

PREPARATION, CHARACTERIZATION AND SWELLING STUDIES OF CARBOXYMETHYL SAGO STARCH HYDROGEL

(Penyediaan, Pencirian dan Kajian Pengembangan Hidrogel Karboksimetil Kanji Sagu)

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Abstract

Carboxymethyl sago starch (CMSS) hydrogel was prepared by dissolving CMSS in hydrochloric acid (HCl) solution under vigorous stirring to form a gel. The parameter studied were the effect of the percentage of CMSS, the concentration of the acid solution, reaction time and reaction temperature to identify the optimum condition of preparation of CMSS hydrogel. 60% of CMSS in 2.0M acid solution for 12 hours reaction time at room temperature were the optimum conditions for CMSS hydrogel. The hydrogel was characterized by using Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). FTIR spectrum of CMSS showed an additional absorption band indicating the substitution of $\text{CH}_2\text{COO}^-\text{Na}^+$ group on the starch molecular chain during carboxymethylation, while the spectrum of CMSS hydrogel showed an additional sharp absorption band indicating that the Na in CMSS being exchanged to H from HCl solution. SEM image of CMSS hydrogel showed pores in structure and connected to form networks. TGA curve showed that the maximum rate of thermal decomposition of CMSS hydrogel was higher than CMSS which could be due to the presence of the cross-linkages in the CMSS hydrogel. CMSS hydrogel gave a high swelling degree in PBS solution at pH 7 and a low swelling degree in acidic medium.

Keywords: hydrogel, carboxymethyl sago starch, crosslinking, characterization, swelling

Abstrak

Hidrogel karboksimetil kanji sagu (CMSS) telah disediakan dengan melarutkan CMSS dalam larutan asid hidroklorik (HCl) di bawah pengadukan kuat untuk membentuk hidrogel. Parameter yang dikaji untuk mengoptimumkan penyediaan hidrogel CMSS, antaranya termasuklah kesan peratusan CMSS, kepekatan larutan asid, masa dan suhu tindak balas. 60% CMSS dalam larutan asid 2.0M dengan masa tindak balas 12 jam pada suhu bilik merupakan keadaan optimum untuk penyediaan hidrogel CMSS. Hidrogel dicirikan dengan menggunakan spektrometer infra-merah Fourier (FT-IR), analisis termogravimetrik (TGA) dan pengimbasan mikroskop elektron (SEM). Spektrum FTIR menunjukkan jalur penyerapan tambahan yang menunjukkan penggantian kumpulan $\text{CH}_2\text{COO}^-\text{Na}^+$ pada rantai molekul kanji semasa tindak balas karboksimetilasi, manakala spektrum hidrogel CMSS menunjukkan tambahan jalur penyerapan tajam yang menunjukkan bahawa Na dalam molekul CMSS ditukar kepada H dari larutan HCl. Imej SEM dari hidrogel CMSS menunjukkan liang-liang dalam struktur dan saling berhubungan untuk membentuk rangkaian. Lengkung analisis TGA menunjukkan bahawa kadar penguraian haba maksimum hidrogel CMSS lebih tinggi daripada CMSS pada 330.22 °C dengan 60.22% penurunan berat utama. Ini mungkin disebabkan oleh adanya rangkaian silang dalam hidrogel CMSS. Hidrogel CMSS memberikan tahap pengembangan yang tinggi dalam larutan PBS pada pH 7 dan tahap pengembangan yang rendah dalam medium berasid.

Kata kunci: hidrogel, karboksimetil kanji sagu, rangkaian silang, pencirian, pengembangan

Introduction

Polysaccharides are polymeric carbohydrate molecules that can be subjected to a wide range of chemical and enzymatic reactions. They have biocompatible and biodegradable properties with low immunogenicity. Polysaccharides can be easily obtained and found in a living organism [1]. Examples of polysaccharides are starch, alginate, cellulose, hemicellulose which originate from plants. Sago starch which is isolated from sago palm is called *Metroxylon sago* that can be found abundantly in marshy areas mostly in Sarawak state in Malaysia [2]. It has outstanding biological properties such as biocompatibility, biodegradable, and nontoxicity. However, in some applications, the usage of starch is confined due to deficiency in its solubility and has a tendency to retrograde. Retrogradation is a non-reversible process that occurs when cooked starch solutions are cooled and molecular interaction between amylose as well as other glucose units abound to form a double helix, resulting in the formation of colloidal crystallites [3].

Chemical modification is introduced to overcome the inherent defects and gain innovation in the utilization of starch. It involves the introduction of various functional groups into a starch structure including cationic and anionic group [4]. Sago starch undergoes the carboxymethylation process to produce carboxymethyl sago starch that has a variety of promising properties like high solubility, thermal stability, ion activity, and higher reaction efficiency [5].

Hydrogels are polymer networks that are able to absorb and retain a huge amount of water due to the presence of its hydrophilic group. The term “network” is implied indicating that cross-links must present for the prevention of the dissolution of the polymer chain before use [6]. Hydrogel has gained considerable attention in recent years due to its unique properties as a biocompatible smart material, which can be designed to achieve desirable properties [7]. It is widely used in various applications such as agriculture, wastewater treatment, food packaging, cosmetic, biotechnology, pharmaceutical, and biomedical including drug delivery applications [8, 9]. Carboxymethyl sago starch hydrogel can be prepared via a cross-linking technique. Crosslinking is a process of forming a network, where the polymers are linked together either by ionic and covalent bonds, van der Waals interaction, hydrogen bonding or physical entanglements [10]. Crosslinking can involve the exchange of sodium with a hydrogen ion, namely hydrogen bond formation. It is a rapid process but proceeds gradually with time [11].

This paper aims to prepare and characterize carboxymethyl sago starch hydrogel and study its swelling behavior. The finding may lead to potential drug and vaccine carriers in future studies. Currently, the use of biopolymers in drug delivery and vaccination has gained huge attention since these polymers can provide better materials due to its compatibility, degradation behavior and non-toxic administration [12].

Materials and Methods

Materials

Carboxymethyl Sago Starch (D.S 0.75) was prepared at Nuclear Malaysia Laboratory, Bangi Selangor. Sago starch was supplied by Song Ngeng Sago Ind. Sibu Sarawak. The CMSS had been synthesized and purified as described in Zainuddin [13]. The degree of substitution was determined as mentioned in Othman et al. [14]. Sodium monochloroacetate (SMCA) was purchased from Sigma-Aldrich. Phosphate buffer saline (PBS), phenolphthalein, diphenylamine reagent, sodium chloride, sodium hydroxide, acetic acid, solvents such as isopropanol, methanol (99%) and absolute ethanol were purchased from R & M Chemicals. Hydrochloric acid (HCl) (Fuming 37%) was purchased from Fisher Chemicals. All chemical reagents were of analytical grade. Deionized and distilled water were used throughout the experiment.

Preparation of CMSS hydrogel

CMSS hydrogel was prepared by dissolving the CMSS in HCl solution under vigorous stirring and left in a petri dish to allow the crosslinking process to complete. CMSS hydrogel was put into a tea bag and immersed in deionized water at room temperature to remove uncross-linked CMSS that were dissolved and extracted into the water. CMSS was also put in a tea bag and immersed in water as a control sample. The successful cross-linked hydrogel was kept inside the tea bag which was then collected out from the deionized water and oven-dried at 60 °C to achieve constant weight. The effect of the percentage of CMSS (40-80% w/v), HCl concentration (0.5-2.5 M), reaction time (12-72 hours) and reaction temperature (RT-70 °C) were studied to obtain the optimum condition in

which one variable at a time was used. The optimum condition of the CMSS hydrogel was determined based on its gel content.

Gel Content and Swelling of CMSS Hydrogel

The measurement of gel content was carried out in deionized water at room temperature. The insoluble part of the CMSS hydrogel after immersion was measured to estimate the content of the hydrogel. The swollen sample after immersion indicates the degree of swelling ability. Samples were removed from the water after immersion and weighed, then dried at 60°C until a constant weight was achieved. Percentage of gel content was calculated by the following equation:

$$\text{Percentage of Gel Content} = \left(\frac{W_2}{W_1} \right) \times 100 \quad (1)$$

where W_1 is the initial weight of the hydrogel before immersion and W_2 is the weight of the dried insoluble part of hydrogel after immersion.

Swelling studies in different media

A completely dried CMSS hydrogel was weighed and immersed in 150 mL deionized water at room temperature for 48 hours. And then, the CMSS hydrogel was removed, weighed and dried. The degree of swelling was calculated by the following equation:

$$\text{Swelling} = (W_s - W_d)/W_d \quad (2)$$

where W_s is the weight of swollen hydrogel and W_d is the initial weight of xerogel. The swelling studies were investigated by using different immersion media such as buffer solution at different pH levels, pH (4, 7, and 10), salt solution (NaCl), 1.0 M of HCl and NaOH solution. Data presented in this study were the mean values of triplicate measurements ($n=3$).

Characterization of CMSS hydrogel

The structures of sago, CMSS, and CMSS hydrogel were identified by FTIR analysis using a spectrometer (100 Series Perkin Elmer) with universal Attenuated Total Reflectance (UTAR) technique. All samples were analyzed in powder form, irradiated with infrared in the range of 4000-280 cm^{-1} . The thermal stability of sago, CMSS, and CMSS hydrogel was examined using TGA (Perkin Elmer TGA7). The samples were heated from 35 to 600 °C with a rate of 5 °C/min in the flow of inert nitrogen gas. The morphology of hydrogel was studied using scanning electron microscopy (FEI, Quanta 400). The hydrogel sample was soaked in 500 mL deionized water and then was freeze-dried to remove the water molecule. All samples were mounted on metal stubs and sputter-coated with gold.

Results and Discussion

Preparation of CMSS hydrogel : The effect of percentage of CMSS

Figure 1 shows the percentage of gel content and degree of swelling of a various percentage of CMSS from 40 to 80% (w/v) in 1.0 M HCl solution. The percentage of gel content increased gradually with the increasing amount of CMSS from 40 to 60%. This indicates that by increasing amounts of CMSS, it provides more crosslinking points thus increasing the gel content. However, when it got higher than 60% (w/v), the gel content started to decrease slightly. The formation of the crosslinked CMSS hydrogel with an acid solution may reach its optimum at 60% (w/v) CMSS. In this experiment, the amount of HCl was fixed for all concentrations of CMSS. Therefore, when the percentage of CMSS was higher than 60% (w/v), there was a possibility that the amount of H^+ in the reaction was not sufficient to promote the crosslinking process through the formation of hydrogen bonding as the same volume of HCl was used. Thus, the gel content did not significantly increase when more than 60% (w/v) of CMSS was used.

Meanwhile, the degree of swelling is inversely proportional to the percentage of gel content. Increase in gel content caused a decrease in swelling capability. It may be due to the degree of crosslinking which affected the water uptake [15]. Higher CMSS concentration produced more crosslinked points in polymeric chains thus increasing crosslinking network which resulted in less swelling when it was brought in water. An increased crosslinking

network led to the formation of a tighter network. This caused difficulty for water to permeate and reduced the amount of water being trapped inside the hydrogel network thus resulted in a low degree of swelling [13, 15]. The highest gel content (62.05%) was obtained from 60% (w/v) of CMSS, which was chosen throughout the experiment to prepare hydrogel as a sorbent.

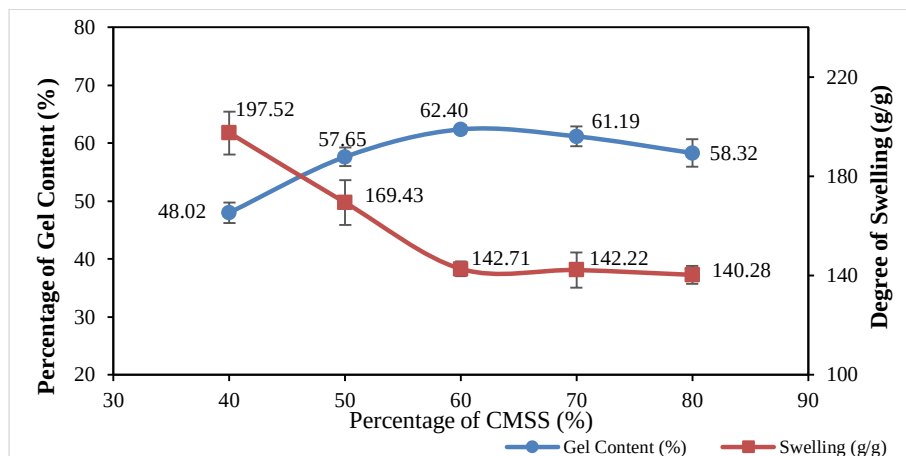


Figure 1. Effect of percentage amount of CMSS in 1.0M HCl on gel content and swelling of CMSS hydrogel at room temperature for 24 hours of reaction time

The effect of HCl concentration

The effect of different concentrations (0.5 to 2.5 M) of HCl solution on gel content and degree of swelling of CMSS hydrogel is shown in Figure 2. The percentage of gel content increased as the concentration of HCl increased. Similar results have been reported by Takigami et al. [11] where the number of crosslinks increased with the increase of acid concentration. However, the graph shows a slight decrease in gel content at 2.5 M that might be due to acid hydrolysis of the linkage at a high concentration of an acid solution. From the data obtained, 2.0 M of HCl gave the highest gel content and had been chosen as a suitable concentration of HCl in preparing the hydrogel in the next parameter. The swelling capability of CMSS hydrogel as expected, decreased as the concentration of the HCl increased. With the increase of the gel fraction, water absorption decreased significantly depending on the acid concentration [11]. A high swelling degree of hydrogel with 0.5M HCl indicates that it has high ability to absorb water.

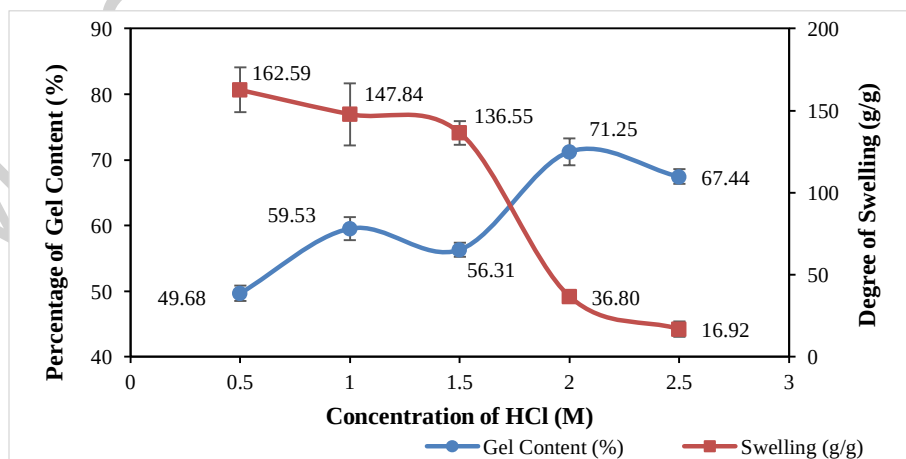


Figure 2. Effect of HCl concentration on gel content and swelling of 60% CMSS hydrogel at room temperature for 24 hours of reaction time

Pushpamalar et al. [16] reported in her study that hydrogels that are relatively weak and consist of a low number of intermolecular bonds can expand by absorbing water and holding it in their void. As the intermolecular bonds and the crosslink density increased, hydrogel becomes tightly packed, lessening the ability of the hydrogel to absorb water. This led to a decrease in the swelling degree of hydrogel with a high concentration of HCl. A similar finding was reported by Sadeghi et al. [17] which stated that higher crosslinker concentration diminished the free space between the polymer chain and subsequently resulted in highly crosslinked rigid structure that cannot be extended and held a vast amount of water.

The effect of reaction time

The third parameter investigated was the effect of reaction time from 12 to 72 hours. From the preliminary study, no gel was formed when the reaction time was less than 12 hours for a low concentration of CMSS. Figure 3 shows that there is no significant difference in gel content of CMSS hydrogel at 12, 24 and 36 hours, and it decreased slightly at 48 and 72 hours reaction time. A possible explanation may be the fact that the prolongation of the reaction time does not affect the gel content as the crosslinking process already achieved the maximum value in a short period. Although 36 hours reaction time gave 71.37% of gel content, 12 hours reaction time is preferable to avoid a waste of time. Usually, swelling capability decreases as the gel content of the hydrogel increases. However, in this parameter, the swelling capability decreased with the increased reaction time although the gel content did not show significant increment. As the reaction time increased, the structure of the hydrogel became stronger and denser. Prolong the reaction time could increase the crosslink density and caused the decreasing swelling capability of the hydrogel [18]. Kabiri et al. [19, 20] mentioned in their studies that there is a possibility of the additional linkage that increased the crosslink density, which resulted in reducing swelling capacity.

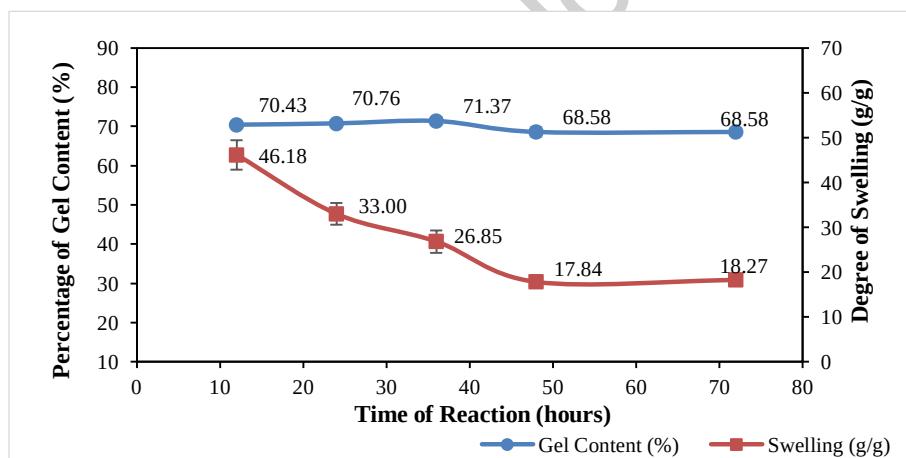


Figure 3. Effect of reaction time on gel content and swelling of 60% CMSS hydrogel in 2.0M HCl at room temperature

The effect of reaction temperature

The effect of the reaction temperature of 60% CMSS in 2.0M HCl at 12 hours of reaction time was examined from room temperature (24 °C) to 70 °C. Figure 4 shows a slight change in gel content as the temperature increased. This could be due to the degree of crosslinking of hydrogel decreased due to acid hydrolysis of CMSS at high temperature, thus reducing the number of long-chain polymers that were available for crosslinking resulting in decreased gel content of the hydrogel. A similar result reported by Takigami et al. [11] indicated that reaction at high temperature will cause starch hydrolysis and gives low gel formation. The swelling capability in this study was different than the expected trend as it decreased with the decrease of the gel content. When hydrogel comes in contact with any solvent molecules, the solvent tries to attack the hydrogel surface and penetrate within the polymeric network structure [21]. However, in this experiment, it was observed that the appearance of the gel

prepared at high reaction temperature was hard and rigid. This could prevent the hydrogel to swell as the water found difficulty to pass through the surface of the hydrogel thus decreasing the swelling capability.

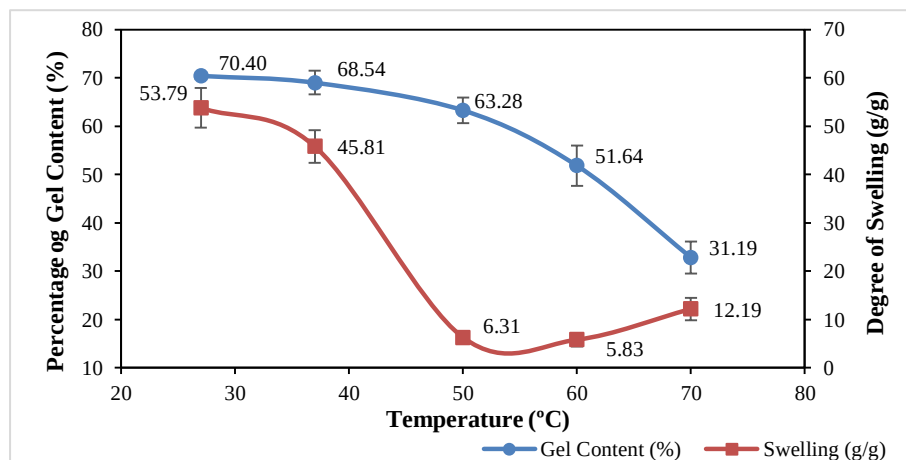


Figure 4. Effect of reaction temperature on gel content and swelling of 60% CMSS hydrogel in 2.0M HCl at 12 hours of reaction time

Swelling properties

A hydrogel is known for its ability to absorb and hold a huge amount of solvent in its network structure. This swelling property is a very crucial factor for its further application. The swelling studies would suggest a good prediction on the ability of the hydrogel to interact with the surrounding media, in which its smart behavior could be used in many applications especially in the controlled release of drug delivery [7]. Figure 5 shows the swelling degree for the CMSS hydrogel at different immersion media. PBS 7 gave a high swelling degree indicating that this CMSS hydrogel swelled well in PBS 7 solution. In acidic media (PBS 4), more -COOH groups were formed, which induced the formation of the hydrogen bond of -COOH with CMSS hydrogel, leading to a more compact network in CMSS hydrogel and caused a decrease in the swelling degree [7, 22]. Similarly, when CMSS hydrogel was immersed in HCl solution, the presence of H^+ ions in the surrounding induced the hydrogen bond formation, resulting in a low swelling degree. In salt solution (NaCl), the swelling ability of CMSS hydrogel significantly decreased compared to the swelling in deionized water. Gupta et al. [23] stated that the phenomenon can be explained on the basis of osmotic pressure developed due to the unequal distribution of ions in the medium and the polymer network. When the polymer immersed in NaCl solution, the development of osmotic pressure is much lower since the external solution contains Na^+ and Cl^- , the presence of Na^+ ions in the outer solution causes a decrease in the osmotic swelling pressure which operates due to the difference of counter ions in a gel phase and solution phase, therefore, reducing the swelling ability.

Sadeghi et al. [17] also reported that this behavior is often attributed to the charge screening effect and ionic crosslinking of monovalent cations. In alkaline solution (NaOH and PBS 10), it was observed that there was no swelling degree recorded as the CMSS hydrogel completely dissolved in the alkaline solution after 48 hours immersion time. This might be due to the degradation of the CMSS hydrogel crosslinked network at high concentration of alkaline solution present in the surrounding resulting in reformation of -COONa thus causing it to be dissolved in the media. These results indicated that CMSS hydrogel prepared in this study exhibited pH-sensitive characteristic and affected by its surrounding environment.

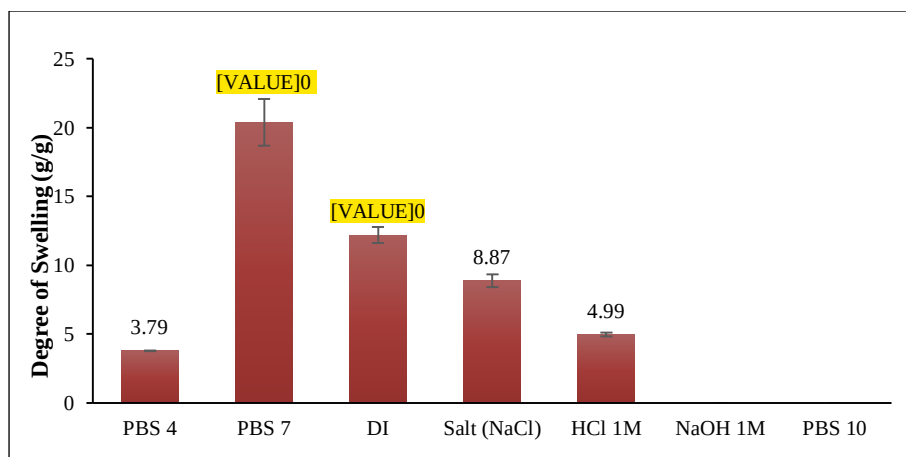


Figure 5. Swelling of CMSS hydrogel at different media at room temperature

Characterization of CMSS hydrogel: Fourier transform infrared analysis

Figure 6 shows the FTIR spectra of sago starch, CMSS, and CMSS hydrogel. The spectrum of sago starch shows the absorption band at 3273 cm^{-1} , which is due to O-H stretching vibration, as well as intramolecular and intermolecular hydrogen bonds in glycosidic bond in the sago starch molecule [7]. The absorption band at 2910 cm^{-1} shows C-H stretching and 1643 cm^{-1} shows a tightly bound water presence in the starch molecule. The appearance of the absorption band at 1350 cm^{-1} belongs to $-\text{CH}_2$ symmetrical band, while the broad band at the range of $1100\text{--}990\text{ cm}^{-1}$ indicates the C-O stretching from C-O-C and C-O-H in a glycosidic ring of the starch molecule [24].

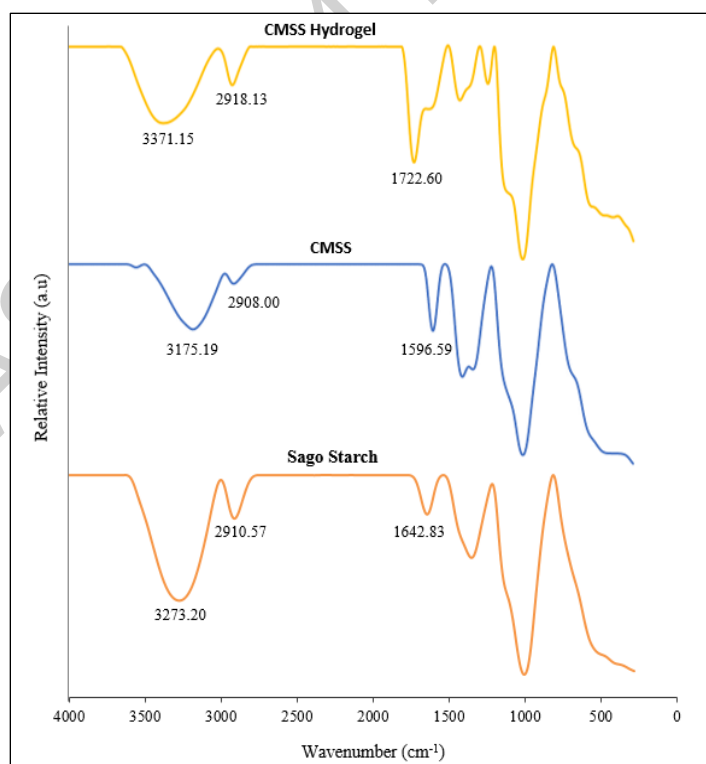


Figure 6. FTIR spectra for Sago Starch, CMSS and CMSS Hydrogel

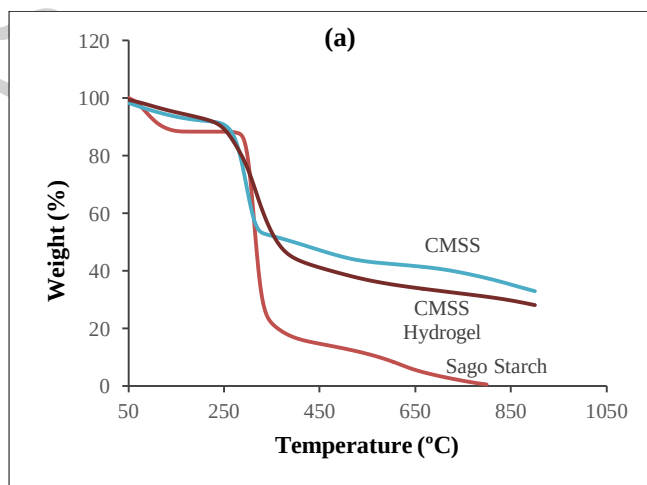
The FTIR spectrum of CMSS shows the shifting of O-H stretching band to lower wavenumber at 3175 cm^{-1} . This was probably due to the low intermolecular hydrogen bonds between the O-H group left in the glycosidic rings of CMSS prior to the substitution reaction take place during carboxymethylation process [13]. The additional absorption band at 1597 cm^{-1} indicates the substitution of the $\text{CH}_2\text{COO}^-\text{Na}^+$ group on the starch molecular chain during carboxymethylation.

CMSS hydrogel spectrum shows a new absorption band at 1723 cm^{-1} indicating the presence of the COOH group. This shows that the Na in CMSS was being exchanged to H from the HCl solution. The concentration of HCl solution used was 2.0 M, thus a sharp absorption at band can be observed indicating that most of the $-\text{COONa}$ have been converted to COOH. The intensity of the absorption band depends on the concentration of the acid used, whereby low concentration of acid shows a weak absorption band of conversion of $-\text{COONa}$ to COOH [24]. A similar result was reported previously where the replacement of Na in carboxymethyl group with hydrogen from acid occurred depending on the type of acid and concentration [11]. The absorption band at 1421 cm^{-1} and 1238 cm^{-1} are respectively due to $-\text{CH}_2$ scissoring and $-\text{OH}$ bending vibration, while OCH-O-CH_2 stretching was represented by the absorption band at 1000 cm^{-1} .

Thermogravimetric analysis

TGA analysis is a convenient analysis to study the thermal decomposition and thermal stability of the polymer. It is a quantitative measurement of the weight change of a sample against the temperature applied. TGA thermograms of native sago starch, CMSS, and CMSS hydrogel are shown in Figure 7. Native sago starch, CMSS, and CMSS hydrogel have two steps of decomposition. The first step below 150°C corresponds to the removal of moisture from the surface and entrapped water in the sample. The second step occurred around 200°C to 500°C due to the decomposition of the starch backbone leaving a residue. Zhang et al. [25] stated that water was the main product decomposition at a temperature below 300°C and further heating up above 500°C resulted in carbonization and ash formation.

For CMSS, the maximum weight loss occurred at 295.33°C which is lower than native sago starch before carboxymethylation (309.57°C) with 49.71% of total CMSS decomposed and 42.37% residue due to the presence of ash and sodium salt in CMSS. It had been found that the carboxymethylation process decreased the thermal stability of starch materials [25]. The maximum thermal decomposition of CMSS hydrogel was 330.22°C with 60.22% major weight loss which indicates slight increase compared to native sago starch and CMSS. An increase in the maximum thermal decomposition of CMSS hydrogel could be due to the presence of cross-linkages in CMSS hydrogel [24]. High cross-linked structure and compact network improve the degree of stability of hydrogel resulted in higher temperature needed to decompose them [7].



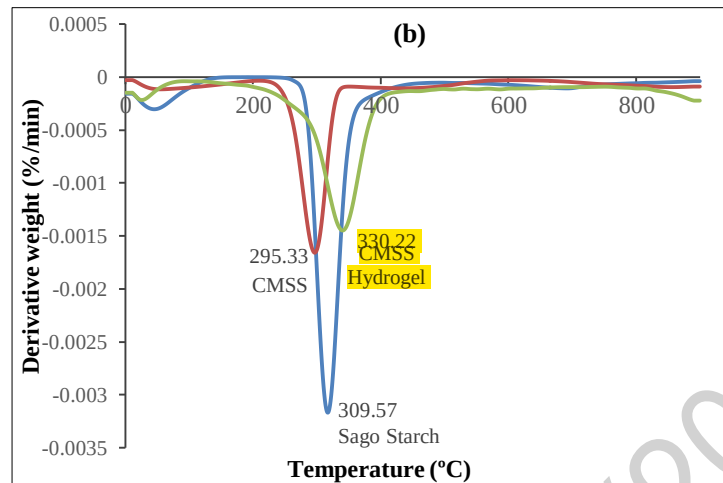


Figure 7. (a) TGA and (b) DTG thermograms of Sago Starch, CMSS and CMSS hydrogel

SEM analysis

Scanning Electron Microscopy (SEM) is an excellent technique in examining the surface morphology of a sample. Figure 8(a) shows a smooth surface and oval-shaped granules sago starch with a diameter in the range of 20-40 μm similar to the previous study reported by Ahmad et al. [26]. Figure 8(b) shows the massive distorted and irregular shape of carboxymethyl sago starch. In comparison with native starch, the surface of CMSS is rough where the groove indicates that the breakage of a chemical bond by a strongly alkaline environment during the carboxymethylation process. Thus, carboxymethylation is not only the reaction on the surface of starch granules but also from within [24]. The micrograph also shows that the CMSS remains intact and agglomerate after preparation with aqueous-alcoholic medium studied. Figure 8(c) shows that CMSS hydrogel has a spongy surface with space called pores in structure and connected to each other to form networks as a consequence of the crosslinking process [27].

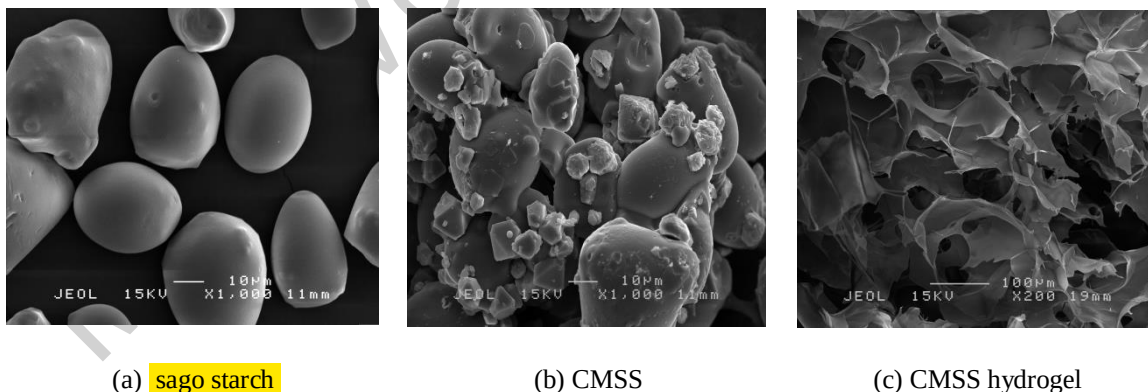


Figure 8. SEM images of (a) sago starch, (b) CMSS and (c) CMSS hydrogel

Conclusion

Carboxymethyl sago starch hydrogel was successfully prepared in the HCl solution. Carboxymethylation decreases the thermal stability of sago starch, but cross-linking increases the thermal stability of CMSS hydrogel. FTIR spectrum of CMSS hydrogel showed that the Na in CMSS is successfully exchanged to H from the HCl solution.

SEM image of CMSS hydrogel shows pores in the structure and connected to each other to form networks. Swelling studies of CMSS hydrogel prepared exhibited a smart behavior as it reacts depending on its surrounding environment and displays pH-sensitive characteristics. The finding of this study suggests that the CMSS hydrogel has potential in broad applications such as heavy metal removal in wastewater treatment and carrier in drug or vaccine delivery system.

Acknowledgment

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References

1. Pushpamalar, J., Veeramachineni, A. K., Owh, C. and Loh, X. J. (2016). Biodegradable polysaccharides for controlled drug delivery. *ChemPlusChem*, 81: 1-12.
2. Pushpamalar, V., Langford, S. J., Ahmad, M. and Lim, Y. Y. (2006). Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste. *Carbohydrate Polymers*, 64: 312-318.
3. Alcázar-Alay, S. C. and Meireles, M. A. A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology (Campinas)*, 35(2): 215-236.
4. Chen, Q., Yu, H., Wang, L., Abdin, Z. U., Chen, Y., Wang, J., Zhou, W., Yang, X., Khan, R. U., Zhang, H. and Chen, X. (2015). Recent progress in chemical modification of starch and its applications. *RSC Advances*, 5: 67459-67474.
5. Kamel, S. and Jahangir, K. (2007). Optimization of carboxymethylation of starch in organic solvents. *International Journal of Polymeric Materials*, 56(5): 511-519.
6. Faheem A., M., Hanif, M. and Ranjha, N. M. (2016). Methods of synthesis of hydrogels: A review. *Saudi Pharmaceutical Journal*, 24: 554-559.
7. Jamingan, Z., Ahmad, M. B., Hashim, K. and Zainuddin, N. (2015). Sago starch based hydrogel prepared using electron beam irradiation technique for controlled release application. *Malaysian Journal of Analytical Sciences*, 19(3): 503-512.
8. Ullah, F., Bisyrul, M., Javed, F. and Akil, H. (2015). Classification, processing and application of hydrogels: A review. *Materials Science & Engineering C*, 57: 414-433.
9. Hoare, T. R. and Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer*, 49(8): 1993-2007.
10. Hamidi, M., Azadi, A. and Rafiei, P. (2008). Hydrogel nanoparticles in drug delivery. *Advanced Drug Delivery Reviews*, 60(15): 1638-1649.
11. Takigami, M., Nagasawa, N., Hiroki, A., Tagichi, M. and Takigami, S. (2012). Preparation of stable CMC-acid gel. *Gums and Stabilisers for the Food Industry*, 16: 175-182.
12. Nitta, S. and Numata, K. (2013). Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. *International Journal of Molecular Sciences*, 14(1): 1629-1654.
13. Zainuddin, N. B. (2003). Carboxymethylation of sago starch and sago waste and the formation of carboxymethyl starch-hydrogel via irradiation technique. Thesis Universiti Putra Malaysia.
14. Othman, Z., Hashim, K., Sabariah, K., Nasir, M. H. A. and Hassan, A. (2015). Synthesis and characterization of carboxymethyl derivatives of sago (*Metroxylon sago*) starch. *Macromolecular Symposia*, 353(1): 139-146.
15. Nagasawa, N., Yagi, T., Kume, T. and Yoshii, F. (2004). Radiation crosslinking of carboxymethyl starch. *Carbohydrate Polymers*, 58: 109-113.
16. Pushpamalar, V., Langford, S. J., Ahmad, M., Hashim, K. and Lim, Y. Y. (2013). Preparation of carboxymethyl sago pulp hydrogel from sago waste by electron beam irradiation and swelling behavior in water and various pH media. *Journal Applied Polymer Science*, 128: 451-459.
17. Sadeghi, M. and Hosseinzadeh, H. (2010). Synthesis and super-swelling behavior of a novel low salt-sensitive protein-based superabsorbent hydrogel: collagen-g-poly(AMPS). *Turkish Journal of Chemistry*, 34: 739-752.
18. Budianto, E., Muthoharoh, S. P. and Nizardo, N. M. (2015). Effect of crosslinking agents, pH and temperature on swelling behavior of cross - linked chitosan hydrogel. *Asian Journal of Applied Sciences*, 3(5): 2321-2893.

19. Kabiri, K., Omidian, H., Doroudiani, S., Mp, T. O. N. and Street, K. (2011). Superabsorbent hydrogel composites and nanocomposites: A review. *Polymer Composites*, 32(2): 277-289.
20. Kabiri, K. and Zohuriaan-Mehr, M. J. (2003). Superabsorbent hydrogel composites. *Polymers for Advance Technologies*, 444: 438-444.
21. Shah, R., Saha, N. and Saha, P. (2015). Influence of temperature, pH and simulated biological solutions on swelling and structural properties of biomineralized (CaCO₃) PVP-CMC hydrogel. *Progress in Biomaterials*, 4: 123-136.
22. Ping Zhao, S., Jie Cao, M., Yan Li, L. and Lin Xu, W. (2010). Synthesis and properties of biodegradable thermo-and pH-sensitive poly [(N-isopropylacrylamide)-co-(methacrylic acid)] hydrogels. *Polymer Degradation and Stability*, 95: 719-724.
23. Gupta, N. V. and Shivakumar, H. G. (2012). Investigation of swelling behavior and mechanical properties of a pH - sensitive superporous hydrogel composite. *Iranian Journal of Pharmaceutical Research*, 11(2): 1-10.
24. Basri, S. N., Zainuddin, N., Hashim, K. and Yusof, N. A. (2016). Preparation and characterization of irradiated carboxymethyl sago starch-acid hydrogel and its application as metal scavenger in aqueous solution. *Carbohydrate Polymers*, 138: 34-40.
25. Zhang, B., Tao, H., Wei, B., Jin, Z., Xu, X. and Tian, Y. (2014). Characterization of different substituted carboxymethyl starch microgels and their interactions with lysozyme. *PLoS ONE*, 2014: 1-13.
26. Ahmad, F. B., Williams, P. A., Doublier, J. L., Durand, S. and Buleon, A. (1999). Physico-chemical characterisation of sago starch. *Carbohydrate Polymers*, 38(4): 361-370.
27. Janarthanan, P., Zin Wan Yunus, W. M. and Ahmad, M. B. (2003). Thermal behavior and surface morphology studies on polystyrene grafted sago starch. *Journal of Applied Polymer Science*, 90(8): 2053-2058