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Characterization of Semidilute κ-Carrageenan Solutions

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ABSTRACT: We have characterized dilute and semidilute κ-carrageenan solutions in the coil conformation (0.1 M NaCl, 20 °C) using static and dynamic light scattering, and viscometry. We have determined the power law concentration dependence of the osmotic compressibility, the correlation length, and the viscosity in the semidilute regime. The results show that κ-carrageenan in the coil conformation behaves like swollen flexible polymers. The power law exponents are close to the theoretical predictions even if the correlation length is less than the persistence length.

Introduction

κ-Carrageenan is a polysaccharide extracted from red algae and is widely used in the food industry as a gelling agent.[1,2] It forms a thermoreversible gel on cooling below the temperature where the conformation changes from a random coil to a helix.[3,4] The ionic strength and the nature of the counterions regulate this conformational change and, consequently, the gelation process.[5,6] The presence of κ-carrageenan in the coil conformation increases the rate of heat-induced aggregation and gelation of globular proteins.[7,8] Recently, we observed that semidilute solutions of κ-carrageenan in the coil conformation causes phase separation and gelation of protein aggregates even at room temperature.[9] It is obvious that a proper understanding of these phenomena necessitates the characterization of semidilute κ-carrageenan solutions.

As far as we are aware, κ-carrageenan has so far only been characterized in dilute solutions. Here we present results of an investigation of κ-carrageenan in the coil conformation using static and dynamic light scattering and viscometry over a range of concentrations covering both the dilute and semidilute regime.

Light scattering experiments on semidilute κ-carrageenan solutions is complicated by the presence of a small amount of aggregated material. Even if the weight fraction of the aggregates is very small, the scattering by the aggregates may be important. In dilute solutions the aggregates can be removed by careful filtration. However, the very viscous semidilute solutions cannot be filtered through small pore size filters, and the aggregates cannot be removed completely. This experimental difficulty is probably one of the reasons for the lack of light scattering studies at higher concentrations. The problem is not limited to κ-carrageenan, but to many other systems that contain aggregates or other spurious scatterers. We will show that nevertheless a proper characterization of semidilute solutions is possible by a combination of static and dynamic light scattering. We will compare the results with theoretical predictions for semidilute solutions of flexible polymers, which we briefly review in the next section.

Theory

The isotropic scattered light intensity of binary solutions of flexible macromolecules is due to concentration and density fluctuations.[10] In dilute and semidilute solutions of macromolecules the density fluctuations may be equated with the solvent scattering so that the scattering intensity due to concentration fluctuations (I) can be obtained by subtracting the latter from the total intensity. For practical reasons one measures the relative intensity compared to a standard like toluene (I_r). I_r is related to the osmotic compressibility ($d\pi/dC$) and the z-average structure factor ($S(q)$):

$$I_r = KC\frac{RT}{d\pi/dC}S(q) \qquad (1)$$

with R the gas constant and T the absolute temperature.

$$K = \frac{4\pi^2 n^2}{\lambda^4 N_a}\left(\frac{\partial n}{\partial C}\right)^2\left(\frac{n_s}{n}\right)^2\frac{1}{R_s} \qquad (2)$$

where N_a is Avogadro's number, λ is the wavelength of the incident light, $(\partial n/\partial C)$ is the refractive index increment, and R_s is the Rayleigh ratio of the standard. $(n_s/n)^2$ corrects for the difference in scattering volume of the solution with refractive index n and the standard with refractive index n_s. $S(q)$ describes the dependence of I_r on the scattering wave vector: $q = (4\pi n/\lambda)\sin(\theta/2)$, with θ the angle of observation.

$S(q)$ is related to the Fourier transform of the pair correlation function of the concentration fluctuations. The distance over which the concentration fluctuations are correlated is characterized by the so-called screening length ξ_s, and $S(q)$ may be written as a series expansion in terms of $q\xi_s$:

$$S(q) = (1 + (q\xi_s)^2 + ...)^{-1} \qquad q\xi_s < 1 \qquad (3)$$

The expansion is done for the inverse of $S(q)$ because for flexible polymers the higher order terms can be neglected up to larger values of $q\xi_s$. In very dilute solutions ξ_s is proportional to the z-averaged radius of gyration: $\xi_s = R_{gz}/\sqrt{3}$. At higher concentrations ξ_s decreases due to repulsive interactions between segments of overlapping polymers, and in semidilute solutions it scales with the concentration: $\xi_s \propto C^{-\alpha}$. Scaling arguments relate α to the so-called fractal dimension of the polymers (d_f): $\alpha = (3 - d_f)^{-1}$.[11] The fractal dimension relates the radius of gyration (R_g) to the molar mass (M): $M \propto R_g^{df}$. The fractal dimension of flexible linear polymers in good solvents is 1.7[12] so that $\alpha = 0.77$.

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In dilute solutions the osmotic compressibility may written in terms of a virial expansion:

$$\frac{d\pi}{dC} = RT\left(\frac{1}{M_w} + 2A_2C + ...\right) \tag{4}$$

where M_w is the weight-average molar mass, and A_2 is the second virial coefficient. In semidilute solutions $d\pi/dC$ scales with the concentration: $d\pi/dC \propto C^{\beta}$. Scaling arguments relate β to the fractal dimension of the polymers:[11] $\beta = d_f/(3 - d_f)$. In good solvents the prediction is thus $\beta = 1.31$.

With dynamic light scattering (DLS) one measures the normalized intensity autocorrelation function: $g_2(t) = \langle I(0)\ I(t)\rangle/\langle I\rangle^2$.[13] Usually, $g_2(t)$ is related to the normalized electric field autocorrelation function ($g_1(t)$): $g_2(t) = 1 + g_1(t)^2$. $g_1(t)$ is a measure of the relaxation of the concentration fluctuations. If $S(q)$ is close to unity, this relaxation is due to cooperative diffusion, and the relaxation rate (Γ) that characterizes the exponential decay of $g_1(t)$ is related to the cooperative diffusion coefficient (D_c): $\Gamma = D_cq^2$. D_c may be written as[13]

$$D_c = \frac{(1 - C/\rho)^2}{f}\left(\frac{d\pi}{dC}\right) \tag{5}$$

where f is the friction coefficient and ρ is the density of the polymers.

In dilute solutions we may use eq 4, and $f^{-1} = f_0^{-1}(1 - k_fC)$ so that D_c becomes

$$D_c = D_0(1 + k_dC) \tag{6}$$

with $k_d = 2MA_2 - 2/\rho - k_f$. D_0 is related to the hydrodynamic radius (R_h) as $D_0 = k_BT/6\pi\eta_sR_h$ with η_s the solvent viscosity and k_B Boltzmann's constant. If the polymers are polydisperse, $g_1(t)$ is characterized by a relaxation time distribution corresponding to the distribution of sizes. In this case one usually calculates the z-averaged diffusion coefficient using the z-averaged relaxation rate Γ_z. At higher concentrations hydrodynamic interactions are screened by the presence of segments from overlapping polymers. D_c may be written in terms of the so-called dynamic screening length (ξ_d) which is proportional to ξ_s:

$$D_c = (1 - C/\rho)^2\,\frac{k_BT}{6\pi\eta_s\xi_d} \tag{7}$$

In very dilute solutions ξ_d equals the z-average hydrodynamic radius (R_{hz}). Polydispersity does not influence ξ_d if it is smaller than R_h of the smallest polymers in the solution.

The viscosity of semidilute polymer solutions increases strongly with increasing concentration due to formation of entanglements.[14] Entanglements act as temporary cross-links, and the viscoelastic behavior of entangled polymers is like that of a transient gel. The number of entanglements is proportional to the number of binary contacts which means that in good solvents the average distance between entanglements (ξ_e) is proportional to ξ_s and ξ_d. The viscosity is related to the gel modulus (G_0) and the terminal relaxation time (τ_r) of the transient gel: $\eta = \tau_rG_0$. G_0 is proportional to the number of elastically active chains, i.e., $G_0 \propto \xi_e^{-3}$, and the terminal relaxation time is the time needed for the polymers to disentangle. In the so-called tube model it is assumed that the polymers disentangle by reptation of the chains through a tube formed by the entanglements. Application of this model to semidilute solutions in good solvents leads to $\tau_r \propto \xi_e^{3(1-d_f)}$ and therefore $\eta \propto \xi_e^{-3d_f}$.[11] From the concentration dependence of the screening length in good solvents it follows that the viscosity of entangled polymer solutions is expected to scale with C: $\eta \propto C^{\gamma}$, with $\gamma = 3d_f/(3 - d_f)$. Using again $d_f = 1.7$, the prediction is $\gamma = 3.9$.

The lower concentration limit where polymers may be considered to be semidilute is determined by the overlap concentration (C^*). The upper concentration limit is determined by the condition that the screening length is sufficiently large so that the polymer chain is still swollen on this length scale and characterized by the fractal dimension $d_f = 1.7$. The concentration where the polymers start to entangle (C_e) is larger than C^* because several binary contacts are necessary to form an entanglement.[15]

Experimental Section

Materials. The κ-carrageenan used for this study is an alkali treated extract from *Eucheuma cottonii* supplied by SKW Biosystems (Baupte, France). The solutions were prepared as follows. A freeze-dried sample of κ-carrageenan in the sodium form was dissolved while stirring a few hours in hot Millipore water (70 °C) with 200 ppm sodium azide added as a bacteriostatic agent. The pH was adjusted to 9 to eliminate the risk of hydrolysis during preparation. The solution was extensively dialyzed against Millipore water containing 0.1 M NaCl. A viscous stock solution was slowly filtered through 0.45 μm pore size Millipore filters. The concentration of the stock solution (14.3 g/L) was determined after filtration by measuring the refractive index. Solutions with higher concentrations were prepared without filtration for viscosity measurements. A separate series of low concentrations ($C < 1$ g/L) were filtered through 0.2 μm pore size Anotope filters. Filtration through 0.2 μm pore size filters was not possible at higher concentrations.

Methods. Light scattering measurements were made using an ALV-5000 multibit, multitau, full digital correlator in combination with a Malvern goniometer and a Spectra-Physics laser emitting vertically polarized light at $\lambda = 532$ nm. The temperature was set at 20 °C and controlled by a thermostat bath to within ±0.1 °C. We have used a toluene standard with Rayleigh ratio 2.79×10^{-5} cm^{-1} at $\lambda = 532$ nm.[16] We found for the refractive index of κ-carrageenan 0.145 g/mL after taking into account the water content, which was about 15%.

The viscosity was measured using the Low Shear 40 rheometer from Contraves for $C < 12$ g/L or the AR1000 rheometer from TA Instruments for $C > 12$ g/L.

Data Analysis. The correlation functions were analyzed in terms of a distribution of relaxation times:

$$g_1(t) = \int A(\log\tau)\exp(-t/\tau)\ d\log\tau \tag{8}$$

using the computer routine REPES.[17] Figure 1 shows the relaxation time distributions at different concentrations for a given angle of observation and at different angles of observation for a given concentration. The area $A(\log\tau)d\log\tau$ is proportional to the scattering intensity. The relaxation time distributions show a well-defined peak at short times plus a number of less well-defined peaks at longer times. The relative amplitude of the fast peak increases with increasing angle of observation and decreasing concentration. The solutions at $C < 1$ g/L that were filtered through 0.2 μm pore size filters only showed one peak. The results at different angles of observation are plotted as a function of $q^2\tau$ and show that the relaxation processes are diffusive. We think that the fast peak is caused by the cooperative diffusion of κ-carrageenan chains, while the relaxation modes at longer times are due to self-diffusion of a broad distribution of large aggregates. At low concentrations

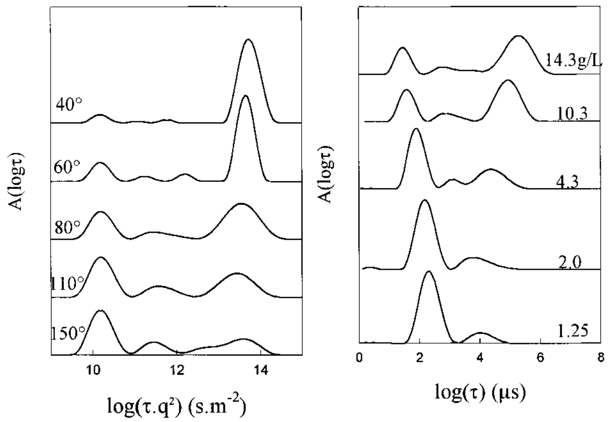

Figure 1. Relaxation time distributions of κ-carrageenan solutions for $C = 10.3$ g/L at different scattering angles (left) and at $\theta = 80°$ for different concentrations (right). The data at different scattering angles are plotted as a function of $\log(\tau q^2)$. In this representation relaxation processes due to translational diffusion are independent of the scattering angle.

the aggregates can be removed by filtration without a significant reduction of the concentration which means that the weight fraction of the aggregates is negligible. The contribution of the aggregates to the scattering intensity is nevertheless important because it is proportional to their molar mass, which is several orders of magnitude larger than that of the κ-carrageenan chains. The relaxation time of the aggregates increases strongly with increasing concentration because the viscosity of the solution increases. The relative amplitude the slow modes decreases with increasing angle of observation because the structure factor of the large aggregates is a strongly decreasing function of q in the range covered in the experiments. It also decreases with decreasing concentration, because the scattering of semidilute polymers increases with decreasing concentration while the scattering of the aggregates is roughly proportional to the concentration.

We have calculated the intensity scattered by the κ-carrageenan chains without the contribution of the aggregates by multiplying the total scattered intensity with the relative amplitude of the peak at short times. The intensity obtained in this way was almost independent of q, which is expected because $q\xi_s$ is much smaller than unity at high concentrations; see below. ξ_d was calculated using the z-average relaxation rate of the peak at short times.

Results and Discussion

Static and dynamic light scattering measurements were done on κ-carrageenan solutions over a range of concentrations between 0.1 and 15 g/L. Dynamic light scattering shows a fast mode and an ill-defined slow mode; see Experimental Section and Figure 1. The contribution of the slow mode is much reduced after filtration and can even be removed by filtration through 0.2 μm filters. The latter procedure is unfortunately not possible for the more viscous solutions at higher concentration. It shows, however, that the slow mode is not due to reversible association. The scattering intensity does not evolve in time, which shows that the slow mode is not caused by irreversible aggregation that usually follows the coil−helix transition. We believe that the slow mode is due to a small weight fraction of κ-carrageenan aggregates in the powder that do not dissociate when we prepare the solution. Using the data analysis described above, we can correct for their contribution to the scattering and in the following we will discuss only the scattering of the κ-carrageenan chains.

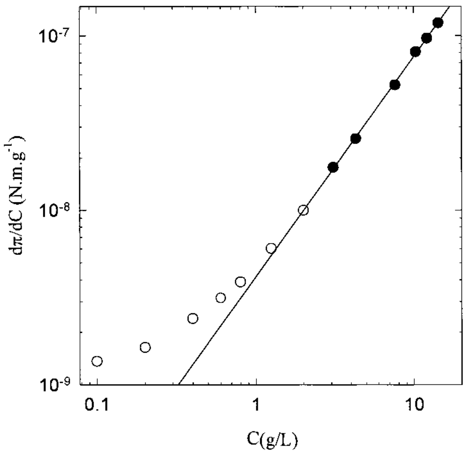

Figure 2. Double-logarithmic representation of the osmotic compressibility as a function of the concentration. The solid line represents a linear least-squares fit to the high concentration data (filled symbols).

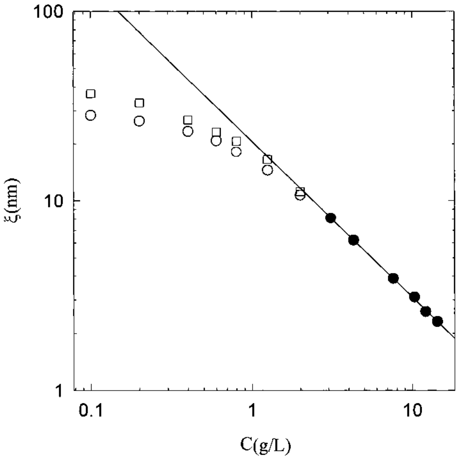

Figure 3. Double-logarithmic representation of the static (squares) and dynamic (circles) screening lengths as a function of the concentration. The solid line represents a linear least-squares fit to the high concentration data (filled symbols).

The osmotic compressibility and static screening length were determined using eqs 1−3. At low concentrations higher order terms in eq 3 could not be neglected over the whole experimental q range, and we used only the data at low q values. Γ_z is proportional to q^2 except at low concentrations where internal modes cannot be neglected over the whole q range. In those cases we used the data at low q values.

Figure 2 shows a double-logarithmic representation of the concentration dependence of the osmotic compressibility. The weight-averaged molar mass and the z-averaged second virial coefficient were calculated from the initial concentration dependence:[18] $M_w = 4.3 \times 10^5$g/mol and $A_2 = 4.5 \times 10^{-3}$ mL mol g^{-2}. At higher concentrations the data have a power law dependence on the concentration. A linear least-squares fit of the data at the highest concentrations gives

$$\log(d\pi/dC) = (1.28 \pm 0.02)\log(C) - (8.38 \pm 0.02) \qquad C > 3 \text{ g/L} \quad (9)$$

where the error is the standard error of the fit.

The concentration dependence of the static and dynamic screening lengths is shown in Figure 3. Extrapolation to $C = 0$ gives $R_{gz} = 72$ nm and $R_{hz} = 30$ nm, and the initial concentration dependence of D_c gives $k_d = 780$ mL/g.[18] For $C > 1$ g/L, ξ_s becomes small and cannot be measured accurately with light scattering. On the

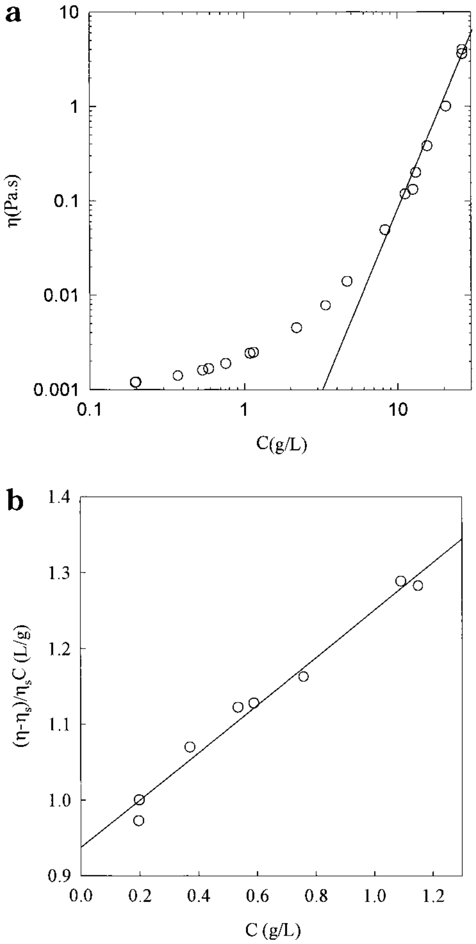

Figure 4. (a) Double-logarithmic representation of the viscosity as a function of the concentration. The solid line represents the theoretical prediction for semidilute, flexible polymers in a good solvent. (b) Initial concentration dependence of $(\eta - \eta_s)/C\eta_s$. The solid line represents a linear least-squares fit.

other hand, ξ_d can be measured over the whole range and has a power law dependence on the concentration at high concentrations. A linear least-squares fit of the data at the highest concentrations gives

$$\log(\xi_d) = (-0.83 \pm 0.02)\log(C) + (1.32 \pm 0.02) \qquad C > 3\ \text{g/L} \quad (10)$$

A rough estimate of the overlap concentration may be calculated using $C^* = 3M_w/4\pi N_a R_{gz}^3$, which gives $C^* = 0.45$ g/L. However, the κ-carrageenan sample is rather polydisperse which broadens the transition to the semidilute regime.

The concentration dependence of the viscosity is shown in Figure 4. The intrinsic viscosity, $[\eta]$, and the Huggins constant, K_H, were obtained from the initial concentration dependence:

$$\frac{\eta - \eta_s}{\eta_s C} = [\eta](1 + K_H[\eta]C) \qquad (11)$$

We found $[\eta] = 940$ mL/g and $K_H = 0.39$; see Figure 4b. An alternative estimate of C^* is obtained using $C^* = [\eta]^{-1}$, which gives $C^* = 1.1$ g/L. Scaling behavior is again observed, but at higher concentrations than for $d\pi/dC$ and ξ_d, which is expected because $C_e > C^*$. We do not have sufficient data in the scaling regime to justify a fit. Nevertheless, the straight line shows that the data are consistent with the predicted behavior for semidilute, good solvent, solutions of entangled polymers: $\gamma = 3.9$. Experimentally, a somewhat stronger concentration dependence ($\gamma = 4.2$) is observed for flexible synthetic polymers, which would also be compatible with our results for κ-carrageenan.

The dilute solution characteristics are coherent with those obtained by Slootmaekers et al. on a lower molar mass sample at the same ionic strength.[19,20] The properties of semidilute solutions of κ-carrageenan in the coil conformation are apparently in good agreement with theoretical predictions for flexible polymers in good solvents. However, it is not evident that the theory is applicable to our results. As mentioned above, the theory assumes that the screening length is large enough so that the polymers may be considered as swollen flexible chains on this length scale. The range of ξ_s where the scaling behavior is observed is from 9 nm down to 2 nm. The persistence length of κ-carrageenan was estimated to be 6.8 nm[19] at very high ionic strength and may be even larger at 0.1 M. This means we have to conclude that the agreement with the theory is fortuitous. A similar fortuitous agreement has been noted earlier for polystyrene in good solvents.[21]

In conclusion, we can say that the viscosity and light scattering of semidilute κ-carrageenan solutions in 0.1 M NaCl are very similar to that of flexible polymers in good solvents even if the persistence length is larger than the correlation length.

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