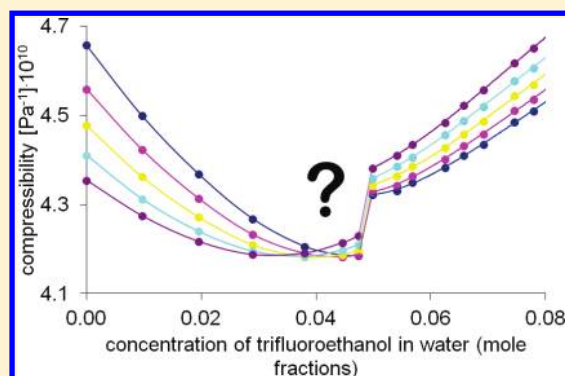


Peculiarity of Aqueous Solutions of 2,2,2-Trifluoroethanol

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S Supporting Information

ABSTRACT: Aqueous solutions of 2,2,2-trifluoroethanol appear to show a structural transition at alcohol mole fraction equal to $x_{\text{TFE}} = 0.05$, which can be concluded from a discontinuity of the speed of sound. At the same concentration, a discontinuity was observed in the parameters of the long-living component of the positron annihilation spectrum. Moreover, the partial molar volumes of components show transition-like behavior in the range of low solute contents, which is significantly different from nonsubstituted ethanol. The peculiarities of the low concentration system correlate with minor infrared spectra changes assigned to a mode composed of the CH_2 bending and CF_3 stretching internal vibrations being sensitive to polarity of the hydration shell surrounding the solute. The majority of the spectral changes arise from a gradual shift of the equilibrium between *trans* \leftrightarrow *gauche* isomers when the composition of the solution is changing. A possible explanation for the peculiar behavior of the system is a thermodynamic equilibrium between hydrated monomers and dimers at that respective mole number.



INTRODUCTION

2,2,2-Trifluoroethanol (TFE) is a very important compound because of diverse applications in molecular biology. It was first used in 1962 by Goodman et al.¹ to generate partially folded states of proteins, and since then, it has been widely used for this purpose.^{2–5} TFE was also found to stabilize β -sheets and β -hairpins,⁶ so it is important to understand the mechanism by which TFE generates partially folded states in proteins in its aqueous solution. This is not possible, however, without knowledge of the hydration of the TFE molecule and the structure of its aqueous solutions.

According to Kundu and Kishore,⁷ after addition of TFE to the protein solution, TFE molecules replace water from the surroundings of the protein, interacting with the amino acid residues and peptide bonds. This concept suggests strong solvation interactions of TFE, competitive with those of water.

In this article, we present results suggesting the existence of a lyotropic structural transition occurring in aqueous solutions of TFE. This phenomenon has been observed by two independent experimental methods: speed of sound and time-resolved positron annihilation. It also seems that it is reflected in the volumetric and infrared properties of this system.

EXPERIMENTAL SECTION

Ultrasound and Volumetric Measurements. 2,2,2-Trifluoroethanol, $\text{CF}_3\text{CH}_2\text{OH}$ (Fluka, puriss, $\geq 99.0\%$), was used without

further purification; water was doubly distilled. Solutions were prepared by weighing. All concentrations will be expressed in mole fraction of TFE, x_{TFE} , throughout this paper. The accuracy of concentration is in the range of $\Delta x_{\text{TFE}} = 0.0001$ or better.

The speed of sound was determined using a computer-steered OPKUD 01/100 apparatus (Optel, Wrocław, Poland), with an absolute accuracy better than $\pm 0.2 \text{ m}\cdot\text{s}^{-1}$ and a precision of similar order. Measurements are based on the determination of the time that acoustic signal needs to pass through the sample of known length. The obtained results are in very good agreement with those of Takagi and Noguchi.⁸

Density was measured using a vibrating tube Ecolab MG-2 (Kraków, Poland) apparatus with an accuracy better than $\pm 0.1 \text{ kg}\cdot\text{m}^{-3}$.

Measurements were performed at temperatures between 288.15 and 308.15 K in intervals of 5 K. The temperature was stabilized by a precision Julabo F25-ME (Germany) thermostat with an accuracy of $\pm 0.01 \text{ K}$.

From the speed of sound and density data, the adiabatic compressibility coefficients, κ_s , were calculated, using the Laplace equation:

$$\kappa_s = -\frac{1}{V} \left(\frac{\partial V}{\partial P} \right)_s = \frac{1}{\rho \cdot u^2} \quad (1)$$

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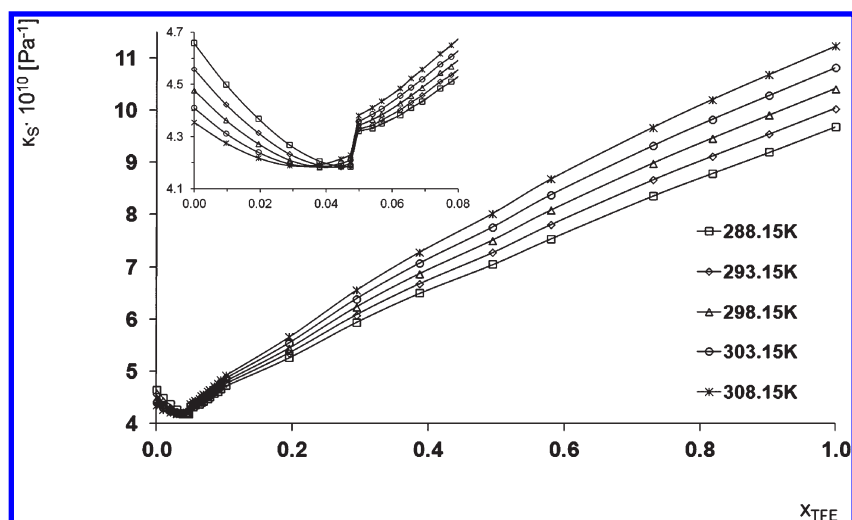


Figure 1. Adiabatic compressibility of TFE + water mixtures vs alcohol concentration. The inset illustrates the compressibility “jump” observed in low TFE contents.

where V is the volume, P is the pressure, ρ is the density, u is the speed of sound; the index S denotes adiabatic condition.

The measurements were performed in two independent series to avoid human error caused by incorrect concentration calculations or sample preparation. When the “jump” (discontinuity) in the speed of sound was found, an additional, independent set of solutions was prepared to increase the density of points in the vicinity of the discontinuity ($x_{\text{TFE}} \approx 0.05$; see below).

Positron Annihilation Measurements. Positron annihilation experiments were carried out at room temperature (~ 298 K) using a standard device based on the “fast-slow” coincidence technique described elsewhere.⁹ Using the POSITRONFIT program, the obtained spectra were numerically resolved into three components, characterized by their lifetimes (τ_1 , τ_2 , and τ_3) and intensities (I_1 , I_2 and I_3) subjected to restriction $I_1 + I_2 + I_3 = 100\%$. For further interpretation, only that denoted as no. 3, corresponding to the longest lifetime, will be analyzed.

Infrared Spectroscopic Measurements. Water used in spectroscopic studies was doubly distilled and additionally passed through a Simplicity 185 Water System (Millipore). The specific resistance of water at 298.15 K was equal to $18.2 \text{ M}\Omega \cdot \text{cm}$.

The mid-infrared spectra of the TFE/water samples were collected by means of total internal reflectance accessory (ATR-Ge, 10 reflections) on a Nicolet Magna 860 FT-IR spectrometer equipped with a Globar source, KBr beam splitter, and DTGS detector at resolution of 1 cm^{-1} and 128 scans.

Before the cluster analysis based on dendrograms and principal component analysis (PCA) were employed, the raw data were subjected to the following procedures: ATR correction, subtraction of water absorption, and normalization.

RESULTS AND DISCUSSION

The adiabatic compressibility coefficients obtained from ultrasound velocimetry and densitometry are shown in Figure 1 as a function of the TFE concentration expressed in TFE mole fractions.

Up to $x_{\text{TFE}} \approx 0.045$, the compressibility behaves similar to aqueous, for example, unbranched or branched short-chained alcohol–water mixtures, such as the aqueous solutions of ethanol¹⁰ or rather *n*-propyl alcohol,¹¹ but this “natural” trend is broken at

$x_{\text{TFE}} \approx 0.05$. The discontinuity seen in Figure 1 has been never reported until now, to the best of our knowledge.

Note that the compressibility isotherms do not intersect at a common point, but the intersection concentration shifts from ~ 0.03 at 35°C to ~ 0.05 at 15°C . According to Endo,¹² existence of a common intersection point for a range of isotherms means formation of clathrate-type hydrates, as is observed for water + ethanol,¹⁰ in which the hydrophobic group of the solute molecule is surrounded by water molecules, forming a cage. Therefore, for aqueous TFE solutions, such a classical clathrate-type hydration model seems doubtful. Let us also add that the compressibility corresponding to the intersection, $\approx 4.2 \times 10^{10} \text{ Pa}^{-1}$, is too high compared with that observed in typical clathrate-forming systems¹³ ($4.0 \times 10^{10} \text{ Pa}^{-1}$, identical to the compressibility of solid crystalline clathrates¹⁴). The above means that hydrates formed in diluted aqueous TFE do not resemble typical ones, known also in the solid, crystalline state, but are supposed to be labile and nonstoichiometric.

Our first idea was that this jump is caused by a rapid structural process occurring in the solution; for example, joining hydrophobic cages of the hydration structures. If two hydration cages containing monomeric TFE molecules stick together forming one big cage around the newly formed dimer, one would observe a local increase in compressibility.

Such a process should also be observed in the long-living components of the positron annihilation spectra. As already shown for aqueous solutions of alcohols¹³ or amines,¹⁵ the lifetime of the long-living component of the annihilation spectrum, τ_3 , exhibits inflection at a concentration corresponding to the stoichiometry of clathrate-type hydrates formed in the system and seen in compressibility as the intersection of its isotherms. The plot of τ_3 vs the TFE content presented in Figure 2 is compared with that for nonsubstituted ethanol.¹⁰ These two dependencies are very similar in shape, but not in the magnitude of changes.

The above suggests that the general mechanism that governs the changes of τ_3 remains unchanged. This seems to support the previous idea about hydrophobic-type hydration.

The annihilation parameter that is much more sensitive to the structural effect is the intensity of the long-living component, I_3 .

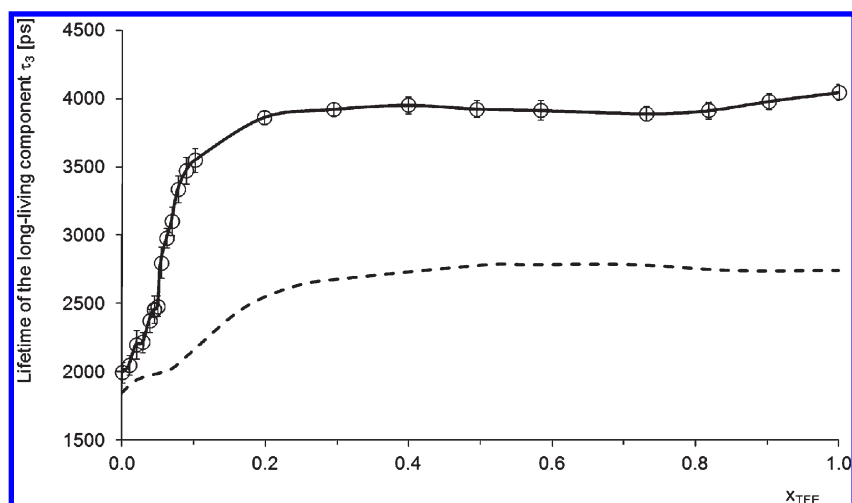


Figure 2. The dependence of the lifetime of the long-living component of the positron annihilation spectrum, τ_3 , on the TFE content in water. The lines are only a guide to the eye, and the broken line represents the ethanol–water mixture.¹⁰

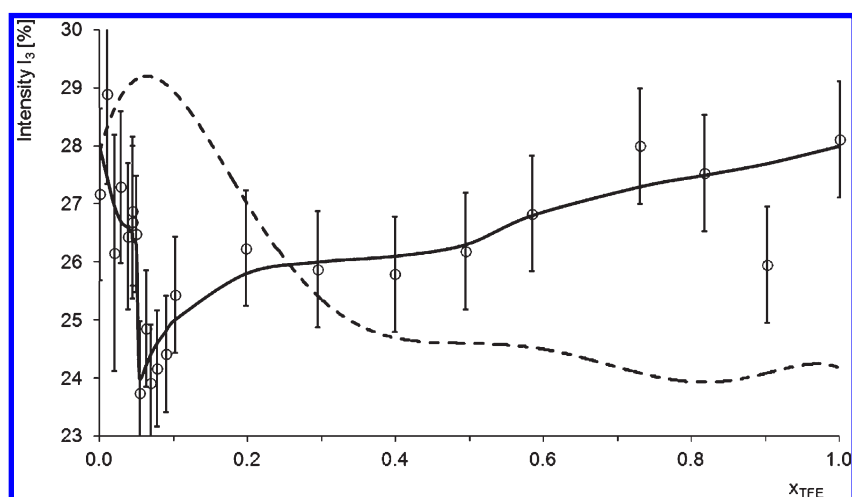


Figure 3. The dependence of the intensity of the long-living component of positron annihilation spectrum, I_3 , on the TFE content in water. The lines are only a guide to the eye; the broken line represents the ethanol–water system mixture.¹⁰

Its magnitude depends on the number (concentration) of voids in the water network. In aqueous solutions of short-chained polar solutes, this parameter increases in the region of the formation of clathrate-type hydrates and rapidly decreases when these structures are broken down. The respective plots comparing the I_3 dependence on TFE concentration with the system water + ethanol are shown in Figure 3. The differences are obvious, as well as the rapid decrease of I_3 at $x_{\text{TFE}} \approx 0.05$.

The rapid initial decrease of I_3 and the discontinuity at $x_{\text{TFE}} \approx 0.05$ suggest that the spatial distribution of water molecules is strongly affected by addition of TFE. The water structure shows many voids that are accessible for electron-positron pairs. The plot in Figure 3 shows that addition of a small amount of TFE reduces the number of these voids. On the other hand, compressibility of the system decreases rather slowly up to $x \approx 0.05$ (see Figure 1). The explanation is that in this region, TFE is hydrophobically hydrated, but its hydrates have compressibility similar to the network of pure water. This is consistent with the idea of very labile and nonstoichiometric hydrates postulated above.

The sharp increase in I_3 above $x \approx 0.05$ can result from lower rigidity of large hydrates (around dimers), which is consistent with increasing compressibility, as well as formation of additional voids between them.

The volumetric measurements are very precise and, thus, often exploited in investigating the liquid structure. The results obtained in the present paper are in perfect agreement with those already published by Harris et al.,¹⁶ Sassi and Atik,¹⁷ and Gente and La Mesa.¹⁸ However, the data of Gente and La Mesa¹⁸ are even more precise than ours, and their results are further used below. From the density data, the partial molar volumes of water, $\bar{V}_1 = (\partial V / \partial n_1)_{T,p,n_2}$, were calculated using the tangent method. The partial molar volume of water, \bar{V}_1 , remains almost unaffected up to $x_{\text{TFE}} \approx 0.05$, and it rapidly decays at higher concentrations, such as illustrated in Figure 4. Note the difference between aqueous mixtures of TFE and ethanol.

The low-concentration plateau means that pure liquid and the hydration shell remain in a similar state. This is again consistent with the concept of hydrophobically hydrated TFE in which the cage surrounding a hydrophobic solute remains an almost

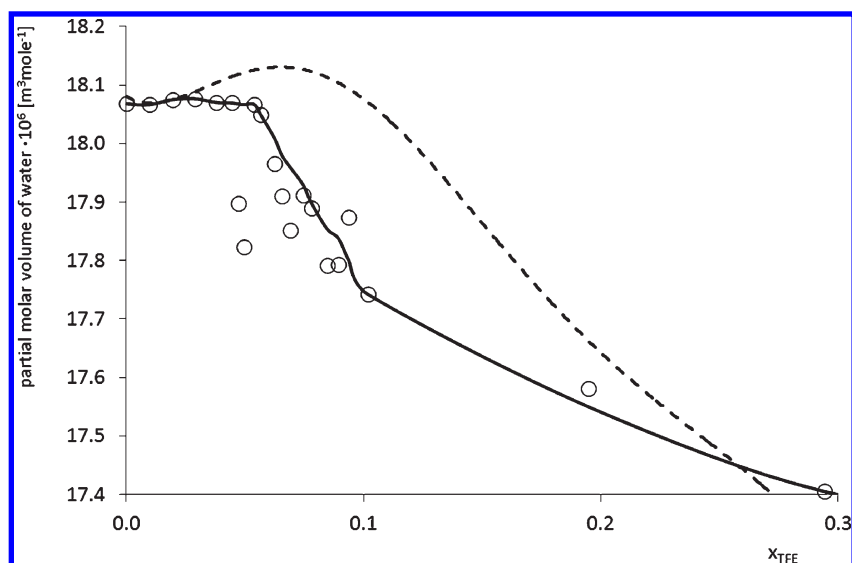


Figure 4. Partial molar volume of water vs TFE content in its aqueous solutions. Density data are from ref 18. The lines are only a guide to the eye; the broken line represents the ethanol–water mixture.¹⁰

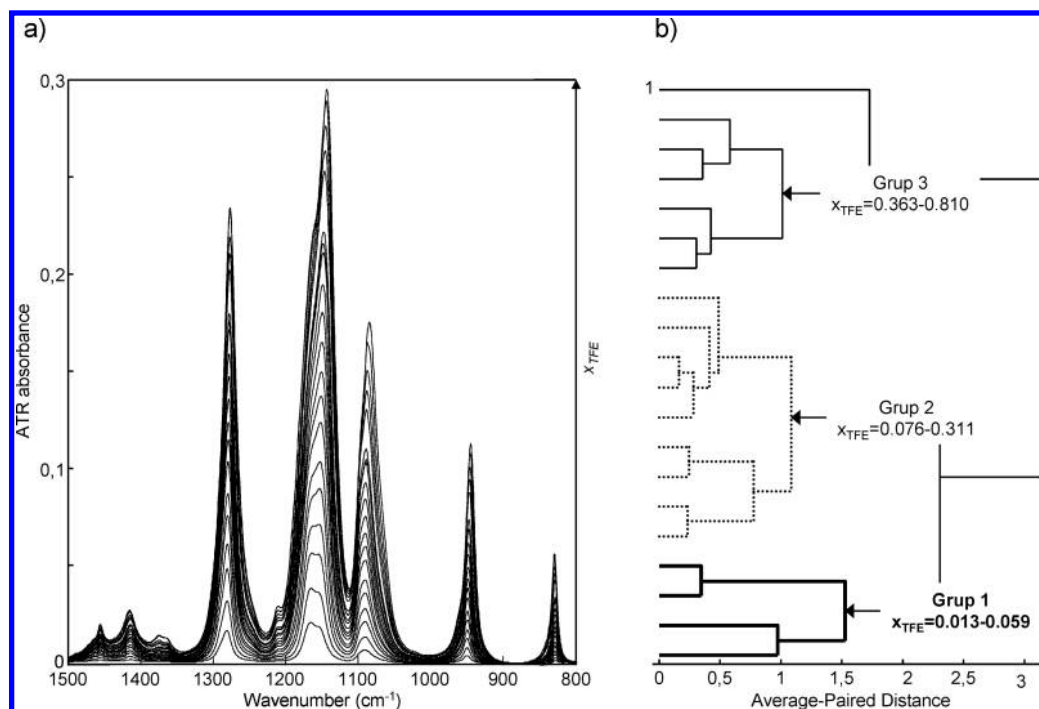


Figure 5. The IR-ATR spectra of TFE + water solutions, after initial processing (a) and their dendrogram with distinct separation into three groups (b).

unaffected bulk water structure. Above $x \approx 0.05$, however, the hydration interaction becomes even weaker. Again, the problem is, why is this transition so sharp?

Finally, we also tested this system using IR-ATR spectroscopy in which a series of solutions was measured in the whole range of x_{TFE} . The spectra of aqueous solution of TFE are very complex, and their interpretation is ambiguous due to the existence of different molecular hydrogen-bonded species in the experimental mixture.¹⁹ Therefore, the detailed analysis of the concentration-dependent spectral changes of bands attributed to different moieties of TFE was assisted by the dendrogram and principal component analysis (PCA).

The use of the two methods for investigation of the whole concentration range allowed separating them into three groups, as shown in Figure 5. The infrared data characterized by relatively low structural resolution subjected to the chemometric procedures provide results consistent with those obtained from the ultrasound speed and the positron annihilation experiments. Of particular interest is the first group, composed of samples with a TFE concentration below $x_{\text{TFE}} = 0.06$. It has to be mentioned that the hydrophobic hydration accompanying the formation of low stoichiometry TFE complexes is observed in different ways. Changes relevant to alcohol modify the infrared data, whereas changes specific for water are best observed by ultrasound speed

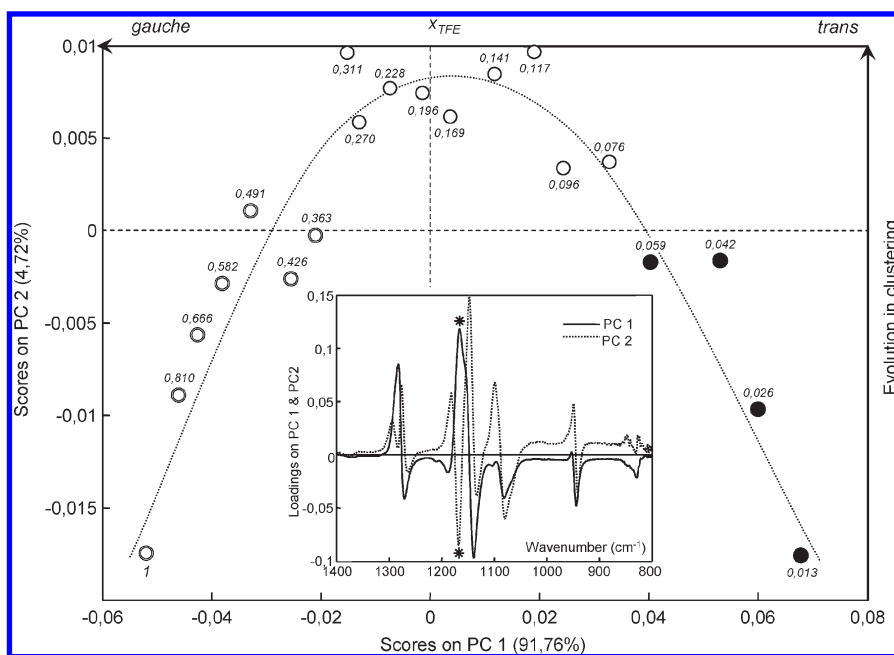


Figure 6. Microheterogeneity in water + TFE mixtures from IR measurements. The numbers are mole fractions of TFE in solutions. The inset shows the loading plots for PC1 and PC2. The star indicates the position of the more pronounced spectral changes characteristic for the samples marked by the ●, where $x_{\text{TFE}} < 0.06$.

and positron annihilation spectroscopy. Obtained results indicate a close relation between spectral properties of TFE itself and the formation of nonstoichiometric hydrates.

In addition to the analysis of spectral changes, PCA was employed for every specific group. This method allows detection of the correlation between samples by scores values, whereas loading values permit selection of wavenumbers at which the absorbance changes are responsible for the correlation. Moreover, PCA helps in distinguishing different mechanisms hidden behind the changes and classifying their relative contributions by eigenvalues. Figure 6 illustrates the results obtained from PCA analysis.

On the basis of the literature data, the trans-to-gauche transition could be the reason for the major changes (92%), whereas evolution in the formation of clathrate hydrates consisting of TFE and water molecules is postulated to be responsible for $\sim 5\%$ of spectral changes. According to the loading plots (see the inset in Figure 6) for $x_{\text{TFE}} < 0.06$, most characteristic are the changes marked by asterisks at 1168 cm^{-1} attributed to the CH_2 bending and CF_3 stretching vibrations.¹⁹ In this range of x_{TFE} , the low-concentration OH polar groups of the *trans*-TFE conformer are involved in hydrogen bonds with water molecules from cages that enclose TFE monomers. The hydrogen bonding interaction for the TFE–water complexes is stronger than that between TFE–TFE molecules.¹⁶ The environment of the clathrate hydrates around the CH_2 and CF_3 groups in the diluted system specifically modulates polarity of the bonds, leading to a meaningful decrease in the absorbance of the band at 1168 cm^{-1} assigned to vibrations of these groups. Such spectral changes are direct evidence of a local polarity decrease in the vicinity of the hydrophobic part of TFE. Around CH_2 and CF_3 groups of the moieties that belong to the group 1 cages are formed whose polar properties differ from those of cages of group 2 formed at the next stage of clustering when $x_{\text{TFE}} > 0.06$.

According to the above-discussed results, formation of dimers is expected in this concentration range. However, the accompanying decrease in the local polarity around the hydrophobic parts of the TFE dimers and higher associates formed with a systematic increase in the TFE concentration is less than that specific for group 1. It is manifested by smaller changes in absorbance at 1168 cm^{-1} for samples classified in group 2. For systems in which $x_{\text{TFE}} > 0.06$, the structural microheterogeneity is changing as the individually solvated TFE monomers undergo segregation from water by clustering. Sizes of the resulting TFE-rich regions with minimized contacts of CF_3 groups with water molecules vary between 5 and 10 \AA .^{20,21} Whenever the monomers are rearranged into clusters, the following changes are expected: an increase in the concentration of the *gauche* conformers and alterations in the properties of the solvation shell without a further pronounced decrease in their polarity.

On the basis of the molecular simulation studies, hydrophobic hydration²² reduces flexibility of the network and “freezes” the solution in the existing hydrogen bond network. Such a picture can be applied only to diluted samples. This picture is in apparent contradiction with the interpretation of Endo, but one should remember that the latter was based on the clathrate structures that can also exist in the solid state; however, less stable structures, which are supposed to exist only in the liquid state or in equilibrium with others, are also possible. The system water + isopropyl alcohol²³ is an example of such deviations from idealized Endo’s hydration.

CONCLUSIONS

Ultrasound speed measurements in aqueous solutions of 2,2,2-trifluoroethanol show a discontinuity at a TFE mole fraction $x_{\text{TFE}} \approx 0.05$. In addition, the intensity of the long-living component of the positron annihilation spectrum shows an analogous behavior in the region of low TFE concentrations,

very different from that of the short-chained alcohols investigated so far. Meaningful differences have been observed in the concentration dependence of the partial molar volume of water, which remains constant up to $x_{\text{TFE}} \approx 0.05$ and afterward rapidly decreases. Moreover, careful IR investigations allowed detection, as a main process, of the gradual shift of the trans \leftrightarrow gauche equilibrium that leads to local microhomogeneities in solution, which reach a maximum at $x_{\text{TFE}} \approx 0.18$. Some additional peculiarity has been observed at low TFE contents. The results suggest formation of less-stable, nonstoichiometric hydrates of TFE in its diluted solutions and, after increasing its concentration to $x_{\text{TFE}} \approx 0.05$, a rapid process of joining these hydrates. This leads to formation of much bigger—but also weaker—hydrates, most probably containing TFE dimers instead of monomeric alcohol molecules. Undoubtedly, further investigations are necessary to explain the nature of this unusual behavior.

■ ASSOCIATED CONTENT

S Supporting Information. Experimental results of dependences of the speed of ultrasound, u ; density, ρ ; adiabatic compressibility coefficient, κ_S ; and the lifetime, τ_3 ; and its intensity, I_3 , of the long-living component of positron annihilation spectrum on TFE content in water are collected in Tables S1, S2, S3, and S4, respectively. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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