A Novel Chiral Nematic Phase in Aqueous κ-Carrageenan

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ABSTRACT: A novel liquid crystalline phase has been identified in the κ-carrageenan/NaI/water system. Solutions of ultrasonically degraded carrageenan were prepared in 0.1 M NaI both by direct mixing and by osmotic compression, and a macroscopic phase separation into one anisotropic bottom phase and one isotropic top phase was found in the concentration range 5−11% (w/w). The properties of the anisotropic phase were investigated by small-angle X-ray scattering (SAXS), ^{23}Na NMR, and polarized light microscopy. The phase could be melted to a completely isotropic solution by heating to 65 °C, and the anisotropic phase slowly re-formed again on standing at room temperature, indicating that the phase is in thermodynamic equilibrium. Intact (nondegraded) κ-carrageenan failed to give macroscopic phase separation but yielded instead birefringent gels for concentrations higher than 2%.

Introduction

Rigid macromolecules in solution are expected[1,2] to form liquid crystalline phases beyond a certain concentration, and this behavior is confirmed for many different types of rodlike particles and rigid polymers: colloidal dispersions of rod-shaped clay particles, rodlike viruses, and polymers with a rigid backbone.[3,4] More closely related to the present work are rods where the rigidity is provided by a secondary helical structure, for example, DNA,[5] poly(benzyl L-glutamate) (PBLG),[5] schizophyllan,[6] and xanthan,[7] all of which show liquid crystalline behavior. The most common type of phase for helical macromolecules in concentrated solution is chiral nematic (cholesteric), but also nematic phases (without the chiral twist) have been reported. There are also reports of more exotic phases in DNA solutions: a "precholesteric" phase, just before the cholesteric in the phase diagram[8] and a columnar hexagonal phase[9] at higher concentrations.

Another feature, common among rigid polymers, is the tendency to form gels. Some of the above-mentioned polymers also form gels under certain circumstances, for example, PBLG,[10] TMV,[11] and DNA.[12] However, there are many well-known gelling polymers with a rigid chain where no liquid crystalline phase seems to have been reported. These include agarose, gellan, and collagen. The competition between phase separation and gelation is an interesting problem[13] that recently has been treated theoretically.[14] In PBLG, the gel was originally suggested to be directly related to the isotropic−nematic phase separation, and the thermoreversible gelation was interpreted[15,16] as a result of the entering into the wide two-phase region (in the classical Flory phase diagram[2]) at low temperatures (large interaction parameter). The current understanding of the PBLG system indicates, however, that also crystallization interferes in the gelation process[17,18] and that the system is rather complex, with many specific effects. The difficulties in experimentally veryfying the existence of a wide two-phase region in most systems may indicate a fundamental physical limitation. The possibility that crystalline phases could become more stable than the nematic phase as the polymer volume fraction approaches unity was suggested by Flory in his original paper,[2] and experimental verifications of the suppression of the two-phase region by crystallization has been reported by Balbi et al.[19] To our knowledge, (hydroxypropyl)cellulose is the only system where a widening of the isotropic−nematic two-phase region with increasing interaction parameter is well documented experimentally, without complications caused by gelling and/or crystallization.[20−22]

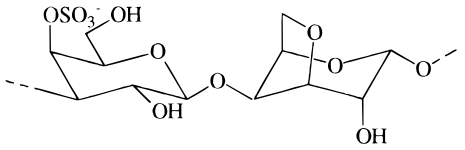

Figure 1. Repeating disaccharide unit of κ-carrageenan.

The present study is the result of a search for a liquid crystalline phase in aqueous solutions of a well-known gelling helical polysaccharide, κ-carrageenan. κ-Carrageenan is a sulfated galactan based on a repeating disaccharide unit (Figure 1). Although its rigid, helical secondary structure has been known since the sixties,[23] no clear observations of a liquid crystalline phase have been reported.[24] This is largely because most experimental investigations have dealt with conditions where gel formation or aggregation of the helices prevents the development of long-range liquid crystalline order. Grasdalen et al. reported long ago, however, that the κ-carrageenan molecule in the presence of iodide can adopt the helical conformation without gel formation.[27] This conclusion has been supported in recent studies in our own laboratory,[28,29] where we could not detect any aggregation of helices in dilute 0.1 M NaI solution. These findings suggest that, under these conditions, it should indeed be possible to obtain a liquid crystalline structure.

The aim of our study is to improve our understanding of the various modes of organization, aggregation, and gelation that are available to helix-forming polymers in solution. There is also a need for experimental studies of liquid crystalline polyelectrolytes[3] where the electrostatic interactions can be controlled by other means than just by addition of inert salt. The κ-carrageenan system is particularly well suited for these types of studies, since the strength of the helix−helix attraction can be tuned by the addition of certain salts.[30] The background

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to this ion specificity is that the κ-carrageenan helix is able to bind both certain cations (notably K^+, Rb^+, and Cs^+) and certain anions (I^- and SCN^-) specifically.[31−33] As the κ-carrageenan helix is negatively charged, the specific binding of cations or anions enhances or impedes, respectively, the helix−helix aggregation.

The molecular weight of semiflexible polymers has an important influence on the thermodynamic behavior. For rodlike particles, Onsager[1] and Flory[2] predicted that the critical concentration for the appearance of the liquid crystalline phase is roughly inversely proportional to the axial ratio of the rod. Indeed, in many polymer systems, the critical concentration is found to decrease with increasing molecular weight of the polymer.[34] The agreement with the theories of Flory and Onsager is often only qualitative, however, since a polymer chain is never completely rigid. Experiments also show that the critical concentration is independent of the chain length above a molecular weight corresponding to one Kuhn length.[35] The polymer flexibility can be included in the Flory lattice model by treating the chains as freely jointed chains with rigid segments of a length corresponding to the Kuhn length of the semiflexible chain.[36] The Onsager virial expansion for rods has also been extended to describe semiflexible polymers.[37,38] A comprehensive review of theories and experimental observations is given by Vroege et al.[3] A practical consequence of a high molecular weight is that the diffusion becomes very slow. The time needed for a concentrated solution to reach equilibrium is therefore very long. Some of the above-mentioned molecular weight effects will be qualitatively investigated in this study by comparisons between a high molecular weight (intact) and a degraded sample of the same polymer.

Experimental Section

Sample Preparation and Concentration Determination. The κ-carrageenan was donated by Sanofi-Bio Industries (ref 12698). Prior to use, it was ion-exchanged to the Na^+ form at 90 °C (cation exchange resin, Dowex-50W, 50 × 8−100, Sigma). A degraded sample was obtained by ultrasonication for 120 min, according to a procedure described earlier.[28] To remove titanium particles from the sonicating probe, the sonicated solution was filtered through a 0.45 μm Millipore filter. From light scattering studies of similar samples[28] in 0.1 M NaI, we estimate Mw to be 5×10^5 and 2×10^5 g/mol for the intact and degraded double helices, respectively. Dry powders of both the degraded and the intact samples were prepared by freeze-drying. The polymer concentrations are given as weight percent (%) throughout the paper.

Osmotic compression was performed according to a procedure described elsewhere.[39] A well-defined dextran (MW = 110 000 g/mol, from Leuconostoc ssp, Fluka, ref 31391) was used to control the osmotic pressure outside the dialysis bags. All experiments were done in 0.1 M NaI at room temperature to obtain the κ-carrageenan in the desired helical state, as verified by optical rotation in an earlier study.[29] Five solutions of dextran in the range 5−25% were prepared and stirred overnight. They were subsequently heated to 50 °C for 30 min to produce clear homogeneous solutions. The initial κ-carrageenan solution for the osmotic compression was 4% and was prepared by mixing the dry powder with 0.1 M NaI solution, stirring, and heating to 80 °C for 15 min.

The dialysis bags (Spectra/Por, with a molecular weight cutoff of 12 000−14 000 g/mol) were washed, soaked in Millipore water for 12 h, and equilibrated in 0.1 M NaI for 24 h before use. The bags were then filled with κ-carrageenan solution and plunged into the dextran solutions. After 1 month, during which time the dextran solutions were exchanged once and more κ-carrageenan solution was added to the bags, the bags were emptied. There were some signs of bacterial attack in the most concentrated dextran solution but the other solutions were clear and clean. The final concentrations, inside and outside the dialysis bags, were determined by optical rotation (after appropriate dilution) at 25 °C for dextran and at 80 °C for κ-carrageenan, using a JASCO DIP-360 digital polarimeter. From the dextran concentration, the corresponding osmotic pressure, Π, was calculated according to the recent determinations by Bonnet-Gonnet and Parsegian:[40]

$$\log \Pi\ (\mathrm{dyn \cdot cm^{-2}}) = 1.385 + 2.185 w^{0.2436} \qquad (w < 10\%)$$

$$\log \Pi\ (\mathrm{dyn \cdot cm^{-2}}) = 1.872 + 1.657 w^{0.3048} \qquad (w < 10\%)$$

where w is the dextran concentration in weight percent.

Solutions of intact κ-carrageenan were prepared by direct mixing and were observed during 1 year.

SAXS. Experiments were performed on a Kratky camera equipped with a position sensitive detector (OED 50M from MBraun). Cu Kα radiation of a wavelength 1.542 Å was used, and the sample to detector distance was 277 mm. The sample was held in a thermostated, 1 mm diameter, capillary holder. The anisotropic sample was subjected to the following sequence of temperatures: 17.8−11−5−11−17.8−25.6−29.2−31.3−37.6−45−50−65−17.8 °C. One hour of equilibration was allowed at each temperature before exposure, and the exposure time was 2 h in all experiments. The sample was then kept at 18 °C for 96 h, after which it was exposed once more. The introduction of the sample into the capillary with a syringe could, in principle, give rise to alignment of the sample, which could affect the scattered intensity measured on a one-dimensional detector. To examine this possible artifact, the experiment was repeated with a paste holder, where the sample is enclosed between two mica surfaces with much less shearing during the insertion. No difference in the shape of the scattering curve could be observed, but the scattered intensity was smaller in the paste holder since the amount of sample was smaller. The data presented here are the raw data from experiments with the capillary holder. No correction for smearing due to the Kratky setup has been performed.

Polarized Light Microscopy. The samples were observed between crossed polarizers in an Axioplan universal microscope (Zeiss, Germany) equipped with a camera. A thermostated board permitted temperature scans similar to the ones performed in the X-ray experiments. At each temperature, the sample was equilibrated for 10 min before the texture was photographed.

NMR. The ^{23}Na NMR experiments were performed on a Bruker DMX-200 spectrometer (^{23}Na resonance frequency 52.94 MHz) with a vertical 10 mm saddle coil probe and a 4.7 T wide-bore superconducting magnet. Experiments were also performed on a Bruker DMX-100 spectrometer (^{23}Na frequency 26.47 MHz) with a vertical saddle-coil probe and a 2.35 T wide-bore superconducting magnet. On both spectrometers, the magnetic field inhomogeneity was less than ca. 3 Hz and the temperature in the sample was constant to within ±0.05 °C. The ^{23}Na spectra were obtained from the single-pulse free induction decay following a 10 μs $\pi/2$ pulse, with an acquisition delay of 50 μs. Prior to acquisition, the sample was equilibrated at the appropriate temperature for about 30 min.

Results and Discussion

Intact Sample. When the intact κ-carrageenan was dissolved in 0.1 M NaI, isotropic solutions were obtained up to around 1%, where a weak, white and streaky birefringent pattern could be observed between crossed polarizers. The strength of the birefringence increased gradually with increasing concentration, and above 2% a colored birefringence was observed up to 3%. The samples were also analyzed between crossed polars in the microscope, but no texture could be observed. Due to the high viscosity and alignment effects on the glass walls of the slide and coverslip,[41] the development of the texture can be very slow in liquid crystalline polymer solutions, but even after several weeks, no

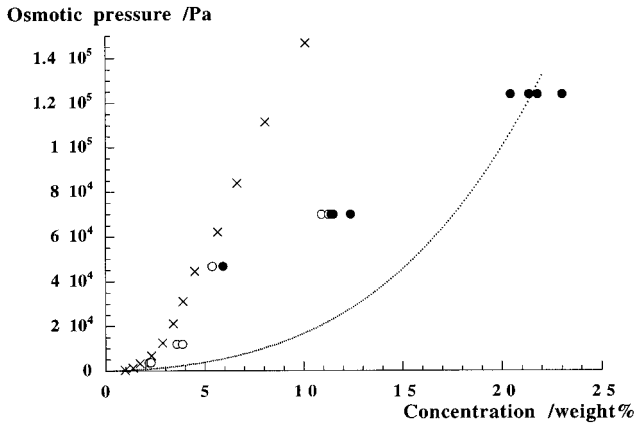

Figure 2. Osmotic pressure vs. concentration (in weight percent) of κ-carrageenan in 0.1 M NaI. Open and filled circles represent isotropic and birefringent samples, respectively. Data[40] for dextran (dotted line) and sodium polyacrylate in 0.1 M salt (×) are given for comparison.

microscopic texture developed in the intact κ-carrageenan samples. The weak birefringence seen between 1 and 2% disappeared after some months.

Another set of solutions was prepared with up to 5% κ-carrageenan in deuterated water and 0.135 M LiI. These solutions were birefringent above 3% and highly viscous. It was not possible to observe any signs of a macroscopic phase separation in these samples, even after centrifuging for more than 24 h at 4000*g*. To check for molecular anisotropy, ^{2}H, $^{7}Li^{+}$, and $^{127}I^{-}$ NMR experiments were made, but no splitting was observed in any case. However, this does not prove that the samples were void of anisotropic regions. The fraction of water molecules or Li^{+} ions sensing an isotropic environment could possibly be too small to give an observable splitting. For the iodide ions, which bind to the κ-carrageenan helix, the large quadrupole coupling may also yield a spectrum with a very large splitting and wide satellite lines, which may be difficult to observe.

Since the degraded polymer yields a macroscopic phase separation (cf. below), a phase separation is predicted at equilibrium also for the intact κ-carrageenan, possibly even at lower concentrations. However, the intact κ-carrageenan is evidently prevented from reaching macroscopic equilibrium. The more detailed investigations were therefore limited to degraded κ-carrageenan.

Degraded Sample. The degraded κ-carrageenan was easier to handle and higher concentrations could be reached by direct mixing. One 7% sample prepared by direct mixing in 0.1 M NaI was found to be birefringent and could be macroscopically separated by centrifugation into one isotropic and one anisotropic phase. Both phases were fluid and clear.

Osmotic compression was performed to prepare concentrated solutions isothermally, starting form a clear, 4% isotropic solution of helices. We thus prepared samples of up to 25% total concentration, and the experimental procedure permitted us to establish an osmotic pressure−concentration diagram, displayed in Figure 2. Except for the most concentrated sample (25%), clear, mono- or biphasic samples were obtained that flowed slowly when the sample tube was turned upside down.

At 5.5 and 11%, the solutions were biphasic, and upon centrifugation they readily separated into two phases. The concentrations of κ-carrageenan in the separate phases were determined. It is clearly seen in Figure 2 that the difference in concentration between the isotropic and the anisotropic solution is very small and that the concentrations of both phases vary with the total concentration of κ-carrageenan. In a true two-component system, the compositions of the phases in equilibrium should be constant in the biphasic region. The degraded κ-carrageenan is, however, polydisperse, and it is well known that polydispersity will smear out the phase transition over a wider concentration interval.[1,42] The longer polymers preferentially go to the anisotropic phase and the shorter enrich in the isotropic phase.

The reversibility of the compression was checked by dilution in 0.1 M NaI, and all samples, except the most concentrated one (25%), formed clear solutions. The 25% sample was also turbid and very stiff, and, as mentioned in the Experimental Section, there were signs of bacterial attack in the stressing dextran solution. No further investigations were done on this sample.

The liquid crystalline behavior reported here has some implications for the rigidity of the polymer constituent. The fact alone that we observe polydispersity effects in the two-phase region suggests that the degradation has reduced the contour length of (a significant fraction of) the helices to below the Kuhn length. This could be used to estimate a lower bond for the persistence length of κ-carrageenan helices. The phase boundaries could also be fitted to some of the theories mentioned in the Introduction, yielding a direct estimate of the persistence length. This would, however, require fractionated κ-carrageenan (to reduce the polydispersity effect) and detailed knowledge about the phase boundaries and is therefore saved for future investigations. Since the persistence length of the κ-carrageenan helix is not accurately known, this information would be valuable. We have assumed here that the κ-carrageenan double helix is the nematogenic structure, but at present we cannot exclude the possibility that superhelical aggregates, which are known to exist at other ionic conditions, could be formed at the high concentrations required for the creation of the nematic phase.[29]

Structure of the Anisotropic Phase at 18 °C. The anisotropic bottom phase with a κ-carrageenan concentration of 11.6% was analyzed by SAXS, ^{23}Na NMR, and polarized light microscopy. The small-angle scattering curve is displayed in Figure 3a together with the scattering curve from a 5% isotropic solution. The shape of the scattering curve of the anisotropic phase is similar to the one obtained in concentrated cholesteric DNA[9,43] and in cholesteric PBLG.[5] In Figure 3a, a very broad correlation peak is found at a scattering vector corresponding to approximately 45 Å, indicative of a local order on this length scale. Such a peak has been interpreted as originating from the local two-dimensional hexagonal array in the ordered phase.[5,44−46] From the position of this peak, the interhelical distance can be calculated to 52 Å ($d_{\text{helix-helix}} = d_{\text{peak}} \times 2/\sqrt{3}$). The isotropic solution at 5% gave a different scattering curve with no clear correlation peak.

In Figure 4a, we show the ^{23}Na powder spectrum of the 11.6% anisotropic phase obtained immediately after insertion of the sample in the probe. As expected for a spin $I = {}^{3}/_{2}$ nucleus in an anisotropic phase in a magnetic field, the ^{23}Na spectrum consists of three transitions.[47−49] The frequency of the central peak (ν_C) is unaffected by the orientation of the local domains in the magnetic field (θ_{LD}), thus producing a single sharp

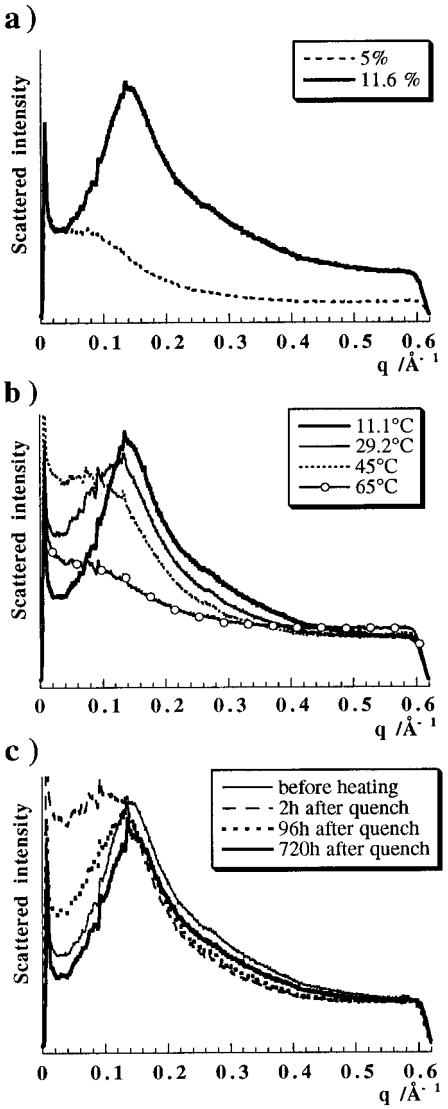

Figure 3. Scattering curves for κ-carrageenan in 0.1 M NaI: (a) 5% isotropic solution at 25 °C and 11.6% anisotropic phase at 17.8 °C; (b) 11.6% anisotropic phase after cooling to 11 °C, followed by heating to 29.2, 45, and 65 °C; (c) recovery of the scattering curve for the 11.6% anisotropic phase after quenching from 65 to 17.8 °C and equilibrating at that temperature during the times indicated. The curve obtained before heating is given for comparison.

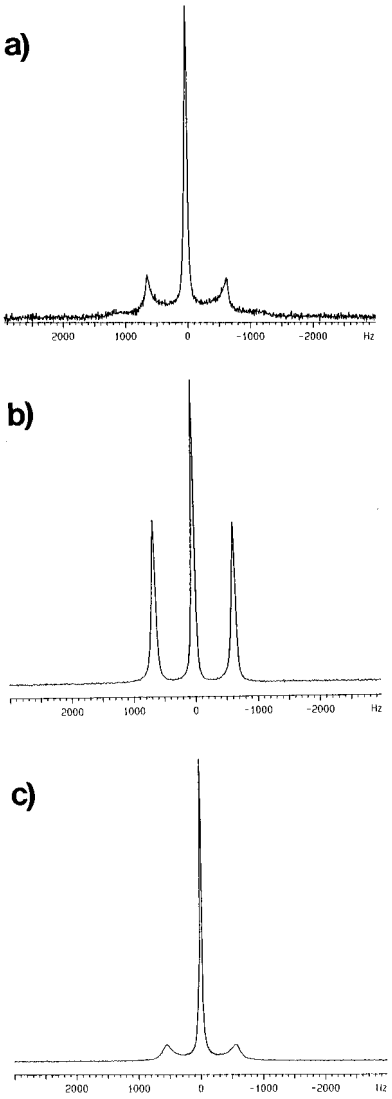

Figure 4. ^{23}Na NMR spectrum of the 11.6% anisotropic phase: (a) initially at 17.8 °C; (b) at 17.8 °C after 30 min in the 4.7 T magnetic field; (c) at 37.6 °C.

line at a frequency given by the Zeeman interaction. The frequencies of the two satellite transitions (ν_S), on the other hand, depend also on θ_{LD} through the residual (after molecular averaging) quadrupole interaction. For an anisotropic uniaxial phase, one thus obtains[47−49]

$$\nu_C = \nu_0 \tag{1a}$$

$$\nu_S = \nu_0 \pm \nu_Q^0(3\cos^2\theta_{LD} - 1)/2 \tag{1b}$$

where ν_0 is the frequency due to the dominant Zeeman interaction between the spins and the magnetic field.

In eq 1, ν_Q^0 is the quadrupole splitting observed from a monocrystalline sample with the director along the magnetic field ($\theta_{LD} = 0$). Depending on the macroscopic distribution of the microcrystalline domains in the sample, the distribution function of θ_{LD} in the sample, $f(\theta_{LD})$, varies from the monocrystalline limit to an isotropic powder. In the latter limit, the weighted superposition of Lorentzian peaks yields a so-called Pake powder spectrum,[50] which is the type of spectrum displayed by the two satellite transitions in Figure 4a. From the two $\theta_{LD} = 90°$ singularities in Figure 4a, we determine the ^{23}Na powder splitting to be ca. 0.65 kHz, which is a rather small value compared to lyotropic surfactant systems.[49] It is, however, within the range observed for other helical liquid crystalline polymers: 0−0.4 kHz for DNA (varying with the concentration and temperature[51]) and 1.2 kHz in salt-free liquid crystalline xanthan (18 g/L).[52] The small splitting is not unexpected since the present system contains excess salt; only a small fraction of the $^{23}Na^+$ ions are in the narrow anisotropic region near the surface of the κ-carrageenan strands (most of them are outside this region probing an isotropic environment). Due to the rapid exchange of Na^+ between these regions, the observed splitting is a weighted average, yielding a small splitting. Using Figure 4a, we conclude that the anisotropic phase (i) is initially a powder and (ii) has a uniaxial symmetry.

In Figure 4b, we show the ^{23}Na spectrum observed after leaving the sample for 30 min in the magnetic field. (Only a slight decrease of the line width of the satellites was observed at longer times and, after 2 h, no further changes could be detected.) This spectrum is markedly different from the one in Figure 4a. In particular, the satellite transitions are now located at two frequencies only ($\pm^1/_2\nu_Q^0$), which implies that the

Table 1. Spectral Parameters Derived from the Aligned ($\theta_{LD} = 90°$) ^{23}Na NMR Spectra vs Temperature

T/°C	$^1/_2\nu_Q^0$/kHz	$\Delta\nu_{1/2}^C$/Hz	$\Delta\nu_{1/2}^S$/Hz	I_C/I_S
11.1	0.62	48	73	
17.8	0.65	40	69	
21.8	0.64	34	67	0.70
25.6	0.64	32	59	
27.1	0.63	29	57	0.71
29.2	0.62	27	55	0.71
31.3	0.55	26	90	0.72
37.6	0.53	25	250	
uncertainty	±0.01	±3%	±3%	±0.05

local directors of the microcrystallites in the anisotropic phase have aligned perpendicular to the magnetic field ($\theta_{LD} = 90°$). The same behavior was observed for synthetic polynucleotides (DNA) while PBLG aligns parallel to the magnetic field.[53]

From a line-shape fit of three Lorentzian lines to the spectrum in Figure 4b, we obtained the width at half-height of the central line ($\Delta\nu_{1/2}^C$) and the satellites ($\Delta\nu_{1/2}^S$). In Table 1, we list these line widths together with the observed splitting ($^1/_2\nu_Q^0$) and the intensity ratio between the central peak and the satellites (I_C/I_S). We ascribe the small systematic deviation from the theoretical intensity ratio $I_C/I_S = 4/(3 + 3) \approx 0.67$ to the transverse relaxation during the dead-time of the receiver (50 μs) and the commonly encountered difficulties in achieving proper intensities from wide Lorentzian lines.

It was not possible to detect any dependence of the quadrupolar splitting on the magnetic field strength as reported by Bezemer et al.[52] for liquid crystalline xanthan. The same splitting was observed on the 2.35 and 4.70 T magnets.

Temperature Effects. By performing temperature scans with the experiments described above, the effect of temperature on the anisotropic phase could be observed. A lowering of the temperature from 18 to 11 °C resulted in small changes in the scattered X-ray intensities (Figure 3b), in the ^{23}Na splitting and relative intensities (Table 1), and in the texture seen in the polarizing light microscope (not shown). However, upon heating, large changes were observed in all experiments. The scattered intensity at low q increased and the peak moved to lower q values (Figure 3b). The texture in the polarized light microscope also changed upon heating. Around 30 °C, a grainy texture was observed (Figure 5a,b). The appearance of the NMR spectrum changed suddenly above 30 °C (Figure 4c) and the splitting also decreased (Table 1). It was also observed, by eye, that the sample turned turbid and that the viscosity increased when the temperature was raised.

These observations led us to suspect that the 11.6% anisotropic sample became biphasic at higher temperatures. The sample was therefore centrifuged at 40 °C and then separated macroscopically into two phases, one isotropic and one anisotropic. Biphasic behavior was observed up to approximately 60 °C, where a remainder of the texture could be seen in the polarizing microscope (Figure 5c). At 65 °C, no texture was observed (Figure 5d), the characteristic correlation peak in SAXS had completely disappeared (Figure 3b), and the form of the scattering curve was similar to that obtained for the isotropic 5% solution (Figure 3a). This indicates major structural rearrangements on the 1−100 nm scale and a loss of local order. The ^{23}Na NMR signal was also completely isotropic (without satellites) at 65 °C (not shown), and the solution was macroscopically isotropic when observed between two crossed polarizers.

Two effects may be responsible for the thermal destabilization of the anisotropic phase. Although the narrow two-phase region is theoretically predicted to be independent of temperature by the original Flory theory,[2] there are several experimental observations of a bending of the two-phase region toward higher concentrations as the temperature is increased.[54,55] This "thermotropicity" in lyotropic liquid crystals can be explained by a temperature dependence of the persistence length, first proposed by Miller et al. for PBLG in DMF,[10] but also other explanations have been provided.[55] The second effect is the melting of the helix, i.e., the gradual shift of the coil−helix equilibrium toward the coil conformation at higher temperatures. The conformational transition interval at these high concentrations is not accurately known, but preliminary extrapolations based on unpublished measurements at our laboratory indicate that the major part of the helix melting at 11% κ-carrageenan should occur just below 65 °C in 0.1 M NaI. Most likely, the helix-to-coil transition is therefore responsible for the ultimate disappearance of the anisotropic phase at 65 °C. However, it could be relevant also at lower temperatures. It should be remembered that the anisotropic phase studied here is the (separated) bottom phase from a biphasic sample and therefore that the concentration of κ-carrageenan is just at the border of the one-phase nematic region. Hence, an infinitesimally small perturbation (in, for example, temperature) should be sufficient to render this sample biphasic.

On cooling, the texture becomes visible in the polarizing microscope at approximately 50 °C (Figure 5e) and more clearly at 45 °C (Figure 5f). At 17.8 °C the texture has more contrast (Figure 5g). The differences in texture seen before and after heating (compare Figure 5a and 5f) indicate that the domains are smaller when they are re-formed, but this does not mean that the re-formed phase is of another type. Another sample was sealed in a flat 0.2 mm capillary, completely melted (80 °C), and then stored at 25 °C for several days. This preparation developed a fingerprint texture, typical for chiral nematic phases (Figure 6). The pitch of the chiral arrangement, p, can be estimated to approximately 20 μm from the distance between two black lines ($=p/2$) in the figure.[56] The pitch is usually concentration dependent.[5] When the pitch is in the range of the wavelength of visible light, selective absorption and scattering of the corresponding wavelength give rise to a strong coloring of the solution. In the present case, the pitch was outside the region of visible light, which was also confirmed by the absence of coloring. Pitches of the same order of magnitude have been reported for other chiral nematic solutions of helical polymers. Values varying from below the resolution of a light microscope to 2 μm have been reported for DNA,[57] and a value of 40 μm for xanthan.[57] Data for PBLG vary from 5 to 200 μm in various solvents.[5,57] It is well known that the texture of a chiral nematic phase is dependent on the orientation and on the presence of defects.[57−59] Observation of a fingerprint pattern can, however, be taken as a safe indication of a chiral nematic structure, since a nematic phase does not show this texture.[56]

The scattering curve obtained after only 2 h of equilibration at 18 °C is rather different from the one obtained before heating (Figure 3c). After 4 days of equilibration at 18 °C, however, the scattering curve

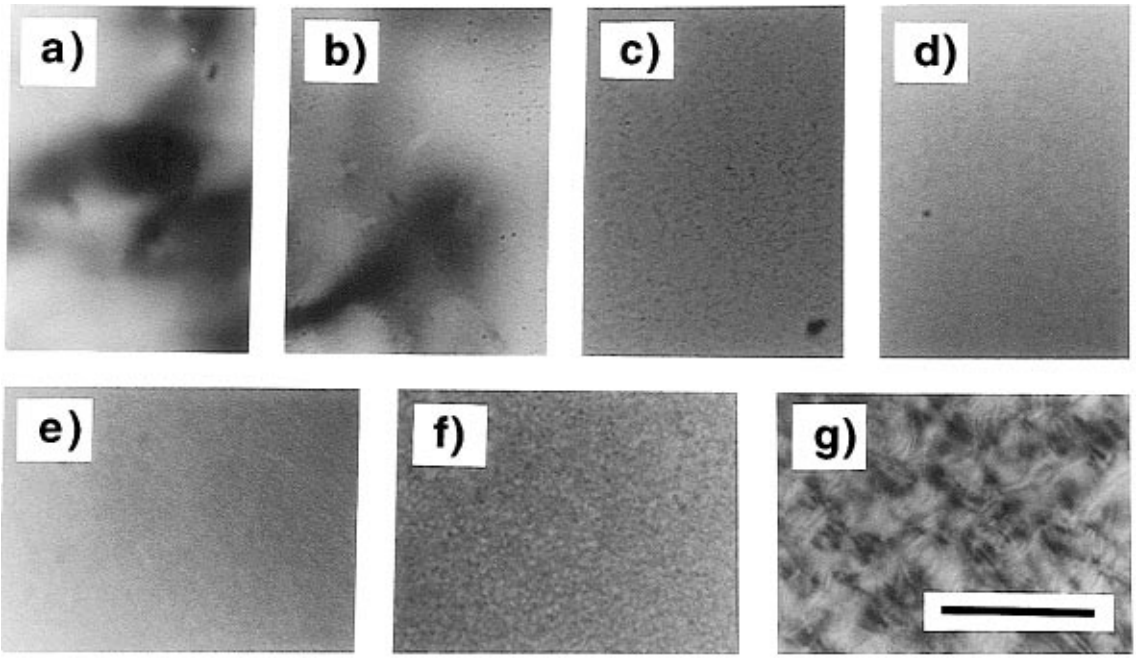

Figure 5. Polarizing microscope texture of the 11.6% anisotropic phase at different successive temperatures: (a) initially at 17.8 °C; (b) 29.2 °C; (c) 60 °C; (d) 65 °C; (e) 50 °C; (f) 45 °C; (g) 17.8 °C. The scale bar is 200 μm.

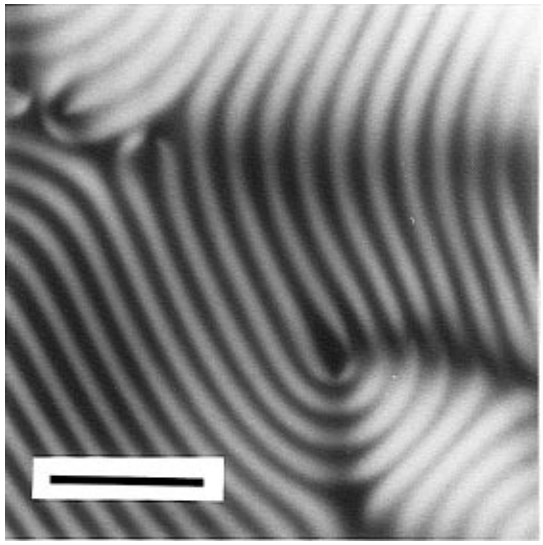

Figure 6. Polarizing light microscope picture of the 11.6% anisotropic phase after heating to 80 °C and cooling to room temperature (25 °C) and aging for several days. The scale bar is 50 μm.

approached the shape originally obtained and finally, after 1 month of equilibration at room temperature, the shape of the scattering curve was identical to the original curve. The reduction in the overall intensity at this last measurement (compared to the original scattering curve) may be an instrumental effect, caused by changes in the sample holder position or X-ray intensity, during the long equilibration time. The results demonstrate that the sample regains its original local structure very slowly, at least on the length scale probed by SAXS.

The NMR spectrum also returned to its original line shape after 5−6 days of equilibration at room temperature. A sample heated to 65 °C in the NMR magnet and then quenched to 17.8 °C permitted the observation of the slow relaxation to equilibrium structure. Spectra were recorded every hour during 48 h and then occasionally during the 5 following days. None of the recorded spectra had a typical powder shape, as was observed before heating (Figure 4a). Instead wide, Lorentzian satellites were formed within 1−2 h at the equilibrium splitting. The satellites then slowly narrowed to finally (after 5−6 days) reach the equilibrium spectrum. The molecular interpretation of this slow relaxation is not straightforward, but the orientation of the domains cannot be the rate-determining step, since no powder shape was observed. Rather, we believe that the slow process is due to recovery of the local structure, which is consistent with the SAXS results showing that after 4 days, the scattering curve had not yet obtained its original shape (Figure 3c).

Concluding Remarks

In light of the experimental evidence presented here, there can be no doubt about the existence of a liquid crystalline phase in the κ-carrageenan system. The phase is observed above approximately 5% up to at least 11.6% of degraded κ-carrageenan ($MW_{\text{double helix}} = 2 \times 10^5$ g/mol) in 0.1 M NaI. It is fluid, clear, and birefringent and has a chiral nematic structure. The experimental observations described above also show that the nematic phase can be melted and re-formed again upon cooling. This demonstrates that it is a thermodynamically stable phase. The melting of the nematic phase is fast (minutes) but the reverse transition is much slower (5−6 days).

The conditions giving the nematic phase have not been investigated in earlier work on κ-carrageenan, where the interest has been focused on ionic conditions where gelling prevents the development of liquid crystalline order. The relation between the nematic phase and the gel is an important question for further investigations.

Interestingly, no macroscopic phase separation could be obtained with the intact κ-carrageenan. Instead a birefringent gel is obtained above 2%. Similar observations have been made on other systems. High molecular weight xanthan forms a birefringent, highly viscous phase that cannot be separated macroscopically by centrifugation.[41,60−62] Upon degrading the xanthan, a

macroscopic separation can easily be obtained.[35,60] It is believed that the macroscopic phase separation is hindered by the high viscosity of the solution but that the solution is biphasic, with anisotropic domains dispersed in an isotropic matrix.[61] Inatomi et al.[35] succeeded, however, in performing macroscopic separation of xanthan up to molecular weights comparable to the intact κ-carrageenan used in this study. As for DNA, the studies are typically performed on biphasic systems,[43,51,63,64] and we have found no report of a macroscopic phase separation, irrespectively of the molecular weight. The birefringence in the intact κ-carrageenan solution could also have another origin. The helices may be immobilized below the critical concentration for phase separation, forming a "birefringent glassy phase" with ordered regions, as reported by Buining et al.[65] for boehmite rods in aqueous solution. Glass transitions of rigid molecules have recently been treated by Edwards et al.[66]

Several authors have suggested that the coil−helix transition can be coupled to a nematic−isotropic phase transition, and theories have been developed to describe this phenomenon.[67−72] The theories predict, among other things, that the cooperativity of the coil−helix transition could be enhanced if the helix is subsequently involved in an isotropic−nematic phase transition. There are only a limited number of experimental observations of the coupling; for example, the reversible disappearance and reappearance of birefringent domains upon heating and cooling of xanthan[61] and DNA.[73] To our knowledge, there are no experimental verifications of the effects of the phase transition on the cooperativity of the coil−helix transition. The κ-carrageenan system would possibly lend itself to such investigations.

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