

Preparation, Physicochemical and Film-Forming Properties of Carboxymethyl/Hydroxypropyl Dual-Modified Tapioca Starches

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ABSTRACT

Water-soluble, dual-modified tapioca starches were prepared via carboxymethylation and hydroxypropylation reactions. The effects of modification sequence on physicochemical and film-forming properties of the modified starches were investigated. The reactions were carried out on the native tapioca starch in specific orders to yield either carboxymethylhydroxypropyl starch (CMHPS) or hydroxypropylcarboxymethyl starch (HPCMS). The degree of carboxymethyl substitution (DS) and molar hydroxypropyl substitution (MS) of dual-modified starches were 0.39 and 0.02 for CMHPS and 0.26 and 0.02 for HPCMS, respectively. Both CMHPS and HPCMS were soluble in water, a 1%w/v solution of which yielded pH of 8.0 and 6.8 and the viscosities of 89.3 ± 0.6 and 82.0 ± 1.4 mPa s at shear rate 500 s^{-1} . SEM images revealed marked differences in the granule surface between the native and modified starches, while XRD showed a decrease in the degree of crystallinity in the modified starches compared to their native starches. The film-formation test by Petri dish method showed that CMHPS and HPCMS formed clear films with good strength and fair flexibility. The addition of plasticizers, PEG 6000 and glycerol, and a crosslinking agent – citric acid – at suitable amount, further improved the texture and flexibility of the films. CMHPS film showed a significant improvement on the water vapor transmission rate (WVTR) compared to CMS and HPCMS and can be developed as new aqueous-based, film-coating agents for pharmaceutical tablets.

Key words: Tapioca starch, Hydroxypropylation, Carboxymethylation, Film-coating agent, CMHPS, HPCMS

INTRODUCTION

Starch is one of the most versatile biopolymers used in pharmaceutical industry as it has been utilized as many types of excipient, including filler/diluent, disintegrant, and, in a paste form, binder. However, some inherent physicochemical properties such as the insolubility in water (Krogar et al., 2002), poor flowability, opacity of cooked paste, etc. have limited applications of native starch in many cases. Modification of starch represents an effective way to utilize starch to its maximum potential.

This can be achieved by chemically or physically modifying the native functional groups of the structure to improve or alter the physicochemical properties such as granule size and crystallinity, chain length, water solubility, swelling capacity, etc. Many types of modified starches have been prepared and used in food, textiles and pharmaceutical industries.

Carboxymethyl starch (CMS), an anionic modified starch, and hydroxypropyl starch (HPS), a non-ionic modified starch, are etherified starches that are less susceptible to retrogradation compared to the native starch and have found uses in the food industry (Arvanitoyannis et al., 1998; Pal et al., 2002). CMS, prepared from a reaction between starch and chloroacetic acid under an alkaline condition in an alcoholic medium (Stojanovic et al., 2000), is hydrophilic and, depending on the degree of carboxymethyl substitution, is either soluble or swellable in water (Yao et al., 2003). The water-insoluble HPS is prepared by a reaction between starch and propylene oxide. HPS is hydrophobic because the $-OH$ groups of native starch are partially replaced by the hydroxypropyl ($-CH_2-CH_2-CH_2-OH$) moieties (Choi and Kerr, 2003).

CMS has successfully been used as disintegrant, binder, suspending agent (Kittipongpatana and Sirithunyalug, 2006) and, most recently, a film former in an aqueous film-coating formulation (Kittipongpatana et al., 2006b) in pharmaceutical dosage forms. The latter reported that CMS formed thin film with greater flexibility and strength than that of the native starch and was used as a film former in an aqueous-based tablet film coating formulation. With the CMS film showing signs of absorbing moisture due to the hygroscopic property of the carboxymethyl group, it is necessary to decrease the water absorption of the modified starch molecules in order to maintain the film integrity. One way to accomplish the goal is to add hydrophobic groups into the hydrophilic CMS molecules. Since hydroxypropylation is done under alkaline condition similar to that of carboxymethylation, a continuous process in which one reaction is carried out after the other can be designed. The significance of the order of the reaction is tested by carrying out first the carboxymethylation, then hydroxypropylation for one sample and vice versa for the other sample to obtain two types of modified starches. In this study, we report for the first time the preparation of dual carboxymethyl-hydroxypropyl tapioca starches, and the physicochemical properties as well as the film-forming ability of these modified starch compared to CMS, HPS and native tapioca starch.

MATERIALS AND METHODS

Materials

Tapioca starch was obtained from ETC International Trading (Thailand). Chloroacetic acid and propylene oxide were products of Fluka (Germany). All chemicals used in the preparation and analysis of modified starches were of AR grade or equivalent

Methods

Extraction of starch and determination of amylose content

Tapioca starch was subjected to starch extraction before use. The method was based on alkaline extraction (Yamamoto et al., 1973). The amylose content of the extracted native tapioca starch was then determined, using colorimetric method (Juliano, 1971).

Preparation of modified starches

• *Hydroxypropyl starch (HPS)*

Starch powder (100 g) was suspended in 120 ml 0.1% NaOH with 15 g Na₂SO₄. After 10 min of reaction, 12 ml of propylene oxide was added and the reaction was maintained at 40°C for 24 h with continuous stirring. The starch suspension was then washed three times with distilled water. The pH was adjusted to 5.5 with 2 M HCl. The starch cake was washed several times with distilled water and dried at 40 °C.

• *Carboxymethyl starch (CMS)*

Starch powder (138 g) was suspended in 260 ml methanol with 40 g chloroacetic acid. Then 79.5 ml of 50% NaOH was added and the reaction was heated up to 70°C. After 1 h with continuous stirring, the reaction was neutralized with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 90% methanol and dried at 50°C.

• *Carboxymethylhydroxypropyl starch (CMHPS)*

Starch powder (138 g) was suspended in 260 ml methanol with 40 g chloroacetic acid. Then 79.5 ml of 50% NaOH was added and the reaction was heated up to 70°C. After 1 h with continuous stirring, the reaction was cooled down to 40°C and 16.6 ml propylene oxide was added. The reaction was maintained at 40°C for 24 h with continuous stirring, then neutralized with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 90% methanol and dried at 50°C.

• *Hydroxypropylcarboxymethyl starch (HPCMS)*

Starch (138 g) was suspended in 165.6 ml 0.1% NaOH with 20.7 g Na₂SO₄. After 10 min of reaction, 16.6 ml of propylene oxide was added and the reaction was maintained at 40°C for 24 h with continuous stirring. The supernatant was decanted, the starch product was washed three times with distilled water and the pH was adjusted to 5.5 with 2 M HCl. Then the starch was resuspended in 260 ml methanol with 39.9 g chloroacetic acid. After 79.5 ml of 50% NaOH was added, the reaction was maintained at 70°C for 1 h before being neutralized with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 90% methanol and dried at 50°C.

Determination of molar substitution and degree of substitution

The determination of hydroxypropyl group in HPS, CMHPS and HPCMS was carried out using a spectrophotometric method (Johnson, 1969). The molar substitution (MS) was calculated according to the formula described by Rutenberg and Solarek (1984)

The degree of substitution (DS) of CMS, CMHPS and HPCMS was determined by the USP XXIII method described for croscarmellose sodium, which included two-step titration and residue on ignition. The DS can be calculated using the following equation:

$$DS = A + S \quad (1)$$

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

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

$$S = \frac{(162 + 58A)C}{(7120 - 80C)} \quad (3)$$

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

Scanning electron micrographs

The surface and morphology of the powder granules were examined, using a JEOL JSM-5910LV Scanning Electron Microscope. The acceleration voltage was 15 kV under high vacuum mode.

X-ray diffraction

X-ray diffractograms of starch powders were obtained, using a Siemen D-500 X-ray diffractometer. Diffractograms were registered at Bragg angle (2θ) of 5 to 60°C at a scan rate of 5°C/min

Solubility and pH of solution

The solubility in water of modified starches was tested by adding 0.1 g of sample into 10 ml of water (1% w/v), mixed thoroughly and observed the solubilization and/or swelling when compared with the native starch. The pH of water-soluble modified starch solution (1%) was determined, using a Waterproof pHScan WP2 (Eutech Instruments, USA).

Viscosity

The apparent viscosities of 1% w/v solutions of CMS, CMHPS and HPCMS were measured, using a Bob-and-Cup format rotational viscometer (Brookfield Engineering LA, R/S-CC). Sample preparation was carried out as previously described (Kittipongpatana et al., 2006). The measuring system was CC48 DIN. The mode used was CSR (controlled shear rate). The measured parameters consisted of three steps: (1) an increase of the shear rate from 0 to 500 s⁻¹ in 1 min, (2) held at 500 s⁻¹ for 1 min and (3) a decrease of the shear rate from 500 to 0 s⁻¹ in 1 minute. 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.

Paste clarity and freeze-thaw stability

The clarity of starch paste was determined as previously described (Kittipongpatana et al., 2006). Freeze-thaw stability was assessed by subjecting the samples through eight cycles in which each cycle consisted of 2 days storage at 8°C and another 2 days storage at 45°C. The clarity was evaluated, using absorbance (A) at 650 nm against a water blank.

Thermal transition study by differential scanning calorimetry

Thermal transition of starch gelatinization was studied, using a DSC-7 differential scanning calorimeter (Perkin Elmer Corp., USA). Starch (2.5-3.5 mg) was weighed directly into a DSC pan. Deionized water (15 µl) was added using a microsyringe, and the pan was sealed. A thermogram was then acquired between 40 and 200°C at a heating rate of 10°C/min. An empty pan was used as a reference. The enthalpy of retrogradation (joules/g of dry starch) was calculated by integrating the area under the peak.

Swelling power and solubility

Five mixtures of 0.5 g native or water-insoluble modified starches and 40 ml distilled water were placed in test tubes and heated at 50, 60, 70, 80 and 90°C, respectively, for 30 min. Lump formation was prevented by stirring. At the end, each mixture was centrifuged at 3000 rpm for 15 min. The supernatant was decanted and the swollen starch sediment was weighed. Swelling power was calculated from the ratio in weight of the wet sediment to the initial weight of dry starch (Subramanian et al., 1994). An aliquot of supernatant was evaporated at 130°C and the residue was weighed. Percent solubility was determined as the ratio in weight of the dried supernatant to the initial weight of the dry starch ×100.

Film formation and characteristics

• Film formation test by Petri dish method

A 3% w/v aqueous solution of CMHPS and HPCMS (30 ml), alone or with varied combinations of the types and/or concentrations of plasticizers (PEG 6000, glycerol) and a crosslinking agent (citric acid), was placed on a plastic Petri dish and dried at 60°C for 17 h. Control films were prepared using native starch solution.

• Water vapor transmission rate (WVTR)

The WVTR of the film was determined according to ASTM E96-94 (ASTM, 1944). All samples were cut to be annular 4 cm diameter. Each sample has to be without a fold, mark or an observable leak. The sample was firmly fixed on top of a 4 cm-diameter glass bottle containing beads of silica gel. Then, the containers were placed in a desiccator at 25°C and 50% relative humidity and were weighed daily on an analytical balance for 7 days. The weight of the film was recorded and the WVTR calculated using an equation:

$$WVTR = \frac{(G / t)}{A}$$

where WVTR is water vapor transmission rate (g/m² day), G/t is weight gain over time, and A is the permeation area. The tests were carried out in triplicate.

RESULTS AND DISCUSSION

Amylose content and determination of degree of substitution and molar substitution

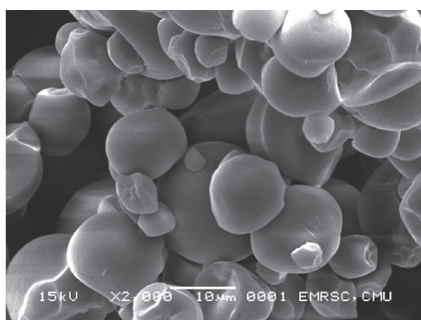
The amylose content of native tapioca starch, as determined by colorimetric method, was 19.7%. The value was in agreement with that previously reported (Yao et al., 2003). The degrees of substitution (DS) for CMS, CMHPS and HPCMS were 0.25, 0.39 and 0.26, respectively. A significantly higher DS observed for CMHPS was probably due to the longer period of time the starch granules were exposed to or reacted with chloroacetic acid as it remained in the reaction vessel during the reaction with propylene oxide. Significant difference among these values indicated the effects of the time of the reaction and the order of which the reaction has taken place. In contrast, the values of molar hydroxypropyl substitution were not affected by either the time or the order of the reaction. The pHs of 1% w/v solution were between 6.8 and 8.0, depending on the DS (Table 1).

Table 1. Degree of substitution (DS), molar substitution (MS), water solubility and pH 1% w/v solution of HPS, CMS, CMHPS and HPCMS prepared from tapioca starch.

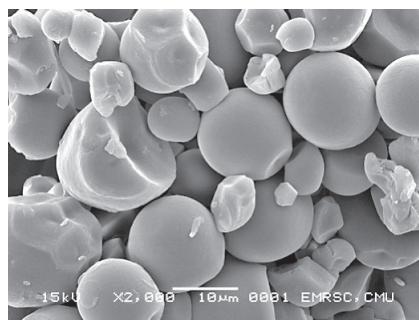
Type of modified tapioca starch	DS	MS	Solubility in water	pH
HPS	-	0.04	×	-
CMS	0.25	-	✓	7.4
CMHPS	0.39	0.02	✓	8.0
HPCMS	0.26	0.02	✓	6.8

Scanning electron microscopy

Scanning electron microscopic (SEM) images of native and four modified tapioca starch (Figure 1) showed that the granules of native tapioca starch were predominantly spheroidal, with size range between 10-15 µm. Hydropropyl-modified starch showed similar SEM image to that of the native starch, while carboxymethyl-modified starch showed slight change in the shape and the size range was broader. The dual modification, both CMHP and HPCM, caused significant changes to the granules. Rough surface was observed on the granules and the shape also became irregular.



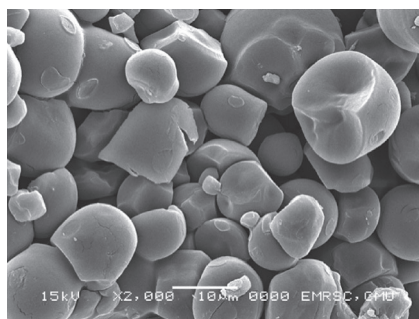
HP-TS



CM-TS



HPCM-TS



CMHP-TS

Figure 1. Scanning electron microscopic (SEM) images of tapioca and modified tapioca starches. (A) HPS, (B) CMS, (C) HPCMS and (D) CMHPS. All images are 2000x magnification.

X-ray diffraction

X-ray diffractograms of native and modified starch powders acquired at Bragg angle (2θ) of 5 to 60°C at a scan rate of 5°C/min are shown in Figure 2. X-ray diffractogram of native tapioca starch showed strong reflections at 15, 17 and 23° of diffraction angle 2θ . These values are in agreement with those previously reported. The modification with hydroxypropylation caused a slight decrease in the intensity of the reflection signal, while those involved the modification with carboxymethylation showed a significant decrease in the signal intensity which corresponds to a decrease in the crystallinity of the granule (Kittipongpatana et al., 2006a).

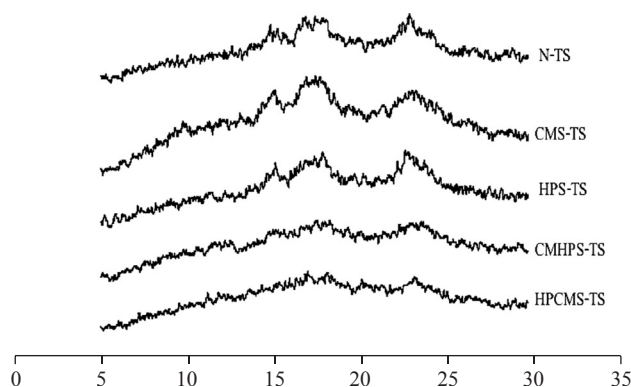


Figure 2. X-ray diffractograms of native and modified tapioca starches.

Viscosity

The viscosities of 1% w/v aqueous solution of CMHPS and HPCMS were significantly lower than that of the CMS (Table 2). However, the decrease in the viscosity of modified starch solution when subjected to FT conditions were 18.78%, 15.22% and 17.07% for CMS, CMHPS and HPCMS, respectively. The results were in agreement with previous report that while hydroxypropylation caused a decrease in the viscosity of the modified starch, it also improved the resistance of modified starch against harsh conditions (Choi and Kerr, 2003).

Table 2. Viscosity ($\pm$ SD at 500 s⁻¹; n=3) of 1% w/v solution of water-soluble modified tapioca starches at room temperature and freeze-thaw (FT) conditions.

Type of modified Starch	Viscosity of 1% w/v solution (mPa s) at 500 S ⁻¹	
	Normal condition	Freeze-Thaw (FT)
CMS	98.5 $\pm$ 2.1	80.0 $\pm$ 1.0
CMHPS	89.3 $\pm$ 0.6	75.7 $\pm$ 0.6
HPCMS	82.0 $\pm$ 1.4	68.0 $\pm$ 2.6

Swelling power and solubility

The swelling powers of the sample at 50, 60, 70, 80 and 90°C are presented in Table 3. Swelling of granules normally took place in native starch when the temperature is increased. This is further enhanced upon the modification with hydroxypropylation. The extent of swelling is positively related to the solubility, i.e., the higher the swelling power, the greater the solubility (Table 4). Hydroxypropylation results in a significant increase in swelling of granules, especially at 60 and 70°C, because the hydroxypropyl groups replaced the original –OH groups and allowed easier access of water molecules into the starch granules (Rutenberg and Solarek, 1984). Hydroxypropylation decreased the temperature at which starch granules started to swell but did not significantly increase the overall swelling.

Table 3. Swelling power of native starches and water-insoluble HPS upon subjecting to heat from 50 to 90°C.

Type of Starch	Swelling Power				
	50°C	60°C	70°C	80°C	90°C
Native- TS	2.25±0.18	6.81±1.57	19.70±0.69	17.95±2.52	17.71±0.65
HPS-TS	3.01±0.64	18.53±2.30*	23.48±0.69*	18.26±1.80	19.34±1.45

*significantly different ($P<0.05$)

Table 4. Solubility of native starches and water-insoluble HPS upon subjecting to heat from 50 to 90°C.

Type of Starch	Solubility				
	50°C	60°C	70°C	80°C	90°C
Native-TS	0.50±0.39	9.88±1.52	74.12±1.76	88.62±0.28	87.76±1.38
HPS-TS	0.47±0.01	22.82±1.33*	69.46±1.90	85.46±2.57	84.12±1.99

*significantly different ($P<0.05$)

Solubility data correlate well with the swelling data. At 60°C, HPS is 2.5 times more soluble than the native starch but at higher temperatures, no significant difference is observed. Both starches are soluble up to ~85-88% at high temperature. Thus, hydroxypropylation caused solubilization to take place at lower temperature but did not improve overall solubility.

Differential scanning calorimetry

DSC thermogram (Figure 3) shows that hydroxypropylation of the native starch resulted in a slight decrease of the onset gelation temperature (T_0) from 59.60±1.45 to 57.23±1.90°C and a marked decrease of the peak gelation temperature (T_p) from 68.61±2.97 to 63.69±1.63°C. The enthalpy (ΔH) of the conformational change was also lower from 10.89 J/g for native starch to 9.25 J/g for HPS which suggested that hydroxypropyl modification allowed easier gelation of starch molecules. This is probably caused by the substitution of the less hydrophilic hydroxypropyl groups in place of the more hydrophilic -OH groups (Choi and Kerr, 2003). With less -OH group to form interchain hydrogen bonds, the strength of bonds in starch chains weakened and so did the gelatinization temperature (Pal et al., 2002).

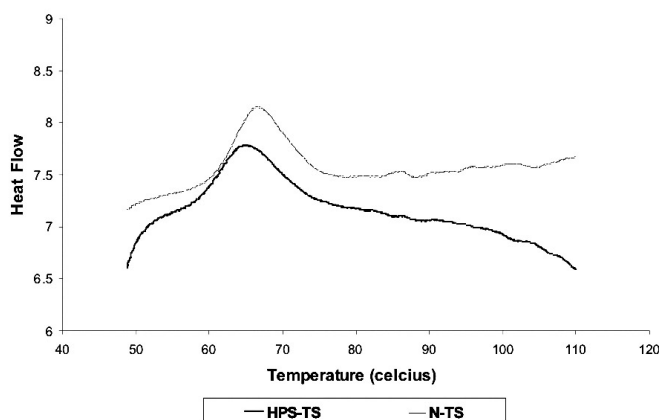


Figure 3. DSC thermogram showing the onset gelation temperature (T_o , °C) and peak gelation temperature (T_p , °C) of native and hydroxypropyl tapioca starches

Film formation and characteristics

The appearance and texture of films prepared from 3% w/v tapioca starch-derived CMHPS and HPCMS (30 ml) with addition of different amounts and ratios of two plasticizers, PEG 6000 and glycerol, and a crosslinking agent, citric acid, are presented in Table 5. Overall, both CMHPS and HPCMS films formed plastic-like films that are not flexible and easy to break, similar to that of HPS and CMS reported previously (Arvanitoyannis et al., 1998; Kittipongpatana et al., 2006b). With the addition of polyol plasticizers and crosslinking agent, the appearance of the films varied but mostly become better and more flexible films.

Table 5. Appearance and texture of films prepared from 3% w/v tapioca starch-derived CMHPS and HPCMS (30 ml) with addition of different amounts and ratios of plasticizers and crosslinking agent.

Starch Type	Amount used per 30 ml			Film Appearance
	Glycerol (ml)	PEG 6000 (g)	CLA (g)	
CMHP-TS	-	-	-	plastic-like film, quite crisp
CMHP-TS	0.300	0.045	0.270	plastic-like film, easy to tear
CMHP-TS	0.300	0.090	0.270	plastic-like film, sticky and absorb moisture
CMHP-TS	0.000	0.045	0.540	plastic-like film, absorb moisture
CMHP-TS	0.300	0.045	0.540	translucent film, absorb moisture
CMHP-TS	0.300	0.900	0.540	translucent film, absorb moisture
HPCM-TS	-	-	-	plastic-like film, quite crisp
HPCM-TS	0.300	0.045	0.270	plastic-like film, flexible
HPCM-TS	0.300	0.090	0.270	plastic-like film, flexible and absorb moisture
HPCM-TS	0.000	0.045	0.540	plastic-like film, not flexible
HPCM-TS	0.300	0.045	0.540	plastic-like film, flexible and absorb moisture
HPCM-TS	0.300	0.900	0.540	plastic-like film, absorb moisture

Water Vapor Transmission

An additional modification to CMS by hydroxypropylation to form CMHPS resulted in a modified starch product which, when formed film, exhibited a slightly but significantly lower WVTR compared to that of CMS. In contrast, HPCMS in which hydroxypropylation was carried out first on the native starch, followed by carboxymethylation, yielded film with similar WVTR to that of CMS (Table 6).

Table 6. Water vapor transmission rate (WVTR) of various types of modified tapioca starch films.

Type of MTS films	WVTR (10^{-2} g/m ² /day)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CM-TS	2.72±0.13	1.49±0.17	1.37±0.13	1.17±0.05	1.15±0.06	1.18±0.11	1.04±0.11
CMHP-TS	2.76±0.59	1.38±0.20	1.18±0.03	1.11±0.14	1.04±0.09	1.10±0.12	0.98±0.09
HPCM-TS	2.76±0.24	1.52±0.01	1.37±0.04	1.14±0.04	1.08±0.04	1.25±0.09	1.12±0.06

CONCLUSION

Two new types of dual-modified starch, carboxymethylhydroxypropyl starch (CMHPS) and hydroxypropylcarboxymethyl starch (HPCMS), were successfully prepared using native tapioca starch. Both modified starches showed different physicochemical properties compared to those of native starch and some combined properties of the single-modified carboxymethyl (CMS) and hydroxypropyl starches (HPS). A particularly-interesting property is the water solubility which is similar to that of CMS and lower water vapor transmission rate characteristic of HPS. The properties of the modified starches were also affected by the sequence in which the two reactions were carried out. CMHPS and HPCMS appeared to be good film formers, especially CMHPS which showed lower WVTR compared to CMS. The variations of DS and MS of this modified starch could further improve its properties and should lead to the development of a new starch-based, aqueous film-coating agent for pharmaceutical tablets.

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REFERENCES

- Arvanitoyannis, I., A. Nakayama, and S. Aiba. 1998. Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydrate Polymers* 36: 105-119.
- Choi, S.G., and W.L. Kerr. 2003. Water mobility and textural properties of native and hydroxypropylated wheat starch gels. *Carbohydrate Polymer* 51:1-8.

- Johnson, D.P. 1969. Spectrophotometric determination of the hydroxypropyl group in starch ethers. *Analytical Chemistry* 41: 859-860.
- Juliano, B.O. 1971. A simplified assay for milled-rice amylose. *Cereal Science Today* 16: 334-340.
- Krogar, K., O. Antikainen, J. Heinamaki, N. Laitinen, and J. Yliruusi. 2002. Tablet film-coating with amylose-rich maize starch. *European Journal of Pharmaceutical Sciences* 17: 23-30.
- Kittipongpatana, O.S., Sirithunyalug, J., and Laenger, R. 2006a. Preparation and physicochemical properties of sodium carboxymethyl mungbean starches. *Carbohydrate Polymer* 63: 105-112.
- Kittipongpatana, O.S., N. Chaichanasak, S. Kanchongkittipoan, A. Panturat, T. Tehkhunmark, and N. Kittipongpatana. 2006b. An aqueous film-coating formulation based on sodium carboxymethyl mungbean starch. *Starch/starke* 58(11): 587-589.
- Kittipongpatana, O.S., and J. Sirithunyalug. 2006. Development of suspending agent from sodium carboxymethyl mungbean starch. *Drug Development and Industrial Pharmacy* 32(7): 609-620.
- Pal, J., R.S. Singhal, and P.R. Kulkarni. 2002. Physicochemical properties of hydroxypropyl derivative from corn and amaranth starch. *Carbohydrate Polymers* 48: 49-53.
- Rutenberg, M.W., and D. Solarek. 1984. Starch derivatives: production and uses. p. 344-349. In R.L. Whistler, J.N. Bemiller and E.F. Paschall (eds) *Starch: chemistry and technology*. New York: Academic Press.
- Stojanovic, Z., K. Jeremic, and S. Jovanovic. 2000. Synthesis of carboxymethyl starch. *Starch/ Starke* 52: 413-419.
- Subramanian, V., R.C. Hosene, and P. Bramel-Cox. 1994. Shear thinning properties of sorghum and corn starches. *Cereal Chemistry* 71: 272-275.
- Yamamoto, K., S. Sumie, and O. Toshio. 1973. Properties of rice starch prepared by alkali method with various conditions. *Denpun Kagaku* 20: 99-102.
- Yao, J., W. Chen, M.M. Robbert, J.G. Klaassien, and J.H. Hero. 2003. Exploratory studies on the carboxymethylation of cassava starch in water miscible organic media. *Starch/Starke* 56: 100-107.