = dilution factor

W = weight of sample (mg)

The molar substitution was then calculated from the following formulation (Pal et al., 2000):

molar substitution (MS) = 162W/5800 – 57W

W = HP group equivalent in 100 mg of dry starch

IR determination

IR spectra were acquired using a Nicolet Nexus 470FT-IR and KBr disc technique. The carboxymethyl substitution reaction was confirmed by the presence of a carbonyl group at wave number 1600-1700 cm^{-1} in the IR spectrum.

Scanning electron microscope (SEM) analysis

A JSM-5910LV scanning electron microscope was used to study granule surface, shape and size. Starch granules were photographed at a 750x magnification.

X-Ray diffraction (XRD)

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

Viscosity

The apparent viscosities of 1% and 3% w/v solutions of CMMS, HPCMMS, CMHPMS and native starch were measured by using a Brookfield Rheometer (Bob-and-Cup format, R/S-CC). The samples were prepared as previously described (Kittipongpatana et al., 2006a). The measuring system was CC48 DIN. The mode used was CSR (controlled shear rate). The measurement parameters consisted of three steps: (1) an increase of shear rate from 0 to 100 s^{-1} in 1 min, (2) held at 100 s^{-1} for 1 min and (3) a decrease of shear rate from 100 to 0 s^{-1} 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 version 2.8. Viscosity was expressed in mPa s.

Water uptake

Water uptake volumes of native and modified starches were measured using a modified Nogamiís apparatus (Nogami et al., 1969). Starch sample (500 mg) was placed in a stainless tube that was covered with filter paper. The sample holder was placed in a sinter glass filter which was connected with a graduate pipette through a silicone tube filled with water. A watch glass was used to cover the sinter glass filter to prevent water evaporation. The water uptake of sample was measured at time 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300 sec, then continued at 10, 15, 20, 25, 30, 40, 50 and 60 min. The sample was measured in triplicate.

RESULTS AND DISCUSSION

Two dual-modified mung bean starches, namely, hydroxypropyl carboxymethyl mung bean starches (HPCMMS) and carboxymethyl hydroxypropyl mung bean starches (CMHPMS), and a single-modified carboxymethyl mung bean starch (CMMS), were prepared based on reactions previously described by Kittipongpatana et al., (2006a) and Pal et al., (2000). The degree of substitution values (DS) of HPCMMS and CMHPMS were 0.27 and 0.31, respectively, compared to DS of 0.25 determined for CMMS. The molar substitution (MS) was 0.05 for HPCMMS, while CMHPMS showed trace amount of molar substitution (Table 2). The discrepancy in the DS and MS was probably due to the sequence of the reactions. For HPCMMS, hydroxypropylation reaction was carried out first and thus successfully substituted hydroxypropyl groups into starch chains before carboxymethylation was performed to substitute the bulkier carboxymethyl groups to the unreacted –OH. In contrast to the case of CMHPMS, carboxymethylation which was carried out first substituted the carboxymethyl groups into the chains. These groups impeded the access of hydroxypropyl groups to the unreacted –OH, while the carboxymethylation continued. Thus, very little hydroxypropyl substitution was detected as opposed to a higher DS for carboxymethylation. The substitution of carboxymethyl group was confirmed by IR spectroscopy that showed a peak at 1600-1700 cm^{-1} in CMMS (Fig.1), HPCMMS (Fig.2) and CMHPMS (Fig.3) spectra, while such peak was less detected in IR of native mung bean starch (Fig.4).

Table 2. Degree of carboxymethyl substitution (DS) and molar hydroxypropyl substitution (MS) of modified mung bean starch.

Type of Modified Starch	DS	MS
CMMS	0.25	-
HPCMMS	0.27	0.05
CMHPMS	0.31	trace

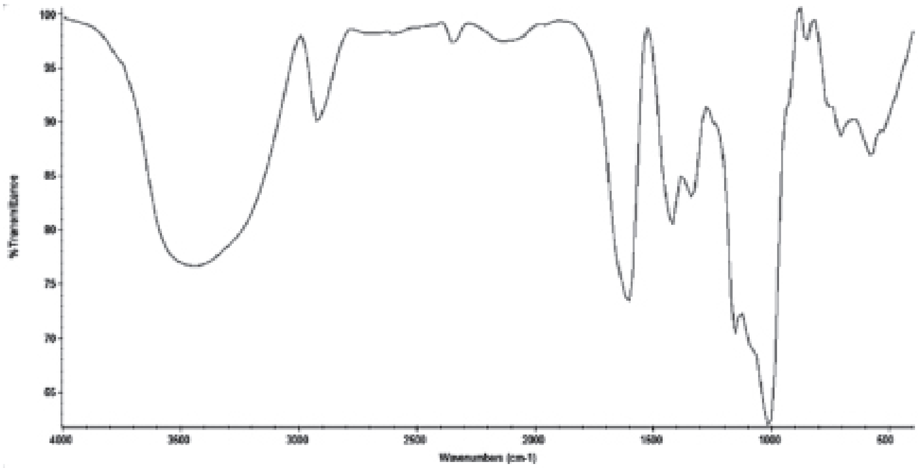

Figure 1. The IR-spectrum of CMMS which showed –COO^- peak at 1600-1700 cm^{-1}.

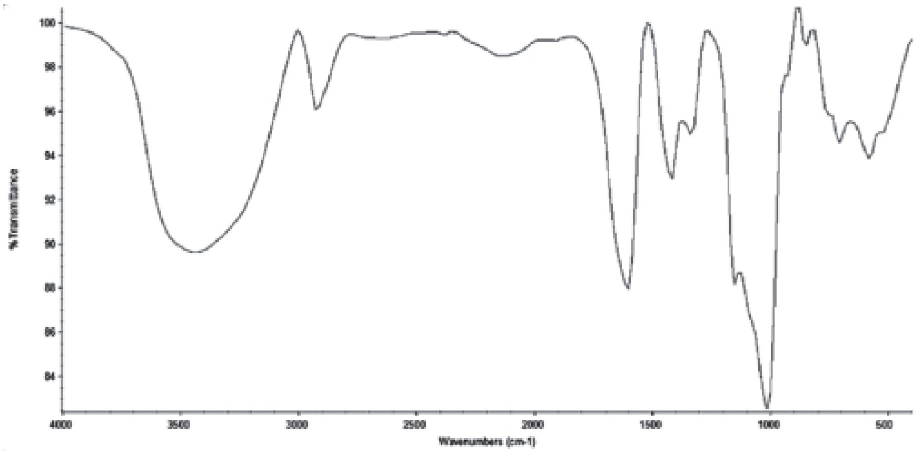

Figure 2. The IR-spectrum of HPCMMS which showed –COO^- peak at 1600-1700 cm^{-1}.

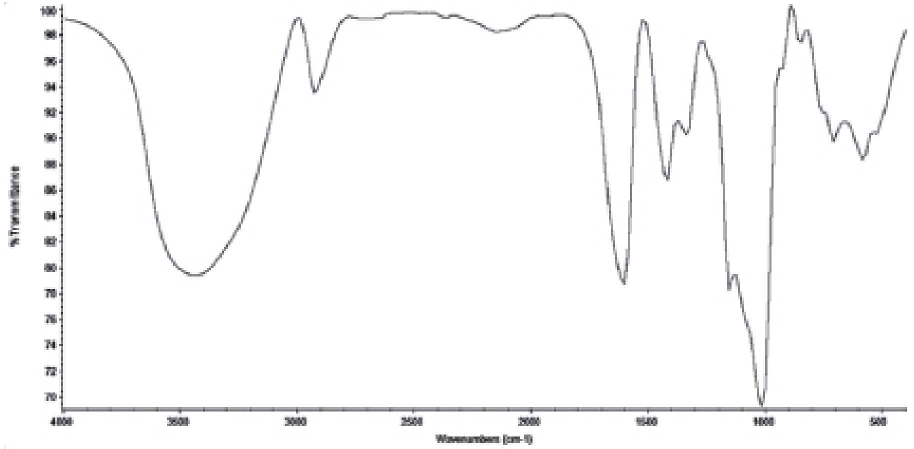

Figure 3. The IR-spectrum of CMHPMS which showed $–COO^-$ peak at 1600-1700 cm^{-1}.

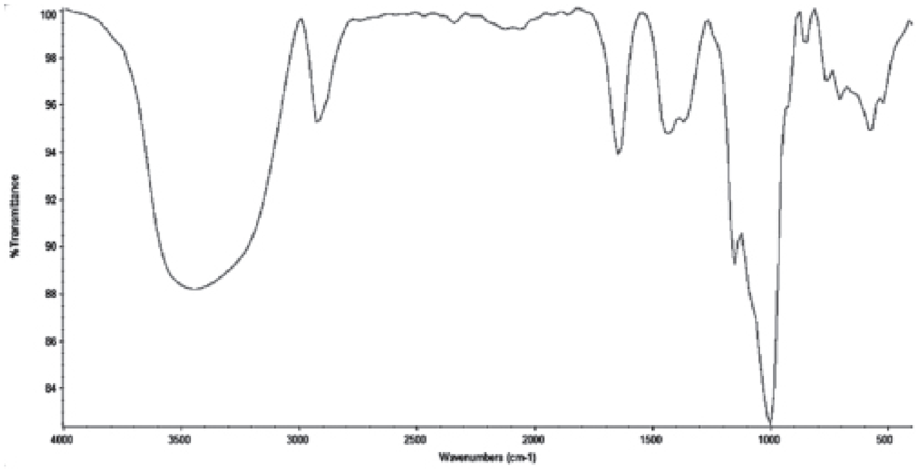

Figure 4. The IR-spectrum of native mung bean starch which showed less intense $–COO^-$ peak at 1600-1700 cm^{-1}.

Scanning electron microscope (SEM) images of CMMS (Fig.5), HPCMMS (Fig.6) and CMHPMS (Fig.7) granules showed similar shapes to that of native mung bean starch (Fig.8) but with more size uniformity with smoother surface. The SEM images in this study are similar to SEM images reported in Kittipongpatana et al., (2006a). This is the indication that the etherification of starch granules occurred on the surface and without fragmentation of the granules which is in agreement with the report by Chuenkamol et al., (2007) that the hydroxypropylation in canna starch did not alter the surface of granules. However, this is in contrast with the report of Kaur et al., (2004) in which hydroxypropylation in potato starch was shown to alter granule morphology. The different results could be due to the conditions of modification and starch types. The solvent used in the reaction could also play an important role in protecting the granule from disrupting.

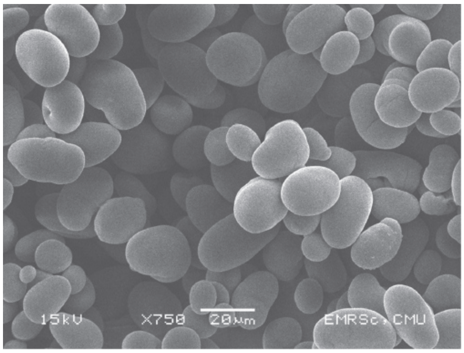

Figure 5. SEM of carboxymethyl mung bean starch.

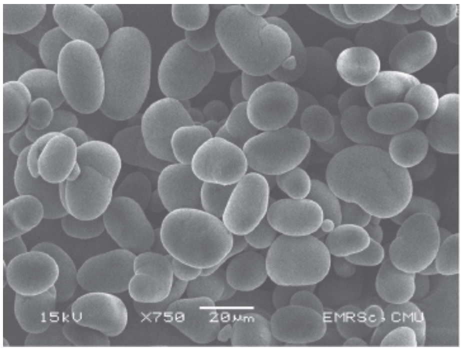

Figure 6. SEM of hydroxypropyl-carboxymethyl mung bean starch.

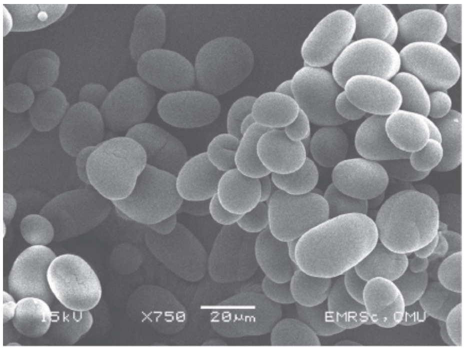

Figure 7. SEM of carboxymethyl-hydroxypropyl mung bean starch.

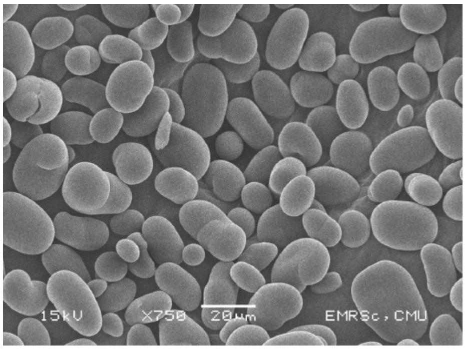

Figure 8. SEM of native mung bean starch.

X-ray diffractograms of native and modified starches are shown in Fig 9. The major peaks were observed at 15, 17 and 23° of diffraction angle 2θ. These values are in agreement with those reported by Kittipongpatana et al., (2006a). According to SEM and X-ray diffraction analysis, these results showed that the modification of carboxymethyl or hydroxypropyl substitution did not change the crystallinity of the starches.

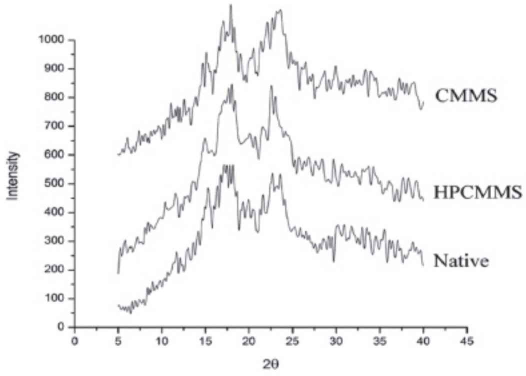

Figure 9. X-ray diffractograms of native and modified starches.

The apparent viscosity of 1% and 3% w/v concentrations of native and modified starches are shown in Table 3. The 1% w/v solution of modified starches yielded viscosity of 507.13±3.75, 301.63±11.67, 255.61±6.16 and 0±0 mPas for CMMS, HPCMMS, CMHPMS and native starch, respectively. All of 3% w/v solutions yielded viscosity more than 2 folds of what 1% w/v solutions did, being 1075.17±42.66, 823.80±51.39, 514.88±38.91 and 44.59±10.68 mPas for CMMS, HPCMMS, CMHPMS and native starch, respectively. The rheograms of 1% w/v solution (Fig 10) and 3% w/v solution (Fig 11) showed a pseudoplastic-type behavior with the formation of hysteresis loop between up curve and down curve but showed a small area of hysteresis loop.

Comparison of the viscosity of CMMS and HPCMMS revealed that HPCMMS had lower viscosity which could be explained by the addition of hydroxypropyl group which showed distinctive point at the increasing of water-holding capacity. HPCMMS could hold more water than CMMS that caused HPCMMS to have lower viscosity although hydroxypropyl group was of hydrophobic property. In the case of CMHPMS which exhibited the lowest viscosity, the higher DS (0.31) compared to that of CMMS (0.25) could be the reason. Higher DS was reported to accompany less viscosity, as a result of the carboxymethyl groups interfering with helical structure of amylose, forming low-strength gel (Kittipongpatana et al., 2006a). Traces of hydroxypropyl MS detected in CMHPMS had no effect on the viscosity.

Table 3. Apparent viscosity (1% and 3% w/v) and pH (3% w/v) of native, CMMS, HPCMMS and CMHPMS.

Type of Starch	Viscosity (mPa.s±SD, SR 100 s^{-1})		pH (3% w/v solution)
	1% w/v	3% w/v	
Native	0.00	44.59±10.68	6.2
CMMS	507.13±3.75	1075.17±42.66	6.2
HPCMMS	301.63±11.67	823.80±51.39	6.3
CMHPMS	255.61±6.16	514.88±38.91	6.7

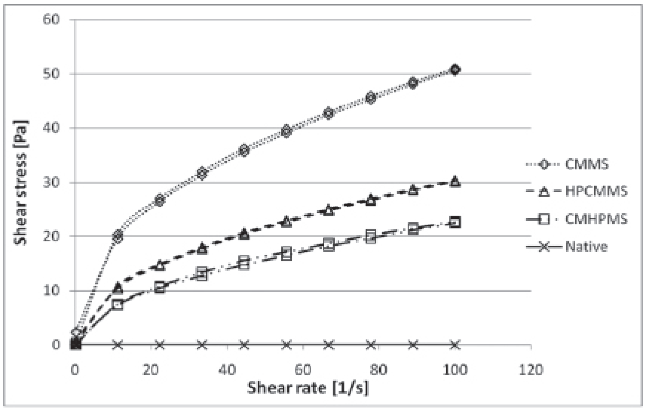

Figure 10. Rheograms of starches at 1 % w/v concentration.

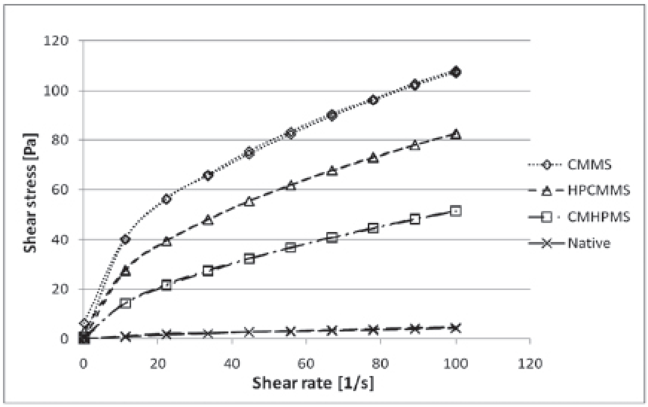

Figure 11. Rheograms of starches at 3 % w/v concentration.

The water uptake profiles of native and modified starches (Fig 12) showed that HPCMMS and CMHPMS took up volumes of water similarly, i.e., approx. 2.00 ml. CMMS took up lower amount of water (1.5 ml) while native starch showed little power of uptaking the water. When considering the water uptake profiles, CMMS could uptake the water very fast in the first period which was different from HPCMMS and CMHPMS that uptook the water slowly. Water uptake diagrams of HPCMMS and CMHPMS showed that they could uptake about 33% of water in 10 min and needed 20 min to reach 50% of water uptake but they could uptake the water more than CMMS at 60 min. The diagram of CMMS showed the difference that CMMS could uptake 50% of water in 10 min but slower after 10 min that could probably be explained by CMMS viscosity. According to CMMS that had the highest viscosity, when CMMS uptook the water, CMMS formed strength gel very fast, then the gel protected water from penetrating into dry starches, causing CMMS to uptake the water lower than the other modified starches. This water uptake property could be related to the drug controlled-release mechanism and could potentially be applied as hydrophilic polymer in the pharmaceutical preparation.

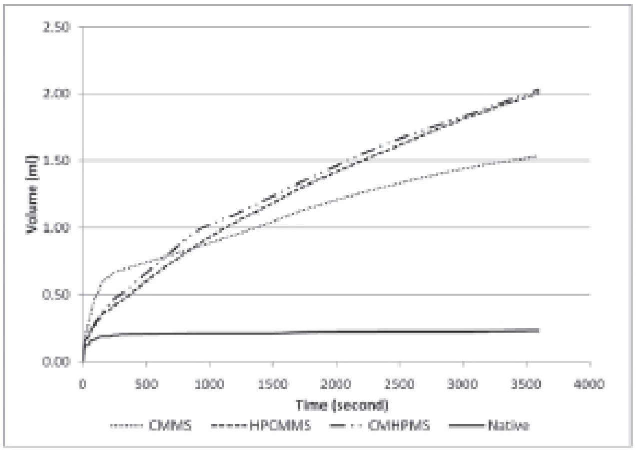

Figure 12. Diagrams of water uptake of starches when using starches approx. 500 mg.

CONCLUSION

This study investigated the effect of the sequential, dual modification by carboxymethylation and hydroxypropylation on the physicochemical properties of mung bean starch. The dual modification was better accomplished when the hydroxypropylation was carried out first, followed by the carboxymethylation (HPCMMS). The reversal of the reaction order resulted in a prolonged, single modification of starch by carboxymethylation, with only trace level of hydroxypropylation detected (CMHPMS). Like CMMS, both HPCMMS and CMHPMS were soluble in water. XRD and SEM analyses revealed no changes in the granule shape, size and crystallinity. Modified starches showed the improvement in viscosity and water uptake ability compared with native mung bean starch. The physicochemical properties of modified starches can be used as a guide to suggest their application. CMMS and HPCMMS were selected for further study. Based on the high viscosity and good water uptake ability, CMMS and HPCMMS are considered to be hydrophilic polymers which can be used as a controlled-release agent because they formed gel networks which entrapped the drug and acted as matrix to control the release of drug to the medium (Onofre et al., 2009). The investigation of both polymers in controlling the release of drug is currently underway.

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