Optimization of Reaction Conditions for Carboxymethylated Sago Starch

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A B S T R A C T

This study involved the optimization of determining parameters in reaction conditions of carboxymethylation process of sago starch (*metroxylum sagu*) against degree of substitution (DS) and reaction efficiency (RE%). Characterizations were carried out by analyzing the spectrum of Fourier transform infrared (FTIR), the thermograms of differential scanning calorimetry (DSC), the X-ray diffraction (XRD) patterns, the scanning electron microscopy (SEM) photomicrographs and viscosity analysis. The optimized reaction conditions were determined by the amount of sodium hydroxide in the system, the time taken for a complete reaction, the temperature used for the reaction to occur and the ratio of the anhydroglucose unit to sodium monochloroacetate produced in the final yield of the product. The optimized reaction conditions of 20% concentration of NaOH, reaction time of 1 h, reaction temperature of 55°C and NaMCA:AGU molar ratio of 1.5:1.0 produced a DS value of 1.05 and RE of 85.9% carboxymethylated sago starch. The FTIR spectrum clearly displayed the new peaks at 1587 cm^{-1} and 1416 cm^{-1}, which indicated the presence of carboxymethyl group. SEM Photomicrographs showed starch granules being distorted, the surfaces were rough and grooved compared to the native starch granules indicating an acid or enzyme-treated reaction took place. The DSC thermograms exhibited an endothermic peak for sago starch but none for the treated sago suggesting the absence of gelatinization, which was later confirmed by the changes of the XRD patterns. These optimization factors allowed higher yields of carboxymethyl sago starch providing plenty of opportunities for its multi-applications.

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INTRODUCTION

The use of natural starch and its derivatives as biodegradable polymers continue to be an area of active research despite the advent of synthetic polymers. It does remain attractive, primarily because they are inexpensive, readily available, degradable, biocompatible and non-toxic [1-3].

From 1969 to 2001, there have been over 23,000 scientific articles in the Food Science and Technology (FSTA) database concerning starch; 2960 of these articles concentrated on amylose, while 1416 papers focused on amylopectin. Earlier papers described methodologies utilized in studying chemical compositions and starch structure. Many of them dealt with the microstructures and fuctional properties of the starch. However, the trend has changed where most of the studies are now focusing on

biosynthesis and modification of the starch structure.

Recently natural starch has gained increasingly importance as a basis for new biopolymer material and functional polymer mainly synthesized by chemical modification reactions. Carboxymethylation process of starch is one of the most versatile functionalization procedures as it provides access to biomaterials with valuable properties. This process was carried out by substituting the hydroxyl groups (O-H) in the starch molecules with carboxymethyl groups (CH_2COOH) to produce carboxymethyl starch (CMS). To prevent gelatinization and to keep the granular structure intact, the reaction was usually carried out in an organic medium [4,5].

Most of the commercially produced CMS especially from cornstarch has a degree of substitution (DS) value of less than 0.3 and the first CMS (sago starch) was produced by Noor Fadzlina et al. [6] and also found to have a DS value of less than 0.3. He also found that the CMS has a high swelling power property, excellent solubility at ambient temperature, low intrinsic viscosity and reduced tendency to retrograde.

Palviainen et al. [7] studied aqueous native cornstarch and found that the material has a relatively good film forming properties. Studies by Krogars et al. [8] on the possibility of using amylose-riched cornstarch as a coating agent for pharmaceutical purposes produced a smooth coating on pharmaceutical dosage forms. However, the need for special and complex equipment for preparing the aqueous starch coating solution and coating with heated solution were the major limitations for this technique. Other drawbacks include poor solubility of natural starch at ambient temperature and its tendency to form a high viscous solution in hot water.

CMS is a water-soluble biopolymer and swells in water. Its application is mainly in food industry. In pharmaceutical industry, it is only used as an auxiliary agent such as a disintegrant and to date no such study has been done to use CMS as an aqueous film-coating agent using sago starch [9]. On the other hand, Pushpamalar et al. [10] studied the optimization factors on the production of carboxymethyl cellulose (CMC) using sago waste, a completely different study and approach compared to our present study. Here, this study worked on the sago starch

extracted from sago to produce carboxymethyl starch which has better physicochemical properties compared to ordinary starch.

The aim of this study is to investigate the optimization factors to allow carboxymethylation process on sago starch (*metroxylum sagu*). The novelty of this work lies on the optimization process that are capable of producing a higher yield of carboxymethylated starch with a higher degree of substitution value which has not been able to achieve before.

EXPERIMENTAL

Materials
Sago starch was supplied by NITSEI Sago Industries Sdn. Bhd. (Penang, Malaysia). The starch was used as provided without any further treatment. Sodium hydroxide (NaOH) and sodium monochloroacetate (NaMCA) were purchased from Scharlau Chemie S.A. (Germany), isopropanol, methanol and ethanol were purchased from BDH Chemical Limited Poole (England). Diphenylamine reagent was obtained from Merck (Darmstadt, Germany). Hydroxypropyl methylcellulose (HPMC) was purchased from BDH Chemical Limited Poole (England). All materials were used as received without further purification and of an analytical grade.

Methods
Carboxymethylation
For the preparation of CMS, the method of Heinze et al. [11] was followed with minor modification. Sago starch was slurred in 300 mL of isopropanol in a 1-L, covered round-bottomed reactor flask equipped with stirrer, reflux-condenser and burette. The reflux-condenser was used to prevent the loss of organic liquid. The estimated amount of aqueous NaOH ranging from 10-30% was added into the reactor flask over a period of 20 min. The mixture was then stirred for 30 min and NaMCA was then added. Subsequently, the flask was heated to the reaction temperature which varied from 35°C to 75°C. After cooling, the mixture was then suspended in methanol and neutralized with acetic acid. It was then washed with methanol, filtered and dried overnight at 60°C.

Degree of Substitution Values

The degree of substitution (DS) value is defined as the average number of substituents per anhydroglucose unit (AGU) and varies between 0 and 3. The DS value was determined by standard method based on acid-based titration with minor modifications [12]. A sample of 4 g of CMS in 75 mL of ethanol was mixed and stirred until good slurry was obtained. The mixture was converted to an insoluble acid by using 5 mL concentrated nitric acid and boiled for 5 min. Ethanol (80%) that had been heated to 60°C was used to wash the precipitate. Diphenylamine reagent was then used to test the removal of acid and salts from the sample. Then, the sample was dried in an oven and the CMS in acid form was weighed and mixed with 100 mL of water and 25 mL of standardized sodium hydroxide (0.5 N). This solution was heated and boiled for 15 to 30 min. The excess of sodium hydroxide was titrated while solution was still hot with 0.5 N of standard hydrochloric acid to a neutral point. Each sample was run triplicate.

The DS for CMS was calculated, using the following equations [12,13]:

$$DS = 0.162A/(1-0.058A)$$

$$A = (BC - DE)/F$$

Where: A as milliequivalent of acid consumed per gram sample; B, sodium hydroxide solution added (mL); C, the normality of NaOH; D, HCl required for titration (mL); E, the normality of HCl; F, CMS in acid form (g); gram molecular mass of the AGU of starch, 162; and net increased in molecular mass of AGU for each CH_2COOH group substituted was 58.

Reaction Efficiency

The percentage of reaction efficiency, RE (%), for carboxymethyl starch was calculated as follows:

$$RE(\%) = (W2 - W1)/W3 \times 100$$

Where, W1, W2 and W3 are the respective weights of initial starch, CMS and sodium monochloroacetate.

Fourier Transform Infrared Analysis

The FTIR spectra were recorded on Perkin Elmer FTIR V2000 spectrometre in the range of 4000-400 cm^{-1}. The attenuated total reflection (ATR) technique was used for analysis purposes.

X-Ray Diffraction Analysis

Diffraction diagrams of samples were recorded using a Shimadzu XRD-6000 X-Ray Diffractometer that generated CuKα radiation. Powder samples were exposed to X-ray beam (40 kV, 30 mA) at 2°/min.

Scanning Electron Microscopy Analysis

Scanning electron micrograph was obtained using a Philips SEM XL 40. The samples were sprinkled on double-sided adhesive tape that attached to specimen stubs, and coated with a thin layer of gold using a sputter coater.

Differential Scanning Calorimetry Analysis

DSC was performed using Perkin Elmer Pyris 1. Samples and distilled water were mixed with ratio of 1:1 placed in an aluminium pan, sealed and the weight was recorded. An empty aluminium pan was used as a reference. The sealed pan was heated from 10°C to 100°C at the rate of 5°C/min.

Viscosity Analysis

The apparent viscosity of CMS was compared together with hydroxypropyl methylcellulose (HPMC) a commercial cellulose derivatives polymer commonly used as an aqueous film-coating agent. Samples were prepared at 1, 2, 3, 4, 5, 6, 7 and 8 w/v% and measured at 25°C using a viscometer (Brookfield Digital Viscometer, USA). Each sample was run triplicates.

RESULTS AND DISCUSSION

Determination of the Optimized Reaction Conditions

The effect of aqueous sodium hydroxide (NaOH) concentration on degree of substitution and reaction efficiency of the carboxymethylated sago starch (CMS) is shown in Figure 1. The carboxymethylation process was carried out at different concentrations of NaOH ranging from 10 to 30% (w/v). The DS value was found to increase significantly when the concentration of NaOH increased to 20% (w/v) with
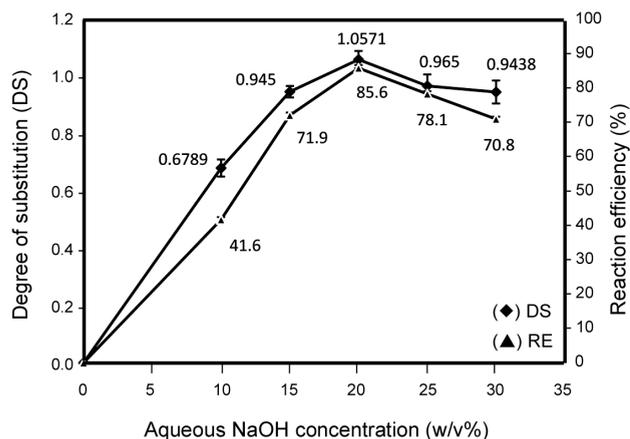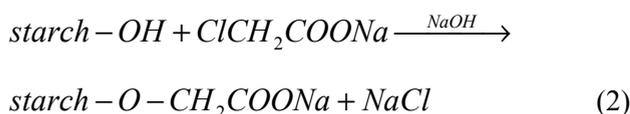
Figure 1. The effect of aqueous sodium hydroxide concentration on degree of substitution and reaction efficiency of the carboxymethylated sago starch.

1.05 maximum degree of substitution and 85.9% reaction efficiency. During the process, aqueous NaOH was used to activate the reaction. The equation below shows the possible chemical reaction that occurred during the process:

$$starch - OH + NaOH \rightarrow starch - O - Na + H_2O$$
(1)
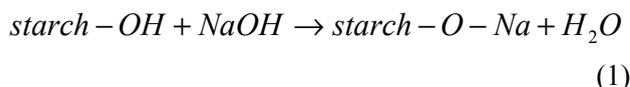
Treatment with NaOH at higher concentration increases the swelling of the starch granules and produces more starch-O-Na resulting in an even more opportunity for carboxymethylation to occur with the hydroxyl group (O-H) in the starch molecules [14,15]. However, further increase in NaOH concentration was accompanied by lowering of DS values. This observation could be explained through the reaction process itself. During the process, two competitive reactions took place simultaneously. The first reaction was the etherification between the starch and NaMCA in the presence of NaOH as shown by the following equation:

$$starch - OH + ClCH_2COONa \xrightarrow{NaOH}$$

$$starch - O - CH_2COONa + NaCl$$
(2)

and the side reaction was between NaOH and NaMCA, which yielded sodium glycolate:

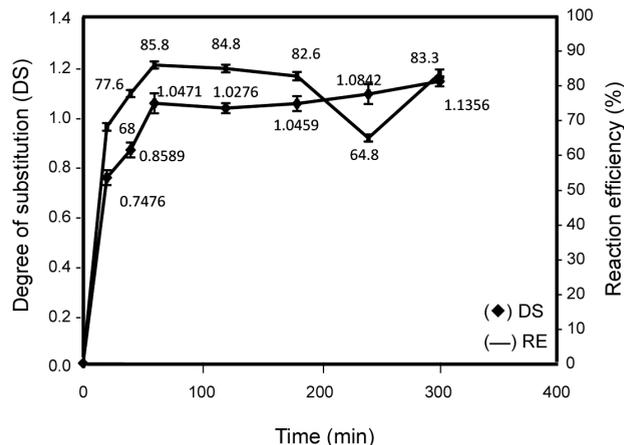$$NaOH + ClCH_2COONa \rightarrow$$

$$OH - CH_2COONa + NaCl$$

Figure 2. The effect of reaction time on degree of substitution and reaction efficiency of the sago starch.

In this study, it was found that above 20% of NaOH concentration, the reaction efficiency decreased. This could indicate that at this stage the side reaction was more dominant to form sodium glycolate than the first reaction. Hence, increasing the concentration of NaOH above 20% lowered the degree of substitution value. Similar results have been reported for carboxymethylation of potato starch [5].

Figure 2 illustrates the effect of reaction time on DS and RE parameters. The time taken for the carboxymethylation process to take place varied from 20 min to 5 h. Following the sudden initial increase in DS, its value remained almost constant at 1.05 after 1 h. The increase in DS value by prolonging the reaction time could be due to the favourable time effect on the swelling ability of the sago starch granules, as well as the diffusion and absorption processes of the reactants with improved contacts between the etherifying agents and starch [13].

The most probable explanation for the DS data reached the plateau at longer reaction times could be due to the fact that the carboxymethylation reaction reaches the equilibrium state after 1 h of the reaction time. This result is consistent with the plot of RE% shown which suggested the activation energy for the main and side reactions reached their peak after 1 h. Stojanovic et al. [14] also reported similar result in carboxymethylation of the other starch.

The influence of the reaction temperature on the DS and RE is shown in Figure 3. The temperature for the carboxymethylation process was varied from
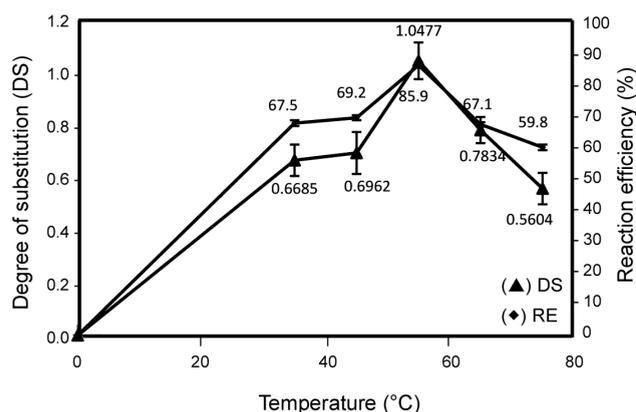
Figure 3. The reaction temperature versus the degree of substitution and reaction efficiency.

Figure 4. The effect of sodium monochloroacetate content on the degree of substitution and reaction efficiency.

35°C to 75°C. The DS value increased with the increases in reaction temperature, approaching its maximum point at 55°C. The maximum values of DS and RE achieved were 1.05 and 85.9%, respectively. By increasing the temperature, the diffusion of NaMCA increased more readily to a reaction point which created more favourable environment for the reaction to take place [14].

On the other hand, the values of DS and RE decreased sharply by further increment in the reaction temperature from 55°C to 75°C. The drop in DS was from 1.05 to 0.70 and that of RE was from 85.9% to 70.2%. This observation could be explained by considering the reduction in the gelatinization temperature of the starch molecules. Although the gelatinization temperature for sago starch is in the range of 69°C to 71°C, it has been reported earlier that in the reaction media, the presence of solvent with high electron polarizability and ions, such as chloride, bromide and iodide, was found to reduce the gelatinization temperature [16]. In this study, isopropanol tends to form hydrogen bonds with the starch molecules, whereas the loose end of this alcohol molecule, which was the alkyl chain, tends to disrupt the neighbouring hydrogen bonds between starch molecules. Both effects tend to decrease the gelatinization temperature of the starch.

At temperature above 55°C, the effect of gelatinization started to be noticed. The product was in agglomerate form rather than in granular structure or semi-transparent mass of gelatinized starch. This could be due to absence of sufficient water that was
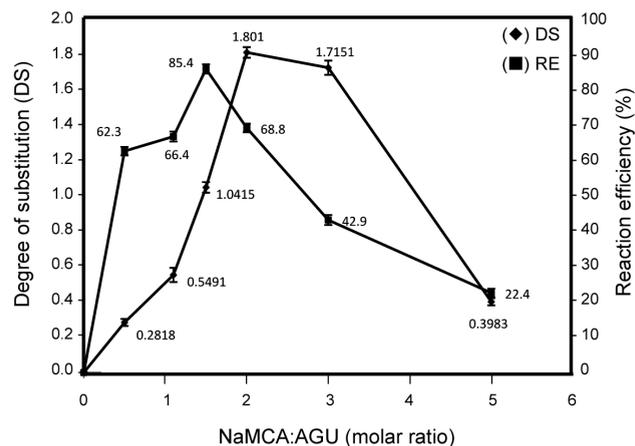
necessary to ensure a complete starch gelatinization [17]. Thus, the starch molecules tend to bind to each other to form agglomerates. Since the water had been used to bind the starch, the amount of water left, which was required in the carboxymethylation process, was insufficient. This reduced the effective sites of carboxymethylation process because less contact was created between the NaMCA and starch granules. This led to lower DS and RE values of the CMS. Hence, during carboxymethylation attempts were made to maintain starch granular structure intact from being gelatinized, which possibley decreased the recovery of the product and the difficulty in drying process.

Hence, the effect of NaMCA content on the DS and RE is shown in Figure 4. The molar ratios of NaMCA to anhydroglucose (AGU), n(NaMCA): n(AGU) were varied from 0.5 to 5.0. The DS values increased with NaMCA content in the reaction, approaching its maximum molar ratio of 1.5:1.0. The highest value of the DS was 1.05.

It was found that beyond these ratios the incremental rate of DS was gradual. There were two possible explanations for this finding. First, the effect of side reaction became more significant with increased NaMCA content in the reaction mixture to yield sodium glycolate. Secondly, the effect of water content in the mixture which helped dissolution of NaMCA in the starch molecules. In this study, it was found that above 1.5:1.0 molar ratio, the NaMCA was not completely dissolved in the reaction mixture. This

might have been due to insufficient amount of water. In addition, it was found that beyond these ratios, the RE% decreased significantly. This reveals that NaMCA side reaction predominated in sodium glycolate formation. A similar result has been discussed by Hebeish et al. [18] on the preparation of carboxymethylation of rice starch.

Fourier Transform Infrared Analysis

The Fourier transform infrared (FTIR) spectra of sago starch show typical absorption bands of a starch backbone (Figure 5). The absorption bands at 3293 cm^{-1} are due to O-H stretching vibration. Usually, the absorption range for O-H vibration is 3700-3500 cm^{-1}, but in sago starch, the O-H stretching peak shifts to lower wavelength. This is probably because the intermolecular hydrogen bonds in the glycosidic ring weaken the O-H bond, thereby shifting the band absorption region to lower frequency between 3400-3200 cm^{-1}. The absorption band at 2929 cm^{-1} shows the C-H stretching.

Weak absorption at 1627 cm^{-1} for sago starch, probably features the tightly bound water molecules present in starch molecules. The -CH$_2$ symmetrical band is found at 1333 cm^{-1}. Broad absorption bands in the range of 1100-990 cm^{-1}, are characteristics of C-O stretching in C-O-C and C-O-H in the glycosidic ring of sago starch. In addition, broad and weak bands occurred in the range of 930-600 cm^{-1}, probably arising from out-of-plane bonded O-H deformation and C-H deformation frequencies [19].

The FTIR spectra of all CMS samples (Figure 5) also show the typical absorption of the starch backbone as well as the additional peaks. New peaks occurred at 1587 cm^{-1} and 1416 cm^{-1}, indicating the substitution of -COO$^-$Na$^+$ group on the starch molecular chains. The frequency of C=O is much lower from the value found for the parent carboxylic acid possibly due to the resonance effect. During the ionization, the formation of COO$^-$ group would give resonance effect between the two C-O bonds. Consequently, the characteristic carbonyl absorption have vanished and are replaced by two bands between 1610 cm^{-1} and 1550 cm^{-1} and between 1400 cm^{-1} and 1300 cm^{-1}, which correspond to the anti-symmetrical and symmetrical vibrations of the COO$^-$ structure. The characteristic absorption band of C-O stretching
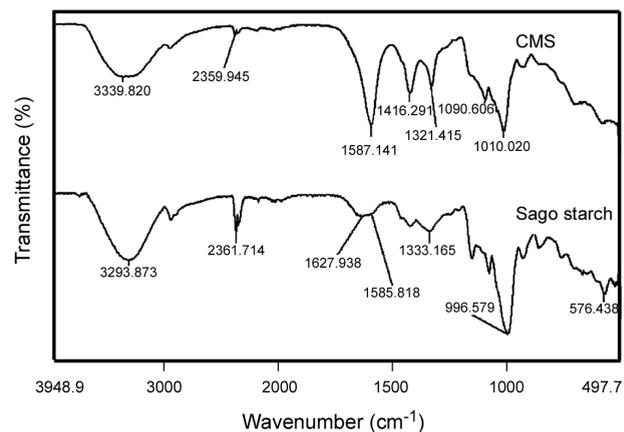

Figure 5. The FTIR spectra of sago starch and carboxymethylated sago starch.

was found at 1004 cm^{-1}. The spectra also showed that the absorption at 1321 cm^{-1} could be due to the overlapping of -CH$_2$ and O-H in-plane bending.

The absorption band of O-H stretching is reduced in intensity and has shifted to 3339 cm^{-1}. This shift in the O-H group could be due to the interaction of O-H group with the carboxylic group. The reduction in intensity of this band may also correspond to partly substituted O-H group with the -CH$_2$COONa group during etherification process. Fang et al. [17] reported similar changes of the O-H vibration during the modification of potato starches.

Differential Scanning Calorimetry Analysis

Differential scanning calorimetry (DSC) is a technique that detects the heat flow associated with order-disorder transitions and gives a quantitative measurement of gelatinization. Gelatinization endotherm obtained by DSC is an overall measure of the progressive loss of long, medium and short-range order in starch granules as they are heated in excess water.

DSC Thermograms for sago starch and CMS are shown in Figure 6. The DSC curve of sago starch shows an endothermic peak at 71.8°C and enthalpy of 2.353 J/g. These endothermic peaks were due to the gelatinization temperature of sago starch. Ahmad et al. [19] have reported similar observation. Native starch contains large amylopectin molecules. Each molecule is a part of many crystallites in the periodically spaced crystalline shell-like layers of the
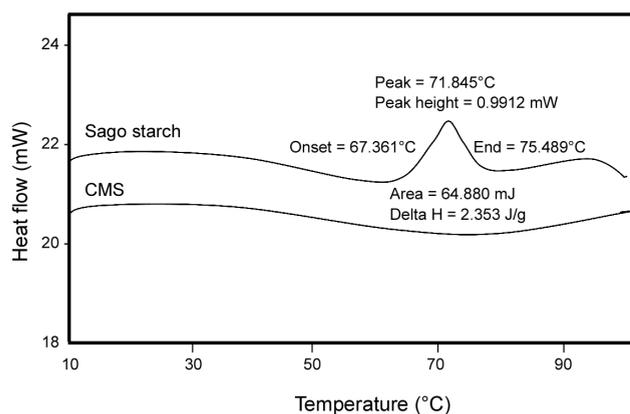
Figure 6. DSC Thermograms for sago starch and carboxymethylated sago starch.

Figure 7. Diffraction pattern of: (a) pure granular of sago starch and (b) carboxymethylated sago starch.

granules and extends through many layers of crystallites. The non-crystalline or amorphous phases of these molecules thus connect the crystalline layers rise to granular birefringence. When the granules were heated with a large amount of water, penetration of water into the amorphous phase and swelling of these regions were limited because the starch chains of the amorphous region end in crystallites. This restricts the movement of the chains and therefore restricts water penetration and swelling process. At sufficiently high temperature, the crystallites, destabilized by the thermal motion and by the swelling force, undergo disruption or melting with simultaneous loss of birefringence. Therefore, DSC records a heat uptake (endothermic transition) of the sample.

However, for CMS samples, no endothermic transition was observed. This is because the CMS has already been gelatinized and dissolved in water. Modification of starch breaks up the crystalline structure and the order of crystallite is destroyed [20]. As a result, there is no gelatinization peak occurring in DSC thermogram of CMS. This observation is confirmed by the X-ray diffraction pattern analysis on the CMS.

X-Ray Diffraction Analysis
X-Ray diffraction (XRD) analysis is a definitive technique for estimating the degree of crystallinity in polymer. Starch is semi-crystalline in nature which is related to the amylopectin fraction. Figure 7 shows diffraction pattern of pure granular of sago starch.
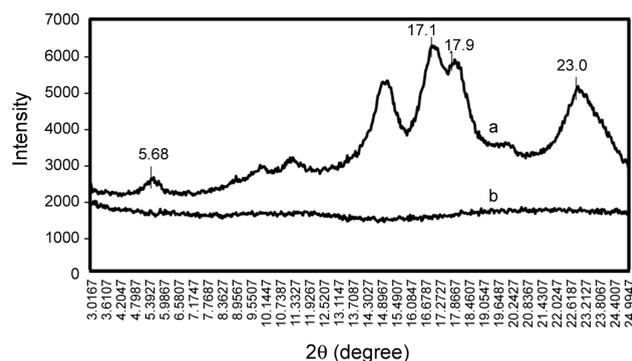
The peaks correspond to the crystalline phase and the background corresponds to the amorphous phase. The peaks are broad due to the small crystallites in starch granules, which is in agreement with the theory of XRD stating that very small imperfect crystals give broadened diffractions. The diffraction spectrum of sago starch is characterized by a single small peak at 5.68°, doubled at 17.1° and 17.9° and peak at 23.0°. Ahmad et al. [19] found a similar spectrum of sago starch and suggested that the crystalline type of sago starch is an intermediate to that of cereal or potato starches.

The diffraction spectrum of CMS shows a complete destruction of the crystalline structure of the original starch. All characteristic peaks for native starch have disappeared and transformed into amorphous phase; therefore it has excellent solubility [21]. During the carboxymethylation process, the starch molecules are placed in alkaline solution. The swelling of the starch granules exert a tension on neighbouring crystalline of starch molecules and tend to distort them. Further swelling leads to uncoiling or dissociation of doubled-helical region and the break up of crystalline structure [17].

Scanning Electron Microscopy Analysis
Scanning electron microscopy (SEM) analysis is a technique for examining granules morphology since it has a large depth of field, which allows a large amount of sample to focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at high magnification. Figure 8a shows the surface
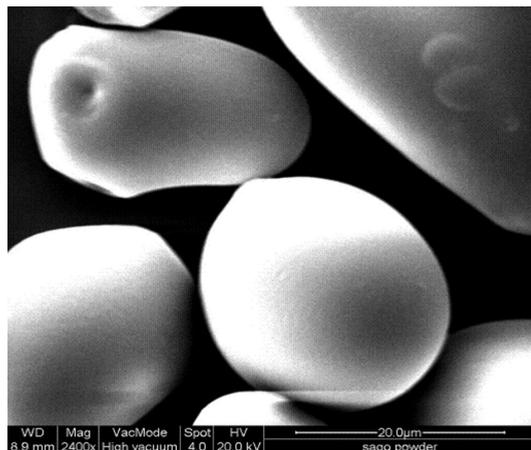
(a)
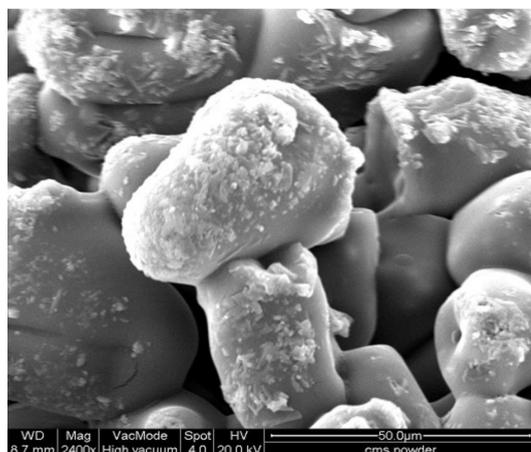
(b)

Figure 8. Surface morphologies of: (a) sago starch and (b) carboxymethylated sago starch.

morphology of sago starch at 2400X magnification (50 μm size bar). It can be clearly seen that the sago starch consists of smooth surface and oval or 'egg-shaped' granules as reported by Ahmad et al. [19] and Karim et al. [22].

The SEM micrograph of CMS (Figure 8b) shows intact, distorted granules compared to the native sago starch. CMS granules remained intact and agglomerated after their preparation in aqueous alcoholic medium. However, the shape of the starch granules is distorted compared to the native starch granules and the surface is roughened, grooved and cracked. This observation is usually found in an acid or enzyme-treated starch granules and sometimes in the gelatinization form of starch granules, in which small amount of amylose is being leached [23,24].
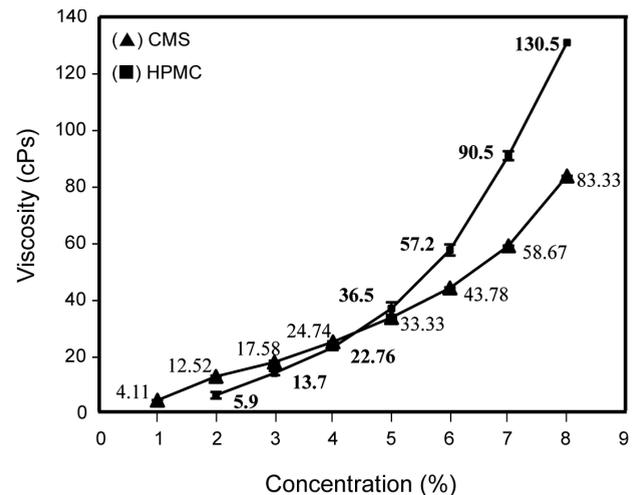

Figure 9. The relationship between the concentration and viscosity of the carboxymethylated sago starch and hydroxypropyl methylcellulose.

Viscosity Analysis

In the application of aquoues film-coating tablet, the rheology of coating solution usually influences the spraying character, as well as the interactions with the core material, as related to the wetting, spreading and coalescence process. This would ultimately affect the final quality of the coated tablet. The viscosity of polymeric solution is known to be directly related to the concentration of the solution. In aqueous film coating process, the concentration of the polymeric solution in the coating formulation is usually higher than the process that uses organic solvents. This is mainly to counteract the effect of the high latent heat of water. Figure 9 illustrates the relationship between the concentration and viscosity of the CMS compared to HPMC. It was found that as the solids content of the solution increases, the viscosity increases exponentially. Regardless of the delivery system, the coating solution must be formulated to have a viscosity of sprayable solution. McGinity [24] reports that, for an aqueous film the coating process is the major concern; concentrations reaching 80-100 centipoise (cPs) are the exact optimum. In this work, the optimized reaction conditions of CMS with a degree of substitution value of 1.05, at 8 w/v% concentration and a viscosity value of 83.33 cPs (±0.1) have fulfilled this requirement.

CONCLUSION

In this study, the optimization of the carboxymethylation reaction conditions of sago starch has been successfully carried out. The optimized reaction conditions are: 20% concentration of aqeous NaOH, 1 h of reaction time, 55°C of reaction temperature and 1.5:1.0 molar ratio of NaMCA to AGU of sago starch which have produced a degree of substitution value of 1.05 and the reaction efficiency of 85.9% of carboxymethylated sago starch. The optimum carboxymethylation reaction conditions shown in this study can be further exploited to prepare industrial products providing much more opportunity for multi-applications of carboxymethyl sago starch.

ACKNOWLEDGEMENT

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