Purification on kappa carrageenan by re-precipitation technique

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Abstract Purification on kappa (ƙ) carrageenan was performed by dissolving ƙ-carrageenan powder in distilled water and re-precipitated in a polar solvent (ethanol) and non-polar solvent (n-hexane) separately during re-precipitation process. The purified kappa carrageenan was analyzed by using Fourier transform infrared (FTIR) spectroscopy and carbon, hydrogen, nitrogen, and sulfur (CHNS) elemental analysis. The outcomes from FTIR showed there are physical changes due to intermolecular interactions which lead to decrease and lower intensity of hydroxyl band at 3345 cm^{-1} after re-precipitated with n-hexane, compared to re-precipitation with ethanol and unpurified ƙ-carrageenan. There were variations observed in the percentages of C, H and S in the CHNS analysis between unpurified and purified ƙ-carrageenan. The successfully purified ƙ-carrageenan are suitable to be used for further application.

Introduction

Carrageenan is a linear sulfated polysaccharide, extracted from red seaweed (*Eucheuma cottonii*) [1]. It is formed by alternate units of d-galactose and 3,6-anhydrogalactose joined by α-1,3 and β-1,4-glycosidic linkages [2]. Carrageenan is a natural biopolymer that are abundantly available in the nature. There has been a lot of interests on biopolymer as they are also sustainable, cost-effective and biocompatible. Hence, apart of commonly used in the food industry as gelling, thickening and stabilizing agents, carrageenans are also used in pharmaceutical, medicine, cosmetics, and industrial applications [3-7] .

There are three basic types of carrageenan, commonly referred to as kappa (ƙ), iota (ι) and lambda (λ) carrageenan. The difference of these carrageenan is the number of sulfate group available per disaccharide, where ƙ-carrageenan has one sulfate group, ι-carrageenan has two, and λ-carrageenan has three [8]. Sulfate groups are known to affect the rheological of carrageenan properties such as viscosity [9]. reported that ƙ-carrageenan produces strong brittle thermo-reversible gels, while ι-carrageenan gives soft elastic thermo-reversible gels and λ-carrageenan is non-gelling [8]. Thus, these showed that carrageenan (ƙ, ι and λ) differ in chemical and physical properties although their chemical structures are almost similar.

Purification of polymer is important in order to reduce the concentration of impurities such as unwanted residue and removal of any insoluble that are not desired [10]. In early study, Jorda and co-workers investigated the ultrafiltration technique for the purification of diluted biopolymer solutions

[11]. Other study reported that carrageenan was purified by centrifugation of aqueous suspensions in order to remove insoluble part, followed by dialysis against 0.1 M NaCl and subsequently against Milli-Q water for 1 day in order to remove excess salt. Finally, the purified carrageenan solution was freeze-dried and stored at room temperature for further analysis [12]. Study on the purification of ι-carrageenan was recently reported. The purification process was done by dissolving ι-carrageenan powder in water. The solution was then filtered and rinsed with ethanol. To end, the carrageenan powder was left to dry at room temperature in a vacuum desiccator [13].

This paper describes the purification of ƙ-carrageenan and the effects of the purification, characterized by ATR-FTIR spectroscopy and CHNS elemental analyzer. Purification of carrageenan is important in order to reduce any impurities in the unpurified carrageenan before further use. Although the purification of ι-carrageenan was previously reported [13], the physical and chemical properties of ƙ-carrageenan and ι-carrageenan are different, thus suggesting dissimilar findings. Additionally, the purification was performed in a diverse manner and the re-precipitation technique was carried out with polar solvent: ethanol and non-polar solvent: n-hexane, in order to observe the significant changes.

Methodology

Chemicals and materials ƙ-carrageenan (molecular weight: 788.7 g/mol) powder was purchased from Sigma Aldrich, Malaysia. Ethanol was used was from HmbG Chemicals (purity: >95%) while n-Hexane was provided by Merck, Malaysia (purity: ≥98.5%). All chemicals were used as received.

Purification of κ-carrageenan Purification of ƙ-carrageenan was done by dissolving the ƙ-carrageenan powder in distilled water to make 1 w/w % concentration. The solution was left to stir for 48 h at 50°C until clear solution discovered. The solvent was extracted out by rotary evaporator until jelly-like solution observed. The solution undergoes re-precipitation with ethanol and n-hexane separately by ratio of 1:5. After the complete re-precipitation process, the solid gel κ-carrageenan was poured into petri dish and dried in the vacuum oven at 60°C until thin film obtained.

Characterization

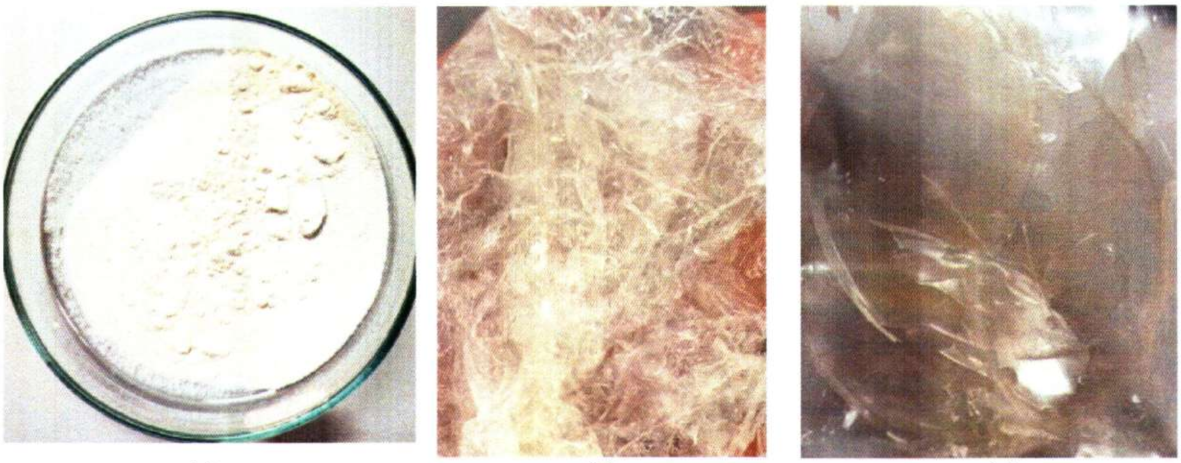
Elemental analyses were done at the Chemical Defence Center (CHEMDEF) of Universiti Pertahanan Nasional Malaysia (UPNM), Malaysia.

Fourier transform infrared (FTIR) The analysis was performed by using Perkin- Elmer Spectrum 2000, USA in the range of 4000-650 cm^{-1} with scanning resolution 4 cm^{-1}. The analysis was completed in order to study the shifting and changes on the spectra of the ƙ-carrageenan film electrolyte.

Elemental analysis The content of carbon, hydrogen, nitrogen and sulfur for purified and unpurified ƙ-carrageenan was analyzed by using Vario el III, ELEMTAR, Hanau, Germany. From this analysis the percentage of the elements before and after purification can be determined.

Results and discussion

Physical observation



(a) (b) (c)

Fig. 1 Physical observation of k-carrageenan (a) before purification and (b) & (c) after purification with ethanol and n-hexane, respectively.

Fig. 1 shows the differences in the physical state between unpurified ƙ-carrageenan with purified ƙ-carrageenan. The purified ƙ-carrageenan was re-precipitated with ethanol and n-hexane respectively. The outcome from the re-precipitation process shows significant changes between those two and discuss further in ATR-FTIR analysis part. The powdery unpurified ƙ-carrageenan is white in color and has a very fine and soft texture. After purification with water, the texture has changed and formed films. The film obtained in Fig. 1(b) is the purified ƙ-carrageenan re-precipitated with ethanol. The film is plastic-like and still in gel form at the end part of the film. It is slightly opaque and elastic. In contrast, purified ƙ-carrageenan re-precipitated with n-hexane in Fig. 1(c) produced thin, more transparent, strong but slightly brittle film. However, there are no notable differential in color for both films. Thin films (b) and (c) undergoes the same procedure and temperature from beginning until drying process. Hence, it can be concluded that re-precipitation using n-hexane has produced a better free standing film compared to ethanol.

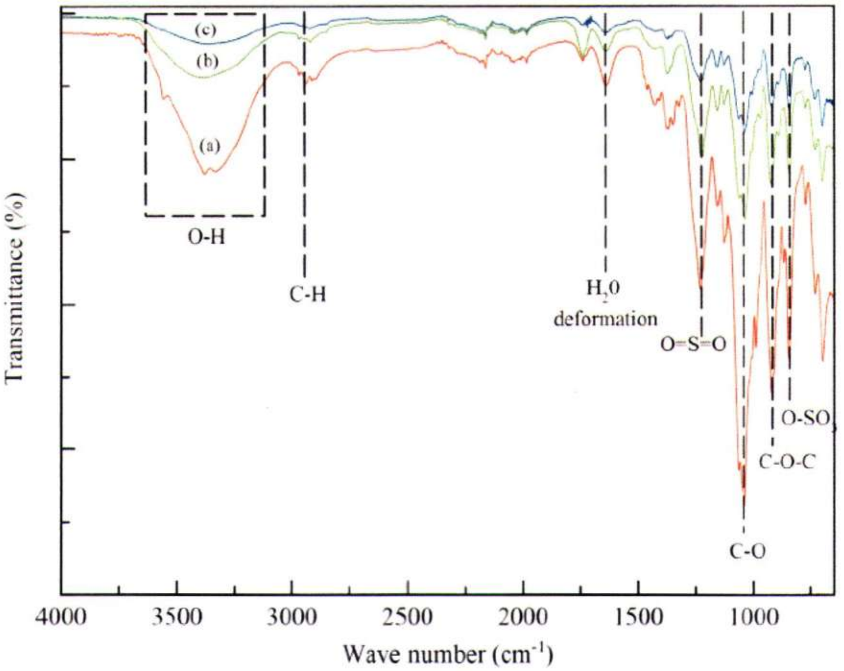


Fig. 2 FTIR spectrum of (a) ƙ-carrageenan, (b) re-precipitated with ethanol and (c) re-precipitated with n-hexane in the region of 4000-650 cm

FTIR spectra of unpurified ƙ-carrageenan and purified ƙ-carrageenan are shown in Fig. 2 with wavenumber region 4000-650 cm^{-1} that shows the overall spectra of (a) unpurified ƙ-carrageenan, (b) purified ƙ-carrageenan precipitated with ethanol and (c) purified ƙ-carrageenan precipitated with n-hexane. Basic bands of ƙ-carrageenan in the FTIR spectroscopy are shown. The attributed bands are C-O-C vibration of 3,6-anhydro-D-galactose at 921 cm^{-1}, ester sulfate symmetric vibration O=S=O at 1231 cm^{-1}, stretching vibration of –O-SO_3 at D-galactose-4-sulfate (G4S) clearly at 844 cm^{-1}. The strong band at 1638 cm^{-1} was correlated with the deformation of the structural water band [15–17]. There are also bands approximately at 1037 cm^{-1}, 2941 cm^{-1}, and 3384 cm^{-1} were reported to be C–O stretch, C–H stretch and O–H stretch, respectively [13-15].

Meanwhile, both purified ƙ-carrageenan re-precipitated with ethanol and n-hexane spectra show significant changes in the wavenumber that can be observed in these figures. FTIR spectra of purified ƙ-carrageenan re-precipitated with ethanol shows same bands as untreated ƙ-carrageenan with shifted wavenumber. There are C-O-C vibration of 3,6-anhydro-D-galactose at 919 cm^{-1}, ester sulfate symmetric vibration O=S=O at 1228 cm^{-1}, stretching vibration of –O-SO_3 at D-galactose-4-sulfate (G4S) clearly at 842 cm^{-1}, C-O stretch at 1037 cm^{-1}, C-H stretch at 2918 cm^{-1} as well as O-H and water deformation stretch at 3377 cm^{-1}, 1635 cm^{-1} respectively [13, 14]. In the meantime, purified ƙ-carrageenan re-precipitated with n-hexane also shows significant changes in the FTIR as shown in the figures. There are C-O-C vibration of 3,6-anhydro-D-galactose at 917 cm^{-1}, ester sulfate symmetric vibration O=S=O at 1228 cm^{-1}, stretching vibration of –O-SO_3 at D-galactose-4-sulfate (G4S) clearly at 842 cm^{-1}, C-O stretch at 1037 cm^{-1}, C-H stretch at 2916 cm^{-1} as well as O-H and water deformation stretch at 3345 cm^{-1}, 1637 cm^{-1} respectively [12, 14]. The shifted wavenumbers of the re-precipitated ƙ-carrageenan with both ethanol and n-hexane in all the regions in the showed that there are strong intermolecular interaction between the solvent and ƙ-carrageenan matrix [16]. The intermolecular interaction such as hydrogen bonding interactions might have formed between polar groups in ƙ-carrageenan and the polar groups or hydrogen atoms in the solvent. Table 1 shows the summarized shifts and assignment of the bands in untreated ƙ-carrageenan and re-precipitation of ƙ-carrageenan with ethanol and n-hexane.

Noticeably, there are changes in the intensities of the peaks, especially for O-H stretching, O=S=O and C-O symmetric vibration. The peaks have become smoother and the intensities of the peaks decreases when re-precipitated with ethanol (Fig. 2(b)) and n-hexane (Fig. 2(c)) compared to the untreated ƙ-carrageenan (Fig. 2(a)), thus suggesting that the purification technique was successfully performed [17]. The reduced intensities might have related to the elimination of impurities, such as unknown residue that was attached to ƙ-carrageenan matrix [17]. However, comparing the re-precipitated peaks with ethanol and n-hexane in Fig. 2(b) and 2(c) respectively, re-precipitation with n-hexane showed reduced intensities, hence suggesting n-hexane as a better solvent. This result is possibly due to the non-polar properties of n-hexane as opposed to ethanol, a polar solvent. Re-precipitation of a polar compound needs to be performed with non-polar solvent. In this case, ƙ-carrageenan is a highly polar biopolymer due to the presence of hydrophilic hydroxyl groups in its chemical structure [16]. Thus, in order to remove the impurities in ƙ-carrageenan, non-polar solvent is required [18]. By adding the biopolymer solution into a non-polar solvent (n-hexene) in the purification steps, it is suggested that only the polar part of the system is forced to reprecipitated due to entropy instability in the system. Therefore, re-precipitation with n-hexane has resulted in reduced intensities in the peaks compared to ethanol due to the elimination of more impurities in ƙ-carrageenan.

Table 1
The assigned bands and wavelength of untreated and purified ƙ-carrageenan with different re-precipitation solvents.

Assignments of bands	Wavenumber of ƙ-carrageenan (cm^{-1})				
	Unpurified	Re-precipitated with ethanol	Re-precipitated with n-hexane	Reference	
O-H	3384	3377	3345	3411	[13-16]
C-H	2941	2918	2916	2933	
C-O	1037	1037	1037	1070	
H_2O deformation	1638	1635	1637	1640	
O=S=O	1231	1228	1228	1250	
C-O-C	921	919	917	928	
-O-SO_3	844	842	842	846	

Elemental analysis Elemental analysis of the unpurified ƙ-carrageenan and purified ƙ-carrageenan re-precipitated with ethanol and n-hexane was conducted to provide the actual composition of carbon, hydrogen, nitrogen and sulfur. There were variations observed between unpurified and purified ƙ-carrageenan as presented in Table 2. Interestingly, the percentage of carbon (C) in unpurified ƙ-carrageenan was 28.03 wt.% changed to 26.27 wt.% and 28.77 wt.% after purified and re-precipitated with ethanol and n-hexane respectively. The reduction in C percentage after re-precipitated with ethanol might be due to the elimination of other polar impurities in ethanol, as ethanol is a polar solvent. There was slight increment in the C percentage after re-precipitated with n-hexane indicating no elimination of non-polar impurities in the polymer [19]. Hydrogen content were reduced in both re-precipitated with ethanol and n-hexane methods, indicating removal of some impurities in ƙ-carrageenan. Nitrogen content in ƙ-carrageenan can be neglected as the percentage is almost zero. Slight increments were also observed in the sulfur percentage indicating the purification process does not alter the percentage of sulfur present in ƙ-carrageenan. Overall, the purification process did not alter the chemical composition in ƙ-carrageenan. Table 2 summarized the carbon, hydrogen, nitrogen and sulfur elements wt. (%).

Table 2
The assigned elements and percentage for untreated ƙ–carrageenan and purified ƙ-carrageenan with different re-precipitation solvents.

Element wt (%)	ƙ-carrageenan	Re-precipitated with ethanol	Re-precipitated with n-hexane
Carbon	28.030	26.270	28.770
Hydrogen	5.340	5.122	5.123
Nitrogen	0.030	0.030	0.020
Sulfur	4.563	4.833	4.756

Conclusion

Purification of ƙ-carrageenan has been successfully performed by re-precipitation technique with n-hexane as a better re-precipitation solvent. The physical appearance of the film has changed from powder to transparent thin film prior to purification. FTIR results showed that re-precipitation of ƙ-carrageenan with n-hexane has resulted in reduced intensities in the peaks compared to ethanol due to the elimination of more impurities in ƙ-carrageenan. CHNS analysis revealed that the purification process did not alter the chemical composition in ƙ-carrageenan.

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