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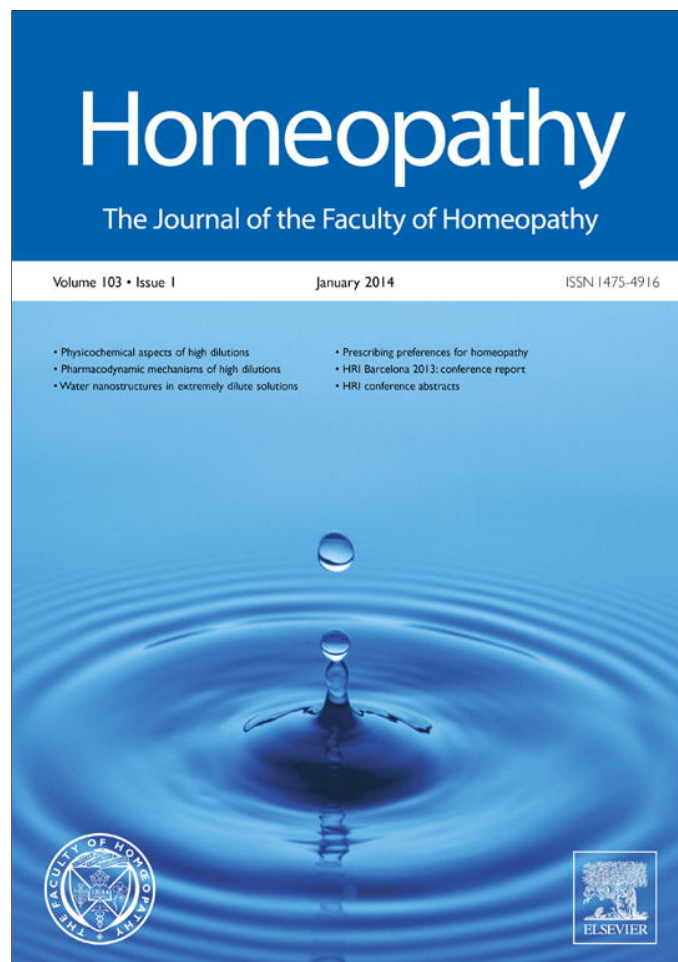
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## ORIGINAL PAPER

# 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.

**Keywords:** EDS; Homeopathic medicine; Dissipative structures; Conductivity; IR spectroscopy; UV–vis spectroscopy; Fluorescence microscopy; AFM

## 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.<sup>1–3</sup> In particular, evidence from conductometric,<sup>1,2</sup> pH-metric<sup>1,2</sup> and calorimetric<sup>3</sup> 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<sup>4</sup> and age of the samples.<sup>5</sup> 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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Received 31 May 2013; revised 28 June 2013; accepted 12 August 2013

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

In recent work,<sup>1–3</sup> 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  $\text{H}_3\text{O}^+$  and  $\text{OH}^-$  ions and the dissipative structures, probably due to favourable steric hindrance and chemical affinity with the aggregates. We conjectured that the  $\text{H}_3\text{O}^+$  or  $\text{OH}^-$  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.<sup>1</sup>

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<sup>9–11</sup>: 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  $\mu\text{m}$ . In previous articles,<sup>12,13</sup> 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.<sup>14–18</sup> 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 ( $5 \cdot 10^{-5}$  to  $1 \cdot 10^{-4}$   $\text{mol} \cdot \text{L}^{-1}$ ). For storing the EDS, we used laboratory glass containers that had been treated with a  $\text{H}_2\text{SO}_4/\text{K}_2\text{Cr}_2\text{O}_7$  solution and subsequently rinsed with double-distilled water until the electric conductivity of the rinsing water reached a value of  $1.2 \pm 0.2 \mu\text{S cm}^{-1}$ , the same as double-distilled water.

### EDS preparation

The EDS were prepared following the traditional technique of homeopathic medicine set out by Hahnemann,<sup>19</sup> 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  $1 \cdot 10^{-5}$   $\text{mol L}^{-1}$ , 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  $\text{Na}^+$  content of these solutions, because sodium oxide, by interacting with the environmental  $\text{CO}_2$ , gives rise to sodium bicarbonate. In any case our measurements are made on samples of EDS aged for long periods in small volumes.

After the above described procedure, the EDS samples were lyophilized to examine the properties of any solid residues.

## Methods

The EDS samples were systematically studied in both the liquid phase and solid phase. In the liquid phase, the following measurements were carried out: conductivity, analytical determination of impurities, UV–vis spectroscopy, FM. In solid state, the following measurements were carried out: FT-IR spectroscopy and AFM.

### Conductivity measurements

Systematic measurements of specific conductivity were performed on the EDS, using a YSI 3200 conductometer, with a conductivity cell having a constant of  $0.1 \text{ cm}^{-1}$ . Before measuring the conductivity of a sample, the cell was calibrated by determining the cell constant  $K/\text{cm}^{-1}$ . The conductometric measurements were performed in a thermostatic room ( $25 \pm 1^\circ\text{C}$ ) using samples whose temperature was conditioned in a measuring cell by a thermostat-cryostat (Grant LTD6) to be within  $\pm 0.1^\circ\text{C}$ .

### Analytical determination of impurities

The sodium concentration,  $M_{\text{Na}^+}/\text{mol L}^{-1}$ , of the EDS was determined through the spectroscopic method of atomic absorption, using a Spectra A Varian instrument. Before measuring the samples, the necessary calibration curve was obtained using standard solutions. To prepare the standard solutions, NaCl was dissolved in water and diluted to 1 L, to obtain  $1000 \mu\text{g}/\text{mL}$  Na. The working conditions were: Lamp current: 5 mA; Fuel: acetylene; Support: air; Flame stoichiometry: oxidizing. The wavelength used, chosen on the basis of the concentration range of sodium, was 589.6 nm. Sodium is partially ionized in the air-acetylene flame. To suppress the ionization, some potassium chloride solution was added, to produce a final concentration of  $2000 \mu\text{g}/\text{mL}$  in every solution, including the blank.

### UV–vis spectroscopy

The UV/vis spectra were monitored using a Jasco Spectrophotometer model V-560 UV/vis. This is a double-beam spectrophotometer with double monochromator, a wavelength range of 190–900 nm and a resolution of 0.1 nm. The photometric accuracy and reproducibility of the instrument are respectively  $\pm 0.002$  and  $\pm 0.001$ ; the recording conditions for each UV/vis spectrum were the following: response: slow; scanning speed: 100 nm/min; sampling interval: 1 nm/data.

### FM

Olympus  $\times 71$  inverted microscope with UPLSAPO  $20\times$  magnification objective was used, it was coupled at  $1.6\times$  magnification lens, the results were the total magnification of  $32\times$ . The illumination source of the microscope was a 100 W Hg lamp. A specific light excitation and emission wavelength was selected through a set of dichroic filters.

Images generated from fluorescence emission were observed through the eyepiece and then acquired by a Hamamatsu cmos photo-camera. The maximum pixel width of the photo-camera was  $1920$  (horizontal)  $\times$   $1440$  (vertical) with a sensor effective area of  $6.97$  (H)  $\times$   $5.23$  (V) mm. Considering the objective magnification we can obtain a ratio of 8.81 pixel/micron. We prepared a dispersion of 1% by weight mixing  $10 \mu\text{L}$  dispersion of polystyrene latex beads 2% by weight in a 0.99 mL EDS sample and in 0.99 mL of bi-distilled water (control) to observe the Brownian motion of the particles dispersed in each medium. The beads, purchased from Thermo Scientific, was made of surface carboxylate-modified polystyrene, had a size of 200 nm with a size standard deviation  $<10\%$ , and each particle carried green-fluorescent fluorescein probes. These are internally dyed microsphere suspensions which emit intense and distinct colours (510 nm) when illuminated by 495 nm wavelength light.

### FT-IR spectroscopy

Samples of EDS were lyophilized before being studied by IR spectroscopy. We recorded IR spectra of the lyophilized samples in solid phase in a NaCl dispersion medium, using an FT-IR Jasco-FT-IR-430 spectrophotometer. The recording conditions for each FTIR spectrum were: 64 scans, scanning speed of 2 mm/sec, a triangular apodization function and a resolution of  $2 \text{ cm}^{-1}$ .

### AFM

A Veeco Digital Instruments Nanoscope IIIa was used to perform AFM topography of the solid residues left behind by a few drops of samples evaporated on mica substrates. The AFM images were obtained by operating the AFM in non-contact mode. The AFM was equipped with an ultra-thin silicon point, with apical radius less than 5 nm, and analyses were evaluated on windows of different areas with an acquisition frequency of 2 Hz. As a control, we used water kept in prolonged contact (months) with Pyrex glass powder and allowed to dry on a similar mica sample holder.

## Results

### Structural investigations in the liquid phase

We carried out systematic measurements to determine the specific electrical conductivities of EDS prepared according to the procedure described in the [Methods](#) section. [Table 1](#) reports: the degree of dilution and succussion of the EDS (Centesimal Hahnemannian (CH)); the nature of the active principle (A.P.); the specific conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ); the molarity of sodium bicarbonate ( $M_{\text{Na}^+}$ ); the excess conductivity,  $\chi^E$  ( $\mu\text{S cm}^{-1}$ ), i.e., the difference between the conductivity of the EDS and that of the solvent employed for preparation; the age and volume of the EDS. Immediately after the preparation procedure no physico-chemical parameter is significantly modified. Only after long periods of ageing in small volumes the changes become significant. The results show that the EDS have systematically higher specific conductivities compared to



**Table 1** Specific conductivity,  $\chi/\mu\text{S cm}^{-1}$ ; the molarity of sodium bicarbonate ( $M_{\text{Na}^+}$ ) and the excess conductivity,  $\chi^E/\mu\text{S cm}^{-1}$ , at 298 K

System	t/days	$\chi/\mu\text{S cm}^{-1}$	$M_{\text{Na}^+}/\text{mol L}^{-1}$	$\chi^E/\mu\text{S cm}^{-1}$
AM 200 cH 2 mL	1784	69	$1.2 \cdot 10^{-4}$	57
AM 200 cH 1 mL	1784	63	$1.5 \cdot 10^{-4}$	48
AM 200 cH 0.5 mL	1784	70	$1.9 \cdot 10^{-4}$	51
AM 200 cH 0.25 mL	1784	273	$1.5 \cdot 10^{-3}$	123
AM 12 cH 2 mL	1784	51	$1.9 \cdot 10^{-4}$	32
AM 12 cH 1 mL	1784	55	$1.7 \cdot 10^{-4}$	38
AM 12 cH 0.5 mL	1784	74	$2.6 \cdot 10^{-4}$	48
AM 12 cH 0.25 mL	1784	155	$8.6 \cdot 10^{-4}$	69
AM 9 cH 2 mL	1745	78	$2.4 \cdot 10^{-4}$	51
AM 9 cH 1 mL	1745	70	$1.8 \cdot 10^{-4}$	52
AM 9 cH 0.5 mL	1745	77	$3.0 \cdot 10^{-4}$	47
AM 9 cH 0.25 mL	1745	107	$6.2 \cdot 10^{-4}$	45
AM 12 cH 2 mL	1745	99	$3.2 \cdot 10^{-4}$	67
AM 12 cH 1 mL	1745	68	$1.6 \cdot 10^{-4}$	52
AM 12 cH 0.5 mL	1745	78	$2.2 \cdot 10^{-4}$	56
AM 12 cH 0.25 mL	1745	108	$3.5 \cdot 10^{-4}$	73
AM 9 cH 2 mL	1696	64	$2.4 \cdot 10^{-4}$	40
AM 9 cH 1 mL	1696	53	$1.8 \cdot 10^{-4}$	35
AM 9 cH 0.5 mL	1696	53	$3.0 \cdot 10^{-4}$	23
AM 9 cH 0.25 mL	1696	81	$4.5 \cdot 10^{-4}$	36
AM 12 cH 2 mL	1696	70	$1.3 \cdot 10^{-4}$	57
AM 12 cH 1 mL	1696	56	$1.9 \cdot 10^{-4}$	37
AM 12 cH 0.5 mL	1696	53	$2.6 \cdot 10^{-4}$	27
AM 12 cH 0.25 mL	1696	78	$4.0 \cdot 10^{-4}$	38
Aspirin 12 cH	1218	130	$6.2 \cdot 10^{-4}$	68

their solvent, i.e., to an aqueous solution having the same chemical composition as the EDS itself.

The same samples studied by conductometric analysis, were also subjected to UV–vis spectroscopic measurements, in the range between 200 and 450 nm. Table 2 reports the detailed results of these UV–vis measurements on the EDS samples. The UV–vis spectroscopy reveals a

**Table 2** Specific conductivity,  $\chi/\mu\text{S cm}^{-1}$ ; and absorption values at 450 and 270 nm

System	$\chi/\mu\text{S cm}^{-1}$	$A_{450\text{ nm}}^{\circ}$	$A_{270\text{ nm}}^{\text{max}}$
AM 200 cH 2 mL	69	0.002	0.0687
AM 200 cH 1 mL	63	0.012	0.0776
AM 200 cH 0.5 mL	70	0.0169	0.1035
AM 200 cH 0.25 mL	273	0.0162	0.2582
AM 12 cH 2 mL	51	0.0017	0.0504
AM 12 cH 1 mL	55	0.0048	0.0477
AM 12 cH 0.5 mL	74	0.0115	0.0729
AM 12 cH 0.25 mL	155	0.0209	0.1994
AM 9 cH 2 mL	78	0.0126	0.0835
AM 9 cH 1 mL	70	0.0184	0.0856
AM 9 cH 0.5 mL	77	0.0228	0.1121
AM 9 cH 0.25 mL	107	0.0384	0.155
AM 12 cH 2 mL	99	0.0335	0.1209
AM 12 cH 1 mL	68	0.0317	0.107
AM 12 cH 0.5 mL	78	0.033	0.1395
AM 12 cH 0.25 mL	108	0.057	0.1771
AM 9 cH 2 mL	64	0.0315	0.0875
AM 9 cH 1 mL	53	0.0286	0.0932
AM 9 cH 0.5 mL	53	0.0375	0.0895
AM 9 cH 0.25 mL	81	0.0415	0.115
AM 12 cH 2 mL	70	0.0384	0.103
AM 12 cH 1 mL	56	0.0385	0.103
AM 12 cH 0.5 mL	53	0.0395	0.106
AM 12 cH 0.25 mL	78	0.0467	0.133
Aspirin 12 cH	130	0.0413	0.1862

new absorption peak in the 225–325 nm wavelength region, at approximately 270 nm: see Figure 1a. We also tested a normalization of the experimental data by subtracting the value of  $A^{\circ}$  from the value of the maximum absorbance. Plotting these values against the previously measured conductivities of the EDS solutions reveals a linear correlation: see Figure 1b.

Examination of the EDS samples by FM revealed an unexpected feature: large-sized structures on which the labelled polystyrene spheres appeared to be clustered, immersed in a surrounding fluid. Figure 2a shows the image of the control prepared with double-distilled water in contact to glass powder for several months, while Figure 2b shows the shape of the structures in which the polystyrene beads were confined in the EDS samples. The images suggest that the latex beads are dispersed in an anisotropic medium, which means it is highly probable that the viscosity inside the aggregates is different from that in the bulk liquid.

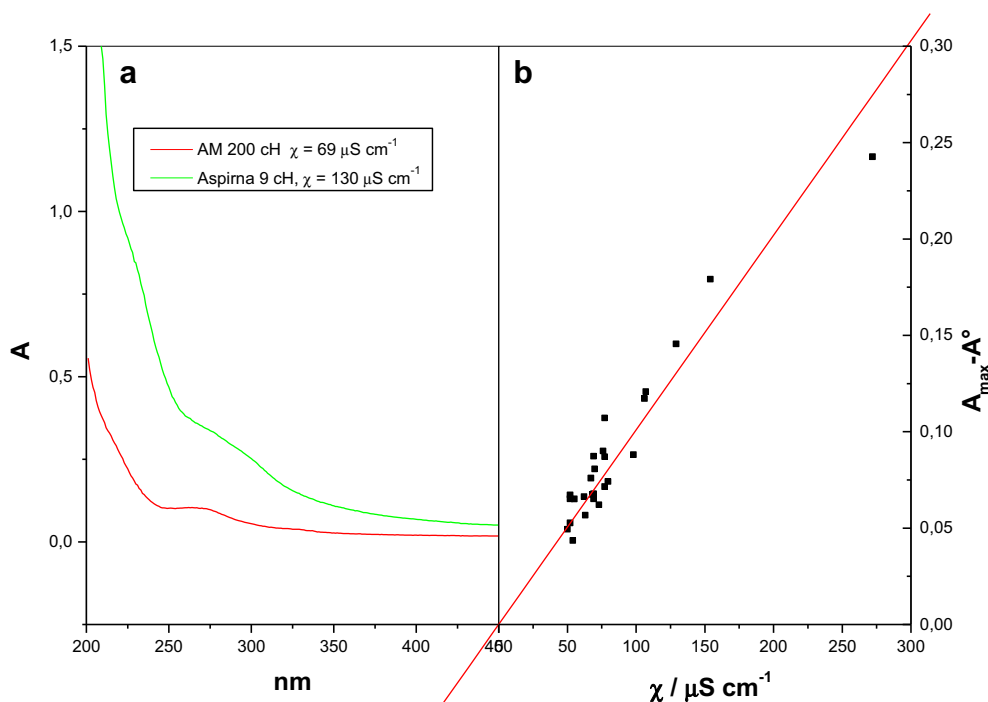
### Structural investigations in the solid phase

Samples of EDS (20 mL) were lyophilized, yielding a solid residue of approximately 1–2 mg: a surprisingly large amount. The production of unexpected solid residues after elimination of bulk water (by evaporation at room temperature, or by evaporation at high temperatures, 90°C) has been described in two papers, which propose the hypothesis that water molecule aggregates are present at room temperature and pressure.<sup>14,20</sup> The solid residue deposits were formed on mica substrates, whose purity was guaranteed by careful cleavage, through evaporation just 3–5 drops of the liquid at room temperature and pressure. AFM images of the deposits were captured in non-contact mode. Figure 3 shows the residues from five drops of an EDS sample having  $\chi = 130 \mu\text{S cm}^{-1}$ : the Z-height is much greater compared with the reference sample of pure water.

The solids obtained by the lyophilization, 20 mL, were used to make NaCl tablets suitable for IR spectroscopy. Figure 4 shows the IR spectrum of the residues of three EDS samples, compared to the spectrum of liquid water in the range 4500–500  $\text{cm}^{-1}$ . The main stretching band of liquid water is shifted to a lower frequency and a bump appears at around 2950–2920  $\text{cm}^{-1}$  (see Figure 4b). As can be observed in Figure 4a, the spectra of the three EDS have very similar shape, and show the same position of the bands. However, the intensity of the signal changes, and is greater for the sample with the highest conductivity. The absorbance is similar for the two EDS presenting very similar conductivity.

## Discussion

The purpose of this study was to validate the hypothesis that the EDS preparation protocol induces a permanent alteration in the supramolecular structure of the water solvent. Our physico-chemical investigations indeed discovered unexpected properties of EDS samples, in both the liquid and the solid phase. The phenomenology is very

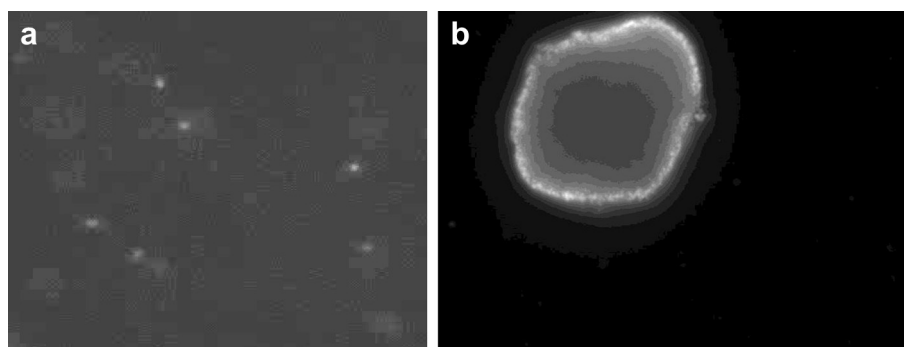


**Figure 1** (a) UV–vis spectrum of two EDS samples. (b) Plot of  $A_{\text{max}} - A^0$  values against the previously measured conductivities of the EDS.

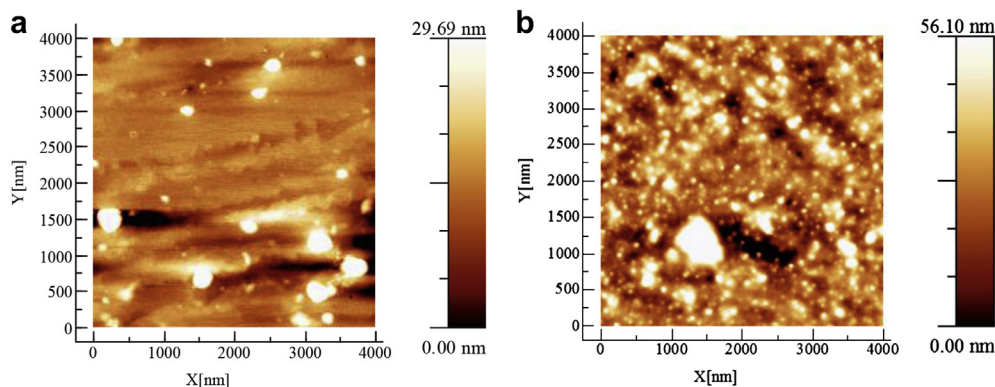
similar to that induced by the contact with strongly hydrophilic polymers, such as Nafion.

In water, the high mobility of  $\text{H}_3\text{O}^+$  and  $\text{OH}^-$  ions under an electrical potential gradient is explained through the hopping mechanism, as proposed by De Grotthuss.<sup>21,22</sup> The migration of ions is strongly associated with nanostructures of water molecules through hydrogen bonds. Thus, a greater structuring of the solvent could favour the aforementioned mechanism, leading to a higher value of conductivity in the EDS. Chemical analyses have found an insufficient quantity of carriers in the samples to justify such marked  $\chi$  increases. Therefore, we posit an increase in the mobility of the ions themselves, stemming from alterations in the supramolecular structure of water. More specifically, we hypothesize that such molecular aggregates of water might augment the proton hopping mechanism, due to the chains of water molecules yielding a higher mobility of ions derived from water dissociation.

We must underline again the peculiar behaviour of EDS, namely the volume and the ageing effects. It seems that in their case the increase of measured parameters depends on the ageing effect, differently from water in contact with hydrophilic surfaces such as Nafion (INW), or Iteratively Filtered Water (IFW). In fact for INW and IFW we obtain the increase of  $\chi$  or  $\Delta H_{\text{mix}}$  and the pH variations in times of the order of some days, while for EDS samples need months or years. If we measure the cited parameters after a short time after the preparation protocol (dilution and succussion) we do not observe any evidence of structural modifications of water. After a long ageing time, especially in small volumes, the variation of supramolecular structure of liquid water is clearly evidenced by variations of the parameters much greater than the experimental errors. It seems that EDS need longer times to change the supramolecular structure of water compared to IFW or INW. Notwithstanding these differences the results obtained with the three different protocols are very similar, even if



**Figure 2** (a). Fluorescent microscope pictures of polystyrene spheres in control. The spheres were labelled with fluorescein. (b) Regular shape of the structure observed by FM in the EDS. The fluorescein-labelled polystyrene beads are clustered in special regions inside the liquid phase.



**Figure 3** The size of the picture is  $10\ \mu\text{m} \times 10$ . Residues from five evaporated drops of a sample of EDS (right) and of the blank (left).

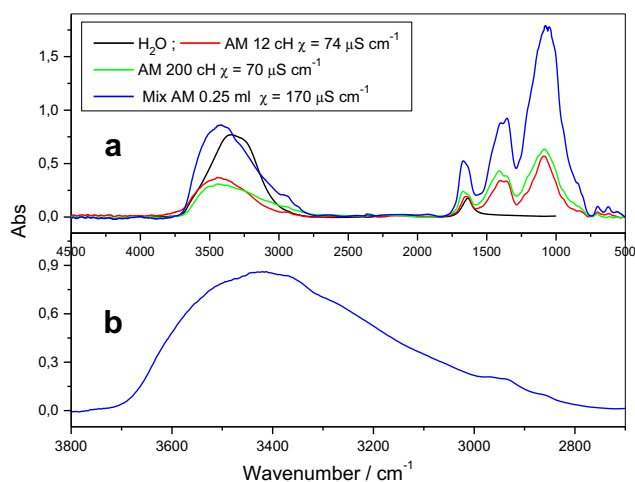
with some peculiarities. It appears that the adopted procedure leads to very similar results, namely the formation of water aggregates. The presence of the aggregates is responsible for the increase of: electrical conductivity (Grotthuss mechanism),  $\Delta H_{\text{mix}}$ , pH etc. In this work we have shown that it is possible to demonstrate, via microscopic measurements, their existence. At the moment we have no evidence of whether and how the size and shape of the aggregates are affected by each of the three protocols. It seems reasonable that such a correlation exists. The images of FM are relative to the largest aggregates, with sizes of the order of micrometres. Through preliminary measures of the variation of the parameters, after filtration using filters of nanometric dimensions of the pores, we can argue that the size of most aggregates is below 20 nm and that the main contribution comes from such low sized nanostructures. Montagnier and Demangeat<sup>15–17</sup> in their work found something similar to what we physically see. But some important differences must be mentioned. In their works there is no direct measure of the presence of the hypothesized nanostructures, but rather an indirect observation. On the contrary in our cases, the long ageing time clearly implies the presence of the aggregates. A very interesting

phenomenon, common to both INW and IFW is the possibility to obtain a highly stable solid via lyophilization, or in the case of EDS also via evaporation at  $90^\circ\text{C}$ . This solid, soluble in water, is able to reproduce the same physico-chemical parameters of the EDS before the evaporation. We think that this solid and the aggregates in the liquid phase are connected with the so called “water memory”. In particular the properties of the solid (high stability at high temperature) and the capability to reproduce the physico-chemical properties of the EDS via solubilization in pure water, are connected with the action mechanism of the globules or granules of the homeopathic medicine (see also the AFM procedure). After the impregnation of the globules with the EDS dilutions, the bulky water evaporates and the solid residue, a very stable one, remains inside the globules. It is possible that the chemical–physical properties of this solid are connected with the therapeutic information of EDS, triggering the formation of the aggregates, whose shape and size could be connected in some way to the therapeutic effect.

## Conclusion

The results of this investigation confirm the hypothesis that formation of molecular aggregates of water occurs in EDS samples. The thermodynamic parameters and structural observations in the liquid phase indicate that a permanent change in the structure of liquid water is induced by the EDS preparation protocol. IR Spectroscopic and AFM further indicate that such molecular aggregates have the property to persist in the solid phase.

In previous works,<sup>12,13</sup> we have shown that changes in the structure of liquid water can also be induced by prolonged contact with Nafion membranes. Those observations were accounted for with the working hypothesis that dissipative structures form within the liquid. Different preparation protocols seem to produce water samples with properties that differ substantially from each other, but which can all be ascribed to the common element of a variation in the supramolecular structure of the water solvent. The physico-chemical properties of these perturbed waters cannot be explained within the paradigm of classical thermodynamics, and must instead be understood with reference to the



**Figure 4** (a) IR spectra of EDS and water at room temperature. The main differences are the overall red-shift of the broad OH stretching peak and the appearance of an absorption line at about  $2926\ \text{cm}^{-1}$ . (b) Zoom of the OH stretching band of Mix AM 0.25 mL in the range between 3800 and 2800 nm.



thermodynamics of far-from-equilibrium systems and irreversible processes.

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