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Effects of Reaction Temperature on the Synthesis and Thermal Properties of Carrageenan Ester

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Abstract: *We report the new evidence of the chemical modification of κ-carrageenan to its ester form using decanoyl chloride and pyridine, and by systematically varying the reaction temperature in the range from 60°C to 90°C. It was found that the percentage of yield of carrageenan esters is increased with the increase of synthesis temperature. Such results are also confirmed by both Fourier transform infrared (FTIR) and* ^1H *NMR measurements. Thermogravimetric analysis (TGA) data showed that carrageenan esters are more thermally stable than κ-carrageenan. Carrageenan ester synthesised at 80°C is thermally more stable than all other carrageenan esters. Glass transition temperature* (T_g) *obtained by DSC analysis showed that the* T_g *values of carrageenan ester samples are lower than κ-carrageenan. The lowest* T_g *value, 83.15°C was obtained for the carrageenan ester sample synthesised at 90.0°C.*

Keywords: κ-carrageenan, carrageen ester, reaction temperature, yield, thermal properties.

1. INTRODUCTION

Among the marine microorganisms, seaweeds possess much importance due to its uses as a large source of valuable materials.[1] Carrageenans are obtained from seaweeds which is a kind of polysaccharide. Among the polysaccharides, carrageenans have attracted intense attention due to its wide range of applications in food industry, such as thickening, gelling, stabilising agents and binder for the manufacture of different kinds of foods.[2] Carrageenans are also used in pharmaceutical, cosmetics, printing and textile industries.[3] To date, three main types of carrageenans have been reported in the literature: kappa (κ), iota (ι) and lambda (λ) and among them κ-carrageenan is a non-toxic sulphated polysaccharide.[4]

The structure of κ-carrageenan is made up of α(1→4) D-galactose-4-sulphate and β(1→3) 3,-anhydro-D-galactose.[5] κ-carrageenan has an important role as antioxidants, free radical scavengers *in vitro* and also in biotechnology sectors.[6–8] However, κ-carrageenan suffers from certain drawbacks like biodegradability and safety of carrageenan containing food, etc.[9] Thus, it may be useful to chemically modify the κ-carrageenan for its better and wider industrial uses and applications.

Several chemical modifications have been proposed in the literature in order to modulate the physicochemical properties of κ-carrageenan.[10–14] Guiseley et al.[10] modified κ-carrageenan by introducing hydroxyalkyl groups. Hosseinzadeh et al.[11] prepared κ-carrageenan hydrogel in order to increase the absorptivity in saline through polyacrylamide followed by alkaline hydrolysis. The synthesis of *O*-maleoyl derivative of κ-carrageenan were reported by the reaction of tetrabutylammonium salt with maleic anhydride, 4-dimethylaminopyridine and tributylamine in DMF.[14] However, several reports can also be found on the acetylation of κ-carrageenan to produce carrageenan ester. Carrageenan ester has already shown some potential as its high anti-HIV activity, low anticoagulant activities[15] and also known to have high inhibitory effect on oxidation.[16]

To produce carrageenan ester, researchers previously introduced carboxylic acid anhydride, Ac_2O and monochloroacetic acid.[15–17] In all of these experiments, κ-carrageenan was subjected to preliminarily depolymerisation process before undergoing acetylation reaction. So, it is of interest to carry out esterification of κ-carrageenan without undergoing any preliminary depolymerisation process. Furthermore, it may be of importance to understand the effects of reaction temperature on such esterification process in order to control the yield and also to realise the thermal properties.

In this work, we are proposing the combined use of decanoyl chloride and pyridine with κ-carrageenan to synthesise carrageenan esters without performing preliminary de-polymerisation process. Moreover, we have also realised the effects of reaction temperature on the yield and thermal properties of carrageenan esters. The synthesised carrageenan esters were characterised by means of Fourier transform infrared (FTIR), ^1H NMR, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) measurements. The results may be promising for the preparation of new functional biopolymer, like carrageenan ester which may have widened applications in industrial and biotechnology sector.

2. EXPERIMENTAL

2.1 Materials

Pyridine (Sigma-Aldrich) was distilled prior to use. κ-carrageenan (99.9%), decanoyl chloride (98%), pyridine (99.8%) and d-dimethylsulfoxide (99%) were purchased from Sigma-Aldrich. Toluene (99.5%) and chloroform (99.5%) were obtained from Qrëc (Malaysia). Sodium hydrogen carbonate and d-chloroform were purchased from Merck. All the reagents were analytical grade and were used as received.

2.2 Esterification of κ-carrageenan

The esterification process of κ-carrageenan was carried out following a slightly modified procedure as previously reported by Mullen et al.[18] The experimental procedure for the synthesis of κ-carrageenan ester samples is presented in Figure 1. The samples were prepared by adding 35 ml of pyridine to the 0.007 mol of κ-carrageenan (as K salt form) at different temperatures ranging from 60°C to 90°C under vigorous stirring in N_2 atmosphere for 30 min. Such pre-heating treatments of κ-carrageenan with pyridine were performed in order to obtain good dispersion between them before the reaction. Then, a desired amount of 0.05 mol decanoyl chloride was added and refluxed at the corresponding pre-treatment temperature (60°C to 90°C) for 6 h to obtain the crude products.

The obtained reaction mixture was left at room temperature for precipitation. The precipitated was collected on a Büchner funnel and washed with ethanol. It is important to note that the solid crude products were grind to small particles and then purified by washing with ethanol in several times until the filtrated liquid became colourless. Finally, the products were allowed to dry at room temperature for 24 h. The obtained products are powder like and white or yellowish colour depending on the reaction temperature used in the synthesis process.

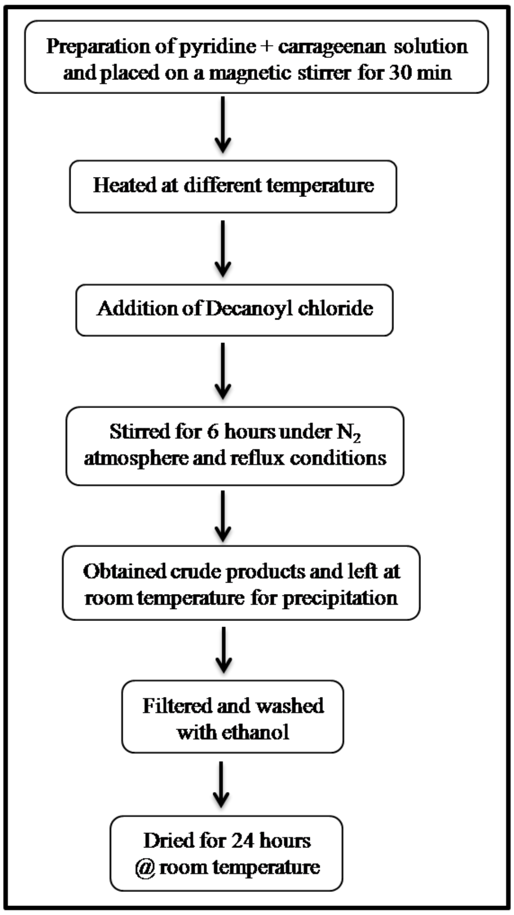

Figure 1: Schematic diagram for the synthesis of κ-carrageenan ester.

2.3 Characterisation

2.3.1 FTIR spectroscopy

Transmission FTIR spectra of κ-carrageenan and carrageenan esters were recorded between 400 cm^{-1} and 4000 cm^{-1} from thin KBr disc of the samples with a model 2000 Perkin Elmer spectrometer (resolution of 0.4 cm^{-1}).

2.3.2 ^{1}H NMR spectroscopy

The 500 MHz ^{1}H NMR spectra were recorded with a Bruker Avance 500 spectrometer for κ-carrageenan, while the 400 MHz was used for carrageenan ester solid samples to obtain spectra on Bruker DPX 400 spectrometer. In both cases, the probe temperature was maintained at 24°C. Solutions of κ-carrageenan for the ^{1}H NMR experiments were prepared using D_2O (99.9%, 1 ml) and the solutions of carrageenan ester were prepared in d-chloroform (99.96%) and d-toluene (99.6 atom D%). Tetramethysilane (TMS) was used as internal standard for the calibration of chemical shift of samples.

2.3.3 TGA analysis

TGA analysis was carried out by measuring the weight loss of the sample against the change of temperature. The sample was heated under nitrogen gas atmosphere between room temperature (30°C) and 900°C at a rate of 20°C min^{-1}. Furthermore, differential thermogravimetric (DTG) analysis was also carried out for all the samples.

2.3.4 DSC analysis

DSC analysis was performed on a Perkin Elmer Pyris 1 instrument under nitrogen flow with a rate of 20 ml min^{-1}. The heating rate of the measurement was maintained at 20°C min^{-1}. The analysis was carried out in the temperature ranging from –50°C to 210°C and –50°C to 170°C for κ-carrageenan and carrageenan ester samples, respectively.

3. RESULTS AND DISCUSSION

3.1 Effect of Synthesis Temperature

3.1.1 Physical properties and yield of carrageenan esters

The carrageenan esters were synthesised at 60°C, 70°C, 80°C and 90°C reaction temperature (synthesis batches α–δ) and the corresponding physical properties and % of yield are reported in Table 1. We obtained white agglomerated powder like products from the synthesis batch, α performed at lowest reaction temperature (60°C). However, when the reaction temperature of the synthesis process increased from 70°C to 90°C, the obtained products are rubbery like and the corresponding colours are light yellow (synthesis batch β) and yellow (synthesis batch γ and δ), respectively.

The reaction temperature not only influenced the physical properties but also the % of yield of the synthesised products. It was found that when we increased the reaction temperature, the percentage of yield of synthesised ester increased from 45.24% for 60°C, 48.3% for 70°C, 63.51% for 80°C and 71.77% for 90°C. Thus, higher reaction temperature is favourable to obtain maximum yield and furthermore, the percentage of yield of carrageenan ester could be directly controlled by varying the reaction temperature of the synthesis process.

Table 1: The percentage of yield and physical properties of carrageenan esters obtained from different synthesis batches.

Synthesis identifier	Reaction temperature (°C)	Colour of the product	Physical state	Yield (%)
α	60	White	Powder	45.24
β	70	Light yellow	Rubbery	48.63
γ	80	Yellow	Rubbery	63.51
δ	90	Yellow	Rubbery	71.77

3.1.2 FTIR spectra and identification

The FTIR spectra of the samples collected for κ-carrageenan and carrageenan esters (synthesis batches, α-δ) are shown in Figure 2. For κ-carrageenan, we observed a broad peak at the region of 3000 cm^{-1} to 3600 cm^{-1} (–OH stretching vibration). The other peaks found at 1643 (polymer bound water), 1241 (asymmetric stretching of O=S=O), 1069 (glycosidic bond in carrageenan), 922 (present of ether group in 3,6-anhydrogalactose of carrageenan), 847 and 701 cm^{-1} (C_4–O–S sulphate ester bonding in galactose) are the characteristic peaks of κ-carrageenan.[19,20] In the case of carrageenan ester samples (α–δ), the characteristic peaks of κ-carrageenan were also observed.

However, the intensity of the broad peak of –OH group around at 3400 cm^{-1} became less intense as compared to κ-carrageenan, indicating the substitution of acyl group in the κ-carrageenan and thus demonstrated the formation of ester. When we compared among the carrageenan ester samples, the intensity of the –OH group peak is reduced with the increase of reaction temperature from 60°C to 90°C.

Furthermore, the spectra for all the synthesis batches (α–δ) showed the presence of ester carbonyl peak at 1742–1749 cm^{-1}, which further confirms the occurrence of esterification process. It is important to note that the intensity of ester carbonyl peak was found to increase for the higher synthesis temperature which shows the maximum peak intensity of the sample synthesised at 80°C (batch γ) and 90°C (batch δ). These results suggest the formation of carrageenan esters for all synthesis batches and also imply that such kind of ester formation is enhanced with the increase of synthesis temperature (60°C–90°C).

However, the sulphate group peak present at the region of 842–844 cm^{-1} was found to become less intense for the sample synthesised at 90°C (batch δ) as compared to the other samples. It is noted that the presence of sulphate group in the carrageenan ester can facilitates its use as a drug carrier and high anti-HIV activity, etc.[15,17] Thus, the ester prepared at 90°C may not be suitable for applications, although the amount of ester formation is higher at this temperature (Table 1). Hence, to obtain comparatively higher yield as well as to avoid the losing of sulphate group in the carrageenan ester, 80°C could be the optimum reaction temperature for this esterification process.

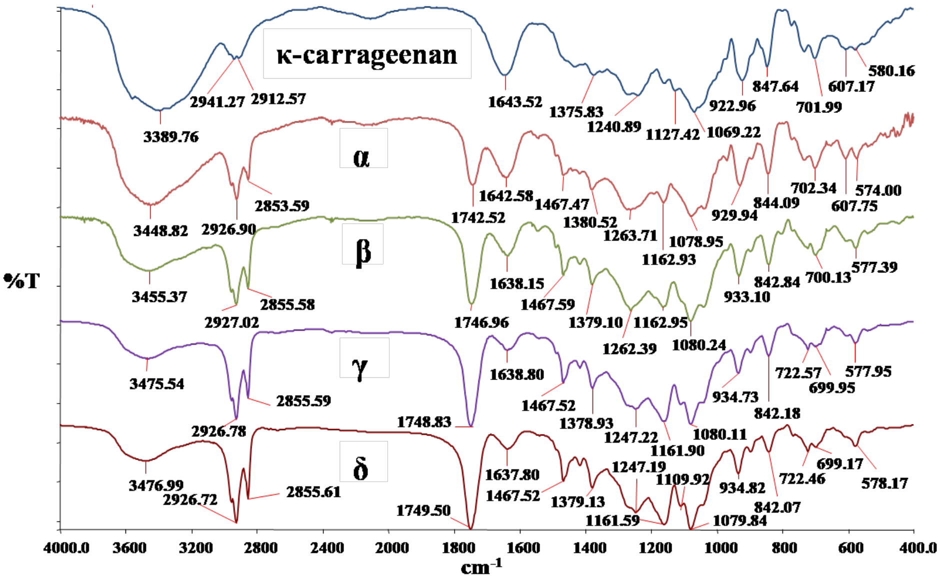

Figure 2: FTIR spectrum of the κ-carrageenan and carrageenan esters. The carrageenan ester samples were synthesised at different reaction temperature. Spectrum α, β, γ and δ correspond to the reaction temperature 60°C, 70°C, 80°C and 90°C, respectively.

3.1.3 ^{1}H NMR spectra and identification

The ^{1}H NMR spectra of κ-carrageenan is shown in Figure 3 and the chemical shift of the corresponding protons is presented in Table 2. The indicated peaks and the chemical shifts are very similar to those already reported in literatures for carrageenan esters.[2,21] The peak at 3.58 ppm corresponding to *O*-methyl proton due to the presence of small amount 3-linked 6-*O*-methyl-D-galactose residue in κ-carrageenan.[22] Similar chemical shift (3.56 ppm) for such *O*-methyl proton was also reported by Campo et al.[2] However, we observed a small variation of the chemical shift value for our samples when compared to the one reported in reference.[2,21,22] This may be due to the several reasons: (i) the samples were collected from different sources; (ii) ^{1}H NMR experiments were performed in different temperature; and (iii) the samples were irradiated before the ^{1}H NMR measurement.[21] In our case, we did not perform any pre-treatment before the ^{1}H NMR experiment.

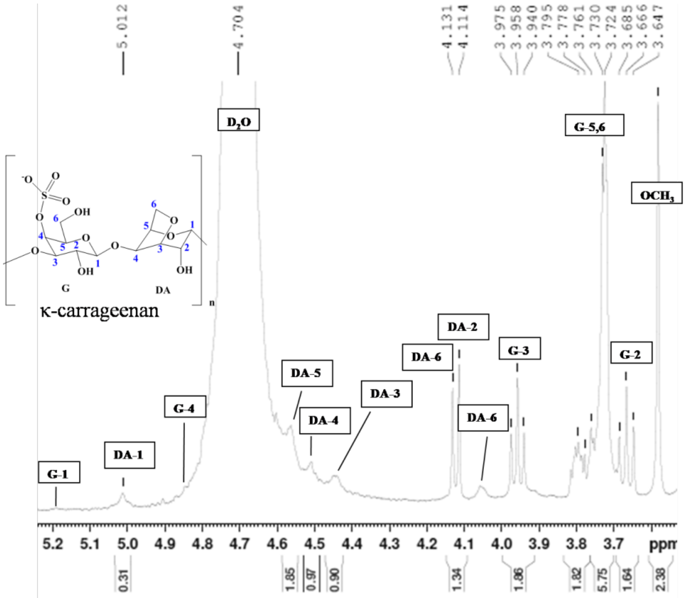

Figure 3: ^{1}H NMR spectrum of κ-carrageenan.

Table 2: Chemical shift of the ^{1}H NMR of κ-carrageenan.

κ-carrageenan		Reference polymer (Abad et al.[21])	Reference polymer (Campo et al.[2])
G-1	5.19	4.57	4.75
G-2	3.67	3.50	3.74
G-3	3.96	3.90	4.10
G-4	4.84	4.83	4.95
G-5	3.72	3.71	3.93
G-6	3.71	3.71	3.93
DA-1	5.01	5.01	5.24
DA-2	4.01	4.05	4.12
DA-3	4.45	4.44	4.30
DA-4	4.52	4.51	4.75
DA-5	4.58	4.56	4.65
DA-6	4.13	4.13	4.25

We performed the ^{1}H NMR measurement of the carrageenan ester samples. Figure 4 shows, for instance, ^{1}H NMR spectra of the sample synthesised at 80°C (batch γ). The peaks found at the chemical shift value of 0.9 ppm (primary proton), 1.3 and 1.8 ppm (secondary proton), and 2.3 ppm (proton adjacent to carbonyl group), indicate the substitution of acyl group to the κ-carrageenan during the esterification process.

It is noted that we did not observe such peaks and corresponding chemical shift values for κ-carrageenan (Figure 3). We also observed peaks at the range of 4.2 to 6.0 ppm, which corresponds to the chemical shift of proton for carbohydrate portion in carrageenan ester. The residue peak found at 0.5 ppm may due to the presence of water impurity in the sample.[23] Thus, all of the results further support the formation of carrageenan ester in the synthesis process.

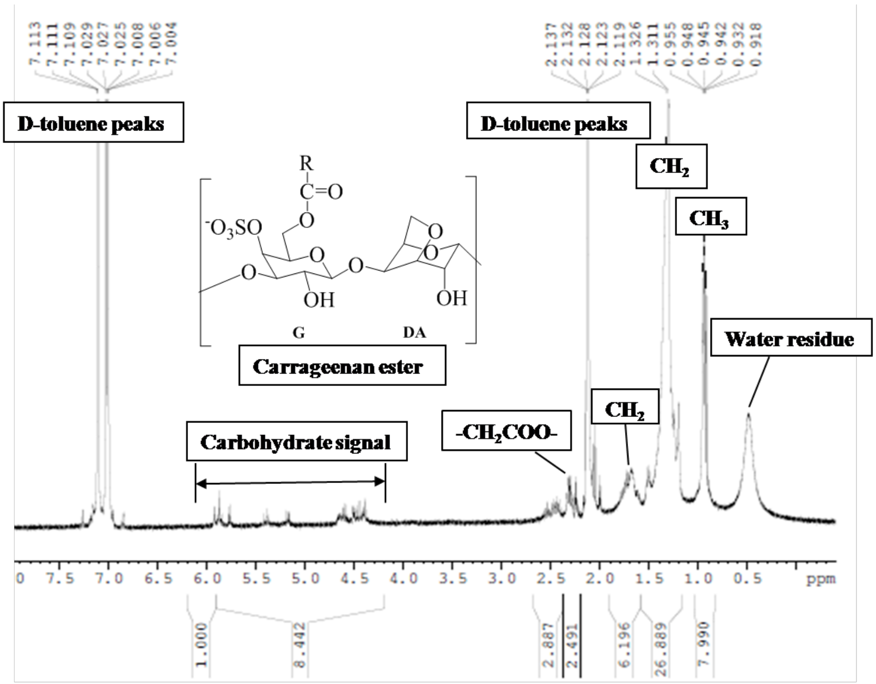

Figure 4: ^{1}H NMR spectrum of carrageenan ester synthesised at 80°C reaction temperature.

3.1.4 Thermal stability

Figure 5 shows the DTG curve of both κ-carrageenan and carrageenan ester samples synthesised at different reaction temperature from 60°C to 90°C (sample α to δ). For κ-carrageenan, we observed five decomposition steps which can be further divided into three main stages. Similar three degradation stages for κ-carrageenan were also observed in reference.[24]

In the first stage, we observed the initial weight loss of 13.96% (decomposition step 1) at about 90°C which may be due to the loss of moisture. The second degradation stage (decomposition step 2, 3 and 4) occurred in the range of 192°C to 350°C. In this stage, the maximum decomposition peak was observed at about 245°C (decomposition step 2), with 17.62% weight loss. This degradation stage may attributed to the loss of –OSO^{3-}group from the pendant chains attached to the polymeric backbone[25] and also may be due to the carbohydrate backbone fragmentation.[26]

In the final and third stage, we appreciated the decomposition step 5 at about 780°C (weight loss 17.35%), which may be due to the decomposition of inorganic salts present in the κ-carrageenan. These results are consistent with a previous report on the thermal behaviour of carrageenan.[24]

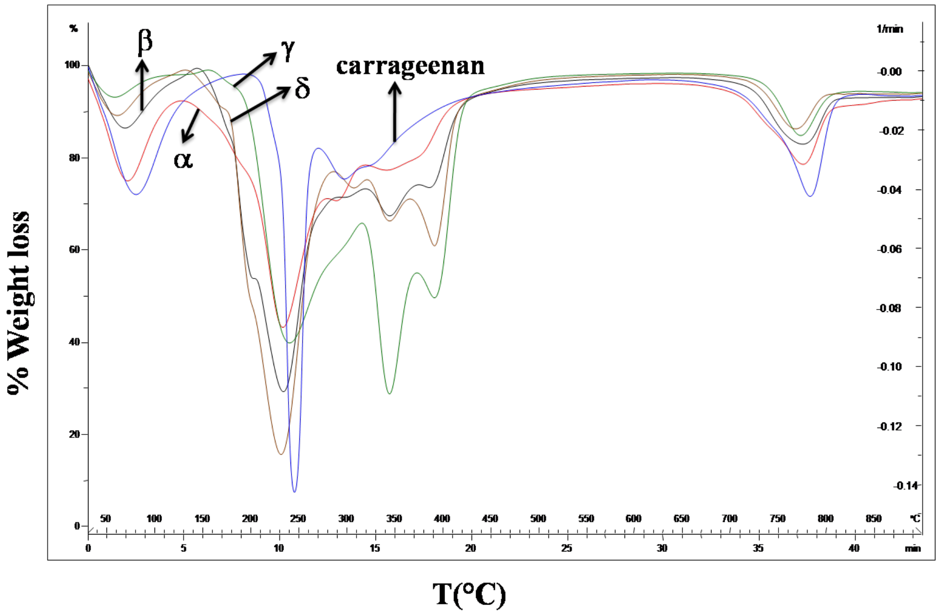

Figure 5: DTG thermogram of κ-carrageenan and carrageenan ester samples obtained from different synthesis batches (α–δ) performed at different reaction temperature (60°C to 90°C).

For all of the carrageenan ester samples (α, β, γ and δ in Figure 5), we also observed five decomposition steps and the whole degradation process can be divided into three main stages like κ-carraggeen. In the first stage, the initial weight loss of 10.6%, 5.3%, 3.8% and to 2.6% were identified at 80°C (decomposition step 1) for the samples synthesised at the reaction temperature of 60°C, 70°C, 80°C and 90°C, respectively. Such kind of weight loss may be attributed to the loss of loosely bounded water present in the carrageenan ester.

Moreover, it is important to note that for all samples, the percentage of weight loss was decreased with the increased of synthesis temperature. These results suggested that at this stage, the percentage of weight loss as well as the hygroscopic nature of all carrageenan ester samples is reduced compared to κ-carrageenan. Moreover, by comparing among the ester samples, we observed that the hygroscopic nature of ester sample decreased with increasing the synthesis temperature.

The second degradation stage (decomposition step 2, 3 and 4) was observed at the range of temperature between 126°C and 450°C. This stage was identified as the devolatilisation stage, where the main pyrolytic process occurred and various volatile components may release gradually.[24] At this stage, the maximum decomposition peak (decomposition step 2) was observed at 230°C and the percentage of weight loss was found to be 32.58%, 46.11%, 43.48% and 39.59% for the sample α, β, γ and δ, respectively.

In the third stage, we observed the last degradation step 5 at about 780°C. This step may be attributed to the fact that the residue of the previous pyrolytic process slowly decomposed to become loose porous residue. Similar pyrolytic process was also observed for marine algae.[27] Furthermore, the percentage of weight loss for the final decomposition step was found 17.88, 13.37, 8.13 and 9.47 for the samples synthesised at 60°C, 70°C, 80°C and 90°C reaction temperature, respectively (sample α–δ). These results imply that the percentage of weight loss became lower for all carrageenan ester samples compared to κ-carrageenan, although the sample α (reaction temperature 60°C) and κ-carrageenan experienced similar weight loss at this stage.

However, by comparing the weight loss among all the ester samples, we realised the reducing trend of percentage of weight loss with the increasing of reaction temperature, except the sample obtained at 80°C (sample γ), which experienced even lower weight loss than sample synthesised following 90°C (sample δ). Thus, the sample synthesised at 80°C is more thermally stable than all of the carrageenan ester samples including κ-carrageenan.

3.1.5 DSC study

The glass transition temperature (T_g) of κ-carrageenan and its carrageenan esters were obtained using DSC measurements. For κ-carrageenan, we performed both first and second heating scan, as shown in Figure 6. The first heating scan was carried out in order to understand the thermal history of the sample while the second heating scan provided the accurate T_g value. For the first heating scan, the T_g was 108.91°C with ΔH of 10.553 J g^{-1}. The ratio of T_m/T_g was found 1.74. These results are consistent with a previous report on semi crystalline polymer[28] which indicate the slightly crystalline nature of the κ-carrageenan sample.

However, for the second heating scan, the T_g was 160.80°C with $\Delta_{Cp} = 3.377 \times 10^{-2}$ J g^{-1} °C which is very similar to the results reported by Mitsuiki et al.[29] Thus, the T_g showed an increase value for second heating scan compared to the first one. The lower T_g value for the first heating scan may be due to the influence of the processing as well as the thermal history of the sample (i.e., presence of water). High T_g value in both heating scans, may be caused by the presence of cross-linking of spiral chains in κ-carrageenan that contain sulphate groups pointed outwards. We did not observe any endothermic peak at 0°C in the DSC thermogram [Figure 6 (a and b)]. This result indicates the absence of bulk water in the κ-carrageenan sample.[30]

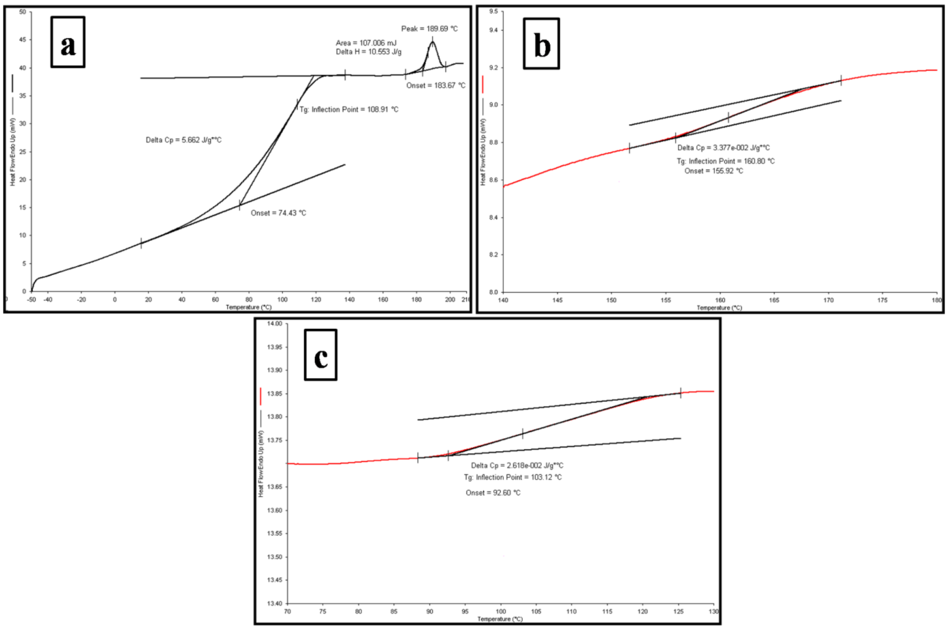

Figure 6: DSC heating scan of κ-carrageenan and carrageenan ester sample. Illustration (a) and (b) correspond to the first heating and second heating scan of κ-carrageenan, while (c) represents the second heating scan of carrageenan ester sample synthesized at 80°C reaction temperature.

We followed the second heating scan on the carrageenan ester samples [Figure 6(c)] in order to obtain accurate T_g value. The T_g was found 144.34°C, 146.19°C, 103.12°C and 83.15°C for the carrageenan ester samples synthesised at reaction temperature 60°C, 70°C, 80°C and 90°C, respectively. These results revealed that the T_g of the carrageenan ester samples are lower than the T_g of κ-carrageenan. We observed the decreasing trend of such value with the higher of synthesis temperature of the esterification process. However, the sample synthesised at 60°C (α) has slightly lower T_g value than the sample obtained at 70°C (β).

The lower T_g value for the sample obtained at 60°C (α) than the sample found at 70°C (β) can be attributed to the presence of large amount of water in the former sample which behaved as a plastiliser. Moreover, the addition of ester group to the κ-carrageenan polymer prevent the polymer chains from forming hydrogen bonds between the chain and thus increase the mobility of the chains and lower the T_g value of the carrageenan esters. Similar lowering of T_g values for polymeric materials was observed and explained by Gröndahl et al.[31] These results further support the observation that as the reaction temperature increases, the formation of ester become more efficient and thus produce higher yield.

4. CONCLUSION

Carrageenan esters have been synthesised from κ-carrageenan using decanoyl chloride and pyridine and by systematically varying the reaction temperature. The synthesised carrageenan esters were investigated by the FTIR, ^{1}H NMR, TGA and DSC measurements. Both FTIR and ^{1}H NMR measurements revealed the formation of carrageenan esters for all synthesis performed at different reaction temperature. It was found that the percentage of yield of carrageenan esters is increased, when the reaction temperature of the synthesis process increased.

TGA results demonstrated that the synthesised carrageenan esters are more thermally stable than κ-carrageenan. The more thermally stable carrageenan ester sample was obtained from the reaction at 80°C. The hygroscopic nature of all carrageenan esters is reduced compared to κ-carrageenan and decreases with increasing the synthesis temperature. DSC analysis showed that the T_g value of the carrageenan esters are lower compared to κ-carrageenan. The lowest T_g value (83.15°C) was possible to obtain for the carrageenan ester sample synthesised at 90°C.

All of the data represent new evidence that chemical modification of κ-carrageenan to its ester form is possible using decanoyl chloride and pyridine, and by systematically varying the reaction temperature of the synthesis process. Therefore, a new technique can be envisaged for the chemical modification of κ-carrageenan which may play important role in its industrial uses and applications.

5. ACKNOWLEDGEMENT

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