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Characterization of macromolecular orientation in κ-carrageenan fibers using polarized Fourier-transform infrared spectroscopy

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Abstract: In the current study, polarized infrared (IR) microspectroscopy was employed to characterize the macromolecular orientation in wet-spun and stretched κ-carrageenan fibers. The fibers were shown to be well oriented by X-ray diffraction, suggesting that the κ-carrageenan molecules were generally aligned along the fiber axis direction. Longitudinal fiber pieces of about 10 microns thick were obtained by focused ion beam (FIB) micromilling. The fiber pieces were examined by polarized IR in transmission mode. Several bands, including those characteristic of κ-carrageenan at 845 and 930 cm^{-1}, were polarization-dependent, demonstrating polarized IR as a useful tool to evaluate macromolecular orientation in carrageenan fibers. Band assignments were discussed by considering the general alignment of molecules and the polarization dependence of vibration modes, and our results agreed well with band assignments from previous reports.

Keywords: Macromolecular orientation; κ-carrageenan fiber; X-ray fiber diffraction; polarized infrared microspectroscopy; Fourier transform infrared spectroscopy; focused ion beam

1. Introduction

Geometrically, fibers have an elongated shape with a length at least 100 times greater than their diameter [1]. More importantly, fiber properties, e.g. mechanical strength, may be anisotropic due to alignment of structural elements, i.e. molecules, in the fiber axis direction. This is particularly true of polymer fibers formed via high-shear spinning processes. Hence, the determination of molecular orientation becomes crucial to polymer fiber properties. There are a number of methods with the potential to evaluate molecular orientation in fibers, e.g. birefringence measurement [2], X-ray fiber diffraction [3], acoustic velocity [4], polarized infrared (IR) spectroscopy [5], and polarized Raman spectroscopy [6].

We previously reported the fabrication of κ-carrageenan fibers and κ-/ι-carrageenan fibers by a wet-spinning method [7,8]. An optical microscope equipped with crossed polarizers and a quarter wave plate was used to evaluate the molecular alignment in the κ-carrageenan fibers. The κ-carrageenan fibers were well oriented as indicated by the presence of birefringence. κ-Carrageenan is known to exhibit low crystallinity probably due to the irregular molecular structure [9]. Therefore, the detection and evaluation of macromolecular orientation in κ-carrageenan fibers by X-ray requires the use of a synchrontron source that is much more intense than the best X-ray laboratory source. Therefore, in the present study, we employed polarized IR microspectroscopy to probe the macromolecular orientation in κ-carrageenan fibers.

The carrageenans are linear, sulfated polysaccharides extracted from various species of red seaweed. The “ideal” carrageenan backbone is based on a repeating disaccharide unit of β-D-galactopyranose (A residue) linked through positions 1 and 3, and α-D-galactopyranose (B residue) linked through positions 1 and 4 (Fig. 1). κ-Carrageenan is one of the three dominant natural carrageenan species, i.e. κ, ι, and λ-carrageenan, which differ in the number and position of sulfate

substitutions and presence or absence of a 3,6-anhydride bridge on the B residue. κ-Carrageenan has a sulfate group on the C4 of the A residue and has the B residue converted to 3,6-anhydro form (Fig. 1a). The presence of this 3,6-anhydride bridge makes κ-carrageenan helix-compatible and able to gel. According to the widely accepted "domain model" [10], κ-carrageenan undergoes a coil-double helix transition when it gels. κ-Carrageenan molecules in the sol state adopt a random coil conformation. Upon cooling and in the presence of certain cations, κ-carrageenan molecules form double helices and multiple double helices aggregate to form one junction zone of the network. The double helical structure of κ-carrageenan was also suggested by modeling from X-ray diffraction data (Fig. 1b) [9].

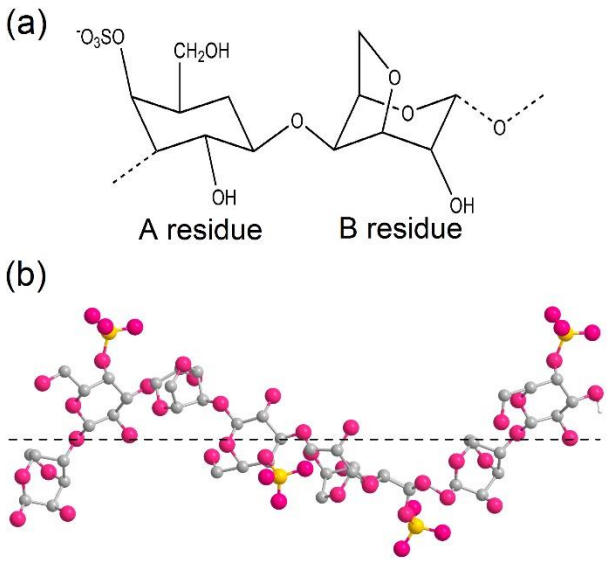


Fig. 1. Molecular structure of κ-carrageenan: (a) repeating disaccharide moieties and (b) one strand of the double helices according to proposed model [9].

The molecular structure of carrageenan presents difficulties in making spectral assignments and for quantitative determination. The regularity of molecular organization in κ-carrageenan is much lower than the highly crystalline cellulose [11] and silk fibers [5,6]. Unlike the silk fiber that

has an amide I stretching mode perpendicular to the fiber axis direction, no vibration vector is positioned parallel or perpendicular to the carrageenan fiber axis. Hence, in the present study, our discussion was based on two assumptions, though they are credible based on current knowledge and evidence: 1) the helical molecules, whether crystalline or not, are generally aligned in the fiber axis direction; 2) both the proposed molecular structure and crystalline structure shown in Fig. 1 are correct. The general direction of the functional groups and their vibrations seen from their molecular structure (Fig. 1) will be used in the discussion.

The main objective of this study was to evaluate the capability of polarized IR to detect macromolecular orientation in κ-carrageenan fibers. Two sectioning techniques, microtoming and focused ion beam (FIB) micromilling, were initially attempted to section wet-spun κ-carrageenan fibers into longitudinal pieces of about 10 μm thick. Polarized IR in transmission mode was used. Though the fiber pieces obtained by both techniques showed similar polarization dependence (The IR spectra of a κ-carrageenan fiber piece from microtome sectioning can be found in Supplementary Materials Fig. S2 & S3), it is worthwhile to point out that raw absorption peaks of the microtome sectioned fiber were all greater than the FIB sectioned fiber, suggesting thinner sections were obtained by the FIB technique. Therefore, only IR spectra of the FIB sectioned fiber were used in the following discussions. The discussions were based on previously reported spectral assignments for carrageenans and by considering the direction of the transition dipole moment for certain vibrational modes.

2. Materials and methods

2.1 Materials

κ-Carrageenan (Gelcarin® GP911 NF) was kindly provided by FMC Biopolymers (New Jersey, USA). The κ-carrageenan was purified from excess salts and low molecular weight carbohydrates according to our established method [7]. Ethanol (200 proof) and potassium chloride were purchased from VWR International and used as received.

2.2 Fiber Spinning

Spinning dope was prepared by dissolving purified κ-carrageenan (6%, w/v) in deionized water at 80 °C for at least 1 hour. The dispersion was homogeneous by visual observation before spinning. Wet-spinning was carried out using a bench-top device. A jacket-type circulating device (Penn State Glass Shop, University Park, PA) was used to maintain the spinning dope temperature at 70 °C in a 3 ml syringe (Becton, Dickinson and Company, Franklin Lakes, NJ). The dope was extruded by a syringe pump (Cole-Parmer 74900, Vernon Hills, IL) through a blunt stainless steel needle (20 G, 0.51 mm) into a coagulation bath containing 0.5 M KCl in 50% (w/v) ethanol. The as-spun fiber was kept in the bath for at least 2 hours to ensure complete ion diffusion into the fiber. Then a piece of fiber was stretched to twice its original length using a Texture Analyzer (TAXT2i, Stable Microsystems, Godalming, UK) with film/fiber clamps. The fiber was then dried and subjected to sectioning.

2.3 Focused ion beam (FIB) micromilling

A longitudinal section of the κ-carrageenan fiber of ≈10 μm thick was prepared using an FEI Quanta 3D DualBeam FIB microscope (FEI Company, Hillsboro, OR). An image of the fiber piece

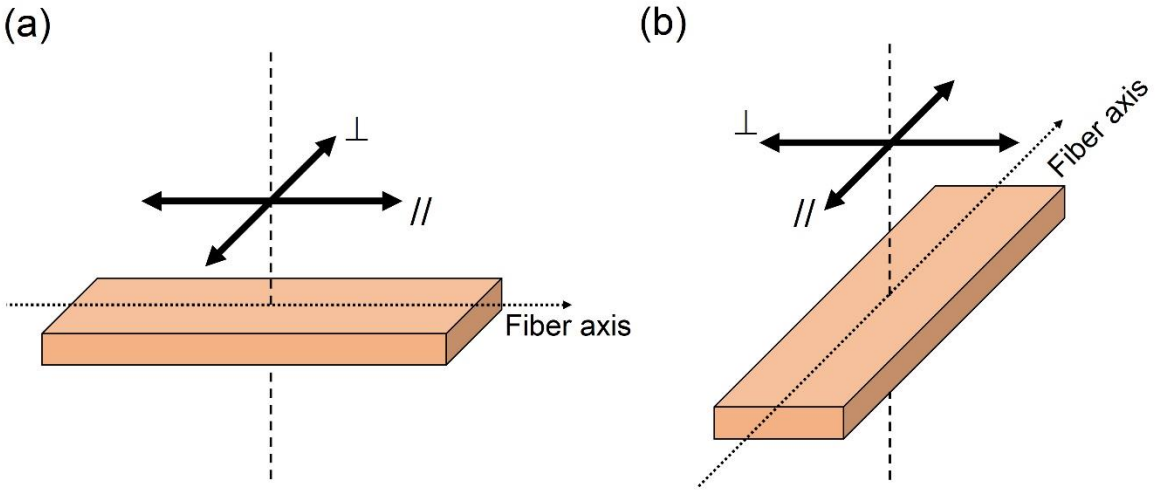
sectioned by FIB can be found in Supplementary Materials (Fig. S1. Electron micrograph of the fiber piece sectioned by FIB).

2.4 X-ray fiber diffraction

Structural organization and orientation of the κ-carrageenan fiber was analyzed using X-ray fiber diffraction principles. Synchrotron intensities were collected at 14-BMC beamline, BioCARS, Argonne National Laboratory, Chicago, IL. The wavelength of X-ray beam was 0.979 Å and the data were recorded on a CCD with 10 s exposure.

2.5 Polarized infrared (IR) microspectroscopy

Longitudinal sections of fiber were analyzed in transmission mode using a Bruker IFS 66/S Fourier transform infrared (FT-IR) Spectrometer (Bruker Optics Ltd., Billerica, MA) coupled to a Hyperion 3000 FT-IR microscope equipped with a 15x objective. The fiber pieces obtained from FIB micromachining were carefully placed on a NaCl window. To cross-check on the directionality of the polarizer on the microscope, the fiber piece can be oriented either parallel (Fig. 2a) or perpendicular (Fig. 2b) relative to the front edge of the sample stage on the microscope by rotating the sample holder. At each position, the polarized IR vector can be either parallel (//) or perpendicular (⊥) relative to the fiber axis. Spectra were acquired using a ~20um x 20um aperture, 100um pinhole condenser aperture, and by the co-addition of 400 scans with a resolution of 6 cm^{-1}.



135

136 **Fig. 2.** Orientation of polarized IR beam relative to the fiber position. The fiber piece can be
137 positioned (a) parallel and (b) perpendicular relative to the sample stage. In both positions, "//"
138 denotes that the IR polarization is parallel to the fiber axis and "⊥" indicates that the IR
139 polarization is perpendicular to the fiber axis.

140 **3. Results and Discussion**

141 A representative X-ray diffraction pattern from κ-carrageenan fibers is shown in Fig. 3. The
142 presence of large arcs indicates that the κ-carrageenan microcrystallites are well oriented in the
143 fiber. But the fiber has low crystallinity, since the Bragg reflections on individual layers are
144 somewhat diffused and not as well resolved as in the case of ι-carrageenan fiber [12,13]. The first
145 meridional reflection is seen on 6th layer line, suggesting that the κ-carrageenan helices contain 6
146 monosaccharide unit per turn of helix, or 3-fold helix of the disaccharide moieties. These features
147 compare well with other κ-carrageenan patterns [9]. The concentric rings are from the excess
148 potassium chloride deposited on the fibers.

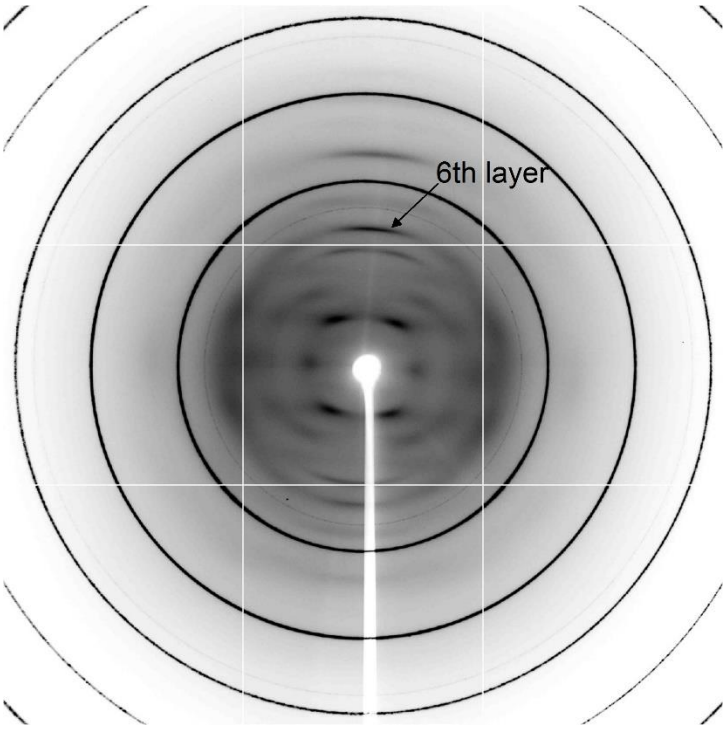


Fig. 3. X-ray diffraction pattern of the wet-spun and stretched κ-carrageenan fiber. The fibers are almost perpendicular to the incident X-ray beam. The first reflection on the meridian (meridian is an imaginary line in the north–south direction passing through the center) suggests the helix fold is six.

The well-oriented κ-carrageenan fiber was successfully sectioned to approximately 10 μm thick pieces by FIB micromachining techniques. Fig. 4 shows IR spectra recorded in the non-polarized and polarized, including parallel (//) and perpendicular (⊥), orientations. Only one set of polarization data was plotted, since identical polarization-dependent spectra were obtained when the specimens were positioned in both parallel and perpendicular directions relative to the sample stage. The spectra were shifted along the y-axis and normalized to the 1640 cm^{-1} intensity, which was assigned to the bending mode of water molecules and independent of polarization. It is worth mentioning that this normalization was applied to the spectra of the same piece of sample

and same spot of analysis, only differing in the orientation angle, but would not be valid when used to compare different samples. The differences between the polarized spectra and the non-polarized spectrum can be observed, which confirms the presence of preferred orientation of vibrations in both fiber samples. The vibrational modes in the range 1030 – 1090 cm^{-1} and 1210 – 1270 cm^{-1} were too strongly absorbing to be useful.

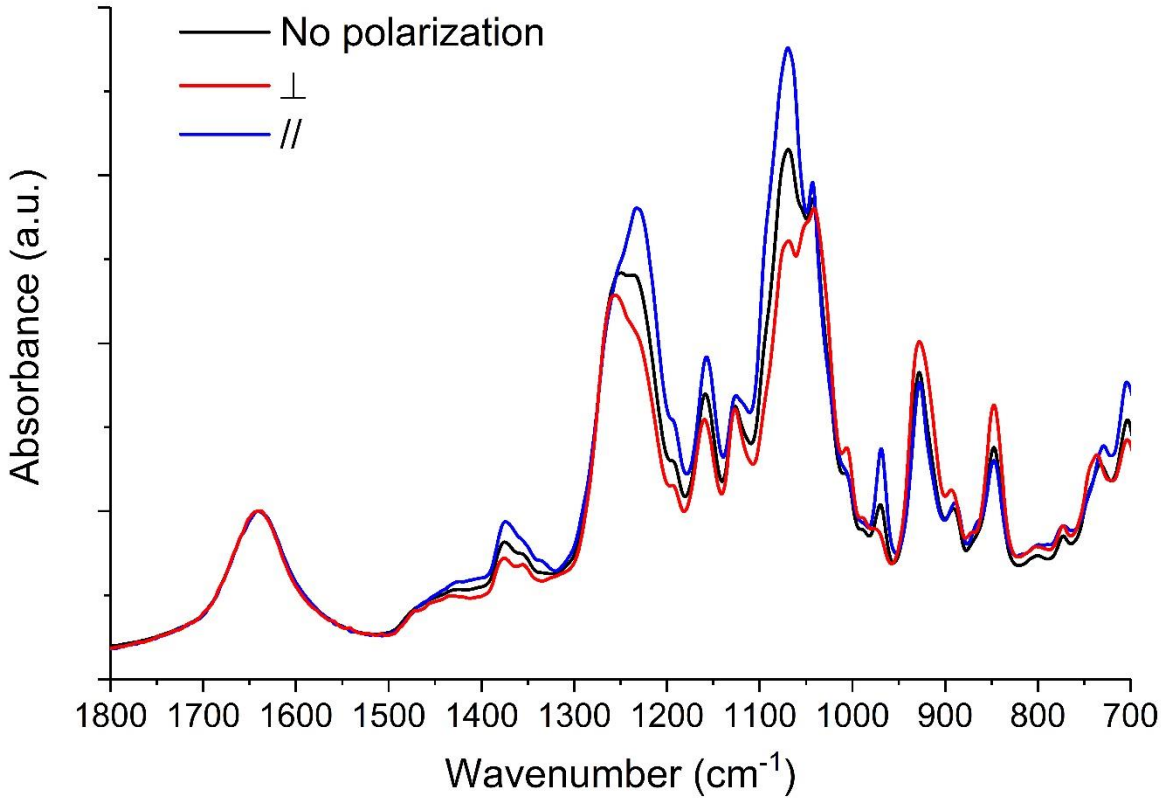


Fig. 4. FTIR spectra of a κ-carrageenan fiber piece from FIB micromachining recorded without polarization and with polarization parallel (//) and perpendicular (⊥) to the fiber axis. Spectra were normalized to the 1640 cm^{-1} intensity.

The bands at 704, 845, 930, 970, 1006, and 1156 cm^{-1} were found to be dependent on the polarization. Among these bands, the 845, 930, and 1006 cm^{-1} bands showed stronger absorbance

when the IR polarization was perpendicular to the fiber axis, while the 704, 970, and 1156 cm^{-1} bands exhibited the opposite polarization dependency. By subtracting the spectra (// - ⊥) of the fiber samples, the polarization dependence in most of the bands became more apparent and new bands could be resolved (Fig. 5). The new bands observed in subtraction spectra may be those shoulder bands, for instance, at 743 and 920 cm^{-1}. Some others were due to band shift, when IR polarization changes, for example, the 716 shifted to 735 cm^{-1} from "//" to "⊥" configuration. The assignments and polarization dependence of bands in κ-carrageenan IR spectra are summarized in Table 1.

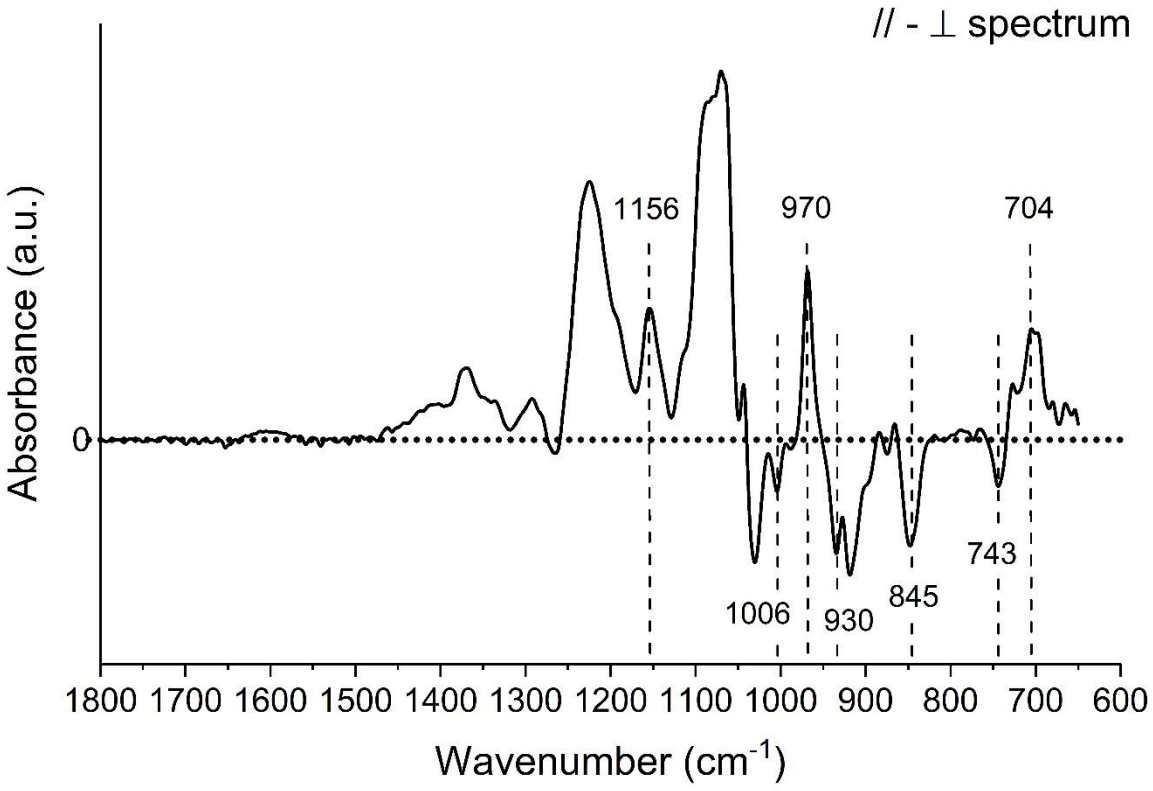


Fig. 5. Subtraction (// - ⊥) spectrum of FIB sectioned fiber by polarized IR in transmission mode.

The IR bands at 845 and 930 cm^{-1} have frequently been used to determine the presence of κ-carrageenan and distinguish it from other carrageenan species [14]. The 845 cm^{-1} band was assigned to the stretching vibration [15], and specifically pseudo-symmetric stretching [16] of C4-O-S on the A residue, which is one of the characteristic and principal vibrations studied in IR spectra of carrageenans. From the structural models, the C4-O-S groups are pointing out from the molecular backbone or the helical axis (Fig. 1 & 6). Since this band showed strong polarization dependence and higher absorbance in the perpendicular configuration, it is more likely to be the asymmetric stretching mode of the C4-O-S group (Fig. 6d). The band at 930 cm^{-1} is another characteristic vibration of κ-carrageenan, which is assigned to the C-O or C-O-C stretching vibration of the 3,6 anhydro bridge on the B residue [16,17]. This bridging group spans across the backbone, so it is obvious that both symmetric and asymmetric stretching will result in a net dipole moment in the plane perpendicular to the fiber axis (Fig. 6e). Correspondingly we see an increase in the intensity of this peak when the IR is polarized perpendicular to the fiber axis.

The rest of the polarization-dependent bands are all associated with vibration modes of glycosidic linkages, including the alternating α(1→3) C-O-C and β(1→4) C-O-C. The 704 cm^{-1} band is close to the assigned bending vibration of β(1→4) C-O-C glycosidic linkage [16]. The 734 cm^{-1} band was assigned to bending vibration of α(1→3) C-O-C glycosidic linkage in κ-carrageenan [16]. However, the two bands have different polarization dependence. The glycosidic C-O-C is generally aligned along the fiber axis direction (Fig. 6). Some bending modes, e.g. scissoring and rocking, would result in a net dipole moment in the same direction as the fiber axis, while other bending modes, e.g. wagging and twisting, would produce a net vibration perpendicular to the fiber axis. Hence, the two bands could be attributed to different bending modes of the two glycosidic C-O-C bonds, respectively. It is also possible that the 704 cm^{-1} band has

some contribution of the sulfate group on B residue as suggested by Rochas et al., (1986). The 1006 cm^{-1} band was suggested to be associated with the α(1→3) C-O-C glycosidic linkage in κ-carrageenan, without assignment to any specific vibration mode [16]. It absorbed more perpendicularly polarized IR, which suggests this stretching vibration may be symmetric (Fig. 6a). The 970 cm^{-1} band was assigned to stretching vibration of glycosidic C-O-C groups. Both the 1126 cm^{-1} band [16] and the 1156 cm^{-1} band [18,19] were assigned to asymmetric stretching of the glycosidic C-O-C group by different researchers. However, the 1126 cm^{-1} vibration was found to be weakly dependent on polarization or even polarization-independent in this study, whereas the behavior of the 970 and 1156 cm^{-1} bands agreed well with their assignments. It suggests that the 1126 cm^{-1} band may represent a group of C-O-C linkages that have their asymmetric stretching vectors close to 45° to the fiber axis.

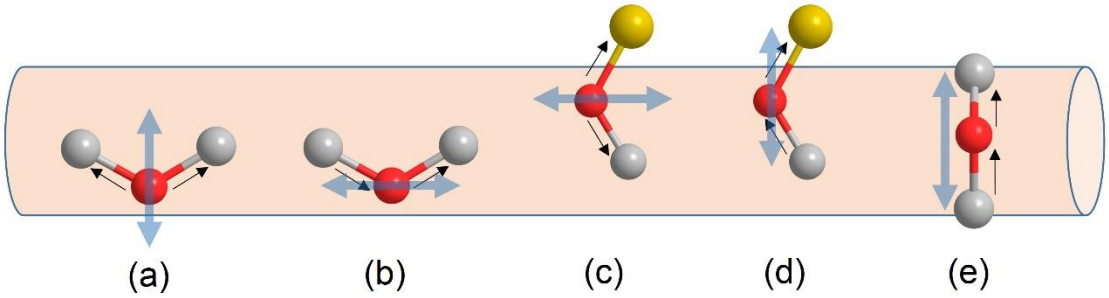


Fig. 6. Schematic drawing of some functional groups of κ-carrageenan, their general alignment relative to the fiber axis, their stretching vibration modes and the overall vibration vectors (blue double arrows): (a) symmetric stretching of glycosidic C-O-C, (b) asymmetric stretching of glycosidic C-O-C, (c) symmetric stretching of the C4-O-S, (d) asymmetric stretching of the C4-O-S, and (e) asymmetric stretching of the anhydride C-O-C.

Table 1. FTIR band assignments

Wavenumber (cm^{-1})[a]	Intensity[b]	Orientation[c]	Assignment[d]
704	m	// S	b[β(1→4)C-O-C] g.l. [16]
726	w	//	
735	w	⊥	b[α(1→3)C-O-C] g.l. [16]
*743	sh/w	⊥ S	
845	s	⊥ S	ν(C4-O-S) [16]
892	w	⊥	b(C1H or C6) [16,20,21]
*920	sh/w	⊥ S	
930	s	⊥ S	νs(C-O-C) anhydro [16]
970	m	// S	ν(C-O-C) g.l. [19]
1006	sh	⊥	α(1→3)C-O-C g.l. [16]
1126	m	No	νas(C-O-C) g.l. [16]
1156	s	// S	νas(C-O-C) g.l. [18,19]

[a] Wavenumbers with "*" are only apparent in the subtraction spectra.

[b] Abbreviations: s = sharp, m = medium, w = weak, sh = shoulder bands.

[c] Abbreviations: S = strong polarization dependence, No = no polarization dependence.

[d] Abbreviations: b = bending/deformation, v = stretching vibration, vs = symmetric stretching, vas = asymmetric stretching, g.l. = glycosidic linkage.

4. Conclusion

In conclusion, we found that polarized FTIR microspectroscopy can be a powerful tool to evaluate macromolecular orientation in κ-carrageenan fibers, because a number of bands, including those characteristic of κ-carrageenan at 845 and 930 cm^{-1} for, were shown to be dependent on IR polarization. Focused ion beam (FIB) micromilling was capable of sectioning the polymer fibers into longitudinal pieces of approximately 10 μm thick for polarized IR measurements in transmission mode. Compared with band assignments by previous researchers,

band assignments, including characteristic and principal κ-carrageenan bands, agreed well with results in this study.

Acknowledgement

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