

Stability of Water/Poly(ethylene oxide)₄₃-*b*-poly(ϵ -caprolactone)₁₄/Cyclohexanone Emulsions Involves Water Exchange between the Core and the Bulk

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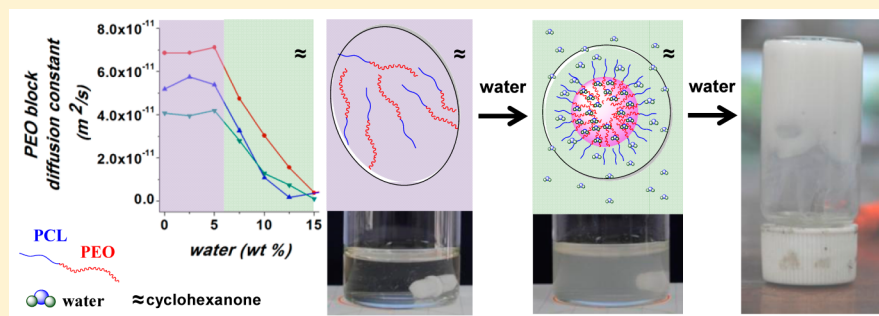
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Supporting Information



ABSTRACT: The formation of emulsions upon reverse self-association of the monodisperse amphiphilic block copolymer poly(ethylene oxide)₄₃-*b*-poly(ϵ -caprolactone)₁₄ in cyclohexanone is reported. Such emulsions are not formed in toluene, chloroform, or dichloromethane. We demonstrate by magnetic resonance spectroscopy the active role of the solvent on the stabilization of the emulsions. Cyclohexanone shows high affinity for both blocks, as predicted by the Hansen solubility parameters, so that the copolymer chains are fully dissolved as monomeric chains. In addition, the solvent is able to produce hydrogen bonding with water molecules. Water undergoes molecular exchange between water molecules associated with the polymer and water molecules associated with the solvent, dynamics of major importance for the stabilization of the emulsions. Association of polymeric chains forming reverse aggregates is induced by water over a concentration threshold of 5 wt %. Reverse copolymer aggregates show submicron average hydrodynamic diameters, as seen by dynamic light scattering, depending on the polymer and water concentration.

1. INTRODUCTION

Amphiphilic molecules have been broadly used in different fields of science and industrial application due to their interesting properties. Widespread attention has been focused on low molecular weight surfactants, which show a high tendency to associate in solution, leading to a variety of structures with different morphology.^{1,2} On the other hand, polymeric amphiphilic molecules may present structural characteristics similar to those of low molecular weight surfactants, which produce similar physicochemical properties.³ In this context, amphiphilic block copolymers, polymers normally composed of two or three blocks with different hydrophilicity, are also found in different media forming part of various aggregates, such as micelles,⁴ vesicles,⁵ and nano-

particles.^{6,7} These aggregates are formed by the self-association of the amphiphilic block copolymer chains, which is normally stimulated by using a selective solvent for one of the blocks. In aqueous solution, the obtained structures have been largely studied as carriers for poorly water soluble compounds, whereas in nonpolar solvents they can be used as carriers for hydrophilic compounds.^{8–10} Self-aggregation of amphiphilic molecules can be studied by means of different techniques, such as fluorescence spectroscopy using probes,^{11–14} dynamic light scattering (DLS),^{15,16} and NMR.^{17–19} The use of fluorescent

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probes can provide information related to the polarity of the environment sensed by the probes, while DLS allows measuring the size of the aggregates when formed. These techniques have been extensively used. However, advanced NMR techniques are less used, despite some advantages they provide; for instance, NMR allows direct exploration of properties of the amphiphilic molecules and solvents, such as relative electronic distributions around different nuclei and molecular dynamics, which normally change upon association.

In contrast to the association of surfactants and polymers in aqueous solution, where the hydrophobic effect is the driving force and an increase of entropy of water molecules is observed, in nonpolar media the hydrophobic effect is absent, and the solvent may play a discrete role in the molecular association process. Association of amphiphilic molecules in nonaqueous solvents is mainly considered as an enthalpy-driven process,²⁰ and dipole–dipole and hydrogen bond interactions between the polar segments of the amphiphiles are often the main forces involved.²¹ Thus, the reverse association process can be stimulated by adding molecules able to form specific interactions with the hydrophilic segments of the amphiphilic molecules.^{22,23}

Block copolymers composed of poly(ethylene oxide) (PEO) blocks are being extensively studied, because they can form aggregates both in aqueous solution and in organic solvents.^{24–27} PEO homopolymer presents a high solubility both in water and in nonpolar solvents, mainly due to the flexibility of the polymeric chain, which can adopt a molecular configuration that depends on the surrounding environment.^{28–30} Thus, in nonpolar media and in the absence of water, block copolymers carrying PEO blocks accompanying hydrophobic blocks can remain in solution as free chains or monomers. However, the addition of small amounts of water to these solutions may induce the association of these amphiphilic molecules,^{23,31} forming the so-called water-in-oil microemulsions. The self-aggregation process of amphiphilic PEO copolymers in apolar environments produces aggregates with a core formed by PEO blocks and a corona formed by the hydrophobic block.^{32–34}

Several reports concerning the self-assembly of poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) (PEO-*b*-PCL) block copolymers in aqueous solution can be found,^{35–39} whereas much less attention has been paid to their behavior in organic media. These polymers are interesting because both PEO and PCL building blocks are biocompatible, and therefore, their aggregates are biocompatible as well. PEO presents a weak interaction with cells and proteins, while PCL is biodegradable due to the presence of ester functional groups, which can undergo enzymatic degradation or hydrolysis at pH values far from neutrality.^{38,40–42} Recently, it has been shown that PEO-*b*-PCL block copolymers may stabilize water-in-oil microemulsions in chloroform in the presence of 2-propanol as cosolvent.⁴³ In the absence of the cosolvent, the dispersion of an aqueous phase in the reported systems was not possible, and microemulsions were not formed. The role of the cosolvent was rationalized to lay on the increase of the polarity and its ability to interact with water molecules. If such is the case, the nature and dynamics of the organic solvent may play a prominent role in the aggregation process of amphiphilic molecules based on PEO blocks, such as PEO-*b*-PCL block copolymers, due to the ability of PEO block to be stabilized both in water and in the organic solvent.

In this work, the formation of water-in-oil emulsions composed of the amphiphilic block copolymer PEO₄₃-*b*-PCL₁₄, cyclohexanone, and water will be described, with special emphasis on the follow-up of the water molecules distribution and dynamics by ¹H NMR (¹H chemical shifts, diffusion constants, and T_1 and T_2 relaxation times). In contrast, we will show that similar emulsions are not formed in organic solvents of comparable polarity, such as chloroform and dichloromethane. As cyclohexanone presents the ability to form hydrogen bonds with water molecules, we hypothesize that the dynamics of water exchange between aggregates and the bulk play a definite role on the stabilization of water-in-oil emulsions based on amphiphilic PEO-containing block copolymers.

2. EXPERIMENTAL SECTION

2.1. Materials. The block copolymer PEO₄₃-*b*-PCL₁₄ has been synthesized by ring-opening polymerization. ¹H NMR and MALDI-TOF characterization are detailed in the Supporting Information (SI) (see Figures SI.1–SI.4 and Table SI.1). The copolymer that resulted was highly monodisperse, showing a molecular mass of 3840 g/mol and polydispersity index of 1.1. The macroinitiator PEO monomethyl ether (M_n 2000 g/mol), ϵ -caprolactone (97%), and stannous(II) octoate (95%) were obtained from Sigma-Aldrich (St. Louis, MO). Tetrahydrofuran and *n*-hexane were obtained from Merck (Darmstadt, Germany). Cyclohexanone was obtained from Tokyo Chemical Industry. Deionized water produced in our laboratory has been used (water resistivity 18 M Ω -cm). ANS was obtained from Invitrogen. Chloroform-*d*₁ and acetone-*d*₆ were obtained from Sigma-Aldrich (St. Louis, MO). The structures of the organic molecules subjected to NMR studies are shown in Figure 1. All reagents were used without further purification.

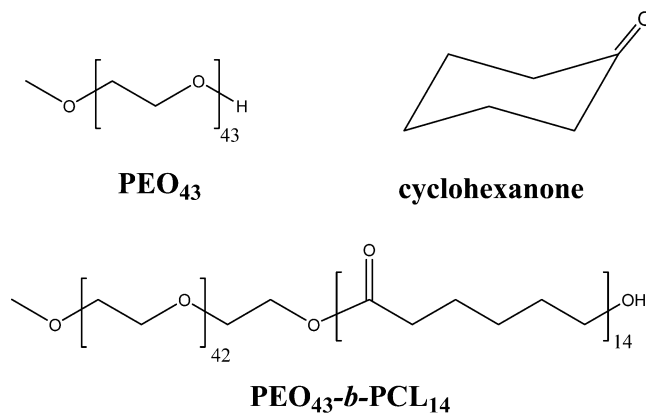


Figure 1. Molecular structures.

2.2. Equipment. NMR measurements were performed with AVANCE 300 and AVANCE 600 spectrometers (Bruker). MALDI-TOF has been done with a Microflex mass spectrometer (Bruker Daltonics Inc.). Fluorescence studies were done with a PerkinElmer LS 55 fluorescence spectrometer. DLS measurements were done with a zetasizer Nano ZS (Malvern) provided with the Zetasizer software (version 7.1). A LCD screen was used as source of polarized light, and a Nikon circular polarizer II 52 mm filter mounted on a Nikon D3000 camera was used for image capturing. Viscosity measurements were done with a vibrational viscometer SV-10 (A&D Co.)



Figure 2. Mixtures of PEO₄₃-*b*-PCL₁₄ (50 mg/mL), organic solvents, and water (10 wt %): (A) toluene, (B) chloroform, (C) cyclohexanone, (D) dichloromethane, and (E) acetone. Insets: corresponding samples after centrifugation.

coupled to a RW-2025G heating bath circulator (Jeio Tech) operating at 25 ± 0.1 °C. Centrifugation was done in a Hitachi CT 15E centrifuge.

2.3. Methods. **2.3.1. Sample Preparation.** Association of PEO₄₃-*b*-PCL₁₄ in cyclohexanone and in the presence of water has been studied using different polymer concentrations, i.e., 50, 105, and 167 mg/mL. Samples were prepared by adding the required amount of the polymer to cyclohexanone, sonicating for 2 min, and stirring for one night to ensure complete dissolution of the polymer in the solvent. Then, the mixtures were titrated with small and consecutive amounts of deionized water, added with the aid of a microsyringe, achieving water contents ranging between 0 and more than 15 wt %. Samples were examined under cross-polarized light to check the formation of nonisotropic solutions. The same procedure was done using toluene, chloroform, dichloromethane, and acetone as solvents. Phase separation was observed by the naked eye. The presence of macroparticles in turbid suspensions has been checked by centrifugation of 1 mL of the suspensions in 2 mL microtubes at 5000 rpm for 30 min. The dynamic viscosity of the samples was measured at 25 ± 0.1 °C by placing 10 mL of the mixtures in a 20 mL glass cuvette; the viscosity was measured until stable values were reached.

2.3.2. NMR Studies of Association. Prepared samples containing polymers, cyclohexanone, and different amounts of water, none of these materials deuterated, were placed in a 5 mm NMR tube. A coaxial tube containing acetone-*d*₆ was then inserted inside the 5 mm NMR tube. This method is called external method referencing.⁴⁴ The probe temperature was fixed at 25 °C. Prior to two-dimensional experiments, samples were equilibrated by rotation inside the NMR magnet for 10 min. The T_1 relaxation times were obtained using the standard inversion recovery pulse sequence.⁴⁵ In the applied sequence, the delay between π and $\pi/2$ pulses is varied, programming eight delay times in the range from 0.1 to 16 s. The repetition time was set to 20 s, and eight scans were made for each delay time. The T_2 relaxation times were obtained by applying the Carr–Purcell–Meiboom–Gill pulse sequence.⁴⁵ In this sequence, eight echo times ranging from 0.2 to 1.6 s were programmed, the repetition time was set to 20 s, and the number of scans was eight for each echo time. DOSY experiments were made under a stimulated echo sequence using bipolar gradients and a longitudinal eddy current delay. Diffusion delays of 40 ms and gradient pulse length of 3 ms were applied in order to obtain appropriate curves (25 points) for inverse Laplace transformation.

2.3.3. Fluorescence Probing. Samples containing the polarity probe ANS (1-anilinonaphthalene-8-sulfonic acid) have been prepared by addition of a stock solution of 1×10^{-3} M ANS in ethanol to an empty flask and subsequent evaporation of ethanol in an oven at 40 °C. Then, polymeric

solutions were added and the mixture stirred for one night to ensure the solubilization of the probe. The concentration of ANS was kept at 1×10^{-5} M. Fluorescence measurements were carried out using a quartz cell with a path length of 1 cm. Excitation wavelength was fixed at 350 nm, and excitation and emission slits were set at 5 nm.

2.3.4. DLS Studies of Association. Samples for DLS were prepared using filtered cyclohexanone. DLS measurements were made with a laser beam operating at 633 nm using a quartz vessel with a path length of 1 cm. The detection angle was fixed at 173° (backscatter). Results of size were considered significant under the Zetasizer software (version 7.1) analytical criteria.

3. RESULTS AND DISCUSSION

3.1. Solubility of PEO₄₃-*b*-PCL₁₄ in Organic Solvents.

The solubility of PEO₄₃-*b*-PCL₁₄ in organic solvents of different relative polarity (see Table SI.2, SI), such as cyclohexane, hexane, toluene, chloroform, cyclohexanone, dichloromethane, and acetone, has been tested. The results indicate that the block copolymer dissolves in toluene, chloroform, cyclohexanone, dichloromethane, and acetone and is insoluble in the remaining solvents, at a concentration of 50 mg/mL. Hansen solubility parameters (HSP) can be used as an approach to explain these results. HSP can help to predict the solubility of solutes in some solvents, taking into account the presence of three major interactions in common organic solvents: dispersion, dipolar, and hydrogen-bonding contributions.^{46–53} If the HPS of a solute are close to the HSP of a particular organic solvent, the solvent will solubilize the solute. In Table SI.2 (SI), the HSP obtained from the literature, corresponding to the homopolymers PEO and PCL and those corresponding to the solvents used in the solubility assay, are shown. PEO and PCL present similar values of HSP, indicating that the solubility of both blocks will be the same in any particular organic solvent. It is clear that toluene, chloroform, cyclohexanone, dichloromethane, and acetone are good solvents for both blocks and, therefore, nonselective for any block. On the other hand, the difference between HPS of both blocks and those corresponding to cyclohexane and hexane is in accordance to the low solubility found.

A recent work of Sereti et al. dealing with the stabilization of water-in-oil microemulsions by block copolymers of PEO-*b*-PCL formulated in chloroform/2-propanol shows the effective solubilization of the copolymer in chloroform.⁴³ However, they also highlight that polymeric solutions of PEO-*b*-PCL in chloroform present a low capacity to dissolve water. Indeed, our own experiments concerning the addition of water up to 10 wt % to solutions of PEO₄₃-*b*-PCL₁₄ in toluene, chloroform, cyclohexanone, dichloromethane, and acetone showed macroscopic phase separation in the case of using toluene,

chloroform, and dichloromethane; clear solutions in the case of using acetone; and stable emulsions when using cyclohexanone, as can be seen in Figure 2. In the case of using the chlorinated solvents, both aqueous and organic phases separate, and oil-in-water emulsions are formed in the aqueous phase. In the case of toluene, polymeric particles are formed that decant in the course of several days, leaving a turbid emulsion in the organic phase. Decantation can be accelerated upon centrifugation, as can be also observed in Figure 2. On the contrary, the emulsions formed in cyclohexanone were stable for at least 1 year, and upon centrifugation, macrophase separation is not observed. These facts give us insights on the characteristics that may present a good organic solvent to obtain emulsions stabilized by this kind of copolymers. In the case of a too low polarity and lack of affinity for water molecules, as in the case of toluene, added water molecules tightly bind to PEO blocks, acting as cross-linkers and inducing the collapse of the system, resulting in precipitation of the polymer. In the case of a too high polarity and high affinity for water molecules, as in the case of acetone, reverse aggregation of the polymer is minimized and normal aggregates are formed upon addition of an excess of water.^{20,31} Interestingly, the polarity of cyclohexanone is intermediate between those of chloroform and dichloromethane, but the chlorinated solvents undergo phase separation upon addition of 10 wt % of water. In this regard, we hypothesize that a solvent that shows a certain affinity for water molecules, allowing higher molecular exchange and faster dynamics for the polar segments and water molecules, may favor the stabilization of water-in-oil emulsions using PEO-based surfactants, such as PEO₄₃-*b*-PCL₁₄, in which the hydrophobic block is smaller than the PEO block. Therefore, we will verify this hypothesis by following the molecular dynamics of water, cyclohexanone, and the polymer in the emulsions. The affinity of cyclohexanone for water is based on the formation of hydrogen bonds. As cyclohexanone is not a selective solvent for either of the blocks, it is considered that the block copolymer chains remain in solution as free monomeric chains, and upon addition of water, a good solvent for the PEO block but not for the PCL block, the assembly of the copolymer in a reverse way is expected.

3.2. Water/Cyclohexanone Mixtures. Previous to studying the physicochemical behavior of the polymer in cyclohexanone, we performed ¹H NMR studies of cyclohexanone in the presence of different concentrations of water. Cyclohexanone is acting as a hydrogen-bond acceptor, due to the presence of a carbonyl group, which can interact with molecules such as alcohols and water.⁵⁴ This interaction is reflected in the solubility of water in cyclohexanone, reported to be 5.6 wt % at 25 °C, which increases at higher temperatures.⁵⁵ Cyclohexanone molecules undergo self-interaction, forming dimers.⁵⁶ In Figure 3, the chemical shift of water as a function of its concentration in cyclohexanone, ranging between 0 and 5.5 wt %, is shown. At a water concentration of 0.5 wt %, the corresponding resonance peak appears at 2.86 ppm, and an increase of the concentration of water produces a downfield shift of its resonance peak, reaching a value of 3.42 ppm at a water concentration of 5.5 wt %. The downfield shift of the water resonance peak indicates that the electronic density around the water proton is decreasing.¹⁷ Since cyclohexanone is found in large excess, it can be deduced that, apart from dipole–dipole interactions and hydrogen bonding with the organic solvent, formation of hydrogen bonds between water molecules is preferred, and the probability to occur increases as

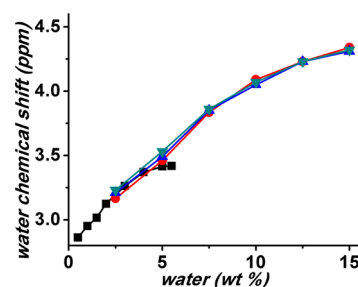


Figure 3. ¹H NMR chemical shifts of water protons in mixtures of PEO₄₃-*b*-PCL₁₄/cyclohexanone at different original concentrations of the polymer as a function of the water content: 0 mg/mL (black squares), 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

the water concentration increases. This explains the low but nonzero solubility of water in cyclohexanone.

By means of DOSY NMR, it is possible to determine the diffusion constants of water and cyclohexanone in these mixtures, which are shown in Figure 4. It can be observed

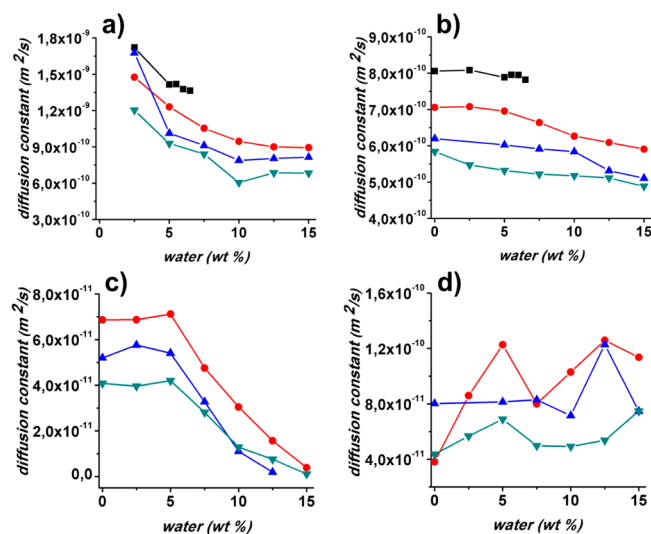


Figure 4. Diffusion constants obtained by DOSY as a function of the water content for (a) water, (b) cyclohexanone, (c) PEO block, and (d) PCL block, at polymer original concentrations of 0 mg/mL (black squares), 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

that the diffusion constants of cyclohexanone are lower than those of water, which may be related to the different molecular weight of the analyzed species, considering besides the tendency of cyclohexanone to dimerize.⁵⁶ Due to the low intensity of water at very low concentrations, reliable measurements of the diffusion coefficients have been obtained from a water concentration over 2.5 wt %. An increase of the water concentration in the mixtures does not produce significant changes in the diffusion coefficients of cyclohexanone. On the contrary, the diffusion constants of water tend to decrease, indicating that water molecules tend to associate with each other, losing mobility as its concentration increases. In addition, it is possible to observe in Figure 5 that the viscosity of the samples remains constant for all the mixtures.

Complementary to the measurements of ¹H NMR chemical shifts and diffusion coefficients, spin–lattice (T_1) and spin–

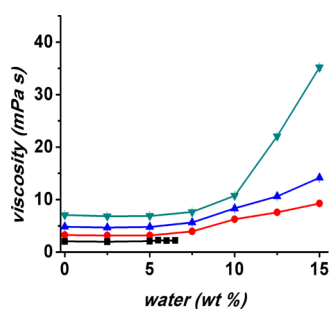


Figure 5. Viscosity of water/PEO₄₃-*b*-PCL₁₄/cyclohexanone mixtures at different original concentrations of the polymer as a function of the water content: 0 mg/mL (black squares), 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

spin (T_2) relaxation times of cyclohexanone and water have been determined as a function of the water concentration. Proton spin–lattice relaxation processes are driven by the magnetic dipole–dipole interaction of the observed protons with their surroundings, or lattice, due to the presence of fluctuating fields generated by surrounding molecules.⁵⁷ This interaction may be modulated by molecular tumbling and by translational diffusion. On the other hand, spin–spin relaxation is related to relaxations upon interaction with protons belonging to the same species and can give an estimation of the strength of the interplay of the functional groups studied separately.⁵⁸ The T_2 relaxation times are highly sensitive to local molecular motion and tumbling, which is related with the molecular packing of molecules in solution. The intermolecular interaction affecting T_2 depends on the distance (d) between two equivalent nuclei, being proportional to d^{-3} . Molecular packing induces a decrease of the molecular motion and higher density of protons of the same species. In Figure 6, T_1 and T_2 relaxation times of water and cyclohexanone are shown as a function of the water concentration. For water, T_1 takes values around 2 s, while T_2 relaxation times take lower values, around 0.1 s. For cyclohexanone, T_1 and T_2 relaxation times take values

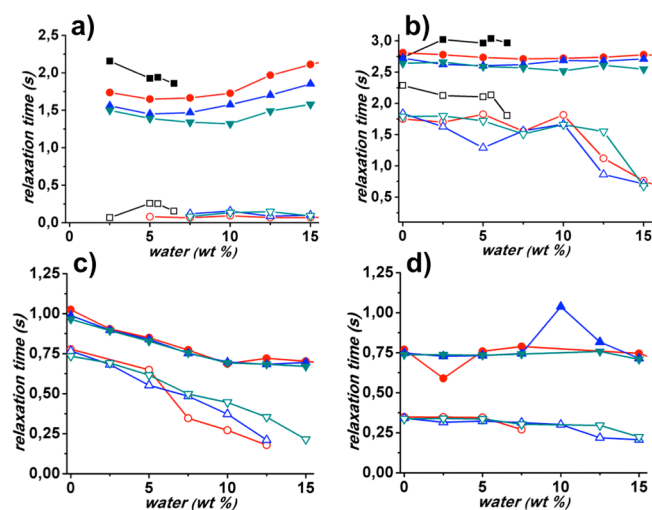


Figure 6. Relaxation times T_1 (filled symbols) and T_2 (empty symbols) as a function of the water content for (a) water, (b) cyclohexanone, (c) PEO block, and (d) PCL block, at original concentrations of the polymer of 0 mg/mL (black squares), 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

of around 3 and 2 s, respectively. The difference between T_1 and T_2 relaxation times of cyclohexanone is low, an indication of that cyclohexanone molecules are present in solution with high mobility. However, the difference between T_1 and T_2 relaxation times of water is high, indicating that the molecular movement of water molecules in cyclohexanone is restricted.

Since the T_1 relaxation mechanism is related with both rotational and translational movements of the observed nucleus and T_2 relaxation is more linked to tumbling, the ratio T_2/T_1 can be used to estimate the contribution of molecular tumbling of inspected molecules in the overall molecular motion.^{22,58} The corresponding values are shown in Figure 7. Values of T_2/T_1

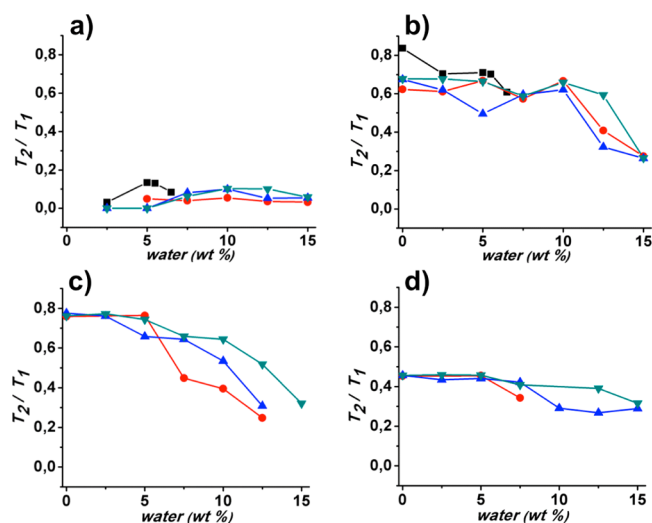


Figure 7. Values of T_2/T_1 ratio as a function of the water content for (a) water, (b) cyclohexanone, (c) PEO block, and (d) PCL block, at polymer original concentrations of 0 mg/mL (black squares), 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

T_1 near 1 indicate that molecular tumbling is high, while a value near 0 indicates that molecular tumbling is restricted. The values of T_2/T_1 corresponding to the cyclohexanone/water mixtures ranged between 0.03 and 0.13 for water and 0.61 and 0.84 for cyclohexanone, indicating that water molecules are present in solution with restricted molecular tumbling, despite their relatively high diffusion coefficients, while cyclohexanone molecules remain in solution with less restricted molecular movements.

3.3. PEO₄₃-*b*-PCL₁₄/Cyclohexanone Mixtures. In the absence of water, the resonance peaks corresponding to PEO and PCL segments and cyclohexanone appear as well-defined signals (see the Supporting Information). The diffusion coefficients of cyclohexanone, and the corresponding to PEO and PCL blocks in the absence of water, obtained by DOSY experiments, are shown in the respective intercepts with the y-axis in Figure 4. It is possible to observe that an increase in the polymer concentration produces a decrease in the diffusion coefficients of cyclohexanone and PEO block, due to a consequent increase of the viscosity of the solutions. The viscosity of the polymer solutions in the absence of water is shown in Figure 5, represented as the intercepts with the y-axis. Applying the Stokes–Einstein equation, the hydrodynamic diameter of the polymers could be calculated from the values of viscosity and diffusion coefficients corresponding to the PEO block, achieving a value of 1.7 ± 0.3 nm. Such a correlation is

not clearly seen from the values of the PCL block. In particular, at the lower polymer concentration, the results may lack accuracy due to the low intensity of the PCL signals and the partial overlap with cyclohexanone signals. The diffusion coefficients for both blocks fall in the range of $(3.8\text{--}8.1) \times 10^{-11}$ m²/s, 1 order of magnitude lower than those of cyclohexanone, which achieve values in the range of $(5.8\text{--}7.1) \times 10^{-10}$ m²/s. On the other hand, T_1 and T_2 relaxation times of cyclohexanone and PEO and PCL blocks can be found as the respective intercepts with the y -axis in Figure 6. Due to the polymeric nature of the copolymer, both relaxation times of PEO and PCL blocks also appear to be lower than that corresponding to cyclohexanone. The contribution of tumbling in the overall molecular motion is high in both cyclohexanone and PEO block, since T_2/T_1 achieved values around 0.75 for the organic solvent and 0.8 for the PEO block, as can be read from the respective intercepts with the y -axis of the plots in Figure 7. A lower contribution is found for the PCL segment, since T_2/T_1 achieved values around 0.45. It can be noticed that no significant differences of the values of T_1 and T_2 relaxation times for each observed protons as a function of the polymer concentration used in these studies are found.

3.4. Water/PEO₄₃-*b*-PCL₁₄/Cyclohexanone Mixtures.

Addition of water to the cyclohexanone/PEO₄₃-*b*-PCL₁₄ mixtures at the three different original polymer concentrations produced changes in the spectroscopic behavior of the samples. The chemical shifts of water protons in NMR analyses change as the water concentration increases. As can be seen in the Figure 3, at a water concentration of 2.5 wt %, the chemical shift of water protons appears at around 3.2 ppm, and further addition of water produces a downfield shift of the resonance peak of its protons, reaching a value of 4.34 ppm at a water concentration of 15 wt %. It is also possible to observe that an increase in the polymer original concentration does not affect the chemical shifts, which remain almost constant at each value of water concentration. Even in the absence of the polymer, the trend and values of the chemical shifts of water protons are coincident in the range of water solubility in cyclohexanone. However, in the presence of the polymer, the solubility of water increases at least up to 15 wt %, without macroscopic phase separation being observed. Additional interactions with the PEO block, which may include dipolar interactions and hydrogen bonding, and even hydrogen bond formation with the ester groups of the PCL block, may justify the higher solubility of water in this system. The lack of macroscopic phase separation, as observed in toluene, dichloromethane, and chloroform, may be attributed to the active role of cyclohexanone, the molecular characteristics of which provide conformational freedom to PEO blocks, as witnessed by the high T_2/T_1 values in the absence of water, and binding sites for water molecules allowing molecular exchange, increasing the entropy of the system. Molecular exchange between water molecules bound to cyclohexanone and to PEO blocks is witnessed by the absence of splitting of the NMR water signal, the chemical shift of which is then averaged. The standing downfield shifting of the resonance peak of water as the content of water increases indicates the increase of the probability of formation of hydrogen bonds with other water molecules, with ethylene oxide moieties, with PCL ester groups, or with cyclohexanone.

During titration, the diffusion coefficient of water decreases, as can be seen in Figure 4, consistent with the higher probability of undergoing hydrogen bonding. A smoother

decrease of the diffusion coefficient of cyclohexanone is also observed, which may be due to differences in the polarity of the samples in the presence of higher amounts of water, provided that there is not a correlation with the changes in the viscosity (see Figure 5).^{59,60}

As an important result of this investigation, we can find that the diffusion coefficient of the PEO block remains almost constant at each polymer concentration up to a water concentration of 5 wt %, almost coincident with the limit of solubility of water in cyclohexanone. A further increase of the water concentration from this threshold produces a stiff decrease in the diffusion coefficients of the PEO block, indicating the aggregation of the polymer through these blocks induced by the excess of water. On the contrary, diffusion constants corresponding to the PCL block remain in the range of 3.9 and 13×10^{-11} m²/s in the whole range of water concentration studied, so that these blocks keep being solvated by cyclohexanone. Although water molecules may be confined in the core of the aggregates, molecular exchange with water molecules bound to cyclohexanone is also witnessed by the smooth decrease of the water diffusion constants, and the smooth increase of the corresponding proton chemical shifts, without showing signal splitting. The diffusion coefficients observed for water molecules are an average of the diffusion constants of the dissolved water in cyclohexanone and that of the water included in the reverse micelles. Fast exchange between both types of water is witnessed, in addition, by the absence of a correlated increase in the viscosity, at least up to a water content of 10 wt %, as can be seen in Figure 5.

The trends of T_1 and T_2 relaxation times for the different species upon titration with water show slight changes for cyclohexanone and the more hydrophobic PCL block, while those of the PEO block continuously decrease, indicating the slow-down of its molecular dynamics. The T_2 relaxation times of cyclohexanone tend to decrease at a concentration of water higher than 10 wt % for all the polymer concentrations studied. Relaxation times corresponding to water molecules present a value of T_1 between 1.5 and 1.7 s, depending on the polymer concentration, when the water concentration is 2.5 wt %. The T_2 relaxation times are not detectable at this low water content. In fact, T_2 relaxation times are only detectable from water contents higher than 5 wt %. Consequently, the molecular tumbling of water is highly restricted, as witnessed by the very low values of the T_2 relaxation times, ranging between 0.1 and 0.2 s, and, more specifically, by the very low values of T_2/T_1 shown in Figure 7. A smooth increase of T_1 relaxation times of water molecules is observed from a 10 wt % of water in Figure 6. The values of T_1 and T_2 for water are, in any case, an average of the corresponding values of water included in the reverse micelles and dissolved in the bulk. The T_2/T_1 values shown in Figure 7 show constant and relatively high values for cyclohexanone, which decrease from a water content of 10 wt %. In the case of the PEO block, T_2/T_1 remains constant until the water concentration achieves 5 wt % and then decreases at concentrations of water higher than 5 wt %, indicating a restriction on tumbling consistent with the reverse aggregate formation from this threshold of water concentration. As said before, for the PCL block, the addition of water does not produce significant changes on the relaxation times, consistent with the higher mobility of PCL segments in reverse aggregates.

The formed microemulsions are isotropic, as indicated by the absence of birefringence under cross-polarized light experiments, as can be seen in Figure 8. The turbidity of the samples

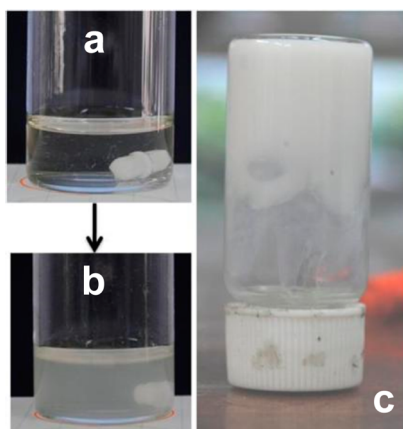


Figure 8. Samples observed under polarized light containing PEO₄₃-*b*-PCL₁₄ at an original concentration of 167 mg/mL in the presence of 0 wt % (a) and 7.5 wt % (b) of water and observed by the naked eye in the presence of 45 wt % of water (c).

increases as the water content increases. Further addition of water from a water content of 10 wt % produces the appearance of milky and creamy mixtures, with an increase in the viscosity, which is pronounced in the case of the most concentrated sample studied, as can be also seen in Figure 5. Indeed, stable emulsions are found even at water contents higher than 20 wt %. As a matter of example, a very viscous emulsion containing 45 wt % of water and an original polymer concentration of 167 mg/mL in cyclohexanone can be seen in Figure 8c. By comparison to the behavior found in toluene, chloroform, and dichloromethane, this is indicating the fact that water exchanges between the copolymer domain and cyclohexanone constitute a major contribution to the stabilization of the mixtures. Under these conditions, NMR signals broaden and the relative signal-to-noise intensity becomes too low to allow further analyses. The emulsions formed remained macroscopically stable for at least 1 year. DLS experiments made at 25 °C show the formation of polydisperse suspensions of aggregates in the submicron range. According to the Zetasizer software analyses, dry solutions of PEO₄₃-*b*-PCL₁₄/cyclohexanone do not form aggregates. Several formulations showing monodisperse apparent droplet size have been found, as can be seen in Table 1. Indeed, yet at water contents of 2.5, 5.0, and 10 wt %,

Table 1. Apparent Size (hydrodynamic diameters) and Polydispersity Indexes (PDI) as a Function of Water and Polymer Concentrations

water concn (wt %)	polymer concn (mg/mL)	apparent size (nm)	PDI
2.5	50	169 ± 2.2	0.19 ± 0.02
5.0	50	96 ± 2.3	0.50 ± 0.02
7.5	105	17 ± 0.2	0.29 ± 0.01
10	105	10 ± 0.0	0.36 ± 0.01
2.5	167	128 ± 4.3	0.47 ± 0.06
5.0	167	251 ± 5.9	0.22 ± 0.03

water droplets appear in the presence of original polymer concentrations of 50, 105, and 167 mg/mL in the range of 10–251 nm.

In order to complement the NMR and DLS observations, fluorescence studies were done. ANS is a polarity probe extensively used to characterize the formation of reverse

aggregates.¹² It presents two important excited states:^{12,61,62} one is a singlet state, S^{*}₁, related to a nonplanar conformation between the benzene and the naphthalene rings of the molecule, and the other is a excited state of lower energy, related to a planar conformation between both groups, with an important charge-transfer character, S^{*}_{1,CT}. The maximum and intensity of emission of this fluorescent probe is affected by the extent of charge transfer from S^{*}₁ to S^{*}_{1,CT}, related to the ability of the molecule to undergo the corresponding conformational change. The result is that under an increasing polarity of its environment, ANS undergoes a red-shift of the fluorescence band and a decrease of the quantum yield. Figure 9 shows the maximum of fluorescence of ANS as a function of

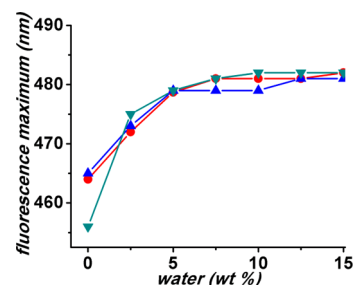


Figure 9. Fluorescence maximum of 1×10^{-5} M ANS as a function of the water content, at original polymer concentrations of 50 mg/mL (red circles), 105 mg/mL (blue triangles), and 167 mg/mL (green inverted triangles).

the water concentration in the emulsions. The decrease of the intensity has not been evaluated due to the appearance of turbidity in the samples. It is clear that an increase of the polymer original concentration does not affect the position of the maximum of fluorescence. However, a strong dependence on the water content has been observed, and shifts to lower energies are found as the water concentration increases, reaching a plateau at around 480 nm when the water concentration reaches a value of 5 wt %. The tendency observed in Figure 9 is related to an original, low polar environment in the absence of water; an increase of the water concentration produces an increase of the polarity of the environment sensed by ANS, achieving an environment of higher polarity at a water concentration of 5 wt %, which does not change as the water concentration further increases, as revealed by the stabilization of the fluorescence maximum. These results are in complete agreement with the observation made by ¹H NMR, where from a threshold of water concentration of 5 wt %, reverse aggregation of the polymer proceeds, consuming added water that may locate in the core of the aggregates.

4. CONCLUSIONS

It has been shown by ¹H NMR (¹H chemical shifts, diffusion constants, and *T*₁ and *T*₂ relaxation times) that the stability of water-in-oil emulsions composed of the monodisperse poly-(ethylene oxide)₄₃-*b*-poly(*ε*-caprolactone)₁₄ block copolymer, water, and cyclohexanone is strongly influenced by the dynamics of water, which undergoes molecular exchange between molecules associated with the polymer and molecules associated with the solvent by means of hydrogen bonding. In the absence of water, the solubility of the block copolymer is high in toluene, chloroform, dichloromethane, acetone, and cyclohexanone, as predicted by the analysis of the Hansen

solubility parameters. All these solvents are nonselective for any of poly(ethylene oxide) or poly(ϵ -caprolactone) segments that compose the block copolymer. The increasing addition of water to these solutions produces macroscopic phase separation in the case of toluene, chloroform, and dichloromethane, solvents unable to produce hydrogen bonds with water. Clear solutions are obtained in the case of using more polar solvents that are able to produce hydrogen bonding, like acetone, while stable microemulsions are obtained when using cyclohexanone, the polarity of which falls between those of chloroform and dichloromethane. Reverse aggregates of poly(ethylene oxide)₄₃-*b*-poly(ϵ -caprolactone)₁₄ in water/cyclohexanone are produced from a threshold of water content of ca. 5 wt % according to NMR data. Polydisperse aggregates have been found in the range of water content between 2.5 and 15 wt %. Water present in these mixtures is found to be highly structured, presenting severe restrictions on molecular tumbling. With the present findings, we aim to contribute to the general picture of association phenomena of nonionic amphiphilic molecules, such as block copolymers, with emphasis on the role of the solvent, and we highlight the convenience of using ¹H NMR to scan molecular characteristics, such as molecular dynamics and electronic environment, in systems where interacting molecules may undergo aggregation and self-assembly.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.5b10274.

Information concerning synthesis and characterization of the block copolymers, as well as the Hansen solubility parameters found in the literature (PDF)

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Notes

The authors declare no competing financial interest.

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