

How to explain microemulsions formed by solvent mixtures without conventional surfactants

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Ternary solutions containing one hydrotrope (such as ethanol) and two immiscible fluids, both being soluble in the hydrotrope at any proportion, show unexpected solubilization power and allow strange but yet unexplained membrane enzyme activity. We study the system ethanol-water-octanol as a simple model of such kinds of ternary solutions. The stability of “detergentless” micelles or microemulsions in such mixtures was proposed in the pioneering works of Barden and coworkers [Smith GD, Donelan CE, Barden RE (1977) *J Colloid Interface Sci* 60(3):488–496 and Keiser BA, Varie D, Barden RE, Holt SL (1979) *J Phys Chem* 83(10):1276–1281] in the 1970s and then, neglected, because no general explanation for the observations was available. Recent direct microstructural evidence by light, X-ray, and neutron scattering using contrast variation reopened the debate. We propose here a general principle for solubilization without conventional surfactants: the balance between hydration force and entropy. This balance explains the stability of microemulsions in homogeneous ternary mixtures based on cosolvents.

microemulsions | aggregation | micelles | hydrotrope | hydration force

Adding slightly hydrophobic compounds to water can lead to structureless solutions, aggregate formation, or even, formation of defined structures, such as micelles, in the case where the added compound is a surfactant. In ternary or quaternary mixtures containing at least one type of surfactant, the formation of microemulsions usually occurs in specific parts of the phase diagram. These macroscopically homogeneous, transparent liquids are composed of well-defined microstructures with specific signatures in scattering experiments (1). It was only recently that similar structures, designated as “pre-Ouzo,” were found and characterized in ternary mixtures of two partly miscible solvents and one hydrotropic cosolvent (2). In this paper, we present a theory that explains and even predicts the existence of such structures in “detergentless” formulations.

Ouzo, Limoncello, and Pommeau liquors are popular in several European countries and produced by maceration of plants with a specific amount of ethanolic solutions containing some water-insoluble compounds (3). Adding water to those solutions leads to spontaneous formation of fine emulsions with a remarkable stability, a phenomenon that is called the “Ouzo effect” (4). Even common mouthwash products show a similar phenomenon. In common, they entirely clear up on addition of ethanol and get milky with the addition of water (5).

Ternary surfactant-free model systems, such as decane-water-isobutoxyethanol [as studied by Shinoda and Kunieda (6)], however, show this Ouzo effect only for specific points in the composition diagram. The precondition for such behavior seems to be the mixture of two miscible (either completely or at least to a large degree) solvents 1 and 2 with a solute that can also be a liquid (7) (e.g., anethole in the case of Ouzo; component 3). This component 3 must be highly soluble in one solvent (e.g., ethanol) but poorly soluble in the other one (e.g., water) (8).

Whereas the Ouzo effect is increasingly studied and partly understood, we focus our attention on the single-phase adjacent region of the phase diagram, where the water content is still low

enough to get a macroscopically homogeneous, stable, transparent one-phase system. Surprisingly, these one-phase systems are structured as revealed by combined X-ray and neutron scattering (2). In analogy to the Ouzo effect, which is related to relatively stable fine emulsions without surfactants, we named the unexpected structuring in these monophasic systems the pre-Ouzo effect, emphasizing that it occurs before the system is diluted with so much water that it phase-separates (9).

There is no quantitative or predictive explanation of this surprising but very common pre-Ouzo solubilization at the mesoscopic scale. The aim of this work is to investigate the underlying mechanism. We use as a model system the generic ternary mixture of water-ethanol-octanol, because detailed measurements of densities and ethanol partition are available (10). Water and octanol present a large miscibility gap, with ethanol being a cosolvent as well as a hydrotrope.

According to the classical theory, based on the balance between the van der Waals and electrostatic interaction combined in the Derjaguin-Landau-Verwey-Overbeck theory (DLVO) approach, it is generally accepted that surfactants in aqueous solutions form micellar and lyotropic liquid crystalline phases (i.e., supramolecular organizations that minimize the free energy above a certain temperature and concentration) (11). Short-chain alcohols, which are surface active but do not fulfill the criteria of micelle-forming surfactants, are used in formulations as a “cosurfactant.” Because they quench the presence of defined surfactant aggregates, they are also known as “hydrotropes” (12, 13). In binary solutions with water, all

Significance

Beginning over 40 y ago, a curious type of microemulsions—as transparent dispersion of two immiscible liquids separated by an interfacial film—at thermodynamic equilibrium has been described as “pre-Ouzo,” “detergentless,” or surfactant-free microemulsions. The experiments in ternary systems containing one hydrotropic cosolvent were ambiguous, and therefore, there was no need to come up with a general theory. Recent evidence obtained by specific deuteration in neutron scattering established the need for the extension of self-assembly theories based on Derjaguin-Landau-Verwey-Overbeck theory (DLVO), bending, or phase transfer energy. Here, we introduce a general free energy expression for weak self-assembly, where solvation effects and entropy compete without the influence of film bending, that explains all experimental results for this class of microemulsions.

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alcohols form loose networks that can be seen as living polymers, and the 3D mesh that they form has a specific signature in small-angle scattering (14).

However, over the last 30 y, occasional papers postulated the so-called detergentless alias “surfactant-free” microemulsions. Their existence was claimed as a working hypothesis to explain some curious observations, such as the fact that enzymes requiring interfaces work well in such systems (15). The oldest papers go back to the 1970s (7, 8). The key observation at that time was the appearance of three optically clear domains in analytical ultracentrifuge. Beyond the single-phase domain near the phase separation line, they show the Ouzo spontaneous emulsification effect on dilution. Moreover, close to the boundary but nevertheless, far from the critical point, these ternary solutions exhibit dynamic light scattering (16) that is experimentally identical to that characteristic of monodisperse micellar aggregates (17). Recently, density contrast variation using three different isotopic labeling conditions in small-angle neutron scattering (SANS) combined with small-angle high-resolution X-ray scattering (SWAXS) could deliver unambiguous structural information (2). It was shown that water, ethanol, and *n*-octanol segregate in water-rich and octanol-rich mesoscopic pseudophase domains and that ethanol partitions between these domains (18) with a scattering pattern different from classical microemulsions (*SI Materials and Methods, Scattering Data and Interpretation*). Clearly, the sample satisfies the international union of pure and applied crystallography (IUPAC) criteria for microemulsions, but the thermodynamic stability of this type of detergentless microemulsions must be of an origin different from classical micelles or microemulsions.

Experimental Results on the Water-Ethanol-*n*-Octanol System

The phase diagram of the water-ethanol-*n*-octanol system is shown in Fig. 1, which also shows the composition for which SANS and SWAXS have been performed (2). (*SI Materials and Methods, Scattering Data and Interpretation*). The partition of ethanol between the two coexisting pseudophases, as inferred from neutron scattering, is also given in *SI Materials and Methods, Scattering Data and Interpretation* (Table S2). The end points of the virtual tie lines representing the two compositions coexisting at the nanometer scale are shown as a virtual tie line in Fig. 1. In the two-phase region, we relate the interfacial tension between octanol-rich and water-rich

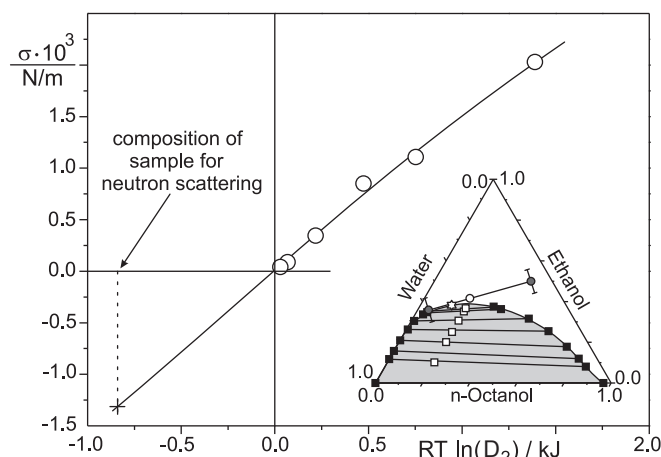


Fig. 1. Experimental interfacial tensions σ between octanol- and water-rich phases in the biphasic domain (46) for the samples shown by \blacksquare in *Inset* with the extrapolation into the monophasic domain. (*Inset*) Ternary-phase diagram (weight %) of the model system water-ethanol-*n*-octanol-1, with \circ the location of the sample investigated by contrast variation in SANS, small-angle X-ray scattering/wide-angle X-ray scattering, and the critical point (\star). Pseudophases of compositions are shown on a pseudotie line. Data recorded at 25° C. RT, gas constant multiplied by temperature.

phases with an evaluation of the reference free energy of transfer per ethanol molecule from the water- to octanol-rich phase (Fig. 1). Because in the single-phase pre-Ouzo domain, the compositions of the two coexisting pseudophases have been determined by neutron scattering, the abscissae of the corresponding data points are known. At compositions containing a smaller percentage of cosolvent (here ethanol) than the translucent sample studied, the sample appears “cloudy,” and pronounced light scattering known as “haze” in chemical engineering is always detected. Extrapolation as a straight line would, for the single-phase pre-Ouzo domain, give a negative surface tension of 1 mN/m, which is shown as a data point in Fig. 1. All surface tensions in Fig. 1 are reported as a function of the molar distribution coefficient D_2 of ethanol, which is defined as the ratio of the molarities of ethanol in water- and octanol-rich phases (18). The x coordinate in Fig. 1 is an estimate of the difference of the solvation free energies of ethanol in water- and oil-rich coexisting phases. As expected, close to the critical point, surface tension vanishes: at that stage, the critical fluctuation between pre-Ouzo aggregates—and not dispersed octanol molecules—is the dominant phenomenon. Vanishing of surface tension is also observed with classical microemulsions (19).

For the composition given in Table S2, assuming a maximum density of 5 OH groups per 1 nm² at the interface between the aqueous and octanol pseudophases and using known partial molar volumes, we infer that the octanol-rich aggregates are composed of 80 octanol and 350 ethanol molecules. The water-rich domains have a composition close to 60/40/0 wt/wt/wt in water/ethanol/octanol, whereas for the octanol-rich domains, the composition is 10/50/40 wt/wt/wt in water/ethanol/octanol. The “internal” volume fraction of octanol-rich domains is 55% (*SI Materials and Methods, General features of SANS and small-angle X-ray scattering patterns*). The SANS experiments show the presence of polydisperse octanol-rich globular domains surrounded by water-rich domains, which can be considered as pseudophases (20). The octanol-rich domains are in thermodynamic equilibrium and separated by a surface film, which contains a slight accumulation of ethanol molecules at the interface (21) as also suggested by molecular dynamics (MD) simulations (22). This microstructure corresponds to the IUPAC definition of microemulsions, because two immiscible fluids are separated by an interface, which in the case described here, is a nanometer-thick diffuse film composed mainly of ethanol (23). However, no “Porod limit” or broad peak characteristic of classical microemulsions is observed (24, 25).

In Fig. 2, we illustrate the features of the weak structuration with the snapshots of an MD structure in the pre-Ouzo region. Octanol-rich domains typically have a radius of about 2 nm, and the interface has a slightly enhanced concentration of ethanol. The phase behavior and the density fluctuations visible in the figures are reminiscent of critical fluctuations in ternary fluids, which also produce Ornstein-Zernike (OZ) scattering functions and diverging scattering intensities near the critical point. Comparison between expected scattering by Fourier transformation of real molecular coordinates is discussed in *SI Materials and Methods, Scattering Data and Interpretation* and shown in Fig. S4.

However, there is a subtle distinction between the critical fluctuations in a ternary molecular fluid and the emerging pre-Ouzo aggregates. Close to a critical point, diverging intensities of static light scattering at low angles are measured, and this divergence is also the case in this ternary system (26). However, in critical fluctuations of an unstructured ternary fluid, fluctuations are featureless changes in molecular concentrations. By contrast, in this case, the hydrotropic nature of ethanol favors the formation of interfaces, and near the critical point, the fluctuations are formed by well-defined aggregates alternating between oil-rich and -poor domains as shown qualitatively by light scattering studies. To interpret SANS at different contrasts, we use the inverse scattering intensity vs. square of scattering vector: the crucial result is shown in Fig. 3. The length scales corresponding to the results are 1.8 nm for the aggregates, 2.1 nm for the “holes” in water-rich solvent, and 1.2 nm for the ethanol-rich interface (Fig. 3). Critical points between aggregates of

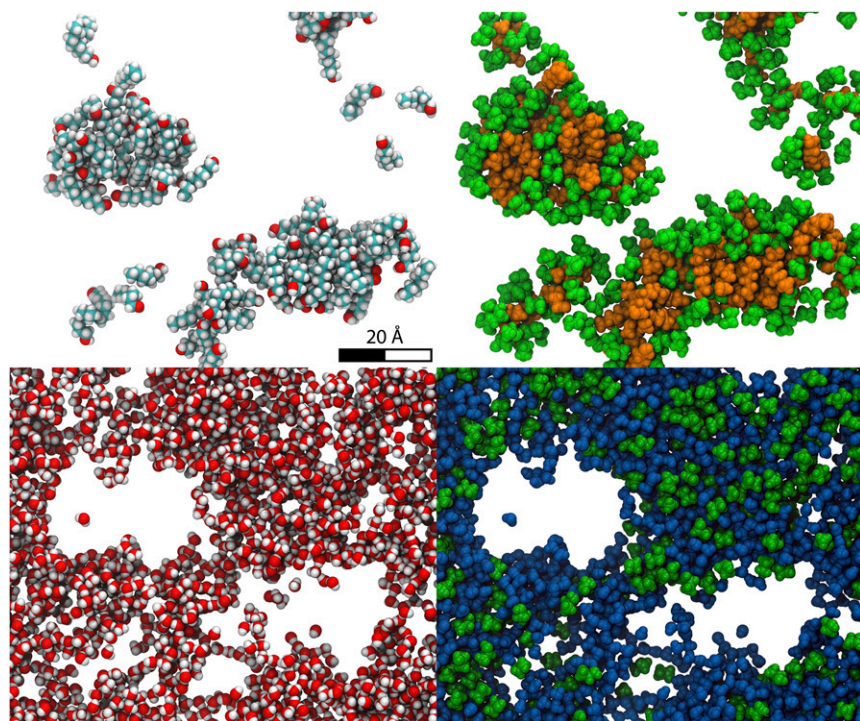


Fig. 2. Snapshots from molecular dynamics (22) in the single-phase pre-Ouzo region (octanol weight fraction $x_E = 0.2$; water weight fraction $x_W = 0.8$). (Upper Left) Only the octanol molecules are shown. (Upper Right) The ethanol molecules that interact strongly with the octanol molecules are shown in green. (Lower Left) Only the water molecules are shown, showing the morphology of the water-rich “external” aqueous pseudophase. (Lower Right) The same snapshot with water molecules represented together with the ethanol molecules partitioned in the water-rich phase. Each slice is 1.5-nm thick.

different morphologies not corresponding to molecular fluctuations have also been described for nonionic micellar systems (27, 28).

Hydration Vs. Entropy Balance

In the previous section, we have shown that, in the pre-Ouzo region near the miscibility gap in the ternary system studied, polydisperse globular transient aggregates with no sharp interface are in dynamic equilibrium. The coexistence of two pseudophases in equilibrium, one octanol-rich and one water-rich, requires that this microstructure corresponds to a free energy minimum. Using the equation of state approach, equivalent to the balance of forces as introduced by Parsegian and coworkers (29) in the case of lipids, the derivative of the free energy vs. correlation length should be zero at equilibrium: any decrease or any increase in average size costs overall free energy. To determine the equation of state, classically, van der Waals vs. electrostatics is considered by DLVO, with entropic terms caused by fluctuations of diluted flexible bilayers considered as thin solids introduced by Helfrich (30) and extended to flexible microemulsions with vanishing interfacial film thickness by Andelman and coworkers (31).

In the case of classical micelles and microemulsions containing at least one surfactant with a well-defined head group “bound” to the oil–water interface, the volume of micelle and microemulsion droplet is controlled by the area per molecule at the interface (as well as topology) (32–34). The free energy of micelle formation vs. the area per surfactant molecule (the lateral equation of state) is a function with a deep minimum (35). This minimum of the free energy varies with the average pseudophase interfacial area per surfactant and controls the micellar volume (36). However, in this case, we have neither electrostatic repulsion nor surfactants bound to the interface and controlling the oil/water-specific surface. Note that octanol does not play the role of a surfactant here, because the interface is enriched in ethanol.

Therefore, the main questions are:

In the absence of electrostatic repulsion, what prevents water-rich as well as octanol-rich nanodomains from coalescence?

What kind of force balance determines the size of these microemulsions?

To obtain insight into the stability of the pre-Ouzo phase, we model the key contributions to the free energy with the structure obtained by a level cut of a random Gaussian field with the OZ spectrum (37, 38). This structure (Fig. 4A) is consistent with the scattering spectrum and morphologically similar to the simulation results (Fig. 2), although the interfaces of the domains are unrealistically sharp. Because our purpose is to illustrate the physical principles behind the observed results, the resulting semiquantitative model is, nevertheless, indicative of the force balance responsible for the stability of these microemulsions that we introduce (for the first time to our knowledge) in this work. Within the model, we look at possible contributions to the free energy:

Mixing Entropy Contribution. The major influence determining the system structure is the entropy of the dispersion. It always drives the system toward smaller domains (i.e., more mixing between the

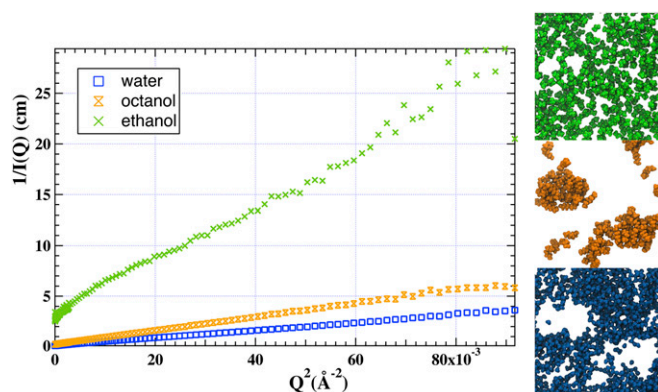


Fig. 3. (Left) Scattering intensity $I(q)$ in OZ representation of the same sample with (Right) three different contrasts: (Top Right) ethanol only (green), (Middle Right) octanol only (orange), and (Bottom Right) water only (blue). The steepest slope is caused by a diffuse layer about 1.2-nm thick. The molecular dynamics (slice is 1.5-nm thick) show the molecules as seen by each specific labeling experiment.

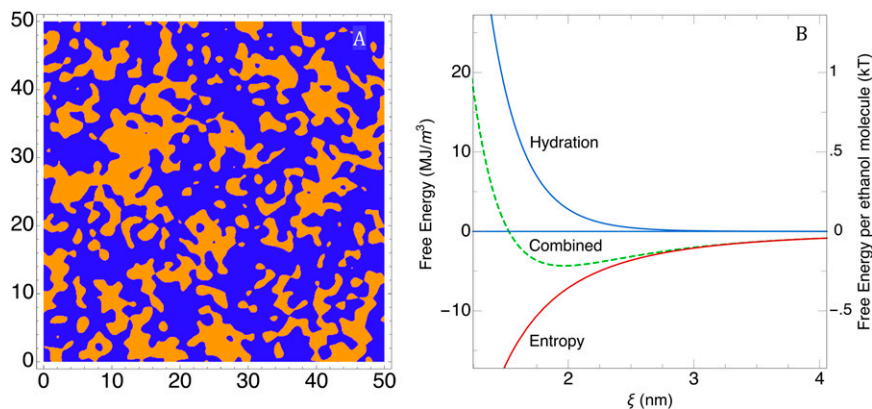


Fig. 4. (A) A snapshot of the real space representation with a cutoff wavelength of 1.8 nm (edge length of 50 nm). (B) Total free energy per unit volume of sample (left scale) and free energy per ethanol molecule (right scale) vs. the correlation length- ξ (nanometers) Also shown is the hydration (solvation) force contribution (evaluated using $\Pi_{\text{hydration}} = 1.5 \cdot 10^{10}$ Pa and $\lambda = 0.27$ nm).

phases). In the monophasic domain, close to phase separation, molecules are not randomly dispersed, and the free energy minimum is obtained for an optimal-length scale of the dynamic clusters. In the case of standard microemulsions, Gaussian random field together with the bending elasticity explained the formation of different bicontinuous microstructures (37, 39). A Gaussian random field with the same OZ spectrum as observed in the experiments results in the maximally disordered configuration (and hence, the highest entropy) consistent with the data. Alternatively, the more ordered matched hard sphere fluid gives the lower bound for the entropy (*SI Materials and Methods, Energetic Aspects*).

Free Energy of Hydration. The contribution to the free energy resulting from the hydration force between surfaces could be positive or negative, depending on the details of the water structure surrounding ethanol clusters. In the case of hydrophilic surfaces that induce solvent orientation, the interaction is normally repulsive, and the associated free energy is the integral of the hydration pressure

$$\Pi_{\text{hydration}} = \Pi_0 \exp[-h/\lambda],$$

where λ is the decay length associated with the hydration (40). The contact pressure Π_0 depends on the binding and positioning of water and ethanol molecules at the interface. The average thickness h of water-rich slabs is obtained using the surface to volume ratio of the leveled Gaussian random field (41). Alternatively, h can be estimated by the tessellation of faceted polyhedrons (42), leading to a similar result: h is close to 1.5 nm for the sample described in Table S2.

The hydration force is significant near the interface, even in the absence of salts (43, 44). The results are consistent with the Marčelja-Radić approach: a structured layer of solvent, with an experimental decay of an order parameter and a slight excess of ethanol at the interface, produces a net repulsion of the aggregates (45).

van der Waals Contribution. The van der Waals term is associated with attractive dispersion forces between octanol-rich domains. Using the average thickness of water domains h mentioned above and the Hamaker constant of the order of 10^{-1} – 10^{-2} $k_B T$, we find that the van der Waals term is negligibly small: at least two to three orders of magnitude smaller than the two other terms.

The equation used for the energy minimization is given in *SI Materials and Methods, Energetic Aspects*. In Fig. 4B, we illustrate the contributions from the hydration force ($\Pi_{\text{hydration}} = 1.5 \times 10^{10}$ Pa and $\lambda = 0.27$ nm), the entropy contribution to the free energy, and the sum of the two terms expressed as the free energy per unit volume of the sample or the free energy per ethanol molecule present in the sample. The free energy of transfer of ethanol molecules between macroscopic “bulk” octanol and water phase is typically 20 times higher than this value (46). In our view, the small difference explains why pre-Ouzo microemulsion domain

thermodynamics have not been evaluated before. Also, non-trivial evaporation paths are observed from the pre-Ouzo region: the paradox is that evaporation does not follow volatility of components (47). It can be expected that similar values of the hydrotrope transfer energy will be obtained in the case of diols and any type of water-insoluble but alcohol-soluble fluids that also form microemulsions. A shallow minimum for droplet sizes around 2 nm allows the formation of a polydisperse microdomain solution. Using the left-hand scale in Fig. 4B, the order of magnitude of the free energy minimum relative to the phase-separated state (when the contributions considered here vanish) is consistent with 5,000 kJ/m^3 (i.e., of the order of 0.2 $k_B T$ per ethanol molecule), which is even lower than the cost in enthalpy of transfer of one ethanol molecule from octanol to binary water–ethanol bulk. Nevertheless, such a low energy is sufficient to ensure activities of enzymes that require the presence of a defined oil–water interface (48).

It is quite remarkable that a shallow minimum exists for a typical size of 2–3 nm dominated by a competition between entropy of mixing and hydration energy (i.e., the solvation energy of the octanol-rich aggregates). We note that taking into account only mixing entropy and bending energy while fixing the area per molecule corresponds to the de Gennes–Taupin expression for the free energy. Moreover, it is known that surfactants with large head groups and intermediary chains produce a broad bump in scattering in binary solutions, even at less than 20% vol water. The introduction of the repulsive hydration term also provides the explanation for scattering of concentrated microemulsions containing nonionic surfactants. Because the minimum is shallow, micelles fluctuate in volume, and the mass distribution is extremely broad: the common broad peak in scattering of microemulsions is not observed (49). Although the average lifetime of microemulsion domains is presently not experimentally determined, the thermodynamic model is valid independent of the lifetimes of the aggregates (50).

The free energy minimization as derived quantitatively in *SI Materials and Methods, Energetic Aspects* unifies the description of the surfactant-free and surfactant-containing microemulsions. The electrostatics or steric-repulsive vs. van der Waals attraction balance valid for classical micellar systems has to be replaced by the hydration vs. entropy balance in the case of hydrotrope-based microemulsions.

The model proposed here is analogous to the classical DLVO approach in the sense that the electrostatic repulsion caused by charged surfactant head groups is replaced by short-ranged but nevertheless, significant repulsion because of to the ordering of water near the interface and a much less pronounced, Gouy–Chapman-like ethanol concentration profile. The resulting hydration force is basically caused by the high OH surface density at the interface as shown in Fig. 2.

Moreover, the “thickness” of the water-rich domains corresponds to typically four layers of water when considered within the frame of water ordering as the key parameter of the hydration

force. The thickness of the interfacial phase, slightly enriched in ethanol, is here close to one-half of the radius of microemulsion domains. The concept of diffuse counterion layers is, therefore, extended from electrolytes (within the Poisson–Boltzmann theory) to partially adsorbed nonelectrolytes, such as ethanol acting as a hydrotrope. To do so, the Marčelja–Radić theory for hydration forces in pure water (45) must be extended to binary solvents much as it has been extended to the case of concentrated counterion layers in the work by Mitlin and Sharma (51).

It should be noted that there is no geometrical constraint on the fluctuating interface, and therefore, influences of curvature may be present but are not responsible for the observed microdomain formation. As mentioned before, our approach is further supported by recent sophisticated molecular dynamics simulations shown in Fig. 2 that are in agreement with the scattering results.

With increasing ethanol concentration, the microdomains progressively vanish, and the mixture becomes mainly a structureless molecular solution (22). Hydration vs. van der Waals balance may also contribute to the stability of emulsion droplets in the Ouzo regime. However, the competition between these two forces alone does not explain the Ouzo effect or the surprising stability of stable fine emulsions without surfactant (52, 53), where other phenomena may be predominant. Finally, it is interesting to notice the absence of lyotropic phases in the pre-Ouzo region in agreement with the general knowledge about hydrotropes (12).

Ubiquity of the Entropy–Hydration Balance in Ternary Systems

We stress that neither the Ouzo effect nor the pre-Ouzo effect discussed here is restricted to ethanol (*SI Materials and Methods, Scattering Data and Interpretation*). Although we considered only one particular system for our detailed SANS/small-angle X-ray scattering/wide-angle X-ray scattering study, on the basis of the agreement with the light scattering results, we are confident that the dynamic light scattering/static light scattering results of many other ternary systems hint at a similar structuring and mechanism (Fig. 5). For example, DMSO, acetonitrile, and other solvents can also play the role of the second water-miscible solvent or hydrotrope (13). Even water can be replaced by glycerol and other solvents, such as ionic liquids and deep eutectics. As stated in the introduction, it seems that the only criterion is that two nonmiscible or only slightly miscible liquids are made compatible by a third one. Solvation vs. entropy plus van der Waals probably is responsible for microdomain formation in perfumes, beverages, pharmaceutical formulations, etc. (9).

To further check the generality of the pre-Ouzo structuring phenomenon and our generalized DLVO approach, we have chosen five other ternary systems near the miscibility gap (Fig. 5). For comparison, the scattering curve of 1-octanol/ethanol/water is also included. In the case where a pre-Ouzo structuring is detected, the characteristic size of the domain, ξ , is indicated. As can be seen in Fig. 5, three samples show a visible structuration at high Q values coexisting with OZ-like scattering at low Q . The “surfactant” enriched in the interface is for either ethanol or 1,5-pentanediol. These sizes are those expected for a reasonable decay of hydration forces as well as contact pressures (*SI Materials and Methods, Energetic Aspects*). In the cases of octane and toluene, no pre-Ouzo structuring as revealed by low Q scattering is observed. Obviously, the ethanol concentration required to get monophasic mixtures is too high, and OZ-like signal can no longer be detected. This behavior is in agreement with our theory, which is further detailed in *SI Materials and Methods, Energetic Aspects*. Nevertheless, the range in volume fraction of ethanol at which the pre-Ouzo effect is still present depends on the particular system. Octane does not produce any pre-Ouzo microemulsion, because it does not form high surface density of OH groups. At a very similar composition, the octanol-1,5-pentanediol-water ternary system has a high hydroxyl surface density, hence developing a repulsive hydration interaction.

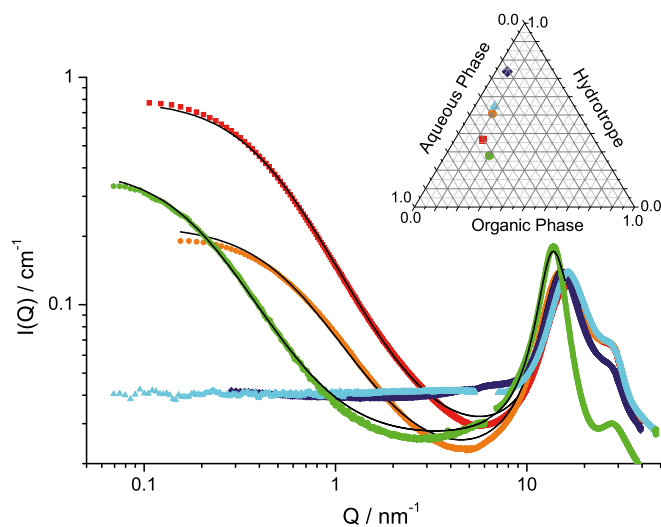


Fig. 5. Five different ternary systems presenting pre-Ouzo structuration and the corresponding SWAXS spectra of different ternary mixtures. Dark blue, toluene/ethanol/water; green, 1-octanol/ethanol/water ($\xi = 2.1$ nm); light blue, octane/ethanol/water; orange, 2-octanol/1,5-pentanediol/water ($\xi = 1.5$ nm); red, *m*-cresol (3-methylphenol)/ethanol/water ($\xi = 4.9$ nm).

To further investigate this point, the system octane-ethanol-water was chosen as the starting point, and 1-octanol was gradually replaced by octane. As we describe in *SI Materials and Methods, Quenching Aggregation by Control of Surface OH Density*, the pre-Ouzo structuration disappears below a certain octanol content, in line with predictions from theory (Fig. S8). In all systems exhibiting pre-Ouzo structuration described until now, the bending energy term can be completely neglected compared with the solvation effects, resulting in polydisperse aggregates (*SI Materials and Methods, Quenching Aggregation by Control of Surface OH Density*). The opposite situation may occur, because highly curved microstructures have been observed very recently by freeze fracture EM in surfactant-free microemulsions, where octanol is replaced by oleic acid (54). Finally, we want to point out that a significant amount of ethanol and even water is solubilized in oil microdomains. This behavior is in analogy to the type of micelles postulated by Menger and Doll (55) and has also been mentioned in the works by Winsor (56) and Lindau (57), which noticed the relation between structuration in detergentless microemulsions and eutectics. Because of the negligible influence of surfactant film bending energy and the ambiguities in the names detergentless or surfactant-free as well as pre-Ouzo or “mesoscopic solubilization,” we propose here to designate these ubiquitous dynamically stable microstructures as “ultraflexible microemulsions,” in contrast to the two previously known types: stiff and flexible microemulsions (1).

Materials and Methods

The phase diagrams and light scattering experiments were established according to the same methods as in the work in ref. 9 and *SI Materials and Methods*. The SWAXS and SANS were performed as in the work in ref. 2 and *SI Materials and Methods*. The surface tensions were determined using a standard spinning drop tensiometer (Krüss GmbH). The use of Gaussian random fields in modeling is detailed in *SI Materials and Methods*.

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