Formulation Development of Carrageenan Based Nanofibers Via Electrospinning Technique

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***Abstract*—Carrageenan, CAR are the natural occuring polysaccharides and have desirable biocompatibility with biological systems especially in biomedical fields. But the effort in developing the carrageenan based nanofibers and film remain unexplored due to its fabrication limitation especially in electrospinning technique. In this work, 3 different methods of preparing the kappa type carrageenan solution were compared based on the viscosity (Electronic Rheometer), conductivity (Conductivity Meter) and FTIR peak (Fourier-Transform Infrared) analysis data. Preliminary data analysis shows carrageenan solution prepared through Method 1 gives the optimum data for PVA cross-link before electrospinning. Two types of PVA polymers, Fully Hydrolyzed and Partially Hydrolyzed PVA types were used as cross-link agent or co-polymer. The cross-link ratio of 70:30 (PVA: CAR) were used, then spinning solution prepared were analyzed in same manner as carrageenan solution previously. PVA FH and CAR spinning solution blend yield optimum properties data that promotes electrospinning technique. The nanofibers were successfully fabricated and the effect of different PVA types on the nanofibers characteristics were investigated through SEM and Contact Angle analysis. Nanofibers with PVA FH and CAR blend produced uniform and smooth nanofiber at 12 w/v % PVA FH concentration. Meanwhile, both PVA type promotes the hydrophilicity of the nanofiber with slight better absorption rate for PVA PH nanofiber blends. The results obtained from this work improve the applications of carrageenan in advancing the biomedical application especially in drug and nutrient delivery system.**

Keywords—Carregeenan, Nanofibers, Electrospinning, Formulation Development.

1. INTRODUCTION

Nanofiber that made up from biomaterials are one of the products or materials that recently received numerous attention by various researchers around the globe [1]. This is because its suitability to be used in many biomedical fields such as tissue engineering, target and release of targeted compound, drug and nutrient delivery, wound dressing, enzyme mobilization and etc [2]. The nanofibers are more desirable because of its high surface to volume ratio, high porosity and the compatibility of its own morphology to natural extracellular matrix [3]. Recently, the effort in fabricate the nanofibers from the natural polymer are increasing to improve the versatility of the nanofibers with the biological systems.

κ-carrageenan is one of the natural polymer, polysaccharides which belong in the sulphonated polysaccharides group that extracted from red algae [4]. According to [5], there are three different types of carrageenan polymer which are kappa, iota and lambda. κ-carrageenan polymer contains 3.6-anhydro-D-galactose and less sulphate group that made it less hydrophilic. Iota-carrageenan have 2-sulphate group which made it more hydrophilic. Meanwhile, lambda is known with high sulphated made it highly soluble under many conditions. The unique structure of carrageenan (kappa and iota only) allowed the molecule segments joint to form double helices which able to bind the molecule network in three dimensional [5]. This joint network was appeared in gels form. This property has given carrageenan an excellence water absorption due to its gelling mechanism. This excellence property become the main reason why carrageenan should be exploited as nanofibers building block for biomedical application [6]. Currently, carrageenan is widely used in the food industries as stabilizer and thickener especially in dairy products. The abundance of this natural polymer across the world has improved its accessibility to be utilize as main building block for nanofibers fabrication in industrial scale in the future.

Many researchers have reported the work in fabrication of carrageenan into nanomaterials such as biofilm but very less in nanofibers especially via electrospinning technique. Moreover, a few researchers have reported the fabrication of carrageenan based nanofibers using electrospinning technique but all of them reported the electrospinning of carrageenan alone as nanofibers main source were unsuccessful because of polyelectrolytic nature of the polysaccharides [7], [8]. Electrospinning of carrageenan alone is impossible to be achieved because of its chemical and biological properties that made it highly unfit for electrospinning. Hence, to improve the electrospinning capability of the carrageenan polymer, a few works reported by blending with electrospinnable carrier polymers such as polyethylene oxide, PEO and polyvinyl alcohol, PVA [6], [9]. In this study, PVA was chosen as co-polymer to blend with carrageenan polymer due to its outstanding properties and most importantly its versatility with electrospinning technique [1], [8], [10].

PVA is a non-toxic synthetic polymer that is water soluble, compatible with biological system, stable, non-expensive and easily obtained resources. Many studies have reported the utilization of PVA polymers as main sources of nanofibers as carriers for drugs delivery in oral system and also in food industries [10]–[12]. Hence, the usage of PVA for biomedical application was perfectly safe. For these reasons, and due to its excellent spinnability, PVA is frequently considered as co-blending polymer in electrospinning processes. There are two types of PVA polymer which are PVA Partially Hydrolyzed and PVA Fully Hydrolyzed.

Nevertheless, due to the limited solubility of the κ-carrageenan polymer [9], other alternatives available for dissolving κ-carrageenan that capable in adjust the properties to be more electrospinning-prone remain unexplored. The solubility of carrageenan polymer can be influenced by the presence of other solutes of inorganic salts that capable to alter the hydration degree of this polysaccharides types [5]. The additions of inorganic salt such as sodium salt and potassium salt highly affect the solubility of the carrageenan polymer. Moreover, the utilization of sonication using ultrasound wave in polysaccharides also help to improve the softening or swelling carrageenan process via hydration by provide high penetration of solvent into the polymer system once the cell wall breaks due to the wave impact [13]. Therefore, this studies aim to preliminarily analyze the optimum solubility alternatives for preparing κ-carrageenan solution to have optimum electrospinning parameters prior electrospinning. Then, also aim to obtain optimum concentration of PVA polymer types in κ-carrageenan based nanofiber synthesis, based on the electrospinning properties and characteristics of the nanofiber formed.

2. METHODOLOGY

2.1 Materials

The semi refined kappa type Carrageenan (κ-carrageenan) purchased from Tacara Sdn. Bhd. was used as natural polymer source meanwhile two types of Polyvinyl Alcohol (PVA) were used as synthetic polymer sources. The PVA used were Partially Hydrolyzed PVA with MW: 70,000 (Merck Schuchardt OHG, Germany) and Fully Hydrolyzed PVA with MW: 145,000 (Merck KGaA Darmstadt). Meanwhile, the main solvent used was distilled water and other additional chemical used in solution preparations was Sodium Chloride powder (Merck Millipore, Darmstadt).

2.2 Methods

2.2.1 Preparation of κ-carrageenan solution

There are three different methods were used to prepare the κ-carrageenan solution at the same concentration of 1.0 w/v %. All of the κ-carrageenan solution prepared from all methods were analyzed in terms of its viscosity, conductivity and FTIR analysis. The concentration of the carrageenan solution was referred to [8], [14]

2.2.1.1 Method 1: κ-Carrageenan dissolve with distilled water.

In this method, the κ-carrageenan powder was dissolved through traditional way by using only distilled water as solvent. Firstly, the distilled water was heated under heating plate until reached temperature of 80°C. Then, the weighed κ-carrageenan powder was added into the hot water and stirred continuously using magnetic stirrer for 4 hours. Ensure the temperature of the κ-carrageenan solution was maintained at 80°C to form opaque yellowish solution at the end. The κ-carrageenan solution was let cooled to room temperature for stabilization and allow for the solution to regain its viscosity. After cool to room temperature, the thermoreversible gel formed and settle at the bottom of the mixture leaving clear transparent solution at the upper layer. The clear upper layer solution was recovered and sent for analysis.

2.2.1.2 Method 2: Sonication of the κ-Carrageenan solution.

Secondly, the 1.0 w/v % carrageenan solution were prepared the same as the first method until the κ-carrageenan solution was cooled to form the reversible gel layer at the bottom. Then, the solution was sonicated with the ultrasonic wave at different contact time of 5,10,15 and 20 minutes. The sonication step was added to break the thermoreversible gel that formed once the κ-carrageenan solution was cooled. The water bath was prepared during the sonication process to avoid vaporization of liquid due to the heat form during sonication process. After sonication, the κ-carrageenan solution was let cooled to room temperature before sent for analysis. The ultrasonic applied was at 50 kHz, meanwhile the pulse was set to on for 1 minute and followed by pulse off for 10 seconds for all sonication period.

2.2.1.3 Method 3: Addition of NaCl salt.

Meanwhile in final method, 1.0 w/v % of κ-carrageenan solution was prepared by dissolving the κ-carrageenan powder in the NaCl solution. The different concentration of NaCl solution was prepared which are 4,6,8 & 10 w/v % by dissolving the NaCl powder with distilled water. Then, the κ-carrageenan powder was added into the NaCl solution and was stirred for 1 hour using magnetic stirrer without presence of heat. The same κ-carrageenan solution concentration of 1.0 w/v % was prepared in different concentration of NaCl solution. Then, the solution was sent for analysis.

2.2.2 Preparation of PVA solution

The fully hydrolyzed PVA flakes was dissolved in distilled water for 5 hours under heat of 90°C at different concentrations of 10,11,12 &13 w/v % with continuous stirring until clear transparent solution was obtained. Meanwhile, for the partially hydrolyzed PVA type, flakes were dissolved in distilled water for 5 hours without presence of heat with aid of continuous stirring at different concentration of 10,11,12 & 13 w/v%. The PVA concentration used for the electrospinning process and cross-link was referred to Yang et. al, that highlight the suitable range of PVA concentration that yield quality nanofiber strands [15].

2.2.3 Preparation of spinning solution

The most stable and suitable carrageenan solution will be chosen based on the preliminary evaluation to be cross-link with both type of PVA solution prepared. The PVA solution and κ-carrageenan solution were cross-linked by mixing it together using magnetic stirrer for 1 hour with mixing ratio of 70:30 (PVA: κ-carrageenan) until the mixture become homogenous. The ratio of PVA: κ-carrageenan mixture were referred on previous studies carried out by [1], [8]. All of the spinning solution were analyzed using the FTIR, Rheometer and Conductivity analysis before it was used for electrospinning.

2.2.4 Electrospinning technique of spinning solution

All of the spinning solution were fabricated into nanofiber form by using electrospinning technique. About 2 ml of spinning solution was loaded into the 5 ml syringe and 23-G needle was used as the nozzle tip. Only 1 ml of the sample were electrospun on the aluminum foils collector. The electrospinning parameters were kept constant throughout the whole experiment. The electrospinning parameters were set as follows: Flowrate: 0.30 ml/hr, Voltage: 12 kV, Distance between tip and collector: 15 cm. The electrospinning parameters were adapted from the previous work reported by [8]. All of the nanofibers collected were characterized using the Scanning Electron Microscopy, SEM and Fourier Transform Infrared Spectroscopy, FTIR analysis.

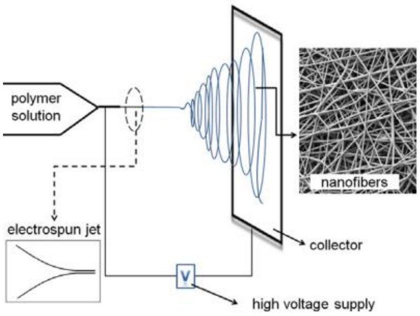


Fig 1. The electrospinning technique configuration in nanofiber fabrication [9]**.**

2.3 Characterization

2.3.1 FTIR Spectroscopy

Fourier-Transform Infrared (FTIR) peak analysis was obtained on a Spectrum One FT-IR Spectrometer, PerkinElmer, USA. The absorbance range that were used in the data analysis was between 500 cm^{-1} until 4000 cm^{-1}.

2.3.2 Electronic Rheometer

Electronic Rheometer, Anton Paar Physica MCR 301, Austria was used to analyze the viscosity properties of the liquid samples that were prepared throughout the experiment. Based on the shear rate and viscosity trend that were obtained during the measurement, the average viscosity of the samples was calculated based on the consistent data obtained.

2.3.3 Conductivity

To measure the conductivity of the liquid samples, SevenEasy Conductivity Metre, Mettler-Toledo, Switzerland was used. All of the conductivity measurement was measured at unit of µS/cm. To improve the accuracy of the data obtained from the measurement, triplicate measurement was done to obtain the final average conductivity value.

2.3.4 Scanning Electron Microscopy, SEM

For morphology analysis, the Benchtop Scanning Electron Microscopy (SEM), Hitachi TM 3030 Plus was used. Three different magnification was done to obtain the clear image of the nanofibers obtained from the electrospinning process which are x10k and x20k. The diameter of the nanofibers formed were measured from the images that were obtained from the x20k magnification.

2.3.5 ImageJ Software

From the x20k SEM images, 50 readings of fiber diameter were obtained manually using the ImageJ Software. From the readings, the histogram was plotted and the mean fiber diameter was recorded.

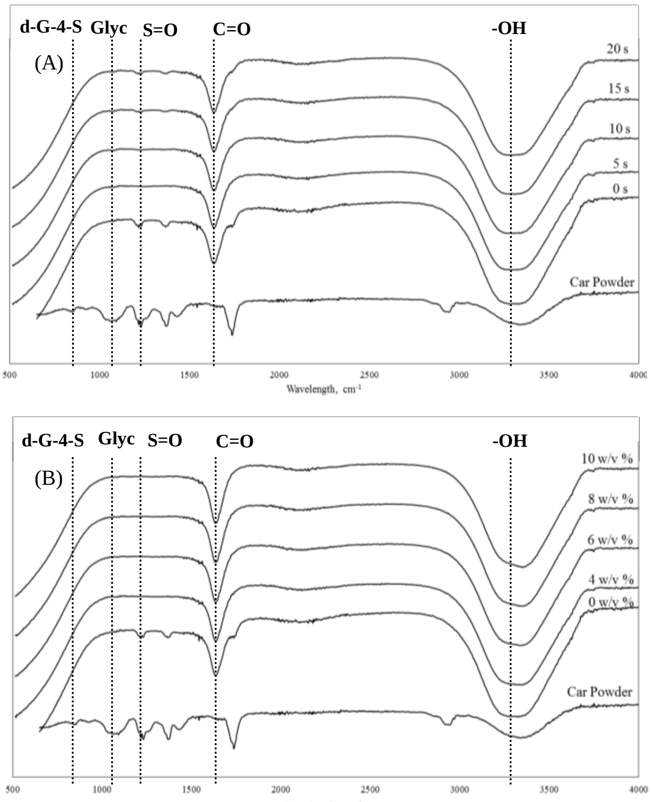
2.3.6 Contact Angle, CA

For contact angle analysis, the equipment used was VCA 3000 Water Surface Analysis System by AST Products Inc. To check the wetting properties that mimic the absorption capabilities of the nanofibers film surface, the water drop was fixed at constant volume of 5µL to ensure uniformity. The time and images for liquid drop start to land on fiber surface and for final steady liquid drop condition were taken for analysis.

3. RESULTS AND DISCUSSION

3.1 The effect of different κ-carrageenan solution preparation method.

In this study, a preliminary analysis was done on the carrageenan solutions which prepared through three different preparation methods. The preliminary analysis was done to analyze the most suitable carrageenan solution that will be cross-link together with the PVA polymer solution. PVA act as the co-polymer to improve the electrospinnability of the carrageenan solution. The data of the carrageenan that analyzed in this section were FT-IR spectra, viscosity and conductivity of the solution. In carrageenan solution preparation, there was only 1 sample obtained from Method 1, 4 samples from Method 2 and also 4 samples from Method 3. Method 1 was the simplest method and have been reported by a few past researchers to prepare the carrageenan solution [8]. Hence, Method 1 was chosen to be compared side by side with Method 2 and Method 3. The FT-IR spectra analysis were done to study the changes that caused due to the interactions between the carrageenan and solvent used in respective method. To analyze the changes occurred to the carrageenan solution, the spectra of pure carrageenan powder was included in the Fig. 2. (A) and (B) so that clear comparison can be made. Fig. 2. (A) and (B) illustrates the FT-IR spectra obtained from the carrageenan solution prepared from Method 2 and Method 3 respectively whilst combine with the FT-IR spectra obtained from Method 1. The FT-IR spectra from Method 1 labelled as 0 min and 0 w/v % were included in Fig 1. (A) & (B) respectively. As it clear illustrated, in both Fig 1. (A) and (B), all of the peak shows obvious broad band at 3300 cm-1 when turn into aqueous state [16]. Compared to the pure carrageenan that exist in powder form, the intensity of band at 3300 cm-1 was deflate and shows less absorbance. When dissolved in distilled water, the –OH group of carrageenan molecule was stretching. The stretching of the –OH vibrations happened due to intramolecular and intermolecular hydrogen bonds that increase when in contact with water molecules during preparation phase [6]. Other than that, all of the carrageenan solution spectra also indicate visible absorbance band peak at 1630 cm-1 due to the stretching of the amide C=O. Compared to those three different methods, the only visible difference that can be detected based on the spectra peaks was the absence of absorption band at range of 1210-1260 cm^{-1} that denoted the S=O sulphate esters stretching that can be seen only on spectra obtained from Method 1 [17]. Meanwhile, for the pure carrageenan powder, the FT-IR spectra shows less absorption intensity and more noticeable small peak. The small noticeable absorbance band peaks were 1092 cm^{-1} [16] that shows easily broken glycosidic linkage and 845 cm^{-1} which indicated the presence of d-galactose-4-sulphate [17]. Side by side comparison shows these weak and small linkages can easily be broken when dissolved in distilled water. Generally, the difference in preparing the carrageenan solution between Method 2 and Method 3 cannot be detected solely using FT-IR spectra because most of the spectra shows the same intensity or similar absorbance peak at very much the same wavelength range. Nevertheless, the comparison between both methods and Method 1 along with pure carrageenan powder still can be made. Hence additional analysis on other solution properties was included to ensure fair comparison between the methods adopted can be made.



***d-G-4-S: d-galactose-4-sulphate, Glyc: Glycosidic**

Fig. 2. FT-IR Spectra (A): 1.0 w/v % of Carrageenan solution sonicated at different sonication times and (B): 1.0 w/v % of Carrageenan solution dissolved in different NaCl solution solvent.

Based on the Table 1 below, Method 1 and 2 yield approximate results of viscosity in range of 30 cP and conductivity in range of (740- 790 µS/cm). Meanwhile, Method 3 yield drastic data by having highest conductivity value and lowest viscosity value. Even though, Method 1 and 2 yield consistent result, these carrageenan solutions are totally different when being differentiate physically. Recall that, in Method 2, the addition of sonication step was to dissolve the forming gel at the bottom layer because ultrasound waves able provides a higher penetration of solvent into the material to facilitate mass transfer [13]. Nevertheless, when being contacted with ultrasonic wave at high frequency, the soft solid gel become suspended throughout the solution and forming small aggregates that can be seen. The intense ultrasonic wavelength able to break the bond between the gel to dissolve it at the beginning. But, due to the condition of the solution that highly stable for the gel to stay in shape, it fail to dissolved into the solution. Furthermore, once the broken gel loses its mass forces, the surface forces dominate the smaller gel body forces and allow it to suspend throughout the solution. The difference between the carrageenan solutions can be seen in Fig. 3. where obvious small gel aggregated can be seen in Fig. 3. (B) and otherwise in Fig. 3. (A). The stable gel aggregates turned the clear carrageenan solution into yellow milky solution as shows in Fig. 3. The carrageenan stable gel did not degrade because of the lack of contact time during sonication process as previously reported by Zhou et. al [18], that reported full degradation of red algae polysaccharides at 240 min contact time. It also reported the intrinsic viscosity decreases as the time increases because of the ultrasonic temperature that behave directly proportional with the contact time [18]. Moreover, during the shear test, the viscosity data obtained shows obvious inconsistency due to the presence of aggregates that disturb the shearing of the spinning load during measurement because sonication did result in shear viscosity reduction of the solution [19]. But due to shorter contact time compared to Zhou et. al, the viscoelastic properties still remain but still capable influence its shear viscosity test due to presence of aggregates. Hence, the sonication did not help in improve in the carrageenan gel solubility and also did interrupt with the existing molecular entanglement of the dissolve carrageenan polymer that give its viscoelastic properties.

Method 3 yield inconsistent data that not suitable for electrospinning process, because both conductivity and viscosity were crucial parameters that highly affect the process performance. Method 3 yield very low viscosity range and very high conductivity range. The conductivity data increases with increasing of NaCl solution, from 29800, 40500, 53600 and 70900 µS/cm for NaCl concentration of 4 w/v%, 6 w/v%, 8 w/v%, 10 w/v% respectively. The higher the NaCl concentration, the higher number of ions disassociates into the solution. This will increase the electric conductivity of the solution. It is because NaCl it is an electrolyte solution that have oppositely charged ions. The NaCl addition allowing for the disassociates of the sodium, Na^+ and chloride, Cl^- ions in the solution to improve the current existing conductivity of carrageenan. According to Tort et al [8], the conductivity of spinning solution that made up of natural polymer must not exceed 1000 µS/cm. Hence, the carrageenan solution conductivity must be lower than that because natural polymers is polyelectrolytic in nature, which the ions easily excited when exposed to electric current [8]. Based on data listed in Table 1, the viscosity of the carrageenan solution shows the lowest range of viscosity data which in (0.96-1.07 cP). The average viscosity obtained increases with the increasing of NaCl solution concentration. Even with the addition of NaCl to improve the solubility of carrageenan powder, the solution supposedly exhibits viscoelastic properties because carrageenan powder is a polymer. But the viscosity data obtained shows the same viscosity value of pure water at room temperature that approximately at 1 cP. It means that the NaCl did not improve the solubility of carrageenan powder. It was proven based on Fig. 5. which illustrates the comparison of solution prepared from Method 1 and Method 3. In the figure, the bottom layer of solution prepared from Method 3, shows visible powder sediment that prove the carrageenan powder insoluble in the NaCl solution. Even with the usage of NaCl solution, the κ-carrageenan solubility decrease tremendously turn the powder remain insoluble to form pale yellow sediment at the bottom layer. Dissolving the carrageenan polymer in the sodium solutes supposedly improve the solubility of the carrageenan powder or the carrageenan gel. Addition of salts are the most effective way to improve the hydration of carrageenan [5]. Unfortunately, the solubility depends on the sulphate group that available in the carrageenan polymer repeating unit. The higher the content of the sulphate group the greater the carrageenan solubility by making the molecule more hydrophilic. Unfortunately, the κ-carrageenan have the lowest sulphate groups in the repeating unit compared to the iota and lambda carrageenan types. The fewer sulphate group in the κ-carrageenan inhibit the competition for available water due to the presence of foreign sodium ions in the solution [20]. The sodium salt increases the sensitivity of the carrageenan as the solutes to dissolve into the solution [5]. The sensitivity of the solute parallel with the solubility of the solute to becoming solution. High sensitivity condition make the sulphate group passive to compete for available water and as the result turn the whole polymer repeating unit more hydrophobic [5]. The function of sulphate group which to promote the hydration of the carrageenan cannot be fulfilled due to low number of sulphate group that did not have enough strength to compete for available water to increase the hydration [21]. Once the hydration does not occur, the carrageenan stays insoluble and not even swell because water did unable to penetrate the protection wall on the carrageenan surface due to hydrophobic effect. When the polymer did not dissolve, the viscosity of the NaCl solution remain unchanged. That explains why the viscosity of the carrageenan solution prepared through Method 3 were extremely unfit to be used prior electrospinning. Due to very low viscosity and very high conductivity value, the carrageenan solution from Method 3 proven not suitable to be cross-link with PVA for electrospinning to fabricate the nanofibers. Meanwhile, the same conclusion applied to carrageenan solution from Method 2 because the presence of suspended aggregates of fractured gel due to the sonication effect. Generally, the Method 1 shows the most promising alternative to be chosen for cross-link step before electrospinning process with optimum conductivity and viscosity data which at 30 cP, 741 µS/cm respectively.

Table 1
The viscosity and conductivity value of the respective carrageenan solutions that prepared via different preparation method.

Method	Sample Description	Avg. Viscosity *(cP)*	Avg. Conductivity *(µS/cm)*
1	CAR 1 w/v% only	33.32±4.23	741±1
2	CAR 1 w/v%, 5 min Sonicate	36.84±16.25	768±3
	CAR 1 w/v%,10 min Sonicate	33.82±13.87	775±4
	CAR 1 w/v%, 15 min Sonicate	32.08±16.05	788±3
	CAR 1 w/v%, 20 min Sonicate	29.32±19.21	791±2
3	CAR 1 w/v%, NaCl 4 w/v%	0.96±3.52	29800±400
	CAR 1 w/v%, NaCl 6 w/v%	0.98±4.66	40500±100
	CAR 1 w/v%, NaCl 8 w/v%	0.99±5.02	53600±300
	CAR 1 w/v%, NaCl 10 w/v%	1.07±3.91	70900±200

[a] The triplicate measurement was done to determine the average conductivity value.

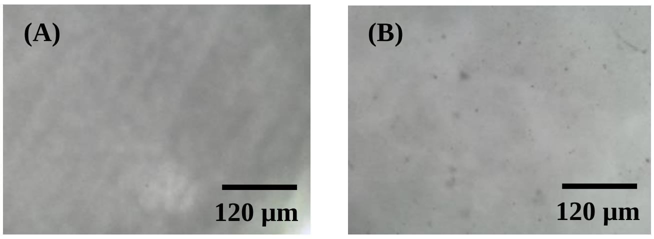


Fig. 3. Microcamera images (A): Images of carrageenan solution without small gel suspension (Method 1) and (B): Images of carrageenan solution with small gel suspension (Method 2)

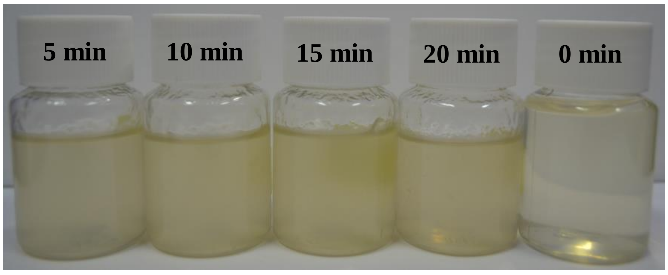


Fig. 4. Comparison of carrageenan solution prepared through Method 1 (Clear transparent solution) between Method 2 (Yellow cloudy solution).

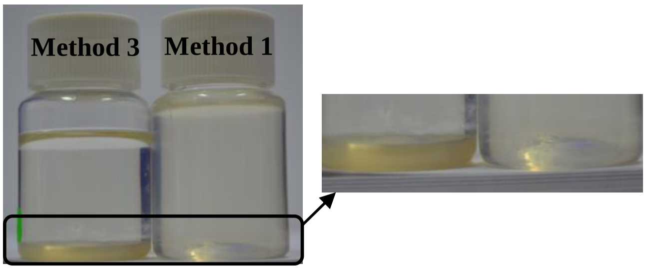
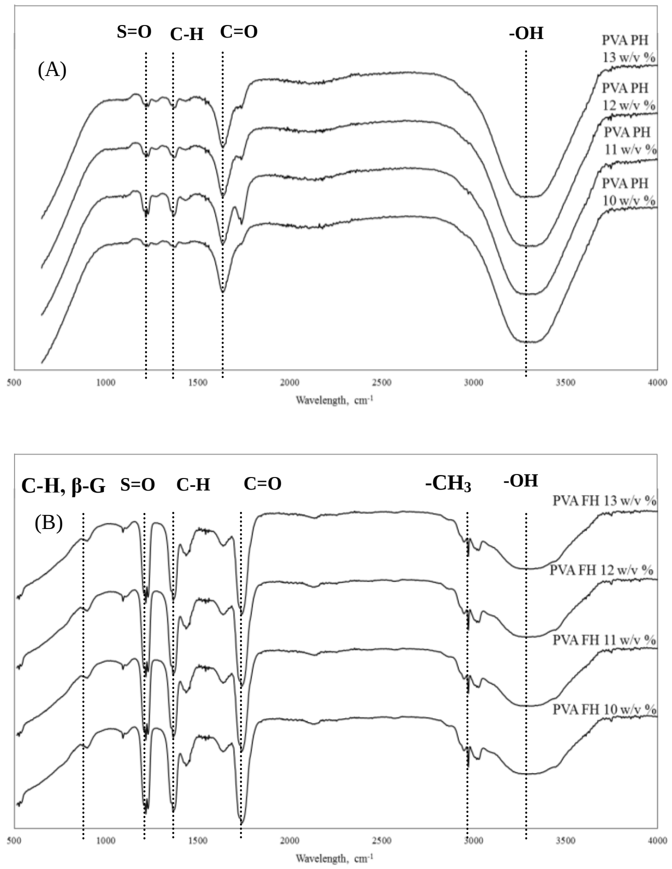


Fig. 5. Comparison of carrageenan solution prepared through Method 1 (Clear transparent solution) between Method 3 (Yellow sediment at bottom layer).

3.2 The effect of different type of PVA solution in spinning solution mixture.

The carrageenan solution from Method 1 were cross-linked with two different types of PVA solution, the spinning solution were sent for FT-IR spectra, viscosity and conductivity analysis. Fig. 6. (A) & (B) depicted the FT-IR spectra for spinning solution that cross-linked with PVA Partially Hydrolyzed (PH) and PVA Fully Hydrolyzed (FH) respectively. Visually comparison clearly shows that FT-IR spectra from PVA PH shows less absorbance peak compared to PVA FH. Spectra of PVA PH have a lot similarity with the FT-IR spectra of carrageenan solution previously as shown in Fig. 2. The spectra from PVA PH spinning solution shows intense stretching at wavelength band of 3330 cm-1 which denoted the –OH groups that stretching due to the presence of water from both carrageenan and PVA solution [6], [12]. Meanwhile, in PVA FH spinning solution (Fig. 6. (B)) shows less absorption peak at the same wavelength range due to less –OH groups in the spinning solution. The –OH groups in the spinning solution intensify when the water molecule increase the hydrogen bonding during the cross-linked process. The PVA FH solution have the higher hydrolysis extent which mean the PVA molecule have stronger hydrogen bond that cannot easily broken and link with water molecule even after cross-linked process [22]. The stronger intermolecular force interaction in the PVA FH molecule that made it require heat assistance during preparation of this PVA solution meanwhile the PVA PH solution preparation did not require heat. This was due to the weaker intermolecular forces between the PVA molecule. Furthermore, the PVA FH spinning solution also illustrates very obvious intense absorbance peak at wavelength range of 1210-1230 cm^{-1} that denoted the (S=O) sulphate esters, 890 cm-1 that abbreviated the C-H anomeric of β-galactose from the carrageenan, 2940-2970 cm-1 that resulting from –CH_3 groups stretch and 1365 cm-1 from the C-H bending which all absence in PVA PH FT-IR spectra [5]. The PVA FH spinning solution spectra shows that all the functional groups that detected in pure carrageenan powder FT-IR spectra (Fig 1 (A) & (B)) were intensify. This prove that there was no new absorption band was form. The subtle or less intense spectra in PVA PH spinning solution suggests that the weaker bond in the PVA intermolecular system has allowed the water presence to be absorbed by the chains and induced the stretching most of the carrageenan functional groups. The difference in the molecular weight between the PVA PH and PVA FH also suggests the difference in interaction between the carrageenan and PVA during cross-link process. The polymer system with longer molecule chain in each repeating unit will promote more bond linkage due to higher number of open chain that available in each repeating unit which allowed for carrageenan molecule to stay sustained its original functional groups when cross-link with PVA FH solution. Generally, cross-linking carrageenan with PVA with high hydrolysis degree that exhibits stronger molecular forces will improve the intramolecular interaction between these two polymer species.

Meanwhile, the change in the properties of the spinning solution were evaluated based on the viscosity and conductivity of the solution. In choosing the suitable PVA type that will improve the electrospinnability of the carrageenan polymer, the parameter that influence the electrospinning technique need to be analyzed. Based on results in Table 2, the average viscosity of spinning solution from mixture of carrageenan from Method 1 and PVA FH solution yield higher range of viscosity meanwhile vice versa with PVA PH solution mixture. All data trends show the spinning solution viscosity increases with increasing PVA solution concentration. the viscosity value for the PVA FH are 315.33, 571.23, 667.13 and 739.31 cP at PVA concentration of 10, 11, 12 and 13 w/v % respectively. Meanwhile for the PVA PH mixture, the viscosity values are at lower range of 35.93, 49.10, 66.00 and 91.90 cP at ascending PVA concentration same as PVA FH. The PVA FH mixture yields more viscous solution due to higher number of molecular weight at 145,000 g/mol compared to PVA PH which only at 70,000 g/mol. Polymer with higher molecular weight give more viscous solution due to longer molecular chain in each repeating unit and degree of polymerization that lead to stronger intermolecular forces Whereas the conductivity values of both types spinning solution were not significantly changed and did not considered as major concern because still in accepted range of (275-492 µS/cm) [8]. Nevertheless, addition of PVA polymer as co-polymer stabilize the conductivity of the carrageenan solution by decreasing it. Carrageenan which natural polyelectrolytic in which have high ions content that increase the charge carrying ability. The conductivity range obtained considered acceptable as the previous work reported successful nanofiber fabrication at conductivity range between (140-980 µS/cm). All of the spinning solution prepared undergo the electrospinning technique to fabricate the nanofibers. Constant electrospinning parameters were implemented to ensure the quality of the nanofibers can be characterized equally. The morphological analysis on all of the nanofibers fabricated from all of those spinning solution were discussed in next section.



***C-H, β-G: C-H of β-Galactose**

Fig. 6. FT-IR Spectra (A): 1.0 w/v % of Carrageenan solution after cross-linked with Partially Hydrolyzed (PH) Polyvinyl Alcohol at different concentration and (B): 1.0 w/v % of Carrageenan solution after cross-linked with Fully Hydrolyzed (FH) Polyvinyl Alcohol at different concentration.

Table 2

The viscosity and conductivity value of the respective solutions before and after cross-link process between CAR, Carrageenan solution and two different type of PVA solution.

Sample Description	Avg. Viscosity (cP)	Avg. Conductivity (μS/cm)
Carrageenan 1 w/v %, CAR only	33.32±4.23	741±1
PVA FH 10 w/v % only	532.00±8.84	177±1
PVA FH 11 w/v % only	1250.44±7.63	202±2
PVA FH 12 w/v % only	1395.80±5.87	225±1
PVA FH 13 w/v % only	1580.00±5.25	206±2
PVA FH 10 w/v % + CAR 1 w/v %	315.33±7.68	275±5
PVA FH 11 w/v % + CAR 1 w/v %	571.23±7.12	304±2
PVA FH 12 w/v % + CAR 1 w/v %	667.13±6.86	313±2
PVA FH 13 w/v % + CAR 1 w/v %	739.31±6.20	297±3
PVA PH 10 w/v % only	54.68±2.35	323±1
PVA PH 11 w/v % only	64.18±2.68	340±4
PVA PH 12 w/v % only	76.56±1.29	363±2
PVA PH 13 w/v % only	94.35±1.08	386±2
PVA PH 10 w/v % + CAR 1 w/v %	35.93±3.54	413±1
PVA PH 11 w/v % + CAR 1 w/v %	49.10±4.21	447±1
PVA PH 12 w/v % + CAR 1 w/v %	66.00±3.98	446±3
PVA PH 13 w/v % + CAR 1 w/v %	91.90±4.57	492±2

[a] The triplicate measurement was done to determine the average conductivity value.

3.3 Characterization of nanofibers morphology

Fig. 7. and 8. illustrates the SEM images of nanofibers formed from electrospinning method that captured at two different magnifications of x10k and x20k. The SEM images data were divided into two main categories which Fig. 7. for CAR-PVA PH and Fig. 8. for CAR-PVA FH respectively. The SEM images were showing the morphological condition and average fiber diameter of the nanofibers. From the images, the comparison between CAR-PVA PH and CAR-PVA FH were done, to find the optimum formulation which fabricated the best nanofiber quality with the optimum viscous and conductive properties. All of the CAR-PVA PH nanofibers images shows uneven size of fibrils formed with large alternate droplet shape presence for every single nano-fibril. The fibrils formed did not uniform diameter dimension due to inconsistent size of the fibrils strand. This appearance suggests that the surface tension was not enough to withstand the columbic stretching force generated from charged molecule to form consistent ejected jet from the needle [10]. Low surface tension of the solution was because of the low viscosity properties that did not provide enough viscoelastic capability that should be able to hold the molecule together and entangled the particle together [9]. Even at ideal conductivity value, the viscosity of the spinning solution still need to match upon the other because the electrospinning highly depends on each other for it to work properly and produce high quality fibers. PVA PH that have lower molecular weight unable to provide enough viscosity to form uniform and smooth fibers due to shorter carbon chain in the repeating unit building block. Meanwhile for the CAR-PVA FH nanofiber SEM images in Fig. 8., the smooth and uniform fiber strands were formed. The diameter of the fibers measure also provided close range accuracy value with lesser difference. Even at lower magnification images of x10k, the uniform fiber surfaces can be seen. Furthermore, the average diameter of the nanofibers consistent in close range with increasing concentration of PVA FH and decreasing in manner for PVA PH solution concentration as shows in Fig. 9. & Fig. 10. Based on Fig. 9, PVA PH produced smaller mean nanofiber diameter of 99.42 nm, 85.79 nm, 88.52 nm and 117.41 nm for 10, 11, 12 and 13 w/v % PVA concentration respectively. Meanwhile for PVA FH based on Fig. 10., the mean diameter shows opposite trend of 136.51 nm, 236.85 nm, 285.55 nm and 207.40 nm for 10, 11, 12 and 13 w/v % PVA concentration respectively. The difference between these were attributed by the viscosity difference. By comparing the morphology of the nanofibers at different PVA FH concentration, the PVA concentration 12 w/v % produce the best quality of fiber strand. It produced the finest fiber strands with no droplet appearance and no sign of breakage that can be seen with nanofiber from PVA concentration 13 w/v %. The fibers depicted breakage due to the excessive stretching of jet because of excess surface tension that hold up on the needle. This denoted the viscosity of solution at 13 w/v % concentration have reached the maximum viscosity. Beyond this point, the fiber will eventually start to breaking apart leaving finer and shorter fiber strands that resulting in smaller fiber diameter size. Meanwhile for the PVA FH 10 w/v % concentration, the nanofiber images depicted very fine droplet that cause little size distortion. In addition, all of CAR-PVA FH were successfully separated from the aluminum foil collector in form of very thin white delicate sheet meanwhile vice versa condition for the CAR PVA PH. The white deposit on the foil collector cannot peeled off due to the large droplets that formed have glued the most of the structure on the foil collector. Hence the viscous and conductivity of the spinning solution at PVA FH 12 w/v % concentration was considered as the most ideal to be use for electrospinning fabrication technique. These statement ultimately proved that the carrageenan based nanofibers were successfully fabricated and further characterization on the nanofiber physical properties were also included in the next section which is the characterization of wetting properties via contact angle analysis.

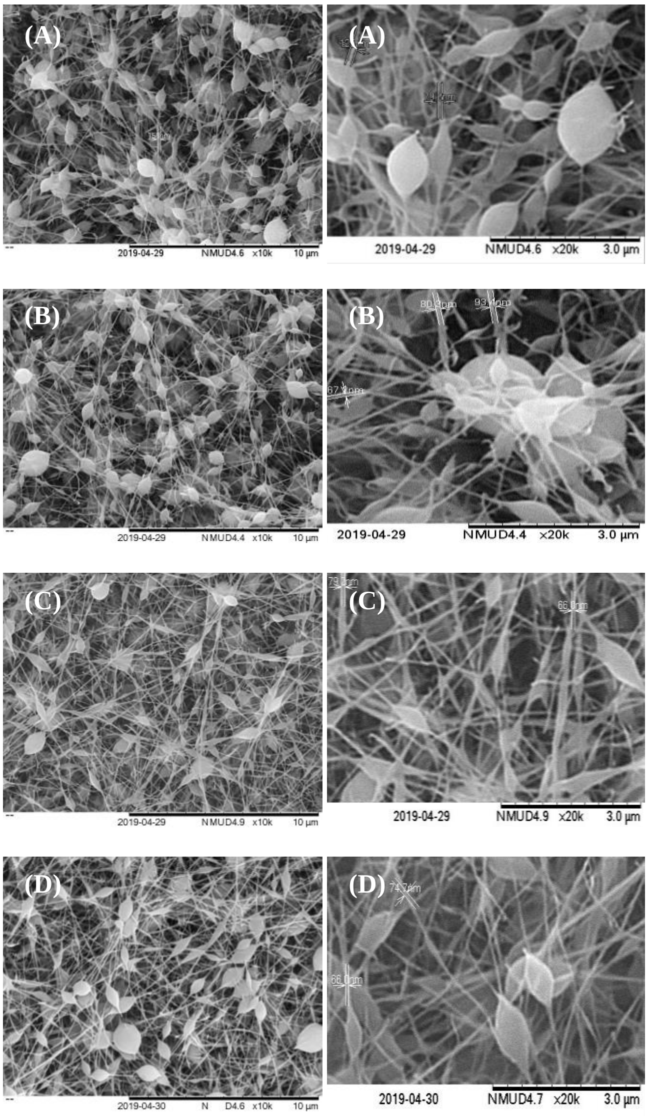
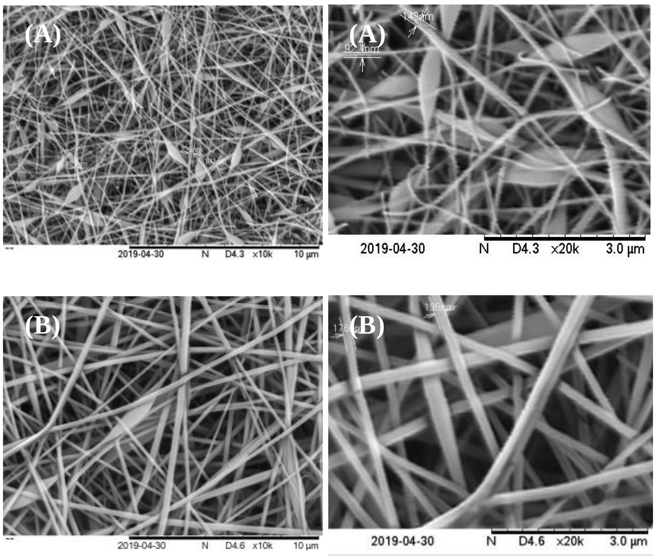


Fig. 7. SEM, Scanning Electron Microscopy Analysis Images (A): PVA PH 10 w/v % + CAR 1 w/v % nanofibers at x10k & x20k magnification, (B): PVA PH 11 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification, (C): PVA PH 12 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification and (D): PVA PH 13 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification.



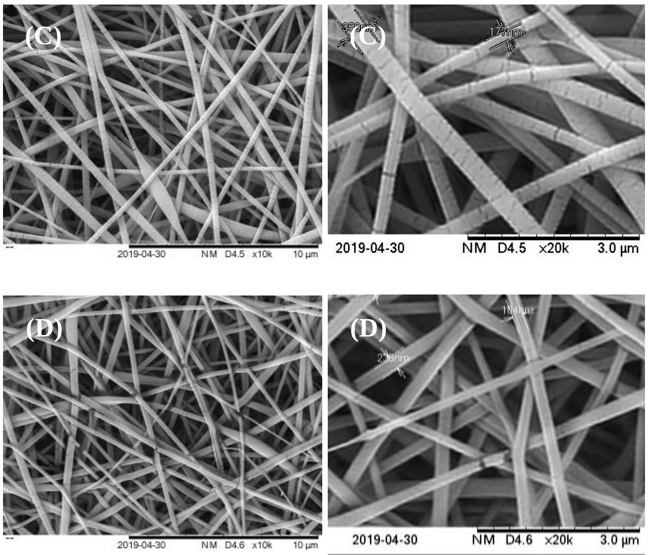
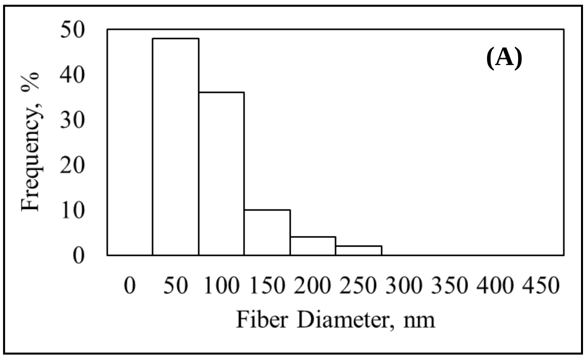
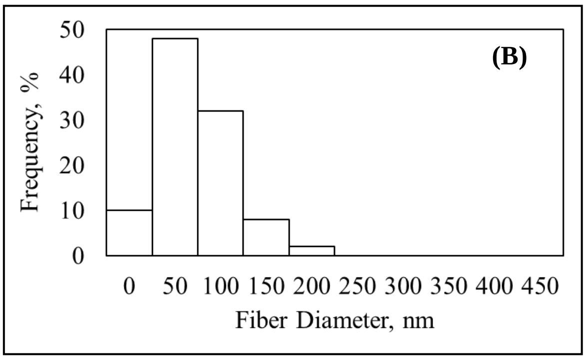


Fig. 8. SEM, Scanning Electron Microscopy Analysis Images (A): PVA FH 10 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification, (B): PVA FH 11 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification, (C): PVA FH 12 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification and (D): PVA FH 13 w/v % + CAR 1 w/v % nanofibers x10k & x20k magnification.

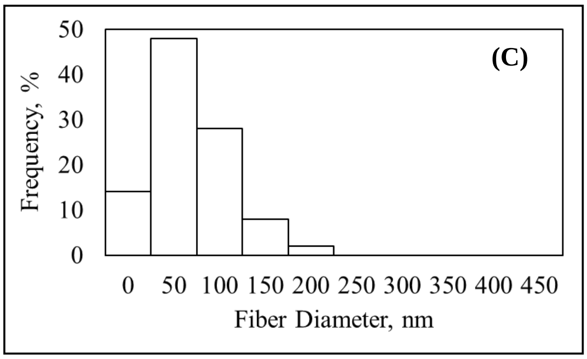
Mean Fiber Diameter: 99.42 nm



Mean Fiber Diameter: 85.79 nm



Mean Fiber Diameter: 88.52 nm



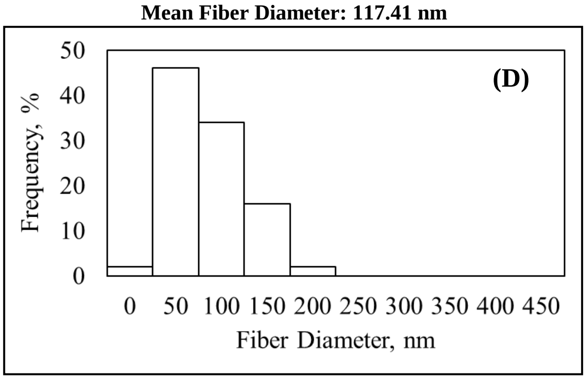
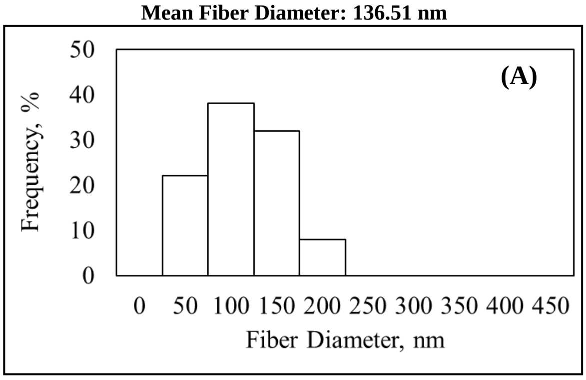
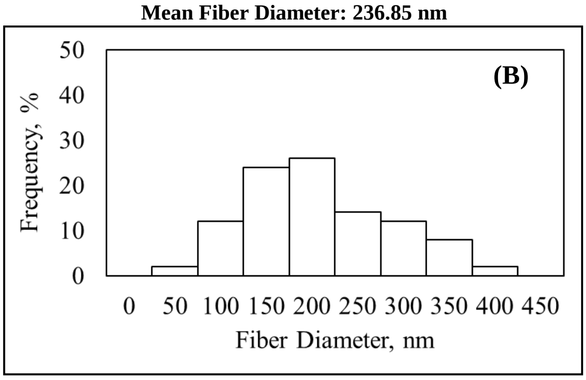
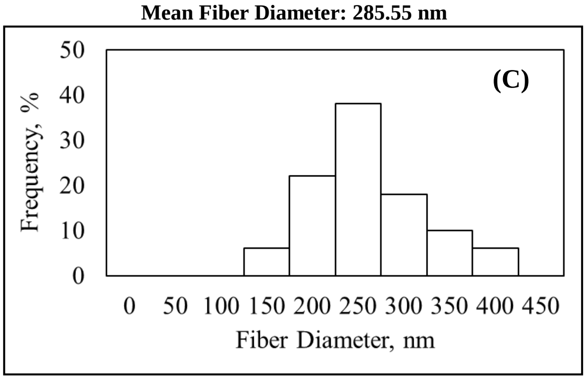

Mean Fiber Diameter: 117.41 nm

Fig. 9. PSD, Particle Size Distribution Histogram Analysis (A): PVA PH 10 w/v % + CAR 1 w/v % nanofibers, (B): PVA PH 11 w/v % + CAR 1 w/v % nanofibers, (C): PVA PH 12 w/v % + CAR 1 w/v % nanofibers and (D): PVA PH 13 w/v % + CAR 1 w/v % nanofibers.


Mean Fiber Diameter: 136.51 nm


Mean Fiber Diameter: 236.85 nm


Mean Fiber Diameter: 285.55 nm

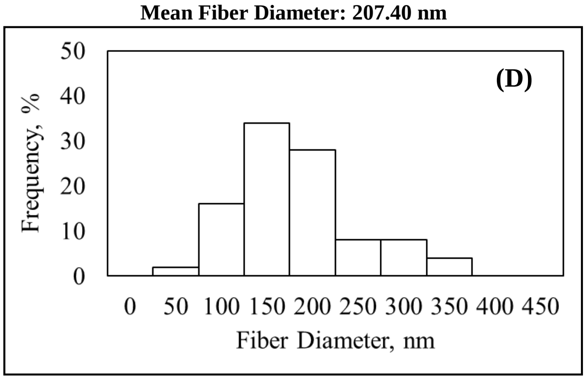

Mean Fiber Diameter: 207.40 nm

Fig. 10. PSD, Particle Size Distribution Histogram Analysis (A): PVA FH 10 w/v % + CAR 1 w/v % nanofibers, (B): PVA FH 11 w/v % + CAR 1 w/v % nanofibers, (C): PVA FH 12 w/v % + CAR 1 w/v % nanofibers and (D): PVA FH 13 w/v % + CAR 1 w/v % nanofibers.

3.4 Contact Angle Analysis

Fig. 11. and Fig. 12. depicted the Contact Angle images for all nanofibers of different type PVA polymer used in cross-linking process. Fig. 11. depicted for nanofiber blend of CAR and PVA Partially Hydrolyzed, meanwhile Fig. 12. shows for nanofiber blend of CAR solution with PVA Fully Hydrolyzed. Contact angle analysis was done to analyze the wetting properties of the nanofibers. The contact angle, θ is defined as angle between tangent drawn on the drop solid surface and solid surface that comprises three point of solid-liquid-atmosphere [23]. The contact angle measurement was highly dependent on the magnitude of adhesive and cohesive molecular forces that available between the solid and liquid surface layers. Based on theory, the contact angle was in value from 0° to 180° [24]. The value range indicates degree hydrophilic and hydrophobic of the nanofibers surface. The contact angle at 0° denoted the perfectly wetting surface meanwhile vice versa condition for contact angle at 180° [25]. Based on the data in Fig. 12., the contact angle values of CAR and PVA FH nanofibers blend were 61.70°, 51.90°, 51.80°, 37.10° for different PVA FH concentration at 10, 11, 12 and 13 w/v %. The contact angle values were decreases with increment of PVA FH concentration in the nanofibers blend. Meanwhile in the Fig. 11., the contact value for the CAR and PVA PH nanofibers blend were at 42.50, 26.60, 25.80, 24.80 at different PVA PH concentration of 10, 11, 12 and 13 w/v %. The same situation applied for these nanofiber where the contact angle decrease with the increment of the PVA PH concentration. The contact angle for the PVA FH nanofibers blend gives higher values range compared to the PVA PH nanofiber blend. This means that the nanofiber with PVA FH blend have higher degree of hydrophobic properties. This phenomenon can explain by the presence of the PVA FH itself in the nanofiber structural framework that have lower degree solubility compared to PVA PH that easily dissolve in water [26]. This phenomenon due to the degree of hydrolysis of PVA polymers that improved the crystallinity of the PVA solid anD strengthen the molecular structure of the PVA species with higher hydrolysis degree. Recall that, in the preparation of PVA solution, the most obvious difference between the PVA FH and PVA PH is the heat addition during dissolving process for PVA FH meanwhile vice versa for PVA PH. Even though PVA FH have lower solubility but its presence still tends to decrease the nanofiber surface hydrophobicity by yield the lowest contact angle for the nanofibers with the highest PVA FH concentration. Meanwhile, for the PVA PH nanofibers blend, the contact angle depicted excellent hydrophilicity properties by gives consistent contact angle values at 11, 12 and 13 w/v % concentration which was at very close range of 24.80°-26.60°. In the analysis, the time taken for the

liquid drop to wet or absorb into the fiber surface were also recorded. Based on Fig. 12., the time taken for angle to reach constant consistent values were decreasing with the increasing of PVA FH concentration. The higher the concentration of PVA FH, the quicker the system to absorb the water [27]. The time taken were at 19.3 s, 16.4 s, 14.9 s and 12.1 s for increasing PVA concentration respectively. Nevertheless, the time taken for PVA PH system revealed shorter absorption time compared to PVA FH nanofiber system due to the same reason justified previously in this section [26]. But, the time data obtained shows inconsistent trend which were 7.5 s, 6.3 s, 7.8 s and 7.9 s for increasing PVA concentration. The uneven thickness of the nanofibers due to the low quality fiber formation explained the uneven absorption period data for the PVA PH nanofiber system. The details on the morphology and quality of nanofiber formation have been discussed briefly in previous morphology discussion. The analysis surely resides with the nanofibers blend with the PVA PH blend proved to have better wetting properties and greater water absorption rate. Nevertheless, based on the studies done by Vogler et. al, the quantitative definition of the hydrophobic and hydrophilic properties surfaces by exhibits contact angle θ> 65.0° and θ< 65.0° respectively [28]. Hence it can be concluded that both PVA PH and PVA FH capable to promote the hydrophilicity and wetting properties of the carrageenan based nanofibers because all of the contact angle values obtained were less than 65.0°.

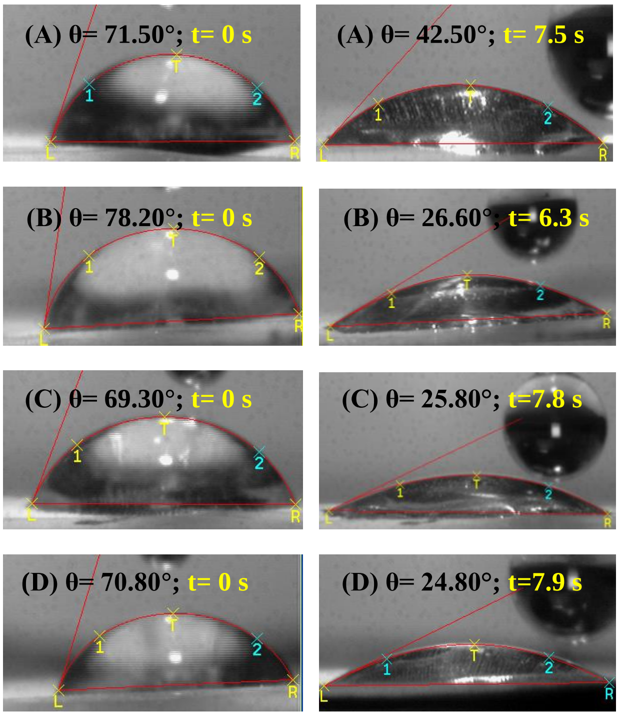
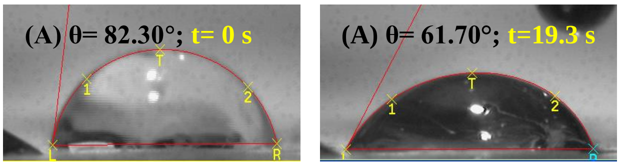


Fig. 11. CA, Contact Angle Analysis Images (A): PVA PH 10 w/v % + CAR 1 w/v % nanofibers, (B): PVA PH 11 w/v % + CAR 1 w/v % nanofibers, (C): PVA PH 12 w/v % + CAR 1 w/v % nanofibers and (D): PVA PH 13 w/v % + CAR 1 w/v % nanofibers.



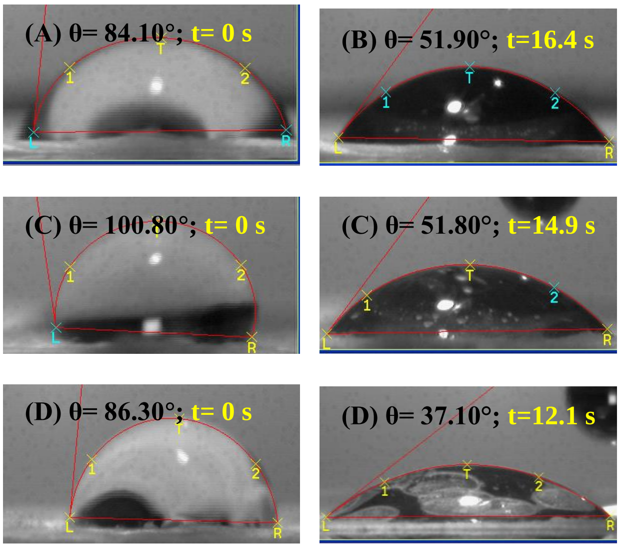


Fig. 12. CA, Contact Angle Analysis Images (A): PVA FH 10 w/v % + CAR 1 w/v % nanofibers, (B): PVA FH 11 w/v % + CAR 1 w/v % nanofibers, (C): PVA FH 12 w/v % + CAR 1 w/v % nanofibers and (D): PVA FH 13 w/v % + CAR 1 w/v % nanofibers.

4. CONCLUSION

This study showed that the carrageenan based nanofibers can be produced with the addition of PVA polymer types. PVA FH and CAR blend yield smooth and uniform nanofibers formation from electrospinning technique due to PVA FH characteristics that allowed the mixture to exhibits suitable electrospinning parameters in terms of viscosity and conductivity compared to PVA PH. The CAR and 12 w/v % PVA FH gives the best nanofibers quality with smoothest and finest strands. Meanwhile PVA PH produced very poor quality nanofibers. Generally, the different type of PVA high influence both electrospinnability and final products quality due to difference in properties such as solubility, molecular weight, degree of hydrolysis and etc. Furthermore, the comparison done on different carrageenan dissolving technique also proved the simplest and conventional method able to produce stable carrageenan solution that exhibits stable electrospinning properties before cross-link were done. The conductivity and viscosity are one of the most important parameters that influence the electrospinning technique success rate. Developing carrageenan solution to have stable electrospinning parameter properties before cross-link will improves the electrospinnability and final nanofibers quality. Furthermore, both PVA FH and PVA FH also capable in improves the hydrophilicity of the carrageenan based nanofibers to promotes the absorption properties.

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