

Brillouin Scattering Study of Polyethylene Glycol/Water System below Crystallization Temperature

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We present the results of a Brillouin scattering experiment on aqueous mixtures of polyethylene glycol polymer cooled below their crystallization temperature. The shape of the registered spectrum changed considerably when the phase transition occurs. The Brillouin profile consisted of two peaks which has been interpreted as a result of the scattering of light on the local regions exhibiting different compressibility. Examination of the temperature dependence of the two Brillouin peak positions allows the identification of the specific microstructures present in the studied system. The existence of a complexlike hydration structure, stable against dilution and temperature agitation, was suggested. The nature and the behavior of the hydration complex is discussed in view of the earlier investigations in similar systems.

1. Introduction

Investigation of the local structure of aqueous polymeric solutions is of great scientific, as well as technological, importance. The motivation comes from the interest in the role of hydrogen bonding in the peculiar properties of water and its ability to interact with polymer chains, resulting in large cluster formation or networking. Aqueous solutions of relatively small amphiphilic molecules can be used as model systems to understand how hydrogen bond interaction can produce mesoscopic structures that are robust against the system composition over a wide range of temperatures. The formation of very strong and stable structures, even though they are made up of individually weak bonds, plays a main role both in the structure and the function of any biological system.

Poly(ethylene glycol) (PEG) represents a very good model system for the study of water structuring effects in macromolecular solutions. The polymeric chain of PEG is able to adopt a number of structural arrangements in the presence of water, including the establishment of local configurations nonaccessible to its anhydrous phase. As an example, the interaction with water can lead to formation of helical structures, micelization, or gelation, depending on the concentration and temperature conditions.¹ Most of the unique properties of PEG are directly related to the peculiar molecular structure of its chain. As indicated by the structural formula, $\text{OH}-(\text{CH}_2\text{CH}_2-\text{O})_n\text{H}$ (n = degree of polymerization), the hydrophobic ethylene units are separated along the polymer chain by hydrophilic oxygen atoms. As a consequence, each chain has a big number of hydrogen bonding sites, which allows the formation of inter- as well as intramolecular hydrogen bonds.² The origin of the hydrogen bonding sites (located at every monomeric unit) are the two unshared outer electronic pairs of either oxygen atom. Such electronic structure allows the development of up to two hydrogen bonds, meaning that up to two water molecules can be directly bonded to each oxyethylene unit.^{1,3–6} Valuable information about water-binding properties of aqueous PEG solutions can be obtained from various methods, including light scattering,^{7–9} ultrasonic,¹⁰ viscosity,⁷ nuclear magnetic resonance

(NMR),^{1,11} and differential scanning calorimetry (DSC),^{1,12} as well as computer simulations.^{13,14} Depending on the technique applied, an estimated number of water molecules participating in the hydration of each polymer repeat unit ranged from 1 to 4.

In the current paper, we present the results of a Brillouin light-scattering experiment. This experimental technique focuses on the analysis of the light inelastically scattered on the thermally induced sound waves (phonons), which propagate through a medium at a velocity that depends on the sample density and rigidity (expressed in terms of the storage longitudinal modulus). Motivated by the technological applications of PEG/H₂O mixtures, all of the previous Brillouin spectroscopy experiments in this system concerned its liquid phase. The method has been found particularly useful in the study of high-frequency mechanical properties of the PEG/H₂O systems, furnishing information about the structure and the dynamics of the polymeric chain in solution.^{8,9,15,16} Recently, however, much attention was generated by the study of PEG/H₂O system cooled down below crystallization temperature because some specific phase transition events (so-called cold crystallization, in particular) correlate well with the biofunctionality of the system.⁶ In addition, the low temperature behavior of aqueous solution of polyethylene glycol has attracted much attention because of their application in cryopreservation of biomedical samples.^{17–19}

When the uniform liquid solution is cooled down below its melting point, the sample phase separates and partially crystallizes. The appearance of local regions that differ in refractive index makes the sample opaque, and multiple light-scattering occurs within the sample. This phenomenon introduces a serious difficulty in using the Brillouin light-scattering technique. From a purely technical point of view, the problem concerns the appearance of strong, elastic scattering, which needs to be efficiently cut out from the spectrum to register just the inelastic component (which is a few orders of magnitude weaker than the elastic one). The major problem, however, is related to the loss of the experimental geometry usually defined by the scattering vector magnitude, q . When the multiply (elastically) scattered light interacts finally with a phonon, its direction and polarization are already random, and the inelastic part of the spectrum contains the contributions from all the scattering

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geometries from the forward- to the backscattering. The spectrum registered under such conditions differs distinctly from the one acquired in transparent media. To the best of our knowledge, there is no analytical formula for a Brillouin spectrum obtained in bulk opaque materials.

In this article, we show experimental results of a Brillouin scattering performed on aqueous mixtures of polyethylene glycol polymer cooled below their crystallization temperature, which, to our best knowledge, is the first experiment of this kind. The analysis of the frequency position of the features observed in the light-scattering spectrum allowed us to indicate specific microstructures present in the examined system. Obtained results correlate well with those from other experimental techniques used to investigate similar systems.

2. Experimental Methods

Poly(ethylene glycol) (PEG) of mean molecular mass 400 g/mol was obtained from Fluka Chemie GmbH and was used without any further purification procedure. The polydispersity index of the polymeric sample was 1.19. Binary mixtures of PEG400 in double-distilled and -deionized water were prepared as weight fractions, w . The samples studied in this work were mixtures of three different concentrations, namely, $w = 0.41$, 0.56 , and 0.82 , as well as pure mixture constituents (PEG400 and water). Because the PEG molecule is composed of the same hydrophilic monomeric units, it is more convenient to express the concentration in terms of a monomer molar fraction, x' , defined as

$$x' = \frac{n_{\text{OE}}}{n_{\text{OE}} + n_{\text{H}_2\text{O}}} \quad (1)$$

n_{OE} being the mean number of oxyethylene units of PEG chains in solution, and $n_{\text{H}_2\text{O}}$, the number of the water molecules. This concentration scale indicates directly how many water molecules are for each ethyleneoxide group. The corresponding monomer molar fractions of the binary solutions were $x' = 0.21$, 0.33 , and 0.65 .

During the Brillouin scattering experiment, the samples were contained in the coldfinger thermostat whose temperature was controlled to an accuracy of ± 0.1 K. The vertically polarized line ($\lambda_0 = 532$ nm) of a Coherent DPSS 532 laser, working at a mean power of about 80 mW, was used as the probe. The randomness of the polarization of scattered light was checked by making a measurement using VV and VH experimental setups. The obtained spectra were almost identical in shape. We then used the VU geometry (the scattered light was collected without unnecessary polarizer in front of the spectrometer), which also allowed reduction of the spectrum acquisition time. The scattered light was collected in the backscattering geometry ($\theta = 180^\circ$) and analyzed by a Sandercock-type (3 + 3) pass Tandem Fabry–Perot interferometer with a finesse, estimated by the line width of the elastic line, of about 80. The cavity length of the interferometer was set to 6 mm, which corresponds to a free spectral range of 25 GHz. The strong elastic component was cut out from the center of the spectrum using a mechanical shutter system synchronized with the scanning ramp of the spectrometer. After the temperature change, the sample was equilibrated for about 30 min before starting the spectrum acquisition. An acquisition time of a single spectrum for each sample was about 30 min.

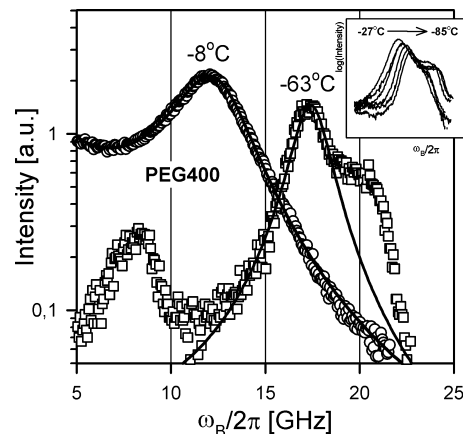


Figure 1. Experimental Brillouin spectra for PEG400 registered above (at -8°C) and below (-63°C) the crystallization temperature. The black solid lines are the outcome of eq 2. The inset shows the temperature dependence of the Brillouin profile.

3. Results and Discussion

As mentioned in the Introduction, the studied samples appeared to be opaque when cooled down below their crystallization temperature. The spectrum obtained for a sample at this state is lower in intensity, and the shape changes considerably as compared to the high-temperature spectra. Figure 1 is an illustration of this effect. It shows the spectra of pure PEG400 registered for transparent (high temperature) and opaque (low temperature) samples.

It can be seen how the usual eq 2, derived assuming a single photon scattering event in a homogeneous medium, perfectly fits the spectrum registered for the sample at high-temperature.^{20,21}

$$I_{\text{VV}}(\omega) = \delta_{\text{R}} + A_{\text{B}} \left[\frac{\Gamma_{\text{B}}}{[\omega - \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}]^2 + \Gamma_{\text{B}}^2} + \frac{\Gamma_{\text{B}}}{[\omega + \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}]^2 + \Gamma_{\text{B}}^2} \right] + \frac{A_{\text{B}}\Gamma_{\text{B}}}{\sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}} \left[\frac{\omega + \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}}{[\omega + \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}]^2 + \Gamma_{\text{B}}^2} - \frac{\omega - \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}}{[\omega - \sqrt{\omega_{\text{B}}^2 - \Gamma_{\text{B}}^2}]^2 + \Gamma_{\text{B}}^2} \right] \quad (2)$$

In the case of transparent media, the values of the frequency shift, ω_{B} , and the Brillouin line HWHM (half width at half-maximum), Γ_{B} , can be obtained from the fitting procedure. These parameters are directly related to the relevant acoustic properties of the medium. The hypersonic wave velocity, v_{B} , and the attenuation of the hypersonic sound (α) per sound wavelength (λ_{B}), $\alpha\lambda_{\text{B}}$, are given by

$$v_{\text{B}} = \omega_{\text{B}}/q \quad (3a)$$

$$\alpha\lambda_{\text{B}} = 2\pi\Gamma_{\text{B}}/\omega_{\text{B}} \quad (3b)$$

where $q = (4\pi n/\lambda_0) \sin(\theta/2)$ is the amplitude of the exchanged wave vector, λ_0 is the incident wavelength, n is the refractive index of the medium, and θ is the scattering angle.

As follows from Figure 1, eq 2 is not a proper description for spectra measured from opaque material. The reason is the multiple scattering process in which the photon undergoes many

scattering events, each changing the direction of propagation.²² In a sense, the photon “diffuses” inside the medium, and the assumption of a single wave-vector, q , appearing in eq 3a, loses its sense.

In the case of transparent media and applied scattering geometry, the peak we observe is the manifestation of light-scattering on longitudinal sound waves. The transverse waves are usually rapidly damped in the liquid phase. In solids or deeply supercooled liquids (or glass), attenuation of the transverse phonons is much lower, and their existence is manifested by additional lower-frequency features in the Brillouin spectra. As can be seen in Figure 1, the registered spectra reveal two main features. The smaller peak, observed at ~ 8 GHz, is probably a quasitransverse mode, and its appearance is related to the polycrystalline nature of the sample. The crystal regions, which appear in the pure PEG below the crystallization temperature, are randomly oriented solids. Therefore, the photons we observe could be scattered on the longitudinal as well as on the transverse phonons. In this paper, however, we focus rather on the high-frequency feature, so we will skip the detailed description of the low-frequency component.

Even if there is no proper analytical equation capable of fitting our spectra correctly, we can still analyze them in a qualitative and comparative way. Figure 1 shows that the high-frequency part of the Brillouin profile seems to be composed of two lines. The second line, which is lower in intensity, occurs as a shoulder on the high-frequency side of the main peak. As indicated in the inset of Figure 1, the frequency position of both lines increases with decreasing temperature. The tentative interpretation of the existence of two Brillouin lines is to ascribe them to scattering of light on local regions of different compressibility. The opaqueness of the sample could be interpreted as a result of the micro phase separation of the sample. In such a case, the lower-frequency peak could be ascribed to the amorphous phase, whereas the higher-frequency component could be a manifestation of the crystalline phase. This is consistent with the universal picture, according to which the low-molecular-mass polymers do not crystallize completely; there is always an amorphous part coexisting with the crystalline one.²³ The relative proportions between both phases depend on the polymer molar mass and the cooling rate.

When the polymer is dissolved in water, the phase behavior of the resulting solution depends on the concentration of the mixture, as well as on the molecular weight of the polymer. The phase behavior of the aqueous solutions of PEG molecules with different molar masses was studied earlier by means of the DSC method.^{1,3,12,17,24,25} The phase diagram was found to be single eutectic, indicating that there is only one concentration, x_E , for which both components of the liquid mixture crystallize simultaneously. This happens at a characteristic temperature called the eutectic temperature, T_E , and the (x_E, T_E) point at the phase diagram is called the eutectic point. If the eutectic point is reached, both components of the mixture separate out simultaneously, and the sample is a mixture of crystals (eutectic mixture). The single eutectic equilibrium solid–liquid phase diagram is schematically presented in Figure 2.

It was found that the phase diagram of PEG400/H₂O was interrupted before reaching the eutectic point.^{1,12} However, an extrapolation of the existing part of the phase diagram enabled us to assume that the eutectic concentration for this system is located around $x' = 0.33$. It is then justified to refer to this particular concentration as a eutectic concentration.

The vertical dashed lines in Figure 2 correspond to the concentrations of the mixtures used in the present study. Two

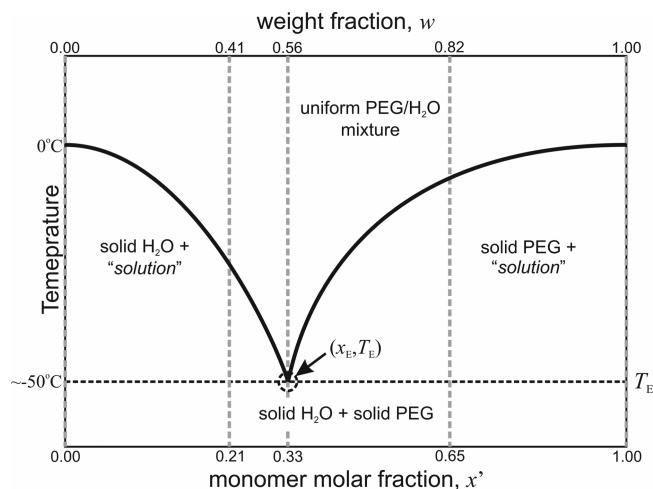


Figure 2. Schematic representation of the solid–liquid phase diagram of PEG/H₂O mixture. The vertical dashed lines correspond to the concentrations of the mixtures used in the present study.

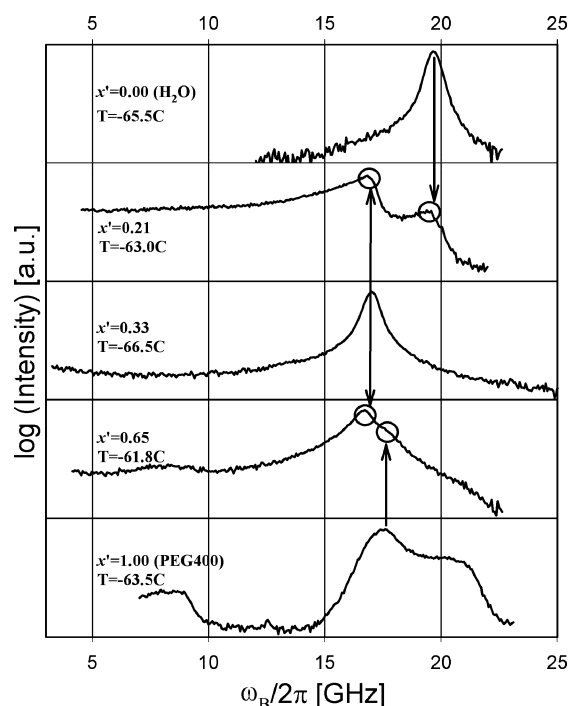


Figure 3. Brillouin spectra for pure constituents of a PEG400/H₂O mixture as well as its solution at different concentrations registered at similar temperature (about -65 °C).

pure components of the mixtures (water and PEG) were used as a reference. The remaining samples are the mixture with a eutectic composition ($x' = 0.33$), the mixture with an excess of water ($x' = 0.21$), and the mixture with an excess of PEG ($x' = 0.65$) with respect to the eutectic composition.

The comparison of the spectra recorded at a similar temperature for each of our samples is shown in Figure 3. In the spectrum obtained for a more diluted mixture ($x' = 0.21$), there are two peaks whose positions correspond almost perfectly to the peaks observed for pure icy water and the peak observed in an $x' = 0.33$ mixture. Similarly, the spectrum obtained for a more concentrated mixture ($x' = 0.65$), consists of peaks corresponding to solid PEG and, once more, to the “pure” $x' = 0.33$ solution.

The special position of the $x' = 0.33$ mixture is evident. If we recall that the eutectic composition reflects the maximum

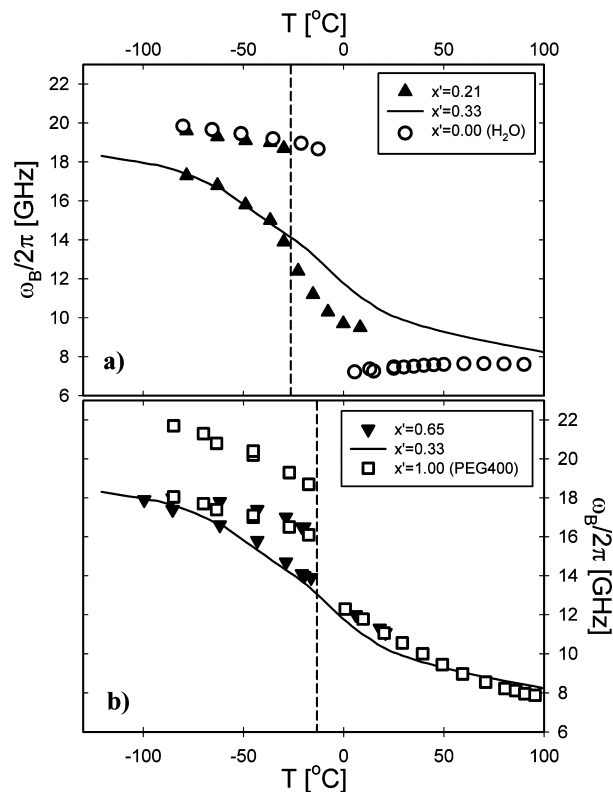


Figure 4. Temperature dependencies of the Brillouin peak positions read from the spectra for PEG400/H₂O mixtures of different concentrations. (a) Data for $x' = 0.21$ solution and for pure water. (b) Data for $x' = 0.65$ mixture and for pure PEG400. The solid line in both plots presents the data for the $x' = 0.33$ mixture. The vertical dashed lines shows the crystallization temperature of a given mixture.

capacity of hydrogen bonding per oxyethylene unit (OE), then for $x' = 0.33$, the maximum number of water molecules bonding each oxyethylene unit is 2. It was already mentioned that each unit of a PEG chain is capable of forming up to two hydrogen bonds. In this sense, it is quite straightforward to think about the $x' = 0.33$ mixture as a sample that contains fully hydrated polymeric chains. Further indication of the special role played by this 2H₂O/1OE hydration structure in PEG/H₂O systems comes from the inspection of Figure 4, in which the temperature dependencies of the Brillouin peak positions have been presented. Because of the lack of a proper mathematical description of the spectrum obtained from the opaque material, the Brillouin peak positions presented in Figure 4 were read directly from the spectra acquired at different temperatures. The uncertainty introduced by this procedure was not higher than 1 GHz. Within the range of this uncertainty, the two peaks observed in diluted PEG/H₂O mixtures ($x' = 0.21$) follow the temperature dependencies of the pure ice and the “pure” $x' = 0.33$ mixture. Similarly, both peak positions for an $x' = 0.65$ mixture evolve with the same temperature dependence as solid (and amorphous) PEG and again, as the “pure” $x' = 0.33$ mixture does. It is then the $x' = 0.33$ mixture that appears as a “solution” on a phase diagram in Figure 2.

As follows from Figures 3 and 4, the 2H₂O/1OE hydration structure appears both in the solvent-excess, and in the polymer-excess compositions (an *excess* refers to the eutectic composition). In this respect, it seems that below crystallization temperature, the mixtures separate into two phases: (1) the solid-state component being in excess and (2) the mixture of both components. This observation is in agreement with DSC results indicating that, indeed, below crystallization temperature, the

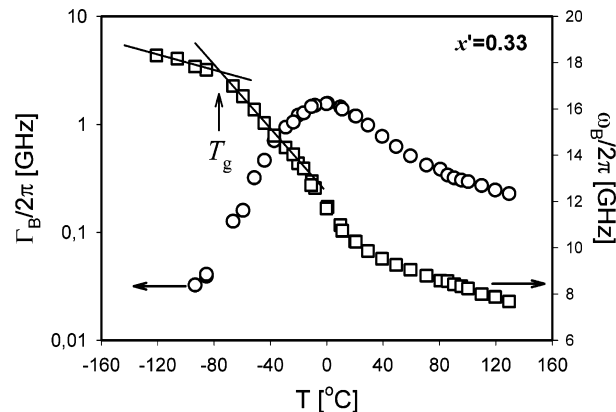


Figure 5. Temperature dependencies of the Brillouin line shape parameters for an $x' = 0.33$ mixture.

component in excess (relative to the eutectic composition) solidifies and precipitates from the solution. Additionally, Figure 4 shows that the concentration of the remaining solution is exactly that of a pure $x' = 0.33$ mixture, which can be interpreted as a 2H₂O/1OE hydration structure. The structure appears just after crossing the phase transition line, and the concentration does not change as the system is cooled down.

It is also worth noting that the sample of the $x' = 0.33$ concentration was the only mixture that showed a single-phase behavior and did not become opaque independent of the temperature (down to the -120 °C). Below the eutectic temperature, the resulting solid should be a mixture of water crystals and PEG crystals independent of the mixture composition. As follows from Figure 4, no sign of eutectic formation was detected. This phase separation process was interrupted by the very high viscosity of the mixture in the low temperatures and, eventually, the glass transition.

The shape of the Brillouin line in this case of the $x' = 0.33$ mixture was Lorentzian, and eq 1 could be used to fit the spectra in the whole temperature range. The temperature dependencies of the Brillouin shift and line width are presented in Figure 5.

A maximum in the Γ_B (connected to the sound attenuation), occurring at the same temperature where the ω_B (connected to the sound velocity) shows an inflection point, can be related to a relaxation process, taking place on the picosecond time scale.^{26,27} This process has already been observed in pure PEG and its mixtures^{9,15,16} and was associated with some conformational rearrangements of the polymer chains. A detailed analysis of the dynamics of this system is beyond the scope of this paper. We just note here that our recent investigation of the relaxation process detected in the liquid PEG400/H₂O system, suggests that the process observed in PEG aqueous solutions of different concentrations concerns a rearrangement of very similar local structure.²⁸ As follows from DSC experiments, the glass transition temperature for the PEG400/H₂O system was found weakly dependent on the system composition.^{12,17} We can find the temperature of this transition for an $x' = 0.33$ mixture from the inflection on the low temperature part of the $\omega_B(T)$ dependence (as indicated in Figure 5). The $T_g \cong -80$ °C is in very good agreement with the calorimetric data.^{12,17} To explain the nontrivial concentration dependence of T_g in PEG/H₂O mixtures, the existence of solute/solvent complexes has been suggested.¹⁷

The Brillouin scattering method, applied to the liquid mixtures of PEG in water, can also be used to show that the complex formation takes place in this system. For this purpose, the concentration dependence of the excess adiabatic compress-

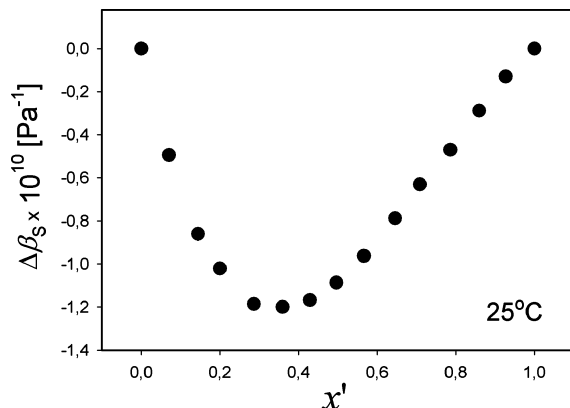


Figure 6. Excess adiabatic compressibility as a function of monomer molar fraction for a liquid PEG400/H₂O mixture at 25 °C.

ibility, $\Delta\beta_s$, is often presented. This parameter is defined as the difference between the compressibilities of the real and the ideal mixtures: $\Delta\beta_s = \beta_s - \beta_{s,\text{id}}$. The value of the compressibility of the real mixture can be obtained directly from the Brillouin measurement of the sound velocity, v_B (eq 3a); the density, ρ ; and the adoption of the Newton–Laplace equation, $\beta_s = 1/\rho v_B^2$. In the case of ideal mixing, when we do not account for any mixing volume, the adiabatic compressibility of the mixture can be written as

$$\beta_{s,\text{id}} = -\frac{1}{V} \left(\frac{\partial V}{\partial p} \right)_s = \beta_{\text{H}_2\text{O}} + (\beta_{\text{PEG}} - \beta_{\text{H}_2\text{O}})\phi \quad (4)$$

where $\beta_{\text{H}_2\text{O}}$ and β_{PEG} are the compressibilities of pure components, and ϕ stands for the polymer volume fraction. We emphasize that in the present case, as well as in many amphiphile/water systems, the assumption of the nonnegligible mixing volume is a very crude approximation.⁹ In general, an attempt should be made to estimate the correct value for a volume fraction. However, we just want to indicate some general behavior without a quantitative analysis of these results, and for this reason, we will stick to eq 4, keeping in mind, however, that the PEG/H₂O system is far from being the ideal case. The monomer molar fraction dependence of the excess adiabatic compressibility for PEG400/H₂O at 25 °C is presented in Figure 6.

The values of $\Delta\beta_s$ in the whole composition range are negative. This indicates that the building up of local coordination between water molecules and the OE units results in a structure tighter than that typical of the bulk water or the pure PEG400. The position of the minimum of the excess compressibility is commonly used to estimate the stoichiometry of the complex.^{8,9,29–31} The striking feature of Figure 6 is that the concentration corresponding to this minimum is very near $x' = 0.33$. The data presented in Figures 4 and 6 clearly show that the complex of about 2H₂O/1OE stoichiometry is more or less stable against dilution and temperature agitation and that it exists in the PEG400/H₂O system above and below the crystallization temperature.

It should be noted here that the observed hydration structure should not be considered a permanent and rigid complex. As follows from dielectric spectroscopy measurements, a process attributed to the dynamics of water molecules has been detected active even below T_g .^{3,32,33} This result served as evidence that the water in the PEG/H₂O hydration structure is not tightly bonded to the polymeric backbone but undergoes some angular

reorientations, even at very low temperatures when the global structure of the solution is basically “frozen”.

As follows from the earlier DSC results in PEG/H₂O systems, the eutectic composition and, therefore, the stoichiometry of the complex change with the molecular weight of the polymer.^{3,12} This result indicates that there exists a molecular structure, connected with a polymeric component, which allows accommodation of more water molecules for higher-molecular-weight samples. There is evidence from the Raman,^{34,35} IR,^{36,37} NMR,^{36,38} and AFM³⁹ experiments as well as from the molecular dynamic simulations¹³ that the PEG molecule in aqueous solution retains to some extent the helical conformation characteristic for its crystalline state. In the crystalline form, the (7/2) helix is formed⁴⁰ meaning that seven monomeric unit turns two times per fiber period. In the aqueous solution however, the (11/2) helix was found.¹³ It is then seen that the PEG chain in water has a more compressed helix with a larger diameter than that of the crystalline polymer. This larger diameter enables accommodating some water molecules inside the helix that further stabilize the helical backbone by an extensive hydrogen bond network formed between the PEG chain and water.

The helical structure can serve as a “local cage” within which water is confined. Because these molecules are not directly connected to the bulk environment, they can still exhibit significant molecular mobility, even though the whole mixture is in the glassy state. The fixed parameters of the helix also introduce some serious restrictions on the phase behavior of PEG with different molar mass. The PEG of mean molar mass 400 g/mol has about 9 OE units. It is then clear that for this oligomer, it is impossible to reach an ideal crystalline state because its chain length is insufficient to form an ordered helical structure. Even if the existence of short helical sections is not excluded, the overall conformation should be rather a quasirandom coil. For a binary mixture with a concentration corresponding to the eutectic composition (or complex formation composition) neither PEG nor water is able to form any crystalline structure. The amorphous structure of the PEG400/H₂O complex is retained down to the glass transition temperature, and there is no sign of the eutectic mixture formation.

4. Conclusions

Aqueous solutions of polyethylene glycol polymer at three different concentrations have been studied below their crystallization temperature using the Brillouin spectroscopy method. The Brillouin profile registered from the partially crystallized and opaque samples consists of two lines, which are a manifestation of the light scattered on the longitudinal phonons propagating at different velocities. Analysis of the concentration and temperature dependencies of the frequency position of both Brillouin lines allowed us to attribute them to the specific microstructures present in the studied system.

It was found that below the crystallization temperature, the studied mixtures separate into two phases. The first one is the solid-state component that is in excess relative to the eutectic composition. The mechanical properties of the second phase correspond almost exactly to the mixture with eutectic concentration ($x' = 0.33$), although no eutectic formation was observed in aqueous solution of PEG400. The solution of eutectic composition was the only sample that has not crystallized and showed single-phase behavior independent of the temperature. An analysis of the excess compressibility of the liquid PEG400/H₂O system indicated the special position of the $x' = 0.33$ mixture and suggested the existence of 2H₂O/1OE hydration

structure. This pseudocomplex should not be regarded as a stable and permanent structure, but rather, it has a dynamic nature. As follows from a comparison of the low- and high-temperature results, the detected hydration structure is robust against the system composition and survives the phase transition event.

The direct observation of how the structure dominated by the interaction through weak hydrogen bonds can endure in the system independent of the external conditions clearly resembles the behavior of many biological systems. Moreover, the ability to drive the aqueous mixture into the low temperatures with an elimination of crystallization is of key importance in cryoprotection applications.

Our findings are in very good agreement with earlier DSC results. In this respect, the Brillouin spectroscopy method can be regarded as an additional valuable tool allowing for identification of microstructures present in the system based on the differences in their mechanical properties.

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