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Biopolymers from the Mauritian Marine Environment

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Summary: To the best of our knowledge, the extraction of biopolymers from algae and seaweeds still remains untapped. Prior studies in this area have been limited to a taxonomic survey of algae and seaweeds found around our coastal regions. In this paper, we report on the extraction of biopolymers from *Hypnea*, *Eucheuma* and *Gracilaria* species collected around the coastal regions of Mauritius. Various extraction conditions were used and their effects on yield and structure of the corresponding biopolymers were investigated. The extracted polysaccharides were characterized by a combination of IR, NMR, SEC, viscometry and elemental analyses. These revealed that polysaccharide extracted from *Gracilaria* is a highly methylated agar and *Hypnea/Eucheuma* contain κ-carrageenan.

Keywords: agar; agarose; algae; biopolymers; carrageenans; seaweeds

Introduction

Seaweeds are classified into three major groups: green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) according to their pigments and coloration. This paper will focus on phycocolloids, that is, polysaccharides of high molar masses obtained from red algae. Phycocolloids are variously substituted galactans which form the dominant constituent of the matrix phase of algal cell wall. Carrageenophytes and agarophytes (Table 1) produce two phycocolloids namely: carrageenans and agar. These two polysaccharides find numerous commercial applications in the food, cosmetics and pharmaceutical industries. The carrageenophytes and agarophytes are found in either tropical and warm waters, e.g. *Eucheuma* and *Hypnea* or in colder waters, e.g. C*hrondus* and *Furcellaria* species. In the year 2001, the carrageenan production from different regions via different precipitating methods amounted to 42 930 tons and the total market has a value of about US$ 300 million.[1]

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Seaweeds and algae are abundantly available on the Mauritian beaches, particularly on the eastern coast. This proliferation constitutes a major environmental problem and causes a lot of inconveniences to the public and hotels in that region. At present, the seaweeds are treated as waste and disposed off by incineration, which involves additional costs. Red algae (*Solieriaceae* family namely Rhodophyta) and seaweeds are major sources of biopolymers such as carrageenans and agarose, which find numerous specialty or high added-value applications in food industry, pharmaceuticals and cosmetics formulations. From an economical point of view, the exploitation of these naturally occurring resources via extraction of their biopolymers can prove to be most beneficial to Mauritius rather than being considered as trash.

Carrageenans

Carrageenan is a natural biopolymer extracted from carrageenophytes. It is a linear sulfated polysaccharide composed of D-galactose and 3,6-anhydro D-galactose units depending on the source and extraction conditions. Carrageenans contain 3,6-anhydro-bridges on the 4-linked galactose residues and may possess varying number of sulphate groups. Commercial carragee-

Table 1.
Red seaweed species classified as carrageenophytes and agarophytes.

Carrageenophytes	Agarophytes
Chondrus Crispus	*Ahnfeltia*
Eucheuma	*Gelidium*
Furcellaria	*Gracilaria*
Gigartina	*Porphyra*
Hypnea	*Pterocladia*
Phyllophora	

nans have a sulphate content within the range 22 to 38% (w/w) and their weight average molar mass (M_w) vary from 400 000 to 600 000. Besides galactose and sulphate, other carbohydrate residues such as xylose, glucose and uronic acids and substituents such as methyl ethers and pyruvate groups can be present in carrageenans. The three commercially most important carrageenans are iota (ι), kappa (κ) and lambda (λ) carrageenans (Figure 1). In the nomenclature proposed by Knutsen *et al.*[2] the letter **G** refers to the 3-linked β-D-galactopyranose, **D** to the 4-linked α-D-galactopyranose, **DA** to the 4-linked 3,6-anhydro-α-D-galactopyranose and **S** to the sulphate ester. The number preceeding the substituent **S** designates the carbon number on the sugar wherein the substituent is positioned.

Agar

Among the agarophytes known, *Gracilaria* is the most abundant resource of agar production. It has more than 150 species and is distributed mainly in the temperate and subtropical zones. America and Asia-Pacific countries are the major producers of agar but Africa's share is not negligible amounting to 14%.

Araki[3] showed that agar consists of a mixture of agarose and agaropectin. Agarose is a linear and neutral polymer with a molar mass of about 120 000. It is composed of agarobiose repeating disaccharide units with alternating 1,3-linked-β-D-galactopyranose and 1,4-linked-3,6-anhydro-α-L-galactopyranose (Figure 2). Agaropectin is an acidic polymer and has a backbone similar to that of agarose. It is a heterogeneous mixture of smaller molecules present in lesser amounts. Its structure is slightly branched and sulfated, and may contain methyl and pyruvic acid ketal substituents. Recent fractionation studies by Yaphe *et al.*[4] on DEAE-Sephadex A-50 column indicated that agar is not made up of only one neutral and one charged polysaccharide but is composed of a complex series of related polysaccharides ranging from a virtually neutral molecule to a highly charged (sulfated) galactan. The neutral polysaccharide has gelling ability and approaches the structure of an ideal agarose but still contains a trace of sulfate (0.1–0.5%) and pyruvic acid (0.02%).[3]

In this paper, we report on the extraction of carrageenans and agar from wild seaweed species collected from the coasts of Mauritius[5–10]. The extraction parameters and characterization of the phycocolloids by NMR, IR and SEC-LS-Viscometry analysis will be discussed.

Experimental

Materials

Commercial carrageenans were used as received from Carrageenan Company (USA). Food grade agar samples from

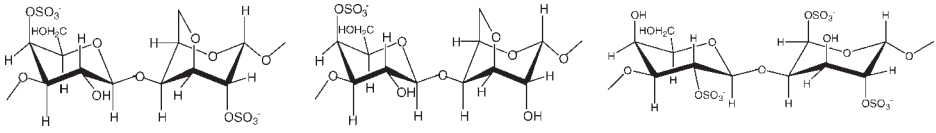

Figure 1.
Schematic representations of the different structures of the repeating dimeric units of (A) ι- (B) κ- (C) λ-carrageenans.

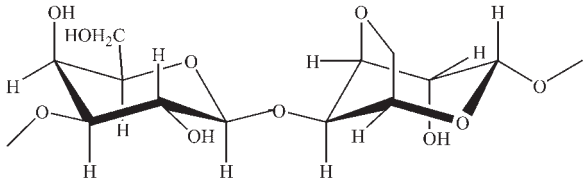

Figure 2.
Structure of an agarose repeat unit.

India, China and Japan available on the local market were used. All other reagents were used as received.

Extraction and Treatment of The Polysaccharides

The seaweeds were washed thoroughly with fresh water to remove sand and adhering corals or shells. The washed seaweeds were cut into small pieces and spread out uniformly over a metallic net with small pores. They were sprayed with fresh water every two hours to enable bleaching and sun dried for 2 to 3 days. The seaweeds were further dried in an oven (80 °C) for two hours to destroy microorganisms thus preventing decomposition of the seaweeds.

Carrageenan extraction

Alkali pretreatment. The seaweeds (5 g) were added to an alkaline solution (1–5% of NaOH or KOH) in a 250 mL conical flask. The mixture was heated at 70–100 °C for 15 minutes. A second method was used in which the alkali pretreatment was performed using 0.1 M NaOH for 1.5 h at room temperature. The treated seaweeds were collected by filtration using nylon mesh, washed thoroughly with water and neutralised with 1 M acetic acid.

Hot Water Extraction at pH 9. Extraction was performed on the alkali pretreated seaweeds (5 g) at pH 9 in 400 ml of H_2O at 85 °C for 1 h. The mixture was hot filtered using nylon mesh (small pores) and the filtrate allowed to cool.

Extraction at different NaOH concentrations. Extraction was performed on the alkali pretreated seaweeds (5 g) using different NaOH concentrations (400 ml, 0.5%, 1.0% and 2.0%) at 100 °C for 2 h.

Precipitation

Method A. As the filtrate cools down and is allowed to stand at room temperature, a gel is formed. It is broken into small pieces, washed thoroughly and pressed to remove impurities (digested materials and colour) and water. The gel is finally lyophilized.

Method B. Potassium chloride (2%) is added slowly to the filtrate and allowed to stand for ten minutes. A gel is formed which is further washed with the salt solution to remove more water, pressed to remove excess liquid and then frozen. During thawing, separation of water occurs by syneresis, the pieces are washed with more potassium chloride solution, chopped up and lyophilized.

Method C. Ethanol or isopropanol is added slowly to the filtrate with continuous stirring until the product precipitates off. It is then separated using a screen (a fine sieve), pressed to remove solvent and washed with more alcohol to dehydrate it further before being lyophilized.

Agar extraction

Alkali pretreatment. The seaweeds (5 g) were treated with NaOH (10–30%) for 1 h at varying temperatures (70–100 °C). The treated seaweeds were collected by filtration using nylon mesh and washed thoroughly till neutral.

Hot Water Extraction. The alkali treated seaweeds (5 g) were boiled in water at 100 °C for 1–4 h.

Filtration and gelation. The hot liquor was filtered through a nylon mesh and the filtrate was allowed to gel at room temperature.

Freeze-thawing. The gel was freezed overnight and allowed to thaw. On melting, the agar seperates as a fibrous material suspended in water solution. The water was decanted and the fibrous product washed before freezing. The freezed product was lyophilized.

Characterization

Nuclear magnetic resonance

^{1}H and ^{13}C NMR spectra were recorded in D_2O/DMSO at room temperature (25 °C) on a FT Bruker Spectrometer 250 MHz at the University of Mauritius. For ^{1}H NMR, 10.0 mg samples of carrageenan were dissolved in 1.0 ml D_2O/DMSO at 90 °C whereas for ^{13}C NMR 20.0 mg sample was used. These solutions were sonificated using a sonicrep 150 apparatus at 10 Amplitude microns for 15 minutes prior to analysis.

FT-IR spectroscopic analysis

IR spectra were recorded at room temperature on an AVATAR 320 Fourier transform-Infrared spectrophotometer at the University of Mauritius.

Molar Mass Determinations

SEC-LS-Viscometry was performed at PSS GmbH, Germany, using an Agilent equipment, 0.1.M $LiNO_3$ as eluent, $3 \times$ PSS Suprema 10 μm linear XL (8×300 mm ea.) as column, RI concentration detector, PSS SLD7000 light scattering detector and PSS eta1001 viscometry detector.

Sulphur content

The sulphur content was obtained from a LECO CHNS-932 analyzer at the University of Mauritius.

Viscometry

A ubbelohde tube of type A was used with polymer concentrations varying in the range of 0.01 to 0.06 g/dL for carrageenan and 0.01 to 0.25 g/dL for agar at a temperature of 25 °C.

Results and Discussion

Three red seaweeds species, *Hypnea*, *Eucheuma* and *Gracilaria* were collected from various locations around Mauritius over the period July to November. *Hypnea* and *Eucheuma* are sources of carrageenans, whereas *Gracilaria* is an agar-containing seaweed.

Extraction and Isolation

Extraction was performed using protocols detailed in the previous section. The carrageenans originating from both *Hypnea* and *Eucheuma* were cream-coloured and fibrous. They were partially soluble in cold water and soluble in hot water. The experimental protocols used for the extraction varies according to the species.

Method A is applicable to κ-carrageenan which is the only one forming strong gel without addition of cations. Some products are lost through washing but the purification method is very easy and no additional precipitating agent is required.

Method B is also applicable to κ-carrageenan. The latter forms strong gel in presence of KCl (2.0%) which prevents the precipitation of other types of carrageenan, for instance lambda. ι-Carrageenan also forms a gel in presence of K^+ ions. However the gel formed is elastic and do not show any syneresis behaviour. Moreover the gel shows thixotropic behaviour, which renders the filtration difficult. The great advantage of this method is that water is lost via the freeze-thaw process. κ-carrageenan is the most hydrophobic carrageenan and thus water is readily squeezed out.

Method C makes use of alcohols which provide an excellent method for obtaining pure products as they do not precipitate other molecules such as sucrose. But the use of alcohol adds on the cost of extraction.

The yield varies in the range 40–45%.

The products extracted from *Gracilaria* were fibrous and off-white in colour. They were soluble in DMSO at room

temperature and in hot water. Various parameters such as pH, solvent and their effects on yield, structure and quality of the extracted biopolymers were investigated. The best yields (20%) of agar appears to result from seaweeds collected during winter season (July-August) which is in agreement with the fact that factors like carbon dioxide concentration, salinity, temperature of seawater, solar intensity and life cycle stage of seaweeds have an impact on their growth, which in turn affect the yield of biopolymer[11].

Characterization

The products extracted from *Eucheuma* (Eu), *Hypnea* (Hp) and *Gracilaria* (Gc) were characterized by elemental analysis, FT-IR, ^{1}H and ^{13}C NMR, SEC and viscometric analyses. The results were compared with commercially available κ-, ι-, λ-carrageenans obtained from Carrageenan Company (USA), agarose and commercial agar. The average molar masses (M_n) were determined by SEC and their pseudoplastic behaviors were observed by viscometric analysis. The sulphur content was determined by elemental analysis.

FT-IR Spectroscopic Analysis

Carrageenans

FT-IR analysis of crude seaweed powder (*Eucheuma* and *Hypnea*) was carried out and the spectra compared with those of commercially available carrageenans (Figure 3). The IR spectrum of κ-Carrageenan showed one band at 849 cm^{-1} which has been assigned to one axial $–O\text{-}SO_3^-$ substituent at C-4 of the 3-linked β-D-galactopyranosyl unit. The spectrum of ι-carrageenan contained one additional band at 806 cm^{-1} which has been assigned to a second axial $–O\text{-}SO_3^-$ substituent at C-2 of the 3,6-anhydro-α-D-galactopyranosyl unit. The spectra of the collected seaweeds were similar to that of κ-carrageenan with the presence of only one band at 855 cm^{-1}. The IR spectra of the biopolymers (Figure 3) extracted from *Eucheuma* and *Hypnea* were similar to the spectra of commercial κ-carrageenan and crude seaweeds powder with the presence of one band at 848 cm^{-1}. Table 2 lists the wavenumbers of the main functional groups of three carrageenans as reported in literature and of extracted biopolymers. A band at 1640 cm^{-1} present in all the spectra has been assigned to O–H bending of H_2O. The intense signal at around 930 cm^{-1} suggests the presence of **DA** in high amounts.

Agar

The infrared spectra of extracted biopolymer, commercial agar and agarose were recorded and compared (Figure 4). The three spectra showed the presence of similar bands. Bands at around 3395 cm^{-1} (OH, axial deformation), 2900 cm^{-1} (CH, axial deformation), 1640 cm^{-1} (OH, bending of H_2O), 1160 cm^{-1} (C–O axial deformation), 1070 cm^{-1} (glycosidic link), 930 cm^{-1} (characteristics of 3,6-anhydrogalactose) and 890 cm^{-1} (CH, angular deformation of β-anomeric carbon) are characteristic of agarose. It can also be noted that no peaks are present in the carbonyl region indicating probable absence of pyruvic acid, a common constituent of agar.

NMR Characterization

Carrageenans

The chemical shift values of α-anomeric proton of the three main types of carrageenans are listed in Table 3.[12] The α-anomeric proton occurs slightly more downfield than the β-anomeric proton due to the field effect of the oxygen on the anhydro group and sulphate group. ^{1}H and ^{13}C NMR analyses of carrageenans are usually reported at temperatures higher than room temperature.[13–15]

The spectra of extracted products and commercial carrageenans spectra were recorded in a mixture of D_2O and DMSO at room temperature. The α-anomeric proton for the commercial κ-carrageenan is seen at 5.06 ppm. Similar chemical shift values are obtained for biopolymers extracted from Hypnea (5.06 ppm, Figure 5) and Eucheuma (5.12 ppm) whereas the

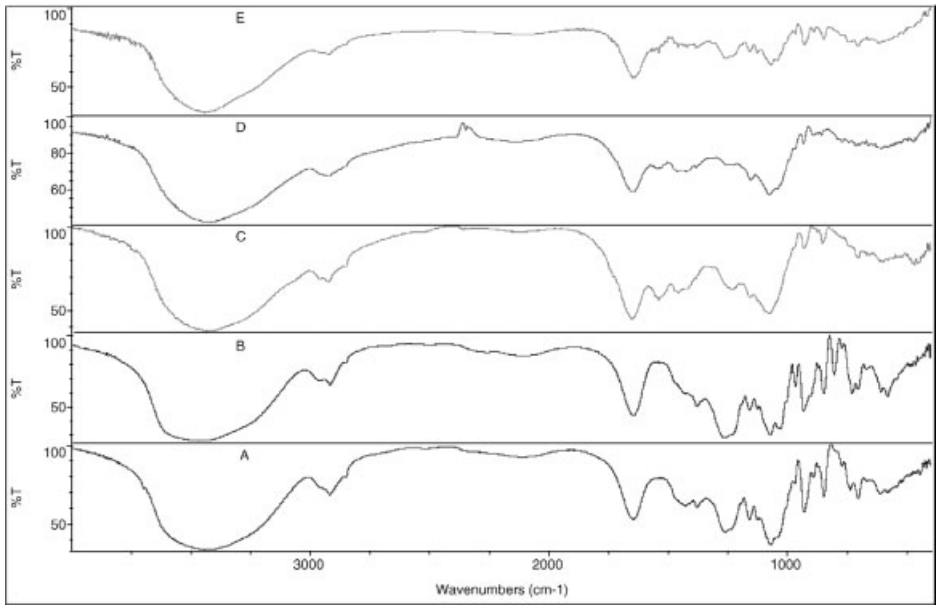

Figure 3.
FT-IR spectra of (A) commercial κ-carrageenan (B) commercial ι-carrageenan (C) *Hypnea* powder (D) *Eucheuma* powder (E) extracted biopolymer.

α-anomeric proton of commercial ι-carrageenan resonates at 5.3 ppm. No change in chemical shift values was observed upon changing extraction parameters. In addition the absence of a peak at 5.2 ppm indicated that μ- (**G4S, D6S**) the precursor of κ-carrageenan was not present in the native carrageenan.

A typical ^{13}C NMR spectrum of an extracted product is shown in Figure 6 and the corresponding chemical shifts are listed in Table 4. The C-4 of **G4S** unit appears more downfield than a normal C(OH due to the presence of the electron-attracting sulphate group. From Table 4, the α-anomeric carbon is found in the same region as that of commercial κ-carrageenan. The signals at 74.3 and 74.7 ppm are assigned to the C-4 of the **G4S** unit. The higher chemical shift confirmed that the G4S is connected to **DA** instead of **DA2S**. DEPT ^{13}C NMR confirmed that the peak at 62.5 ppm is a $-CH_2OH$. No peak was detected at 97.3 ppm (α-anomeric carbon of **G4SD6S**) indicating the absence of μ-carrageenan, precursor of κ-carrageenan.

Table 2.
Wavenumber of functional groups present in commercial carrageenans and extracted biopolymers.

Wavenumber cm^{-1}	Functional groups	Kappa	Iota	Lamda	Eu 1	Eu 2	Hp 1	Hp 2
1210–1260	Ester Sulphate	vs	vs	vs	vs	vs	vs	vs
1010–1080	Glycosidic link	vs	vs	vs	vs	vs	vs	vs
928–933	3,6-anhydro-D-galactose	s	s	a-l	s	s	s	s
840–850	D-galactose-4-Sulphate	m	m	a	m	m	m	m
820–830	D-galactose-2-Sulphate	a	a	m	a	a	a	a
810–820	D-galactose-6-Sulphate	a	a	m	a	a	a	a
800–805	3,6-anhydro-D-galactose-2-Sulphate	a-l	m	a	a-l	a-l	a-l	a-l

vs: very strong; s: strong; m: medium; l: low; a: absent

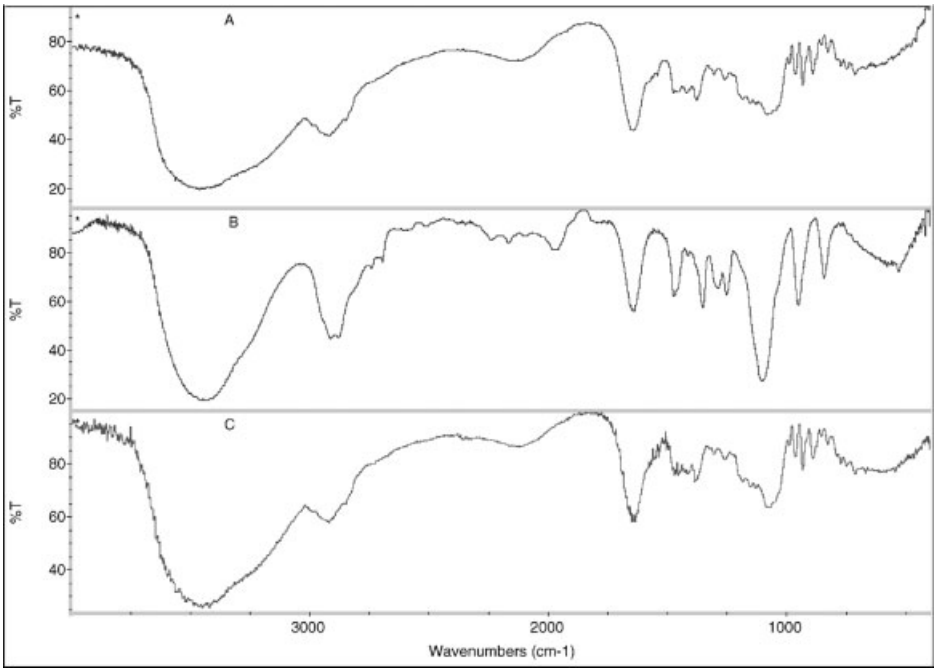

Figure 4.
FT-IR spectra of (A) extracted biopolymer (B) agarose and (C) commercial agar.

Agar

^{1}H-NMR spectra were recorded for agarose, commercial agar and the extracted biopolymer in DMSO. In an agarose repeat unit, the protons on C_2-C_6 for the GLA diad have almost similar chemical environment as they are all linked to an oxygen atom. Hence, it is quite difficult to assign the individual peaks. The anomeric protons appear at 5.1 and 5.3 ppm respectively[17]. Similar results were obtained for the standards and extracted biopolymer (Figure 7). An additional signal is observed for extracted agar samples at 1.3 ppm. It was attributed to $OC\underline{H}_3$, which is expected to be present in the precursor of agarose (agarobiose).[11] Pure agarose does not exist in nature as methylated, pyruvated and sulfated galactoses were also proved to be constituents of the agar molecule. Pyruvic acid is also known to form a cyclic acetal at positions 4 and 6 of the 3-linked galactose residues and can be detected in the ^{1}H-NMR spectrum by a peak at 1.45 ppm. The absence of this peak for the extracted biopolymers suggests the absence of pyruvic acid substituent. ^{1}H-NMR confirms that the extracted products have a similar backbone to that of agarose.

^{13}C-NMR spectra of agarose, commercial agar and the extracted biopolymer (both the crude and pure extract) were recorded in DMSO. Typical spectra of one commercial sample and an extracted sample are shown in Figure 8 and the peak assignments are listed in Table 5. The ^{13}C-NMR spectrum recorded for agarose is quite similar to that of extracted and commercial agar except for the presence

Table 3.
Chemical shift* values of α-anomeric protons of carrageenans.

Carrageenan	α-unit	Chemical shift (δ ppm)
λ	D2S,6S	5.52
ι	DA,2S	5.32
κ	DA	5.11

* The chemical shift values were recorded in a 400 MHZ NMR at 50–60 °C using D_2O as solvent.

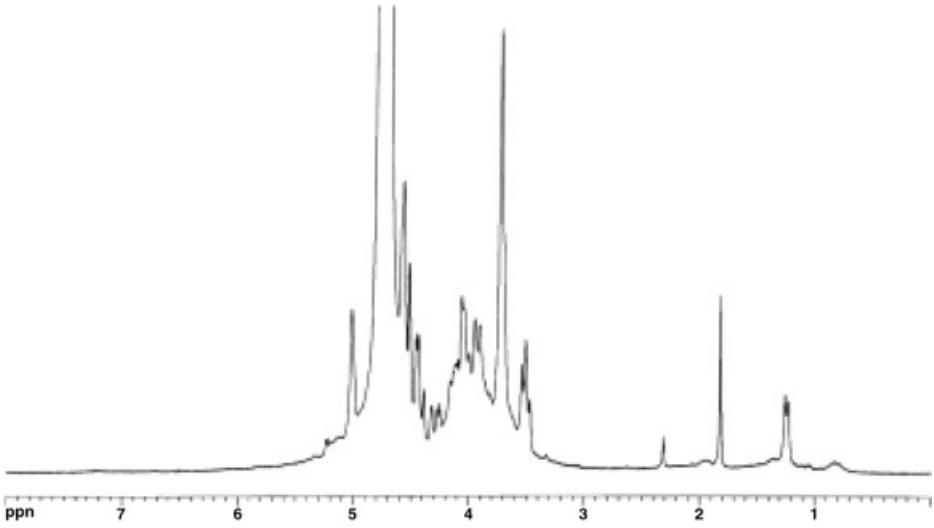

Figure 5.
^{1}H NMR spectrum of product extracted from *Hypnea* recorded in D_2O at 25 °C.

of two peaks at 59 and 83 ppm (Figure 8). The extracted biopolymer was purified with water and re-analyzed by ^{13}C NMR but the peaks were persistent. Methylated galactose unit is known to be a common feature in agars from some species of Gracilaria.[18] Chivotti *et al.*[19] have also reported the presence of a similar peak in their highly methylated polysaccharide obtained from red algae of the Rhodophyta family. Thus these peaks can be attributed to a $O\underline{C}H_3$ group attached to C-6 and the resulting

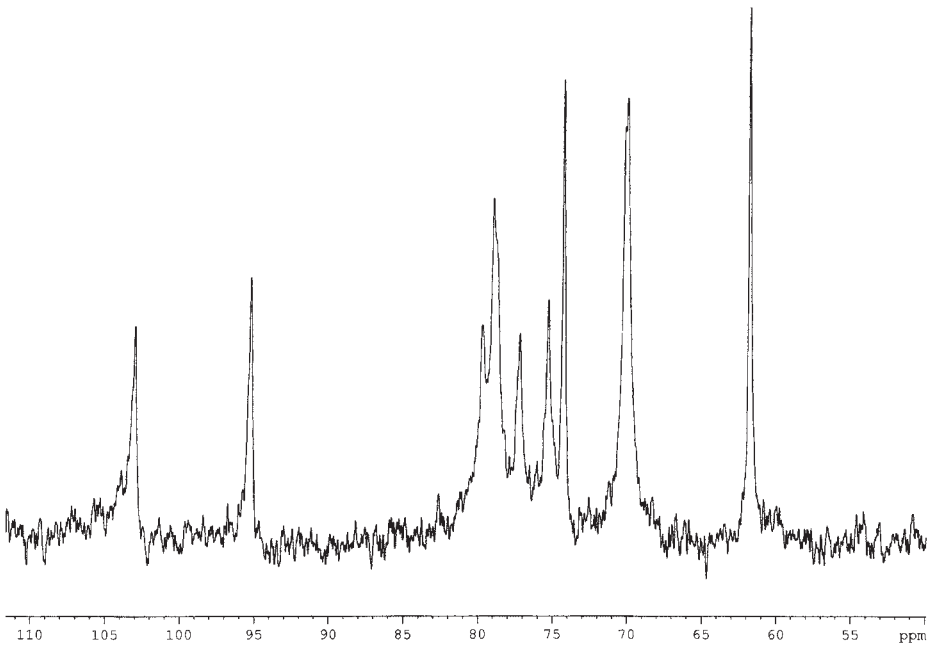

Figure 6.
^{13}C NMR spectrum of product extracted from *Hypnea* recorded in D_2O at 25 °C.

Table 4.
Chemical shift values of κ-and ι-carrageenans from literature[16], commercial κ- and ι-carrageenans, and extracted carrageenans obtained experimentally.

Diad	C-1	C-2	C-3	C-4	C-5	C-6
			κ-carrageenan from literature[16]			
G4S	102.4	69.6	78.9	74	74.7	61.2
DA	95.2	69.9	79.1	78.1	76.7	69.4
G4S	102.8	69.9	79.3	74.5	75.1	61.8
DA	95.7	70.4	79.6	78.8	77.3	69.9
			ι-carrageenan from literature[16]			
G4S	102.1	69.4	76.7	72.1	74.7	61.2
DA,2S	91.9	75.9	77.7	78.1	77	69.7
G4S	102.8	69.9	77.3	72.5	75.1	61.8
DA,2S	92.5	75.1	78.3	78.8	77.3	70.4
			Commercial κ-carrageenan			
G4S	103.5	69.9	79.4	**74.7**	75.7	62.6
DA	**95.7**	70.4	80.2	78.2	77.6	69.9
			Commercial ι-carrageenan			
G4S	103.3	70.5	77.9	**72.8**	75.9	62.4
DA,2S	**93**	75.9	77.9	79.3	77.9	70.5
			Eu1			
G4S	–	69.5	79.5	**74.8**	76	62.3
DA	**95.8**	70.2	80.1	–	77.7	69.5
			Hp1			
G4S	103.5	70.3	79.3	**74.3**	75.6	62.3
DA	**95.6**	70.6	80.1	–	77.5	70.3

–$\underline{C_6H}$(OH)(OCH_3). DEPT analysis performed on the extracted sample confirmed the identity of the peaks at 59 and 83 ppm.

SEC Analysis

The molar masses of our extracted carrageenans were determined by SEC-LS-

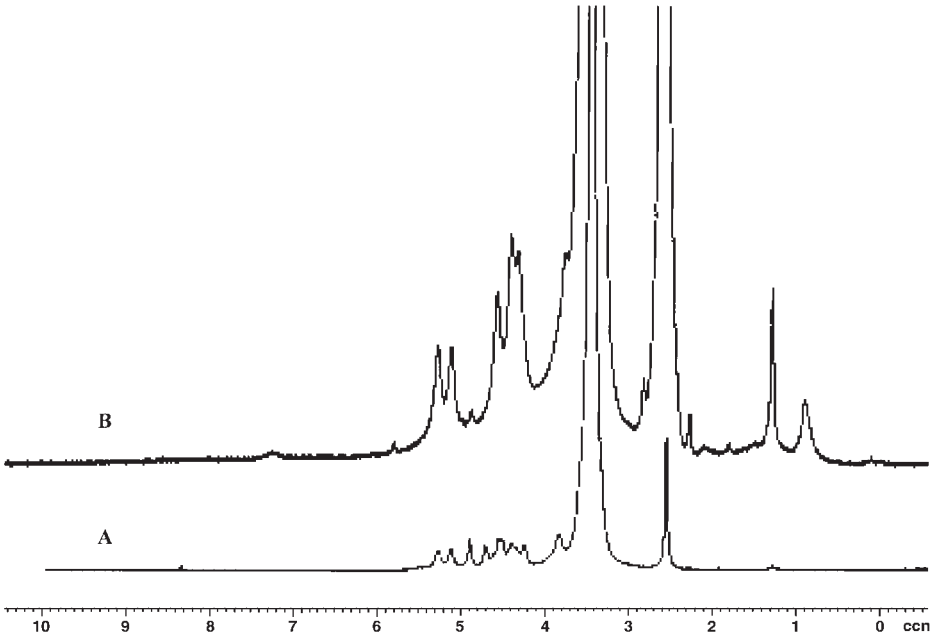

Figure 7.
^{1}H-NMR spectrum of (A) commercial agar and (B) extracted agar.

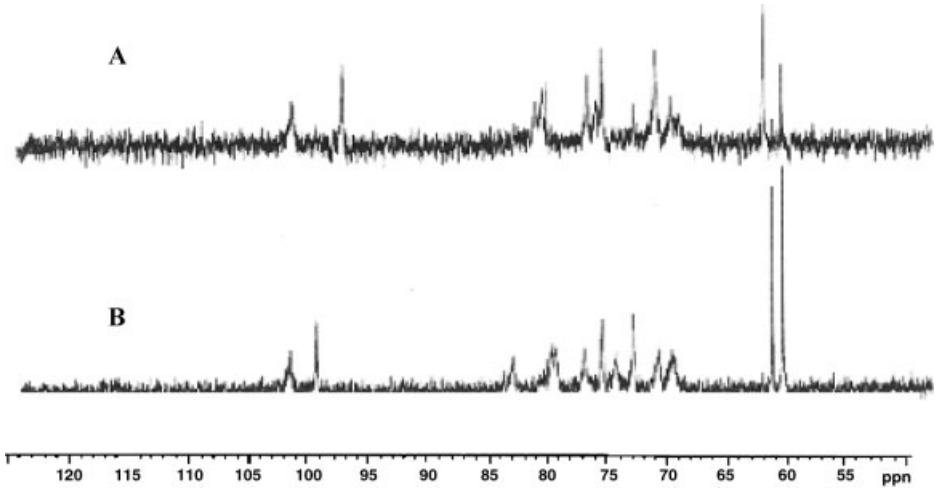

Figure 8.
^{13}C-NMR spectrum of (A) commercial agar and (B) extracted sample.

viscometry, using $LiNO_3$ as eluent, and compared with the commercial κ- and ι-carrageenans. The results are summarized in Table 6. As can be seen, all the samples have molar masses in the same order of magnitude. A close look at the results reveals that the molar masses determined by light scattering for the *Eucheuma* species are higher than that of the *Hypnea* species. Moreover, quite a good correlation is observed between the molar masses (M_w LS) from light scattering and the online viscometry data ([η] bulk) for the extracted samples. In the absence of a light scattering detector, viscometry could thus be used as a first approximation for molar masses. The radii of gyration of the commercial and extracted (Eu 9) κ-carrageenan are also

Table 5.
^{13}C-NMR assignments for agarose, commercial agar and extracted agar.

Product	Diad	Unit	Chemical shifts (ppm)					
			C-1	C-2	C-3	C-4	C-5	C-6
Agarose	G-LA	G	102.4	68.6–70.5	81.1	68.6–70.5	75.2	61
		LA	98	68.6–70.5	80.5	76.5	75.7	68.6–70.5
Commercial agar	G-LA	G	102.4	68.6–70.5	81.1	68.6–70.5	75.2	61
		LA	98	68.6–70.5	80.4	76.5	75.8	68.6–70.5
Gc 1			103	69.2–70.5	79.8	69.2–70.5	75.5	60.3
			100.3	69.2–70.5	79.4	77.2	74.2	69.2–70.5

Table 6.
SEC-LS-Viscometry results for commercial and extracted carrageenans.

Sample name	M_w (LS) [D]	M_n (LS) [D]	M_w (conv)*	M_n (conv)*[D]	[η] bulk [mg/g]	$\langle R_{g^*} \rangle$ [nm]
Iota	316 00	140 000	820 000	233 000	638	68
Kappa	268 000	184 000	666 000	135 000	513	48
Eu 15	315 000	173 000	626 000	127 000	481	89
Eu 9	202 000	135 000	436 000	119 000	293	46
Hp 15	169 000	84 000	272 000	83 000	170	52

* conv stands for conventional SEC data analysis based on pullulan standards.

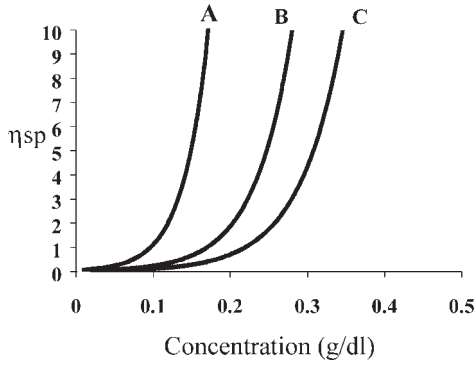

Figure 9.
Specific viscosity as a function of concentration. for: (A) commercial agar; (B) extracted biopolymer Gc 25 (20% NaOH pretreatment), and (C) extracted biopolymer Gc 8 (10% NaOH pretreatment).

very close, which denotes a similar folding of the chains. SEC analyses were not performed on extracted agar due to insolubility of the product in the eluent.

Elemental Analysis

Elemental analysis carried out on the extracted biopolymers confirmed the presence of sulphur in all the samples.

Viscometric Analysis

Carrageenans

Viscosity measurements were carried out in water to observe the pseudoplastic behavior of the carrageenans extracted from *Hypnea* and *Eucheuma* with respect to commercial κ- and ι-carrageenans. The reduced viscosity v/s concentration curves confirmed the ionic character of the polymer. It is found that the reduced viscosity of ι-carrageenan is twice that of κ-carrageenan, which is in accordance with the greater number of sulphate groups present on ι-carrageenan.

Agar

The gel point, P_g was calculated from the gradient of the tangent to the curve of specific viscosity against concentration (Figure 9). P_g for commercial agar and extracted biopolymers are listed in Table 7. A low value of P_g, indicates that the polymer gels at a lower concentration. Molar mass also influences P_g and probably accounts for the difference in P_g between extracted biopolymer and commercial samples.

The gelling temperature was determined for agarose, commercial agar and extracted biopolymers (Table 8) and was found to be lowest for agarose (A), since it does not contain any OCH_3 groups. Thus, the presence of OCH_3 in product B increases its gelling temperature compared with pure agarose.[20] It was also found that seaweeds treated with NaOH gave harder gels after extraction. These findings are consistent with literature as it is known that NaOH improves the quality of the gel by promoting the formation of the anhydro ring.[11]

Conclusion

In this study, the chemical extraction of biopolymers – carrageenans and agar – from seaweeds (*Hypnea*, *Eucheuma* and *Gracilaria*) around Mauritius has proved

Table 7.
Gel point of agar from (A) Japan; (B) India; (C) China; (D) Gc 25 (20% NaOH pretreatment); (E) Gc 8 (10% NaOH pretreatment); (F) Gc 7 (5% NaOH pretreatment), and (G) Gc 9 (no alkali pretreatment).

Product	Gel point (g/dl)
A	0.10
B	0.15
C	0.16
D	0.18
E	0.25
F	0.27
G	0.28

Table 8.
Gelling temperature of: (A) agarose; (B) agar from China; (C) agar from Japan; (D) Gc 9 (no alkali pretreatment); (E) Gc 7 (5% NaOH); (F) Gc 8 (10% NaOH), and (G) Gc 25(20% NaOH).

Product	Gelling temperature (°C)
A	35 ± 1
B	39 ± 1
C	39 ± 1
D	38 ± 1
E	40 ± 1
F	42 ± 1
G	45 ± 1

to be successful on a small scale (lab scale). A number of sophisticated physico-chemical techniques, mostly available at the University of Mauritius, were essential to fully characterize the biopolymers. Thus, their structures were confirmed by NMR. SEC analyses have shown that the average molar masses of κ-carrageenans obtained from *Hypnea* and *Eucheuma* are of the same order of magnitude as the commercial samples. *Gracilaria* species yield a biopolymer which is similar to commercial agar but with a higher methoxy content as evidenced by ^{13}C-NMR and DEPT spectra as well as from the gelling temperature.

The optimization of the extraction process from an economical and ecological point of view as well as its scaling-up is currently under study. We also believe that the expertise developed by our group can be made available to other research groups in the region and in particular in Madagascar where red algae are abundantly available.

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