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Investigation of Mixing Time on Carrageenan-Cellulose Nanocrystals (CNC) Hard Capsule for Drug Delivery Carrier

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Abstract— Present global market demands for plant based capsule to replace the animal based product. However, plant capsule produces a lower mechanical strength in comparison to gelatin based capsule. In this work, at constant 65 °C mixing temperature, the solution mixing time was varied to investigate the final carrageenan-CNC hard capsule mechanical properties, moisture content and chemical properties. The mixing time increased the solution viscosity and mechanical strength of hard capsule, while reduced the moisture content of the product. High tensile strength was obtained at higher mixing time might indicates uniform dispersion of CNC and increment of hydroxyl bonding in the carrageenan matrix. The disintegration time of hard capsule starts to rupture also increased as the mixing time increased but still disintegrated in less than 15 minutes.

Keywords—Carrageenan, Cellulose Nanocrystals, Capsule Loop Strength, Breaking Force, Ultrasonication.

I. INTRODUCTION

Hard capsule is defined as a shell consisting of two prefabricated cylindrical sections that fit together [1]. The contents of hard capsules are usually in solid form, either powder or granule. Thus, it is paramount importance to control the final hard capsule properties such as mechanical strength, moisture content and stability. In hard capsule making, most of them are made of gelatin and hydroxypropyl methyl cellulose (HPMC) [2,3]. The hard capsule production using HPMC requires a new and complex technology, equipment and process which leads to high cost of the hard capsule [4].

Carrageenan is a potential raw material to replace the gelatin and HPMC in hard capsule. There are abundant of seaweed in Sabah, East of Malaysia [5]. At present, carrageenan is regularly consumed in various field such as food, pharmaceutical, nutraceuticals, cosmeceuticals, medical, and other industries but as a thickener and filler only [6,7]. While in this research, carrageenan was used as the main raw material for hard capsule formulation. However, carrageenan tends to become brittle upon drying process due to its low mechanical stability and formation of double helices in the carrageenan matrix [8].

It is a high demand to utilize the raw materials that is renewable, biodegradable, non-toxic, recyclable, and sustainable non-petroleum based resources such as cellulose for sustainability of healthy lifestyle and environment while achieving an improvement in mechanical properties of materials or composites[9–11].

Cellulose nanocrystals is a nano size of cellulose which can be isolated by several methods such as acid hydrolysis and mechanical processes. CNC has a rod-like shape with aspect ratio of diameter to length at range from 1 to 100 nm [12,13]. There are increasing efforts to develop materials from nanocellulose due to its potential competitive advantages which include mechanical, functional, biocompatible and biodegradable properties [14]. CNC is widely used in material science as filler and it is proven to increase the mechanical strength and stability of various materials such as polyvinyl alcohol (PVA), cement, gelatin hydrogel, and others [15–17]. In this work, CNC was incorporated as a filler in the formulation solution to increase the strength of carrageenan hard capsule.

Mixing is an important parameter in process control as it can intensify mass and heat transfer thus affect the process reaction [18]. Mixing is directly linked to the viscosity and solution properties. Longer mixing time commonly produce a large viscosity difference in of formulation solution and it is also dependence on the mixing speed [18,19]. Mixing time is also related to the homogeneity of solution. In most of process condition, longer mixing time are required for the homogenization of viscous solution [19]. Additionally, increment of mixing time has effect on the size of the dispersed phase [20]. These parameters need to be considered

carefully during the process control to monitor the product quality and energy requirements [18,19].

Mixing time is manipulated to study the best process condition in the making of the best carrageenan hard capsule. Increasing the mixing time of solution formulation will increase the mechanical properties of the final product due to solution homogeneity, without affecting the disintegration time of the hard capsule as regulated by international standard.

II. METHODOLOGY

A. Materials

Semi refined carrageenan was purchased from TACARA, Sabah, Malaysia. Food grade crosslinker, toughening and opacity agents, and microcrystalline cellulose (MCC) were obtained from Sigma-Aldrich, USA. Polyethylene glycol (PEG) as a plasticizer was purchased from Merck, Germany.

B. Cellulose Nanocrystals (CNCs) Preparation

Approximately 5.0 g of microcrystalline cellulose (MCC) was dispersed in 500 mL deionized water and stirred overnight[21]. The MCC dispersion was sonicated using a QSonica (Q700, USA) ultrasonicator for 50 minutes at an amplitude of 20%. The bottom layer of the solution was removed after rested for 10 minutes and the solution was left to rest overnight. To increase the concentration of CNC, the top clear layer of the solution was removed and the solution was reduced to 300 mL. Remaining solution was designated as CNCs solution.

C. Carrageenan-CNCs Film and Hard Capsule Preparation

Semi refined carrageenan and crosslinker were mixed with distilled water in a double jacketed glass reactor at 65°C. Then, 1.6 wt./v% CNC and plasticizer were mechanically stirred for a total of 2 hours mixing time. 1.6 wt./v% is chosen in this experiment because previous study proves that it is the best concentration to be incorporated in the carrageenan matrix. The mixing time were manipulated from 2 to 4 hours. Next, approximately 20 mL of solution was poured into a stainless steel tray before it was dried overnight in an oven at 40°C for film making. Meanwhile the other portion of the solution was dipped using capsule dipping machine using size "1" capsule shell to make hard capsules. The dried film and hard capsules were then employed for characterization and analysis.

D. Characterization

a). FTIR Spectroscopy Analysis

To examine chemical bonding occurred between carrageenan, crosslinker and CNCs, FTIR spectra of the formed hard capsule were undertaken. A Perkin Elmer ATR-FTIR spectrometer (Frontier) was used with the spectra range of 400 to 4000 cm^{-1}. A total of 32 scans were acquired at 0.15 s/scan and with a spectral resolution of 8 cm^{-1}. The spectra was analyzed using OMNIC software.

b). Moisture Content Analysis

The moisture content of the hard capsule was determined using a moisture analyzer (AND MS-70, Japan). Initially, approximately 0.1 g of sample was placed on the heating pan of the moisture analyzer. The moisture from the sample evaporates because of continuous heating. The analysis stopped automatically once the mass of the sample attained a constant moisture content value.

c). Viscosity Measurement

The viscosity of carrageenan formulation solutions was measured using a rotational measuring block Rheometer (Brookfield, Rheo 3000, USA) equipped with LCT 25 4000010 geometry. Approximately 16.5 mL of solution was programmed at speed of 300 revolutions per minute, with 100 MPoints at a constant temperature of 50 °C in triplicate.

d). Mechanical Properties of Carrageenan-CNC Film and Hard Capsule

The tensile strength and elongation at break of 2 cm x 10 cm strips of carrageenan control and composite films was determined using an electronic Universal Testing Machine (VEW 260E, Victor, Malaysia) fitted with a 5 kN load cell. The method was in accordance to ASTM D882-12 and a crosshead speed of 50 mm min−1 was employed. The tensile machine was operated with an initial grip separation of 50 mm, and a crosshead speed of 50 mm/min [22].

Capsule loop tensile test and breaking force test were conducted using CT3 Texture Analyzer (Brookfield, USA) and it was loaded with a 50 kg load cell. For capsule loop tensile test, the instrument is fitted with TA-CLT separating rod fixture (Figure 1a). Upon analysis, TA-CLT protrudes horizontally from the fixture. The starting position for the two rods is 2 mm apart (as the fixture can hold the cap of the hard capsule). The capsule was mounted onto the pair of rods. The test was conducted using a test mode of "tension" and target option of "distance". The probe travels upwards with target value of 5.0 mm at a speed of 0.50 mm/s until the hard capsules were pulled apart and the applied force was recorded [23].

Meanwhile breaking force was conducted using a flat-ended probe with the diameter of 12.7 mm (TA-10). The probe was individually attached to the upper arm of CT3. The individual capsule was placed on the lower stationary platform and positioned centrally under the probe (Figure 1b). The texture analyzer was programmed to measure force in the "compression" mode and target option of "distance" (4 mm) [24]. The probe was then advanced onto the capsule at the speed of 1.0 mm/s before the breaking force was recorded [3]. An average of at least three measurements was taken for each formulation.

e). Disintegration Test

Disintegration test was carried out using the disintegration tester (Pharma Test, Dist. 3, Serial No: 11580, Germany) at

temperature of 37 ± 2 °C, in 600 mL distilled water, in accordance to U.S. Pharmacopeia standard [25]. Hard capsule was filled with lactose as placebo and assistance for disintegration process. Disintegration time was immediately recorded when the lactose was released into the water.

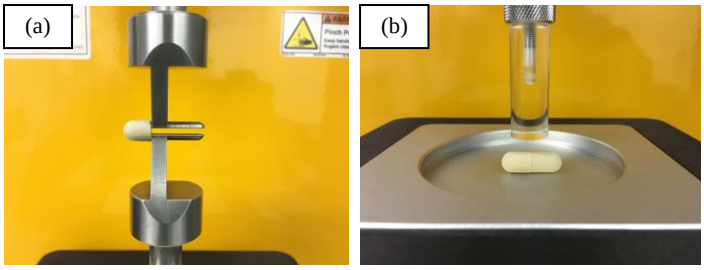

Fig. 1 The Texture Analyzer Equipped with (A) Capsule Loop Testing and (B) Flat-Ended Probes

III. RESULTS AND DISCUSSION

A. *FTIR Spectroscopic Analysis*

Figure 2 demonstrated the FTIR spectroscopy of carrageenan-CNC hard capsule at different mixing time. The peaks of C-O of 3,6-anhydrogalactose and C-O-SO_4 on galactose-4-sulphate in kappa carrageenan is attributed at 925 and 842 cm^{-1} respectively [26]. Peaks at 1033 and 1219 cm^{-1} represented the D-galactose-4-sulphate, 3,6-anhydro-D-galactose, glycosidic linkage and ester sulphate, respectively [27].

Peaks at 3331 and 2917 cm^{-1} shows the presence of the OH stretching vibration and C-H stretching groups respectively. The intensity of these functional groups shows an increment with the increase of mixing time. The absorbance peaks at 892 cm^{-1} represents the glycosidic C-H deformation with ring vibration contribution and OH bending which represents the typical structure of cellulose [28].

It is expected that the hydroxyl groups (-OH) available on the surface of CNC plays an important role in the carrageenan matrix by reacting with the sulphate group and in the network formation via hydrogen bonding. The influence of the network formation was determined by calculating the area of corresponding peaks as shown in Table 1 [29]. The result

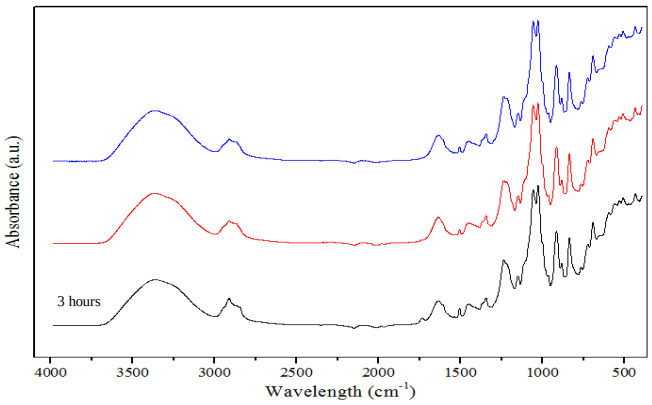

Fig. 2 FTIR Spectroscopy of Carrageenan-CNC Hard Capsule at Different Mixing Time

proves that as the mixing time increase, the hydroxyl bonding in the carrageenan matrix increase. The increment will lead to increase in composite strength as summarized in Table 2.

Mixing time (hrs)	Peak are for 'bonded' -OH	Moisture Content (%)	Disintegration Test (min)
2.0	134.30	19.69 ± 1.71	7.77 ± 1.47
2.5	142.16	17.62 ± 0.65	10.82 ± 1.15
3.0	143.70	17.42 ± 1.46	10.51 ± 1.28

Table 1: Peak Area of Bonded Hydroxyl Group at Different Mixing Time

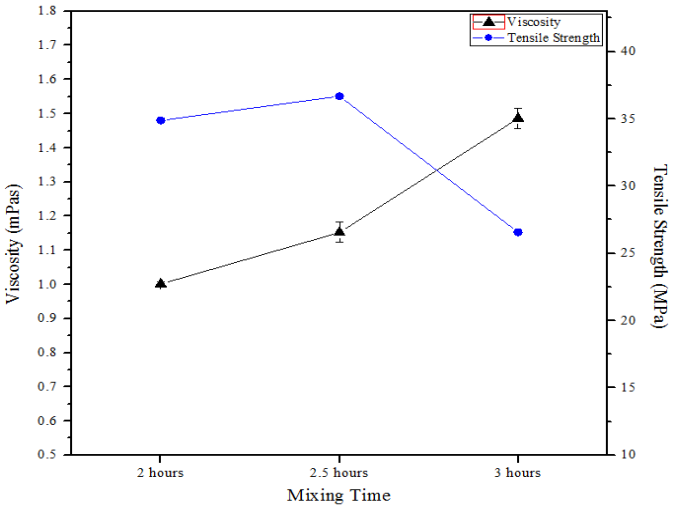

Fig. 3 Viscosity and Tensile Strength of Formulation Solution and Film at Different Mixing Time

B. *Rheology and Mechanical Strength Analysis*

Figure 3 represents the line graph of viscosity and tensile strength of Carra-CNC1.6 at different mixing time. The triangle symbol represents the viscosity of sample solution meanwhile the circle symbol represents the tensile strength of the sample film. The mixing time of 65 °C shows that the viscosity of formulation solution increased from 1.001 mPas to 1.152 mPas and 1.486 mPas. The tensile strength of the sample films is also increased from 34.87 MPa to 36.67 MPa from 2 to 2.5 hours but it is decreased to 26.56 MPa at 3 hours of mixing time. The result is presumably due to more water loss in the composite matrix that lead to the formation of wrinkle film. If the mixing time is increased up to 4 hours, the viscosity will increase but it is impossible to cast a film from the solution due to too viscous solution and high wrinkle issue. As the mixing time increase, the tensile strength of sample film increases due to the increment of hydroxyl group formation throughout the mixing process that create a stronger ionic bonding in the carrageenan matrix.

The moisture content analysis in Table 1 shows that as the mixing time increase, the moisture content decrease. This is due to the homogeneity of the solution is improved and the water loss to surrounding throughout the mixing process.

Table 2 shows the hard capsule thickness, capsule loop strength and breaking force of carrageenan-CNC at different mixing time followed by commercial gelatin and hydroxypropyl methyl cellulose (HPMC). The results indicate that as the mixing time increased, the capsule thickness, capsule loop maximum load and breaking force are relatively increased. The breaking force test simulates the situation which may take place during the packaging, warehousing and handling of hard capsules after production process [3]. The result represents the ability of the hard capsules to withstand any load such as during stacking on shelf in warehouse or in container during transportation process. Increment of 58% and 295% for hard capsule loop strength and breaking force were obtained when the solution was mixed for 2 to 3 hours at 65°C.

Mixing temperature (°C)	Mixing time (hour)	Hard capsule thickness (mm)	Hard capsule loop strength (N)	Hard capsule breaking force (N)
65	2	0.12	46.54 ± 1.46	4.46 ± 0.050
	2.5	0.15	59.75 ± 1.56	6.81 ± 0.344
	3	0.34	73.78 ± 1.44	17.64 ± 0.251
Commercial HPMC capsule		0.10	68.89 ± 0.63	7.89 ± 0.422
Commercial Gelatin capsule		0.13	115.01 ± 1.49	13.93 ± 1.011

Table 2: The Hard Capsule Thickness, Capsule Loop Strength And Breaking Force

Fig. 4 Physical appearance of carrageenan-CNC hard capsule at different mixing time- 2 hours (a), 2.5 hours (b) and 3 hours (c).

The capsule loop strength and breaking force of carrageenan-CNC hard capsule are lower than the HPMC and gelatin hard capsule. The HPMC hard capsule can be considered comparable with the carrageenan-CNC hard capsule at mixing time of 2.5 hours. Additionally, the hard capsule at 3 hours is not favorable even if it has the highest hard capsule loop strength and hardness because it has thick hard capsule shell (0.34mm) and the moisture content is too low that lead to shrinkage in hard capsule structure as shown in Figure 4.In fact, the regulated hard capsule thickness should be less than 0.25 mm, according to Indian Pharmacopeia [30].The size of the capsule becomes shrink upon drying process due to too much moisture loss during mixing process and high viscosity of solution. The hard capsule samples disintegrated in less than 15 minutes in water at pH 7 as summarized in Table 1. Disintegration time less than 15 minutes is required for drug delivery carrier application [1].

IV. CONCLUSION

Manipulation of mixing time significantly increase the mechanical properties of carrageenan-CNC film and hard capsule. Increment of mixing time affect the hydroxyl bonding, homogeneity and loss of water during the reaction process. As the mixing time increase, the moisture content decrease that lead to increase in viscosity, thickness and tensile strength of sample solution and film Additionally, the capsule loop strength is also increase in parallel with the increment of breaking force of hard capsule. The mechanical strength increase in parallel with the increment of hydroxyl group in the composite matrix as proven by FTIR analysis. All of the hard capsules fulfill the disintegration test requirement according to European Pharmacopeia. The desirable hard capsule is the sample with at mixing time of 2.5 hours as it shows the best mechanical properties. As the capsule loop strength and breaking force test of the formulated hard capsule is still lower than the commercial gelatin and HPMC hard capsules, this study recommends that some improvement is still required by incorporation and manipulation of additives and plasticizer to improve the mechanical properties of the hard capsules.

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