

## Properties of 2,2,2-Trifluoroethanol/Water Mixtures: Acidity, Basicity, and Dipolarity

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In this report, we focus our attention on the characterization of 2,2,2-trifluoroethanol(TFE)/H<sub>2</sub>O mixtures and describe their intrinsic parameters; *i.e.*, solvent acidity (SA), solvent basicity (SB), and solvent dipolarity/polarizability (SPP), by the probe/homomorph-couple method for a range of mixtures from 0–100% (*v/v*) TFE. Variation of these parameters is not linear and has a singular and unpredictable behavior depending on the precise composition of the mixture. Based on these parameters, we describe the TFE-induced changes in some physical properties; *i.e.*, viscosity ( $\eta$ ), partial molar volume ( $\bar{V}$ ), density ( $\rho$ ), dielectric constant ( $\epsilon$ ), vapor pressure ( $p_v$ ), and spectroscopic properties; *i.e.*, NMR chemical shifts ( $\delta(^1\text{H})$ ) of TFE Me group for all molar fractions studied. In addition, by means of CD studies, we report that formation of the secondary structure, as percentage of helical content,  $\theta$ , of a polypeptide, poly(L-lysine), in several TFE/H<sub>2</sub>O mixtures is adequately described by these mixture parameters. SA, SB, and SPP of TFE/H<sub>2</sub>O mixtures provide an excellent tool for the interpretation of formation and stability of intramolecular H-bonds, and, thus, of secondary structures in polypeptides.

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**Introduction.** – The alcohol most widely used to promote the formation of secondary structure in polypeptides and proteins is 2,2,2-trifluoroethanol (TFE) on account of its ability to substantially increase secondary structure [1–3], to produce and/or stabilize partly denatured states [4–7], and to alter the folding/unfolding kinetics of proteins [8–10]. It has also been used as a standard solvent in establishing scales of helix propensity for the amino acids [2][11]. As a result, it is a co-solvent widely employed in biophysical and biotechnological investigations [12].

Most of these effects have been ascribed to the presence of H-bonds involving alcohol molecules [13]. First chemical evidences of TFE involvement in H-bond formation were the decrease in the rate of aqueous hydrolysis of picryl fluoride with the addition of TFE to H<sub>2</sub>O [14] and that of the racemization of biphenyls [15]. TFE undergoes self-association to a lesser extent than aliphatic alcohols [16]. Theoretical simulation studies show a possible implication of TFE aggregation [17] in these effects on H-bond formation. However, to date, the actual mechanism by which TFE alters the

H-bond arrangement of a protein or a polypeptide chain still remains obscure. Its elucidation may decisively contribute to the understanding of the mechanisms of biological processes such as folding/unfolding of peptides and proteins.

Although TFE is miscible with H<sub>2</sub>O in all ranges of molar fractions [18], the quantitative acidity and basicity properties of the TFE/H<sub>2</sub>O mixtures have scarcely been studied. Previous studies in this area have provided viscosity ( $\eta$ ), partial molar volume ( $\bar{V}$ ), density ( $\rho$ ), dielectric constant ( $\epsilon$ ) [19], and vapor pressure ( $p_v$ ) [16] throughout the whole range of molar fractions. None of these properties has an ideal behavior; in fact, the variations of  $\eta$ ,  $\bar{V}$ ,  $\rho$ , and  $p_v$  exhibit positive deviations with respect to the ideality, and  $\epsilon$  exhibits negative ones. In practice, this non-ideal dependence hinders the interpretation of physical quantities measured in this type of mixtures. Hence, interpolated values obtained from those for pure H<sub>2</sub>O and TFE lack any physical significance.

By <sup>1</sup>H-NMR measurements, *Llinás* and *Klein* [20] concluded that the important effect of TFE in protein and peptide structure must be a result of alcohol molecules being more acidic and less basic than H<sub>2</sub>O. In addition, the lower  $\epsilon$  than for H<sub>2</sub>O must also result in electrostatic interactions becoming favored [21].

We assume that it is the local interactions between molecules of H<sub>2</sub>O and TFE that are determinant in the formation of specific structures, and, therefore, that the particular variation of the physical properties of the mixture is crucial to explain the results obtained from different mixtures. In the characterization of TFE/H<sub>2</sub>O mixtures, we have quantified the parameters SA, SB, and SPP, by using the probe/homomorph method developed by *Catalán et al.* [22–27] initially applied to pure liquids. Recently, *Gajewski* [28] expressed the inadequate use of solvatochromic effect, supported by the unclear physical meaning of a change in UV wavelength of a dipolar dye to a non-polar excited state, when the solvent is in a non-equilibrium distribution about the neutral excited state due to the vertical nature of the UV transition, when using *E<sub>T</sub>30* dye. However, this dye has a dipole moment in the ground ( $\mu_g = 14.7$  Debyes) as well as in the excited state ( $\mu_e = 6$  Debyes), as has been reported by *Reichardt* [29]; and, in our case, the dye used considerably increases in polarity during the electronic transition ( $\mu_g - \mu_e = 18$  Debyes) [22][23], so we are able to make use of solvatochromic parameter to analyze the equilibrium data.

According to *Taft* and *Kamlet* [30], acidity represents the ability of solvent molecules to contribute as H-bond donors, and basicity represents the ability of solvent molecules to contribute as H-bond acceptors on a H-bond between solvent and/or solute molecules. SPP is the ability to form non-specific interactions. Recently, we have used the parameters with success to rationalize change of several chemical properties in both aqueous and non-aqueous media of different compounds such as the kinetics of *t*-BuCl hydrolysis, the decarboxylation kinetics of 3-carboxybenzisoxazoles [31–33], and a new characterization of ACN/H<sub>2</sub>O mixtures [34]. We have also successfully explained the photophysical behaviors of the probes ACRYLODAN (=6-acryloyl-2-(dimethylamino)naphthalene), ANS (=8-anilinonathalene-1-sulfonate), and PRO-DAN (=‘6-propano-2-(dimethylamino)naphthalene’), of their use in biological studies, in aqueous mixtures of various alcohols; *i.e.*, MeOH, EtOH, PrOH, *i*-PrOH, *t*-BuOH, and 2,2,2-trifluoroethanol, all solvents of wide application in structural studies of peptides and proteins [35].

In this work, we describe the variation of physical properties  $\eta$ ,  $\bar{V}$ ,  $\rho$ ,  $\varepsilon$ , and  $p_v$ , experimentally obtained, by several authors [16][19], from the TFE/H<sub>2</sub>O mixtures, as a function of the solvent-mixture parameters SA, SB, and SPP.

To extend the use of these parameters, we have also measured and rationalized the change on chemical shift  $\delta(^1\text{H})$  of the TFE Me group in different H<sub>2</sub>O concentrations and the generation of helical structure,  $\theta$ , of a polypeptide, a poly(L-lysine), used as a model to study the formation and stability of  $\alpha$ -helix in these mixtures, at pH 7.

**Experimental.** – SA, SB, and SPP: The intrinsic solvent parameters of acidity (SA), basicity (SB), and dipolarity/polarizability (SPP), were measured experimentally by the suitable probe/homomorph method developed by *Catalan et al.* [22–27] initially applied to pure liquids. The TFE used was of the highest available purity and purchased from *Merck* in *Uvasol*. Solvent mixtures were prepared from freshly opened bottles, using *Brand II* 25.00-ml burettes, previously calibrated, to transfer the liquids. The SA was evaluated from the solvatochromism of the probe *o*-(*tert*-butyl)stilbazolium betaine dye and its homomorph *o,o'*-di-(*tert*-butyl)stilbazolium betaine dye. The acidity of solvents more acidic than MeOH (SA = 0.605) is evaluated by applying the solvatochromic comparison method [36] to solvatochromism measurements of the probe 3,6-diethyl-1,2,4,5-tetrazine. The SB was obtained from the solvatochromism of the probe 5-nitroindoline and its homomorph 1-methyl-5-nitroindoline. SB values range from 0 for the gas phase to 1 for tetramethylguanidine. The SPP was characterized from experimental measurements of the solvatochromism of the probe 2-(dimethylamino)-7-nitro-9H-fluorene and its homomorph 2-fluoro-7-nitro-9H-fluorene. The SPP values range from 0 in the absence of solvent (gas phase) to 1 for dimethyl sulfoxide. Absorption spectra were recorded on a *Shimadzu 2100* UV/VIS spectrophotometer, the monochromator of which was calibrated with respect to wavelength by using the 486.0- and 656.1-nm lines of a deuterium lamp. The instrument was routinely checked for wavelength accuracy by using holmium oxide and didymium filters. All measurements were performed at 25°, using a pair of matched quartz cells of 1-cm light path.

**NMR.** <sup>1</sup>H-NMR Spectra for the TFE/H<sub>2</sub>O mixtures were recorded on a *Bruker DXP-300* spectrometer. TFE was purchased from *SIGMA Chemical Co.* The deuterium lock was performed by adding 10% D<sub>2</sub>O to H<sub>2</sub>O solvent. Bandwidth was adjusted to 3591.954 Hz, and 131072 TD points per spectrum (128k) were collected with a final FIDRES digital resolution of 0.03 Hz. The acquisition time was 18.24 s per spectrum. *Fourier* transforms were obtained by using the XWINNMR software package, also from *Bruker*, following addition of 128k (to an overall size of 262144 points). LB was 0.30. All chemical shifts were internally referenced to TSP (sodium 3-(trimethylsilyl)(2,2,3,3-<sup>2</sup>H<sub>4</sub>)propanoate) using a small proportion (1%) of TSP in every sample. Water resonance was eliminated by irradiation during 1 s.

**CD.** CD Measurements were performed on a *JASCO (J-710)* spectropolarimeter between 200 and 250 nm. Calibration of the instrument was made using (+)-10-camphorsulfonic acid,  $\Delta\varepsilon = +2.37 \text{ M}^{-1} \text{ cm}^{-1}$  at 290.5 nm and  $-4.95 \text{ M}^{-1} \text{ cm}^{-1}$  at 192.5 nm. Resolution was 0.2 nm. Poly(L-lysine), polymerization degree of 200, was from *SIGMA Chemical Co.* Eight spectra were accumulated in all cases. The helical content,  $\theta$ , was calculated according to *Greenfield* and *Fasman* [37].

*Fit.* In principle; any molecular property of a mixture can be analyzed by using *Eqn. 1*, as discussed in [38].

$$\text{Property} = a + b \text{ SA} + c \text{ SB} + d \text{ SPP} \quad (1)$$

Software MINITAB was used to calculate the non-linear regression fit of the change of properties [39]. Coefficients *b*, *c*, and *d* pertain to the property in question,  $\eta$ ,  $\bar{V}$ ,  $\rho$ ,  $\varepsilon$ ,  $p_v$ ,  $\delta$ , and  $\theta$ , and they describe the sensitivity of the corresponding property to the SA, SB, and SPP. Standard deviation (sd) and correlation coefficient (*r*) were also calculated with this program.

**Results.** – We have measured the solvent parameters SA, SB, and SPP for TFE/H<sub>2</sub>O mixtures reflecting the whole range of molar fractions. The results obtained are shown in *Fig. 1*. The properties studied vary differently and non-ideally with  $X_{\text{TFE}}$ , so no qualitative or quantitative conclusion about the behavior of mixtures of these components can be derived solely from the knowledge of parameter values for the pure solvents.

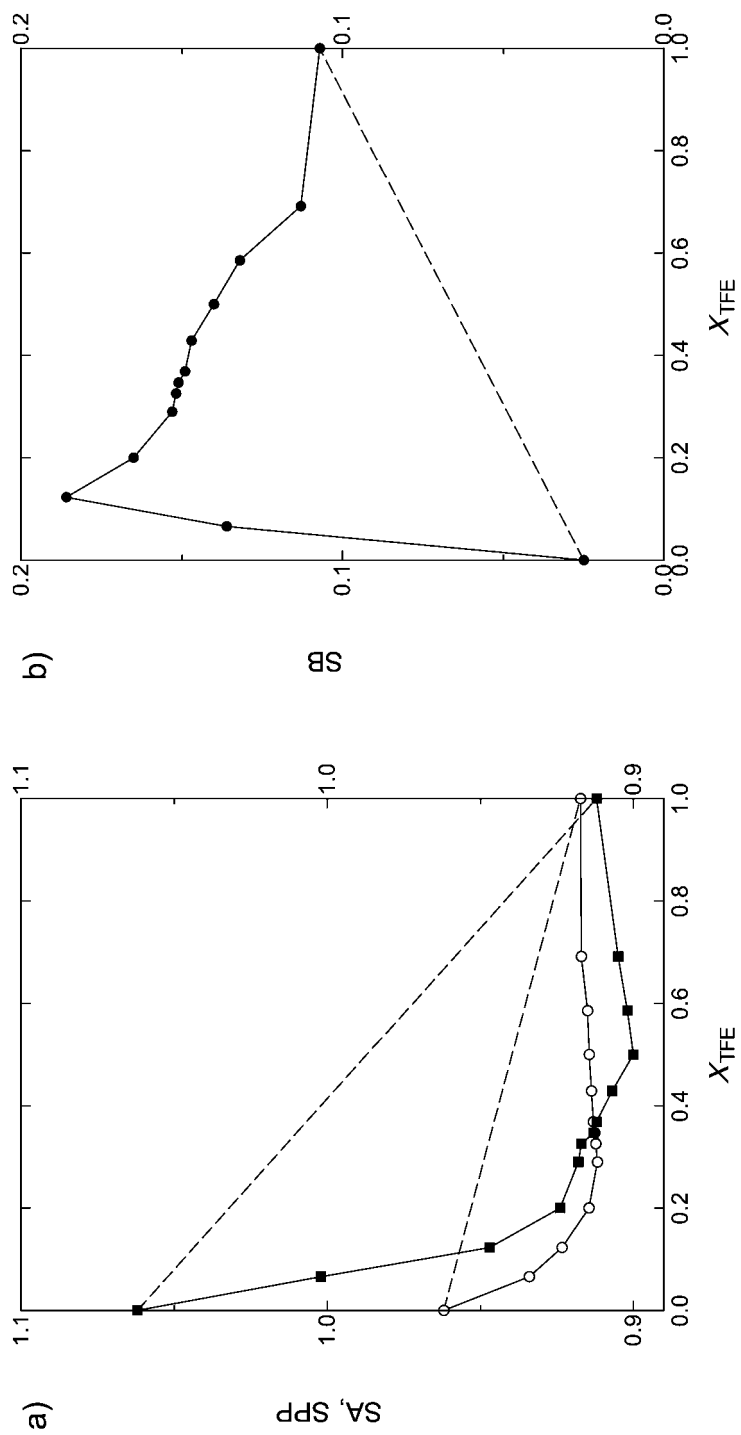


Fig. 1. Dependence of intrinsic mixture parameters: a) SA (filled squares) and SPP (open circles), b) SB (filled circles), on the composition for the binary mixture TFE/H<sub>2</sub>O. Symbols represent experimental data. Broken lines represent the ideal behavior of the corresponding property in the mixture.

Values obtained for SA and SPP present negative deviations with respect to the ideality in the whole composition range and decreases rapidly up to  $X_{\text{TFE}} = 0.20$  (50% (v/v)). Beyond that composition, the variation is less marked, almost constant, and slowly increases to reach the values measured for pure TFE (SA = 0.92 and SPP = 0.91). On the other hand, values for SB present positive deviations with respect to the ideality over the whole composition range. It reaches a maximum at  $X_{\text{TFE}} = 0.1$  (31% (v/v)) beyond which it decreases, reaching a final value for pure TFE (SB = 0.11) larger than that of pure H<sub>2</sub>O (SB = 0.025). Hence, SB shows dual behavior. In the entire composition range, TFE/H<sub>2</sub>O mixtures are always less acidic, less polar, and more basic than H<sub>2</sub>O. That is, enriching the mixture with TFE decreases its acidity (*i.e.*, its donor H-bonding capacity, SA), decreases its SPP, but increases strongly its SB with respect to pure H<sub>2</sub>O.

Therefore, the mixtures exhibit a dual variation that must influence solutes sensitive to the studied mixture's parameters. Several authors studying the folding of peptides and proteins have previously described a change on TFE behavior for values above and below  $X_{\text{TFE}} = 0.2$  (50% (v/v)).

In summary, at each  $X_{\text{TFE}}$  value, SA, SB, and SPP vary in a different manner that is not possible to predict and quantify from reported values of pure solvents.

The SA, SB, and SPP of TFE/H<sub>2</sub>O mixtures must result from molecular interactions in their bulk. Consequently, they could be of assistance in interpreting changes in their physical properties at each mole fraction used. Nevertheless, the weight of the terms in the corresponding fitted equation cannot be known *a priori*.

To demonstrate the usefulness and applicability of the data obtained in this work, we set out to rationalize the change on physical properties ( $\eta$ ,  $\bar{V}$ ,  $\rho$ ,  $\epsilon$ , and  $p_v$  [16][19]) as function of concentration of TFE. From NMR data, we determined the change on  $\delta(^1\text{H})$  for Me group of TFE with H<sub>2</sub>O, and from CD experiments the generation of helical structure,  $\theta$ , for a poly(L-lysine) chain in TFE/H<sub>2</sub>O mixtures at pH 7. All data were fit as a function of mixture parameters.

Viscosity,  $\eta$ , of TFE/H<sub>2</sub>O mixtures are dramatically non-linear functions of mole fraction, as reported by *Murto* and *Heino* [19]. This behavior can be successfully described as a balance of SA and SB mixture parameters (*Fig. 2*) expressed in *Eqn. 2*.

$$\eta = 5.17 - 4.05 \text{ SA} + 2.20 \text{ SB} \quad (r = 0.989, \text{ sd} = 0.05\text{cp}) \quad (2)$$

where  $r$  is the correlation coefficient and sd the standard deviation.

The  $\eta$  value of TFE/H<sub>2</sub>O mixtures should increase with increasing interactions between its components. As observed experimentally (*Fig. 2*), there is a maximum at a mole fraction of TFE of 0.2, which we explain as a simultaneous dependence and difference of  $\eta$  with SA, which decreases in the whole range, and SB, which increases up to 0.1 and decreases above it.

The variation on partial molar volumes ( $\bar{V}$ ) in TFE/H<sub>2</sub>O [19] mixtures can be explained as a function of the change on SA values for these mixtures. Thus, experimental values were adequately fit with *Eqn. 3*, and a representation of both experimental and calculated data is given in *Fig. 3*.

$$\bar{V} = 41.6 - 45.9 \text{ SA} \quad (r = 0.989, \text{ sd} = 0.4 \text{ cm}^3/\text{mol}) \quad (3)$$

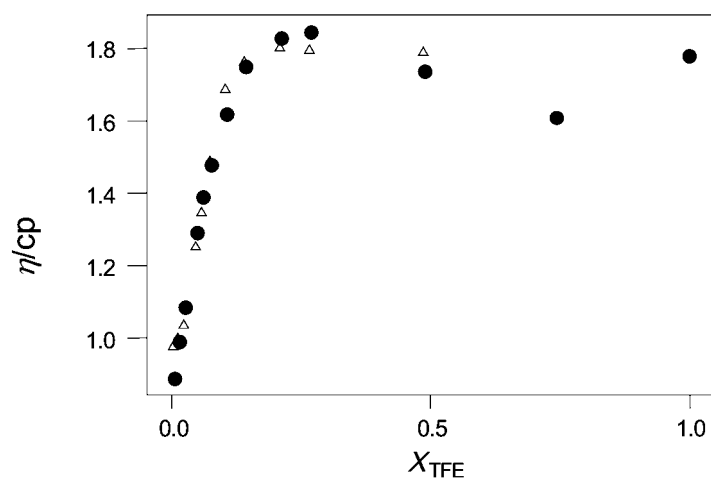


Fig. 2. Dependence of viscosity,  $\eta$ , on the composition of TFE/H<sub>2</sub>O mixture. Filled points represent experimental data [19]. Open triangles represent data calculated with Eqn. 2, dependent on SA and SB parameters.

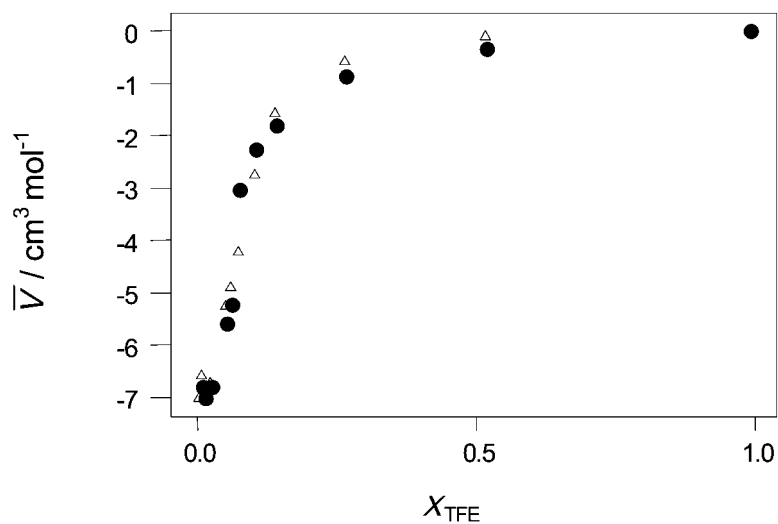


Fig. 3. Dependence of molar volumes,  $\bar{V}$ , on the composition for TFE/H<sub>2</sub>O mixtures. Filled points represent the experimental data [19]. Open triangles represent data calculated with Eqn. 3, dependent on SA parameter.

$\bar{V}$  depends only on SA of the mixture and increases when acidity decreases; that is, when the interactions between ‘solute and solvent’ molecules diminish, and when solvation of basic centres of TFE diminish.

Density,  $\rho$ , values [19] were fitted to Eqn. 4.

$$\rho = 4.10 - 2.87 \text{ SA} - 1.16 \text{ SB} \quad (r = 0.989, \text{ sd} = 0.02 \text{ g/cm}^3) \quad (4)$$

Therefore,  $\rho$  depends on SA and SB parameters. Fit and experimental values are shown in *Fig. 4*. As previously explained, increase in TFE concentration decreases SA, so in diminishing the solvation of acid and basic centres  $\rho$  increases. We can conclude that aggregation of TFE molecules must play an important role in the behavior of these mixtures, as suggested by *Chitra and Smith* [40].

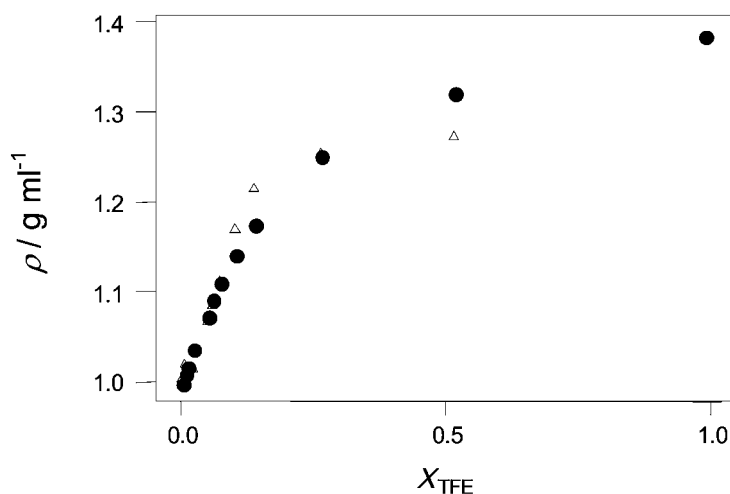


Fig. 4. Dependence of density,  $\rho$ , on the composition for TFE/H<sub>2</sub>O mixtures. Filled points represent the experimental data [19]. Open triangles represent data calculated with Eqn. 4, dependent on SA and SB parameters.

The experimentally determined values [19] of  $\epsilon$  were fit to Eqn. 5, and results are shown in *Fig. 5*.

$$\epsilon = -1197 + 202 \text{ SB} + 1323 \text{ SPP} \quad (r = 0.994, \text{ sd} = 2) \quad (5)$$

This suggests a dependence of SB and SPP on  $\epsilon$ , with the latter as the most influential factor. Therefore,  $\epsilon$  does not only refer to dipolarity but also to SB, which should not be surprising, since the dielectric constant of H<sub>2</sub>O is higher than that of TFE, and H<sub>2</sub>O is a much stronger acid than TFE.

*Smith et al.* [16] reported vapor pressures,  $p_v$ , for TFE/H<sub>2</sub>O mixtures spanning the whole composition range. Their data were fit to the SA and SB values (*Fig. 6*) using Eqn. 6.

$$p_v = 382 - 330 \text{ SA} - 150 \text{ SB} \quad (r = 0.994, \text{ sd} = 1.50 \text{ mm Hg}) \quad (6)$$

The change in  $p_v$  with TFE mixtures is a function of two microscopic properties, SB and mainly SA.  $p_v$  increases with TFE concentration, mainly with the decrease of SA; that is, with the decrease of H-bond formation.

Although several NMR studies of proteins and peptides have shown TFE-induced changes on  $\delta$  for the amide H-atoms of all residues [41], little is known about the change of  $\delta$  of TFE/H<sub>2</sub>O mixtures. In this report, we have characterized the change on

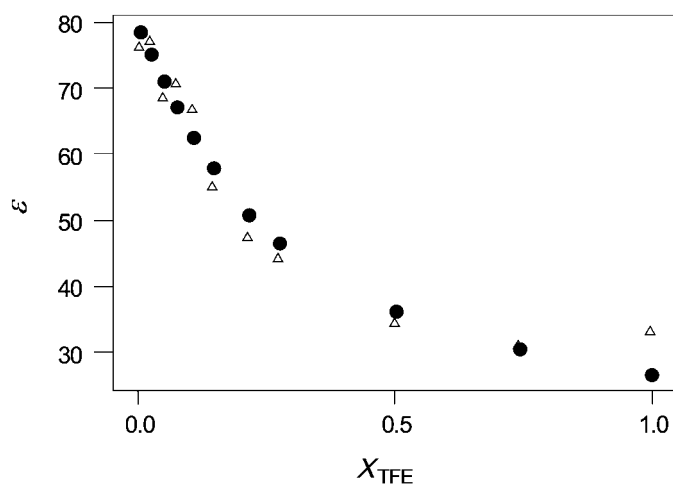


Fig. 5. Dependence of dielectric constant,  $\epsilon$ , on the composition of TFE/H<sub>2</sub>O mixtures. Filled points represent the experimental data [19]. Open triangles represent the data calculated with Eqn. 5, dependent on SB and SPP parameters.

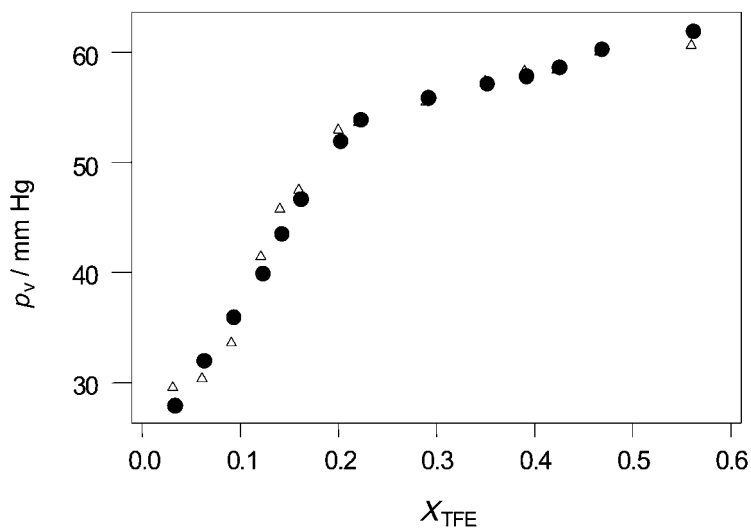


Fig. 6. Dependence of vapor pressure,  $p_v$ , on the composition of TFE/H<sub>2</sub>O mixtures. Filled points represent the experimental data [16]. Open triangles represent data calculated with Eqn. 6, dependent on SA and SB parameters.

the four bands making up the *quadruplet* of the Me group in the <sup>1</sup>H-NMR spectrum of TFE, in TFE/H<sub>2</sub>O mixtures. As shown in Fig. 7, there is an upfield shift of  $\delta(^1\text{H})$  with TFE concentration, which exhibits a non-linear dependence. A similar behavior was already observed for other H-atoms in several molecules and several solvents, as for example, the amide H-atoms in random peptides at different TFE concentrations [42].



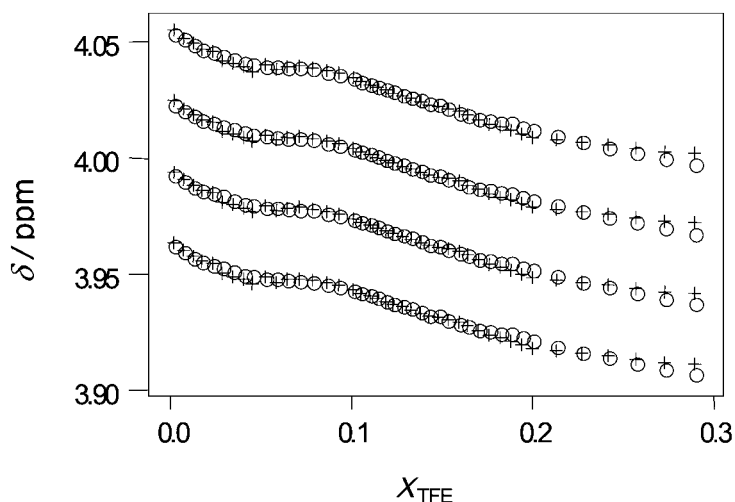


Fig. 7. Dependence of the chemical shift,  $\delta(^1\text{H})$  of the four bands making up the quadruplet of TFE Me group in TFE/ $\text{H}_2\text{O}$  mixtures. Opened circles represent experimental data, measured on a 300-MHz spectrometer. Plus represent calculated values obtained with Eqns. 7–10, dependent on the intrinsic parameters SA, SB, and SPP.

We were able to describe this upfield change on the *quadruplet's*  $\delta(^1\text{H})$  of the Me group of the TFE as a function of all mixture parameters (Eqns. 7–10), what demonstrates that microscopic mixture parameters allow explaining the change in  $\delta(^1\text{H})$  of TFE signal with the mixture, indicating that they are a suitable ensemble to represent the environment of the nucleus in solution. Measured values together with the calculated data obtained are shown Fig. 7.

$$\delta_1 = 2.78 + 0.210 \text{ SA} + 0.231 \text{ SB} + 1.09 \text{ SPP} \quad (r = 0.994, \text{ sd} = 0.002 \text{ ppm}) \quad (7)$$

$$\delta_2 = 2.78 + 0.211 \text{ SA} + 0.228 \text{ SB} + 1.06 \text{ SPP} \quad (r = 0.994, \text{ sd} = 0.002 \text{ ppm}) \quad (8)$$

$$\delta_3 = 2.75 + 0.206 \text{ SA} + 0.226 \text{ SB} + 1.07 \text{ SPP} \quad (r = 0.994, \text{ sd} = 0.002 \text{ ppm}) \quad (9)$$

$$\delta_4 = 2.70 + 0.200 \text{ SA} + 0.227 \text{ SB} + 1.09 \text{ SPP} \quad (r = 0.994, \text{ sd} = 0.002 \text{ ppm}) \quad (10)$$

Analysis of Eqns. 7–10 show that, owing to the weight of the SPP term, TFE induced changes in  $\delta(^1\text{H})$  are primarily dependent on the SPP of the medium. However, the variation of  $\delta(^1\text{H})$  in the mixture is simultaneously dependent on the variation of SA and SB and, in a minor proportion, on the variation of SPP, since this parameter is almost constant in these ranges of concentration. Consequently,  $\delta(^1\text{H})$  for of the Me group of TFE molecules in these mixtures is sensitive to specific and non-specific interactions with the environment. The magnitude of  $\delta(^1\text{H})$  variation will depend on the number and type of interactions. Several studies have already related the variation of the chemical shift for amide protons to the ability of molecules to form H-bonds [43]. Thus, for amide H-atoms, an upfield shift indicates a decrease in H-bonding with the

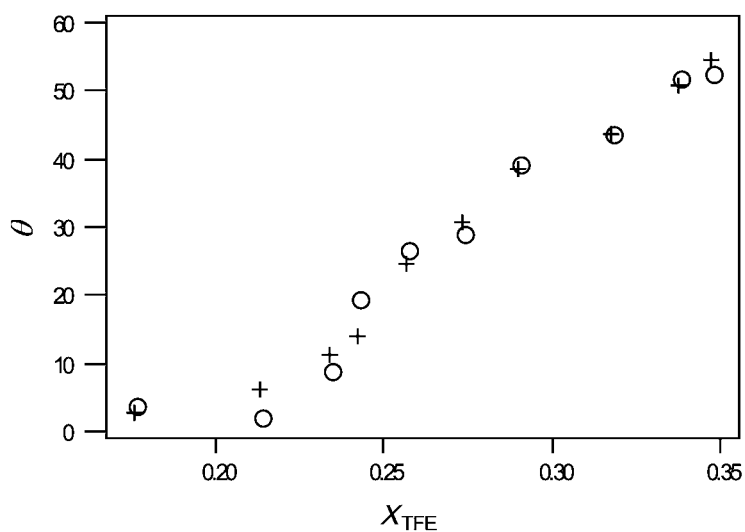
solvent, and a downfield shift an increase in H-bonding of the solvent to the connected C=O group.

In another study, the upfield shift for TFE H-atoms has been related to a decrease in the ability of the solvent to form H-bonds with solute molecules, reflecting the ability to form bifurcated H-bonds [43]. Also, theoretical studies by *Thomas* and *Dill* [44] confirm the importance of these polar interactions as a better way of modeling TFE behavior.

Poly(L-lysine) is a polypeptide known to adopt helical conformation in aqueous solution at pH 9 [37]. In this work, we studied the formation of secondary structure at an apparent pH of 7, as a function of composition of TFE/H<sub>2</sub>O mixtures. Measurements of the effect of TFE concentration on CD spectra permit calculation of the magnitude of the TFE-induced changes in % of helical content,  $\theta$ , of the poly(L-lysine). For values below 50% (v/v) of TFE ( $X_{\text{TFE}} = 0.2$ ) in the alcohol/H<sub>2</sub>O mixture, no secondary structure was detected, and the polypeptide chain was found as a random coil. Between 50 and 70% (v/v) of TFE ( $X_{\text{TFE}} = 0.2-0.4$ ) the poly(L-lysine) acquires an  $\alpha$ -helix conformation, which increases with the concentration of TFE (*Fig. 8*). The variation of  $\theta$  with TFE concentrations in the range of  $X_{\text{TFE}} = 0.2-0.4$  is described by *Eqn. 11*:

$$\theta = -5841 - 4760 \text{ SB} + 7248 \text{ SPP} \quad (r=0.99 \text{ and } \text{sd} = 3.00) \quad (11)$$

A representation of the experimental values together with those obtained from *Eqn. 11* is shown in *Fig. 8*. The coincidence of experimental and calculated values indicates the usefulness of the microscopic parameters in predicting the folding as a function of solvent-mixture composition for the polypeptide. From *Eqn. 11*, we can derive that  $\theta$  in



*Fig. 8.* Plot of the % of  $\alpha$ -helix of poly(L-lysine) chain,  $\theta$ , as a function of TFE mole fraction in TFE/H<sub>2</sub>O mixtures. Experimental values are calculated from CD measurements. Filled points represent experimental data, and open triangles represent the values obtained with *Eqn. 11*, dependent on the intrinsic parameters SB and SPP.

poly(L-lysine) depends on the SPP of the solvent-molecules, which, although having the largest coefficient, cause the smaller changes in this range of compositions, and, mainly, on the SB parameter. A large term for SB in the corresponding equation means high solvation of acid groups in the solute molecules in this mixture.

The TFE-induced variation in  $\theta$  is determined by the relative values of SB and SPP for TFE/H<sub>2</sub>O mixtures, as the first independent term and SB terms are negative, and SPP term is positive. As SB reaches a maximum on 30 % (v/v) TFE, the absolute value of the corresponding negative term in Eqn. 11 increases, which, thus, explains that the  $\theta$  helix is not formed at concentrations below 30% TFE. Physically, this means that the acid centers of the solute molecules, NH groups of the backbone and/or NH<sub>3</sub><sup>+</sup> groups on the side chains, are solvated and cannot form the necessary intramolecular H-bonds needed to adopt a stabilized secondary structure. It is necessary for the SB of the solvent to reach a minimum limit value to form an  $\alpha$ -helix and for the solute acid centers to be less solvated than in pure H<sub>2</sub>O. SPP also diminishes with respect to pure H<sub>2</sub>O, although in a lower proportion than SB, indicating 'hydrophobic solute centers' are also less solvated than in pure H<sub>2</sub>O. Once it is formed, the increment on  $\theta$  depends principally on the decrease of SB of the solvent, because SPP remains almost constant for this range of mixtures. To compare, SB diminishes by 10%, whereas SPP diminishes only by 0.2%.  $\theta$  in poly(L-lysine) does not depend on the SA of the solvent molecules. The solvation of basic groups in the solute molecules is the same in H<sub>2</sub>O as in TFE/H<sub>2</sub>O mixtures.

**Discussion.** – In summary, the viscosity,  $\eta$ , density,  $\rho$ , and vapor pressure,  $p_v$ , of the mixture depends on two parameters, SA and SB, both parameters are determined by number of H-bonds in the mixture. Dielectric constant,  $\epsilon$ , and % of helical contents,  $\theta$ , also depends on two parameters, SB and SPP, with SPP as the most influential. In contrast, the partial molar volume,  $\bar{V}$ , depends only on SA. Chemical shift,  $\delta$ , depends on all three parameters.

The previous examples reveal that the variation of physical properties can be rationalized with acceptable accuracy as a function of experimentally measured solvent parameters: acidity, SA, basicity SB, and dipolarity/polarizability, SPP. As shown above, the properties of TFE/H<sub>2</sub>O mixtures examined in this work can be interpreted on the basis of a multiparametric equation for each property throughout the composition range. The apparent deviations from ideal behavior in the properties studied ( $\eta$ ,  $\bar{V}$ ,  $\rho$ ,  $\epsilon$ ,  $p_v$ ,  $\delta$ , and  $\theta$ ), and their seemingly dual behavior, can be avoided by expressing them in terms of the SA, SB, and SPP parameters, instead of  $X_{\text{TFE}}$ .

Our results demonstrate that specific and non-specific interactions between solvent and solute play a determinant role in secondary-structure acquisition and stability of a polypeptide. The knowledge and quantification of the microscopic properties of the solvent to form H-bonds, as well as the sequence effects of solute, are determinant in understanding how polypeptides or proteins fold according to previous studies [45][46].

These results should be highly useful with a view to establishing and understanding the underlying effects of structuring processes involving H-bonding and/or polar interactions (e.g., those observed in peptides and proteins), and explains the ability of TFE/H<sub>2</sub>O mixtures to induce regular structures. A future paper will deal in detail with

the use of these systems, which behave differently depending on the particular composition of the mixture and solute properties.

The authors dedicate this manuscript to the memory of Professor *José Laynez*, who passed away (24.12.2002), for his outstanding contributions to calorimetry and for being an extraordinary good friend. This work was funded within the frameworks of *Projects BQU2003-07281, BQU2003-07658-C02-01, and BQU2002-02016*. <sup>1</sup>H-NMR Spectra were recorded on a *Bruker DPX 300* spectrophotometer of the Spectroscopy Center (CAI) of the University Complutense of Madrid.

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