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ORIGINAL PAPER

Experimental evidence of stable water nanostructures in extremely dilute solutions, at standard pressure and temperature

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This paper presents the results of several experimental methods (FT-IR spectroscopy, UV–vis spectroscopy, fluorescence microscopy (FM), Atomic Force Microscopy (AFM)) evidencing structural changes induced in extremely diluted solutions (EDS), which are prepared by an iterated process of centesimal (1:100) dilution and succussion (shaking). The iteration is repeated until an extremely high dilution is reached, so that the composition of the solution becomes identical to that of the solvent—in this case water—used to prepare it.

The experimental observations reveal the presence of supramolecular aggregates hundreds of nanometres in size in EDS at ambient pressure and temperature, and in the solid state. These findings confirm the hypothesis—developed thanks to previous physico-chemical investigations—that formation of water aggregates occurs in EDS. The experimental data can be analyzed and interpreted with reference to the thermodynamics of far-from-equilibrium systems and irreversible processes. *Homeopathy* (2013) **103**, 44–50.

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Introduction

A number of studies published in recent years have investigated the physico-chemical properties of aqueous solutions prepared by an iterated process of dilution and vigorous vertical shaking (called 'succussion') whose end result is an 'extremely diluted solution' (EDS). The chemical composition of solutions obtained by this protocol is identical to that of the solvent used to dilute them: it follows, therefore, that an EDS should behave exactly like its solvent, at least from a physico-chemical point of view. Yet systematic experimental investigations into such solutions show that EDS exhibit markedly different properties from those of the water used to prepare them.[1–3] In particular, evidence from conductometric,[1,2] pH-metric[1,2] and calorimetric[3] measurements supports the hypothesis that the EDS preparation protocol causes an alteration in the 'structure' of the solvent, and that EDS behave as complex systems, with a path-dependent sensitivity to certain aspects of the preparation technique. Among the many such factors that can affect the physico-chemical properties of an EDS, the most important appear to be storage container volume[4] and age of the samples.[5] However, all the preparation variables—which also include the nature of the solutes, degree of dilution and the presence of electrolytes—act in concert, making the EDS a complex system that exists in a far-from-equilibrium state. EDS are systems that, under the action of various kinds of perturbations (e.g.,

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dilution, succussions, electromagnetic fields), are capable of taking on a structural organization characterized by variations in local order, through the formation of dissipative structures,[6–8] with variations of the physico-chemical properties that can be either permanent, temporary or oscillating.

In recent work,[1–3] we carried out conductometric, pH-metric and calorimetric titrations of EDS by adding HCl or NaOH, used as probe solutions. The results of those experiments were interpreted as denoting favourable interactions between the H_3O^+ and OH^- ions and the dissipative structures, probably due to favourable steric hindrance and chemical affinity with the aggregates. We conjectured that the H_3O^+ or OH^- ions from the probes were able to form complexes with the supramolecular structures in the EDS – dissipative structures. Acids and bases differ in their capacity to interact with such structures, thus producing different titration curves. The entire phenomenon hints at the presence of dissipative structures with a 'concentration' that can be determined using conductometric titrations with strong acids.[1]

Also water close to hydrophilic surfaces shows very interesting properties, which differ significantly from those of ordinary bulk water. For instance, water close to Nafion exhibits these noteworthy features[9–11]: it is unable to host solutes, hence the name Exclusion Zone (EZ); its viscosity is much higher than that of normal water, suggesting the presence of a strong interaction among the molecules; it exhibits a fluorescent response in the UV region at 270 nm. The existence of EZ water suggests a major reorganization of the supramolecular structure of water. The observed thickness of the EZ could be as high as 500 μm. In previous articles,[12,13] has been shown that the properties of such water, named INW, Iteratively Nafionized Water (INW) are quite similar to those of the EDS.

In the literature, there are many works on properties of water prepared by iterative dilutions and succussions.[14–18] It must be underlined that the procedure that we used to put in evidence the presence of molecular aggregates of water molecules are very different from those in the literature. The use of different approaches makes it difficult to compare them with our results, with apparently similar ones obtained by other authors. The main difference is related to our observation that the physico-chemical parameters of homeopathic dilutions of the samples increase with age (ageing effect) and with smaller volumes of ageing (volume effect). These observations have opened up a new possibility to study the physico-chemical properties of homeopathic dilutions, since the changes in the measured parameters have become measurable, i.e. significantly higher than the experimental error.

When we prepare a homeopathic dilution and, without waiting a long period of time, we measure its chemical and physical parameters, we do not see any numerically significant change in them. This includes parameters such as: electrical conductivity, pH, heat of mixing with acid or basic solutions, electromotive force of suitable galvanic cells (emf, mV), density, light scattering, microscopy, Atomic Force Microscopy (AFM) or fluorescence microscopy (FM), absorbance in the visible or infrared spectroscopy etc. Only after a long period of ageing in small volumes it becomes possible to measure differences that have physical meaning.

The aim of the present study was to confirm the hypothesized presence, in EDS, of molecular aggregates of water molecules (dissipative structures), by means of other kinds of experimental methods. This paper accordingly reports the structural measurements (FT-IR analysis, UV–vis spectroscopy, FM, AFM) alongside the thermodynamic data. Spectroscopic measurements provide important information about the shape and size of the hypothesized supramolecular structures, in both the liquid and solid state. We were here able to observe the formation of supramolecular aggregates, measuring hundreds of nanometres in size, in samples prepared by the procedure of successive dilutions and succussions.

Experimental

Materials

The solutes, produced by Carlo Erba, Sigma or Fluka, were of the highest purity commercially available. The EDS were prepared using, as the solvent, either water or a solution of sodium bicarbonate at low and known concentration ($5\cdot10^{-5}$ to $1\cdot10^{-4}$ mol·L^{-1}). For storing the EDS, we used laboratory glass containers that had been treated with a $H_2SO_4/K_2Cr_2O_7$ solution and subsequently rinsed with double-distilled water until the electric conductivity of the rinsing water reached a value of 1.2 ± 0.2 μS cm^{-1}, the same as double-distilled water.

EDS preparation

The EDS were prepared following the traditional technique of homeopathic medicine set out by Hahnemann,[19] which essentially involves the iteration of two steps: dilution (centesimal or decimal) and succussion. The process of succussion (also called 'dynamization') consists of vigorous agitation of the solution, by means of a mechanical apparatus (such as DYNA HV 1 by Debofar N.V.S.A. Belgium). For a centesimal dilution, the starting point is a 1% by mass solution. As an example, to prepare a centesimal dilution of sodium chloride (NaCl), 1 g of NaCl is added to 99 g of solvent (dilution step), and the resultant solution is then shaken (succussion step). These two steps produce what is called a 'first centesimal Hahnemannian' dilution, abbreviated 1 cH. So, in the preceding example, NaCl 1 cH was obtained. To prepare the second centesimal Hahnemannian dilution (NaCl 2 cH), 1 g of the 1 cH solution is added to 99 g of solvent, and the resulting solution is again succussed. These dilution and succussion steps are repeated until the desired degree of dilution is reached. Given that from 3 cH onward the concentration of the original NaCl solute is less than $1\cdot10^{-5}$ mol L^{-1}, it follows that an EDS should exhibit identical physico-chemical properties to those of its solvent. In practice, however, since the glass containers release alkaline oxides, we also need to know the Na^+ content of these solutions, because sodium oxide, by interacting with the environmental CO_2, gives rise to sodium bicarbonate. In any case our measurements are made on samples of EDS aged for long periods in small volumes.