

## Analysis on the Seasonal Variations in Carrageenans of *Hypnea flagelliformis* and *Sarconema filiforme* by FTIR Spectroscopy

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Carrageenans are the most common and abundant cell wall polysaccharides of red algae and are commercially important sulphated galactans. In the present study, polysaccharides extracted from *Hypnea flagelliformis* and *Sarconema filiforme* of summer and winter collections, were examined by FTIR spectroscopic analysis. The spectra of *Hypnea flagelliformis* collected in both seasons are correspondingly similar to that of kappa carrageenan. FTIR spectra of *Sarconema filiforme* showed a peak at  $805\text{ cm}^{-1}$  which confirms the presence of 3,6-anhydro galactose-2-sulphate. This is characteristic of iota carrageenan. The seasonal variations occur in the absorption bands of polysaccharides.

**Key Words:** Carrageenans, *Hypnea flagelliformis*, *Sarconema filiforme*.

### INTRODUCTION

Carrageenans are commercially important hydrophilic colloids which occur as matrix material in numerous species of red algae. Chemically they are highly sulphated galactans. Due to their half-ester sulphate moieties they are strongly anionic polymers. The solubility properties of carrageenans have led to their widespread commercial use in food and other industries<sup>1-3</sup>. Carrageenans are used as thickening, suspending and gelling agents. The commercially important carrageenans are kappa (k), iota (i) and lambda ( $\lambda$ ). The type of carrageenan could be identified based on the intensity of absorption bands exhibited by the spectra. The consistent occurrence of kappa carrageenan in the red algal family Hypneaceae was also confirmed by IR spectral analysis<sup>4,5</sup>. The polysaccharides of some members of Solieriaceae, Cystocloniaceae and Phylloporaceae were characterized as iota carrageenan by spectroscopic method<sup>6,7</sup>. The present study aims to bring out the types of carrageenan and seasonal variations in the spectra of *Hypnea flagelliformis* and *Sarconema filiforme* by FTIR spectroscopy.

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## EXPERIMENTAL

As the cell wall polysaccharides are soluble in water, the hot water extraction method was adopted. The extracted polysaccharides from summer and winter collections of *Hypnea flagelliformis* and *Sarconema filiforme* were dried in dessicator. The dried polysaccharide was powdered and passed through 1 mm IS sieve. The powdered polysaccharide was transferred to air tight glass bottle and stored for quality analysis. 1 mg of the dry sample powder was mixed in a mortar with 99 mg of KBr. A thin KBr disc was obtained by pressing the powder in French press and Fourier transform infrared (FT-IR) spectra were recorded using Bruker Vector 20 FT-IR double beam spectrophotometer in the range of 4000-600  $\text{cm}^{-1}$ . Spectra were taken under identical conditions and compared with spectra of authentic kappa and iota carrageenan.

## RESULTS AND DISCUSSION

Seasonal variation exhibited in the carrageenans and their band assignments are presented in the Tables 1 and 2.

### Vibrational band assignments

**O-H stretching vibrations:** The O-H stretching occurs in the present study at around 3400  $\text{cm}^{-1}$  in general with very strong intensity of bands. This O-H stretching is unique and common, occurs in samples taken for the investigation as reported in earlier work<sup>8</sup>.

**Methyl group vibrations:** The FTIR spectra of samples, exhibited weak bands for methyl group *i.e.* symmetric stretch band at 2860  $\text{cm}^{-1}$  and asymmetric C-H stretch band<sup>9</sup> at 2925  $\text{cm}^{-1}$ . In the present work, the bands of strong to weak intensity observed at around 1450 and 1370  $\text{cm}^{-1}$  are attributed to asymmetrical and symmetrical bending vibrations of  $\text{CH}_3/\text{CH}_2$  group, respectively supported by previous report<sup>10</sup>.

**Ester-sulphate link vibrations:** The ester-sulphate link vibration is exhibited by the peaks<sup>11</sup> at 1370, 1250, 1180 and 1060  $\text{cm}^{-1}$ . In this investigation, the intensity of the absorption in these regions varies from strong to weak. The variation in the intensity of absorption bands depends upon the species and the seasons. The carrageenans have wide and strong absorption bands in 1100-1000  $\text{cm}^{-1}$  region, which are typical of polysaccharides<sup>12</sup>. In the present study, the maximum absorption is given by 1080-1020  $\text{cm}^{-1}$  for both carrageenan types except in summer spectra of *Sarconema filiforme*, in which there is a weak absorption in the same bands. The sulphate stretching occurs at 850  $\text{cm}^{-1}$  mainly due to C-O-S in C-4 link vibration<sup>13</sup>. The strong and weak intensity of bands were observed in the FTIR spectra of samples studied.

**Carbon-sulphur link vibrations:** The literature study reveals that the carbon sulphur link vibration of polysaccharides occurs<sup>12</sup> at 770  $\text{cm}^{-1}$ . The spectra of present study showed strong to weak band over the range 780-703  $\text{cm}^{-1}$  attributed to the deformation of sulphate. The peaks at 670  $\text{cm}^{-1}$  represent C-S deformation in the spectra of all the species.

TABLE-1  
FTIR BAND ( $\text{cm}^{-1}$ ) ASSIGNMENTS OF STANDARD KAPPA CARRAGEENAN AND  
CARRAGEENAN OF *Hypnea flagelliformis*

Kappa carrageenan (FMC)	<i>Hypnea flagelliformis</i>		Band assignments
	Summer	Winter	
674 ms	666 m	668 m	C-S link vibration/S-O stretch/C-S deformation
703 s	704 s	703 mw	C-S link vibration/S-O stretch /C-S deformation
763 mw	773 mw	777 w	C-S link vibration/S-O stretch /C-S deformation
847 ms	845 s	847 w	Ester-sulphate in C-6 link vibration/C-C/C-O/C-O-S stretch
929 s	931 s	921 m	3,6 anhydro galactose vibration/C-C/C-O stretch
1042 vs	1040 vs	1030 s	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1069 vs	1074 vs	1060 ms	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1160 vs	1162 vs	1160 s	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1264 vs	1266 vs	1262 s	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1383 ms	1383 s	1384 ms	Methyl group vibration/S=O symmetric stretch
1442 m	1450 s	1441 m	Methyl group vibration/S=O asymmetric stretch
1549 w	1550 mw	1541 mw	C=O symmetric stretch/N-H deformation
1641 s	1643 s	1637 vs	C=O asymmetric stretch/N-H deformation
1724 w	1750 vw	1760 vw	C=O stretch
2828 vw	2866 w	2867 vw	Methyl group vibration/C-H symmetric stretch
2917 vw	2923 mw	2925 vw	Methyl group vibration/C-H asymmetric stretch
3416 vs	3434 s	3446 vs	OH/NH stretch

TABLE-2  
FTIR BAND ( $\text{cm}^{-1}$ ) ASSIGNMENTS OF STANDARD IOTA CARRAGEENAN AND  
CARRAGEENAN OF *Sarconema filiforme*

Iota carrageenan (FMC)	<i>Sarconema filiforme</i>		Band assignments
	Summer	Winter	
678 m	666 ms	669 m	C-S link vibration/S-O stretch/C-S deformation
776 m	778 ms	774 w	C-S link vibration/S-O stretch /C-S deformation
806 mw	805 w	805 vw	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
853 m	849 m	847 vw	Ester-sulphate in C-6 link vibration/C-C/C-O/C-O-S stretch
919 ms	929 vw	932 w	3,6 anhydro galactose vibration/C-C/C-O stretch
1019 vs	1018 w	1029 m	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1058 vs	-	1071 m	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1146 s	1114 vw	1159 mv	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1255 s	1227 w	1270 m	Ester-sulphate link vibration/C-C/C-O/C-O-S stretch
1383 ms	1385 w	1385 mw	Methyl group vibration/S=O symmetric stretch
1460 m	1469 m	1457 mw	Methyl group vibration/S=O asymmetric stretch
1547 w	1547 vw	1541 mw	C=O symmetric stretch/N-H deformation
1640 s	1642 ms	1637 vs	C=O asymmetric stretch/N-H deformation
1721 w	-	1760 vw	C=O stretch
2838 vw	2863 vw	2863 vw	Methyl group vibration/C-H symmetric stretch
2925 w	2924 vw	2924 vw	Methyl group vibration/C-H asymmetric stretch
3400 vs	-	3419 vs	OH/NH stretch
-	3464 vs	3475 vs	

**3,6-Anhydro-galactose bridge vibrations:** The peak at  $805\text{ cm}^{-1}$  was attributed to  $-\text{O}-\text{SO}_3$  vibration on C-2 of a 3,6-anhydrogalactose ring<sup>14</sup>. This weak band at  $805\text{ cm}^{-1}$  was observed in the study only in *Sarconema filiforme*, which was characteristic of iota carrageenan. The strong to weak absorption bands occur at  $930$  and  $1070\text{ cm}^{-1}$  in all the spectra of the present study attributed to C-O ether bond of 3,6-anhydro-galactose vibration<sup>14</sup>. These two peaks are typical for phycocolloids.

**Carbonyl vibrations:** In the present work, very weak band was obtained at around  $1740\text{ cm}^{-1}$  in both the species studied. The strong absorption bands at  $1640\text{ cm}^{-1}$  was assigned to carbonyl vibration of carboxylic group (COO) due to asymmetric stretching (C=O). The medium to very weak intensity of band at around  $1550\text{ cm}^{-1}$  was assigned to symmetrical stretching of C=O. These assignments are in agreement with previous observations<sup>15</sup>.

**NH<sub>2</sub> group vibrations:** The presence of protein in polysaccharides shows carbonyl absorption as amide group. Primary and secondary amides display a band in the region  $1650$  and  $1540\text{ cm}^{-1}$  due to amine deformation vibrations and CO-NH peptide link vibrations, respectively<sup>16</sup>. The spectra of the present investigation show strong to medium band at  $1640\text{ cm}^{-1}$  and medium to weak band at  $1540\text{ cm}^{-1}$  thus confirming the previous observations. A sharp peak centered at around  $3500\text{ cm}^{-1}$  may be due to NH stretching vibration<sup>17</sup>. These bands are observed in the characteristic region in the spectra of present study.

**C-C stretching vibrations:** The band observed in the region  $1200-800\text{ cm}^{-1}$  may be due to overlapping of C-C stretching and C-H bending modes<sup>18</sup>.

**Qualitative analysis:** The FTIR spectra of members of carrageenophytes exhibited more or less some common peaks at  $3500$ ,  $3400$ ,  $2920$ ,  $2860$ ,  $1740$ ,  $1640$ ,  $1540$ ,  $1460$ ,  $1380$ ,  $1260$ ,  $1150$ ,  $1070$ ,  $1030$ ,  $930$ ,  $850-840$ ,  $770$  and  $670\text{ cm}^{-1}$  (Table-1). The bands for carrageenophytes are found in the region  $850-840\text{ cm}^{-1}$  as expected. Some additional peak may be attributed, which serve as a criteria to differentiate the type of carrageenans.

**Kappa carrageenan:** The commercial kappa carrageenan (Gelcarin GP 812) obtained from FMC Biopolymer, USA was analyzed using FTIR spectral measurements. The spectrum exhibits peaks at  $3416$ ,  $2917$ ,  $2828$ ,  $1724$ ,  $1641$ ,  $1549$ ,  $1442$ ,  $1383$ ,  $1264$ ,  $1160$ ,  $1069$ ,  $1042$ ,  $929$ ,  $847$ ,  $763$ ,  $703$  and  $674\text{ cm}^{-1}$  (Table-1). The spectrum of *Hypnea flagelliformis*, collected in both seasons are correspondingly similar to that of the commercial sample of kappa carrageenan showing sharp peak at  $930\text{ cm}^{-1}$  for 3,6-anhydrogalactose and at  $845\text{ cm}^{-1}$  for 1,3 linked galactose 4- sulphate. The broad band at  $1240\text{ cm}^{-1}$  is common to all sulphated polysaccharides. This indicates an index of the degree of sulphation, its intensity increasing with sulphate content. The intensity of absorption was more in summer than in winter which indicates the effect of seasonal variation in the synthesis of carrageenan.

**Iota carrageenan:** The FTIR spectrum of iota carrageenan (Viscarin SD 389) obtained from FMC Biopolymer USA showed peaks at  $3400$ ,  $2925$ ,  $2838$ ,  $1721$ ,  $1640$ ,  $1547$ ,  $1460$ ,  $1383$ ,  $1255$ ,  $1146$ ,  $1058$ ,  $1019$ ,  $919$ ,  $853$ ,  $806$ ,  $776$  and  $678\text{ cm}^{-1}$

(Table-2). The FTIR spectra of *Sarconema filiforme* showed more or less same peaks. However a broad band occurs at 1227, 1114 and 1018  $\text{cm}^{-1}$  and absence of peak at around 1720  $\text{cm}^{-1}$  was observed in summer. Comparatively less intensity of peaks is exhibited by the spectrum of summer collection than in winter. This reflects the seasonal variation in the molecular level of carrageenan. The spectrum of winter collection shows resolved peaks at 3475 and 3419  $\text{cm}^{-1}$ . Apart from these peaks, a peak was found at 805  $\text{cm}^{-1}$  more or less with the same level of absorption both in summer and in winter, indicating the presence of 1,4 linked 3,6-anhydrogalactose 2-sulphate. This is characteristic of iota carrageenan. It is quiet evident that the absorption bands of polysaccharides from *S. filiforme* are typical of iota carrageenan.

**Quantitative analysis:** A comparative study was made for all the samples to determine the variation in the absorbancy of major functional groups like hydroxyl group, total sulphate and  $\text{CH}_2\text{OH}$ , CH and amide groups. In each case normalized absorbancy show considerable variation between species and seasons. The results are given in Table-3.

TABLE-3  
ABSORBANCE VALUES OF DIFFERENT GROUPS IN CARRAGEENANS

Group	Kappa carrageenan (FMC)	<i>Hypnea flagelliformis</i>		Iota carrageenan (FMC)	<i>Sarconema filiforme</i>	
		Summer	Winter		Summer	Winter
OH	0.260	0.120	0.190	0.25	0.350	0.130
Total sulphate	0.210	0.140	0.170	0.22	0.170	0.160
Ester	0.110	0.040	0.080	0.09	0.110	0.130
$\text{CH}_2\text{OH}$	0.310	0.190	0.210	0.31	0.320	0.250
CH	0.020	0.011	0.007	0.19	0.007	0.005
Amide	0.105	0.030	0.060	0.07	0.080	0.060

## Conclusion

Kappa, iota and lambda carrageenans are having their, own peculiar properties. Mostly in food industries, lambda carrageenan is preferred because of its non-gelling and high viscosity properties. The kappa and iota carrageenans are widely used in the industries where they are required as gelling agent. In cosmetics they are applied as stabilizer, as suspending agent for cocoa in chocolate milk and a gelling agent for milk puddings, water-gel desserts and air fresh gels and binding agents in tooth paste, skin ointments, cough syrup, *etc.* But there is no carrageenan manufacturing unit in our country. Carrageenan production must be commenced in India, using algal resources available in our country.

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