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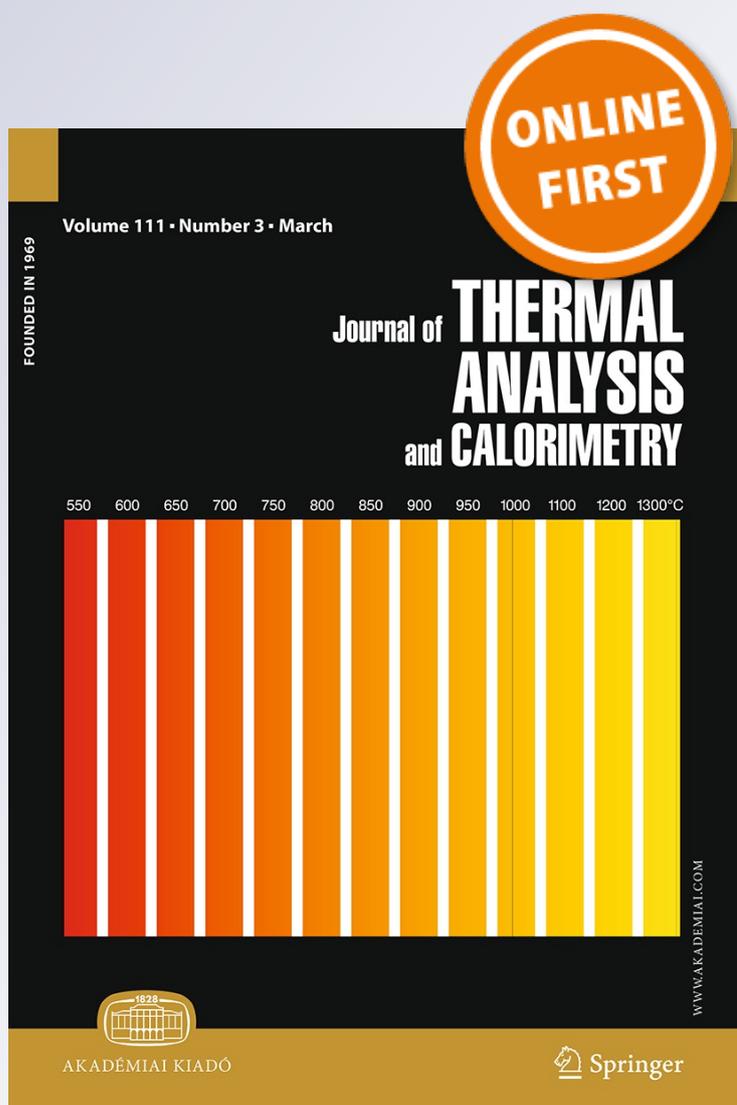
# *Calorimetric, conductometric and density measurements of iteratively filtered water using 450, 200, 100 and 25 nm Millipore filters*

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# Calorimetric, conductometric and density measurements of iteratively filtered water using 450, 200, 100 and 25 nm Millipore filters

Vittorio Elia · Nadia Marchettini · Elena Napoli · Marcella Niccoli

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**Abstract** This study presents some experimental results on the variation of the physico-chemical properties of pure MilliQ water, when subjected to a procedure of iterated filtration through Millipore filters with porosity ranging from 450 to 25 nm. The parameters measured were: calorimetry, electrical conductivity, density, and pH. Release of chemical impurities can be ruled out due to the nature of the materials used. As in the case of iteratively filtered water prepared using Pyrex glass filters, the specific electrical conductivity and the pH were found to increase with increasing number of iterations. There was also a dependence on the average pore size of the filters. The idea of water as a system capable of self-organization triggered by various perturbations (mechanical and/or electromagnetic) is gaining momentum. It responds to such perturbations by forming dissipative structures, i.e., far-from-equilibrium systems.

**Keywords** Pure water · Conductometry · Densitometry · Calorimetry · Iterative filtered water (IFW) · Extremely diluted solutions (EDS) · Water molecules aggregates · Dissipative structures

## Introduction

Studies dealing with water and aqueous solutions are increasingly appearing in the scientific literature [1–4]. The

idea of water as a system capable of self-organization triggered by various perturbations (mechanical and/or electromagnetic) is gaining momentum [5–11].

Previous studies on the physico-chemical properties of extremely diluted solutions (EDS) have shown that pure water in itself possesses extraordinary properties [12–21], giving rise to a phenomenology that depends on the age of samples and their storage volume in a way that is not explained by classical thermodynamics.

This study continues the study of samples of water when subject to a procedure of iterative filtrations. To prevent any such contamination due to the formation of mold within the liquid, the samples of EDS stored for prolonged periods were filtered with a porosity of 450 nm. The electrical conductivity of the clear filtrate was systematically higher than that of the initial solution, often by a large margin. This fact suggests that also procedure of filtration through Millipore filters could be able to modify physico-chemical properties of water. For this reason, we have carried out a systematic study of MilliQ water samples iteratively filtrated through filters with porosity ranging from 450 to 25 nm.

In recent studies, we have confirmed the hypothesis that the filtration through Pyrex glass filters is able to induce the formation of aggregates of water molecules or “dissipative structures” [22–25]. The measured physico-chemical parameters are: specific electrical conductivity  $\chi$  ( $\mu\text{S cm}^{-1}$ ) [22], pH [23], NIR spectroscopy [24], calorimetric and conductometric titration [25]. All the cited methodologies are self-consistent with the work hypotheses of the presence of water molecules aggregates in the IFW liquid. Linear correlation exists between electrical conductivity and density for IFW [22], pointing to a single cause underlying the variations of the studied parameters. The fact that different methods, based on dissimilar phenomena, converge upon an

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unexpected scientific unity, is very interesting. In fact, electrical conductivity is linked to the transport of electric charges under a gradient of electric potential, while density is linked to the bundling of water molecules in the liquid phase. Furthermore, the importance of filtration for the formation of “aqueous nanostructures” has been recently highlighted by Luc Montagnier [26]. In this study, we are trying to extend the complex phenomenology to filters of different chemical nature and porosity lower than the synthesized glass filters, used in preceding studies, to show that the phenomenon is a generalized one.

Many of the properties discovered by these new studies on water cannot currently be explained in terms of classical thermodynamics and quantum mechanics. However, the thermodynamics of far-from-equilibrium systems [27–29] and coherent quantum electrodynamics [30, 31] may open up new ways of understanding these novel results.

The thermodynamics of far-from-equilibrium systems described by Prigogine can help explain apparent anomalies arising after long periods of time [14, 16, 17], or associated with the use of small volumes [21]. Time is indeed of paramount importance in Prigogine's dissipative structures [27–29]. Phenomena in which both time and interface interactions in confined environments play a role could create a bridge between the thermodynamics of far-from-equilibrium systems and the chemistry of interfaces, for a vision of classical physical chemistry capable of incorporating the formation of dissipative structures, on the one hand, the formation of water structures [31] on the other.

## Experimental

### Background

The process of iteratively filtering a given volume (1–10 mL) of MilliQ water simply consists in: filtering the liquid in a vacuum; taking the resultant filtrate, and putting it through the filtering step again; repeating this filtration up to 250 times.

The following filters were used: Millipore filters made of cellulose nitrate, with porosities of 450, 200, 100, and 25 nm. In a preceding study, we determined that the qualitative effects on water are the same regardless of the filter type: e.g., Pyrex glass Büchner, disposable Millipore, or ceramic filters. Upon examining the repeatability of the phenomenon, we decided to first use Pyrex glass Büchner filters [22–25]. Given the novelty of the investigated phenomenon, we paid careful attention to the impurities released by the glass filters which might affect electrical conductivity, calorimetry, and density. The main chemical impurities present are derived from alkaline oxide ( $\text{Na}_2\text{O}$ ) released by the glass. In contact with water, they transform into sodium hydroxide ( $\text{NaOH}$ ), and the last

substance, due to atmospheric carbon dioxide ( $\text{CO}_2$ ), turns into sodium bicarbonate ( $\text{NaHCO}_3$ ). We therefore systematically determined the sodium concentration of the samples, and subtracted the contribution of sodium bicarbonate from the conductivity and calorimetric readings. The concentrations of impurities deriving from the other components of the glass— $\text{SiO}_2$ ,  $\text{B}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ —are very low compared to sodium bicarbonate. They neither contribute significantly to electrical conductivity at the low alkalinity of the water medium nor do they affect the density, due to their low concentration [22]. Using Millipore filters for the iterative vacuum filtration process requires use of a sintered glass filter as support. The findings of previous research indicate that, after filtration, electrical conductivity increases by two orders of magnitude, while density shows variations on the fourth decimal digit. Approximately 10–30 % of the observed increases can be attributed to impurities released by the glass filters. At the porosity of the R1 filter (90–120  $\mu\text{m}$ ) used as a solid support for the Millipore membrane filter, the contribution to electrical conductivity is so slight that its effect does not need to be taken into account [22].

To exclude the contribution of chemical impurities from inside the Millipore filters, they were rinsed with abundant water until they produced a filtrate with electrical conductivity of 1.2–2.0  $\mu\text{S cm}^{-1}$ . This procedure is equivalent to or better than the soaking which is suggested sometimes. In fact, soaking tends to diminish the release of impurities, while our goal is to remove all the impurities of electrolyte nature that can increase the electrical conductivity. After the rinsing, we can be sure that all the soluble impurities were removed from the filters. We found that the quantitative reproducibility was improved by rinsing the filter with MilliQ water after each iterative filtration step, rather than replacing the filter with a new one. It is in fact worth repeating that, during the experiment, no extraneous chemical substances were introduced into the water, other than those deriving from the partial dissolution of the glass solid support. In other words, there was no route for contamination of the filters when using only samples of MilliQ water.

### Methods

#### *Conductivity measurements*

Systematic measurements of specific conductivity were performed on the samples, using a YSI 3200 conductometer with a conductivity cell constant of 1.0  $\text{cm}^{-1}$ . Before measuring the conductivity of a sample, the cell was calibrated by determining the cell constant  $K$  ( $\text{cm}^{-1}$ ). The specific conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ), was then obtained as the product of the cell constant and the conductivity of the solution. For a given conductivity measuring cell, the cell constant was determined by measuring the conductivity of

a KCl solution having a specific conductivity known to high accuracy, at several concentrations and temperatures. All conductivities were temperature-corrected to 25 °C, using a pre-stored temperature compensation for pure water.

Calorimetry

The heats of mixing were monitored using a thermal activity monitor (TAM) model 2227, by thermometric (Sweden) equipped with a flow mixing vessel. A P3 peristaltic pump (by Pharmacia) envoys the solutions into the calorimeter, through Teflon tubes. The flow rates of the two liquids are the same, and are constant in the inlet tubes, so that the solution coming out of the calorimeter has a concentration half the initial one. The mass flow-rate, constant within 1 %, amounts to  $3 \times 10^{-3} \text{ g s}^{-1}$ : it was the same for all the experiments. The values of the mixing enthalpies,  $\Delta H_{\text{mix}}$ , were obtained using the following formula:

$$\Delta H_{\text{mix}} \left( m_x^i, m_y^i \rightarrow m_x^f, m_y^f = \frac{dQ}{dt} P_w \right)$$

where  $(dQ/dt)$  is the heat flux (W),  $P_w$  is the total mass flow-rate of the solvent ( $\text{kg s}^{-1}$ ), and  $m_x^i, m_y^i$  and  $m_x^f, m_y^f$ , are the initial and final molalities.  $\Delta H_{\text{mix}}$  is given in  $\text{J kg}^{-1}$  of solvent in the final solution.

Density measurements

The solution densities were measured using a vibrating-tube digital density meter (model DMA 5000 by Anton Paar, Austria) with a precision of  $\pm 1 \times 10^{-6} \text{ g cm}^{-3}$  and an accuracy of  $\pm 5 \times 10^{-6} \text{ g cm}^{-3}$ . The temperature of the water around the densitometer cell was controlled to  $\pm 0.001 \text{ K}$ . The densitometer was calibrated periodically with dry air and pure water.

Results

Tables 1, 2, 3, 4, and 5 show the volumes of the MilliQ water samples subjected to iterated filtrations, with the number of iterations  $n$  and the measured electrical conductivity  $\chi$  ( $\mu\text{S cm}^{-1}$ ), for Millipore type filters of different porosities.

In Table 6, we report: the studied IFW, their initial conductivity,  $\chi^i/\mu\text{S cm}^{-1}$ ;  $\Delta H^E$  (see the calorimetry section) in  $\text{J kg}^{-1}$  of water in the final solution with NaOH  $0.01 \text{ mol kg}^{-1}$ . The values result systematically higher than those of the reference solvent, i.e., an aqueous solution at the same chemical composition and concentration of the sample. Every experimentally measured excess heat turned out to be exothermic. Such measures put in evidence a systematically higher value of conductivity with respect to that of the solvent. For the solutions under study, measures

**Table 1** Volume of MilliQ water filtered,  $V$  (mL), number of iterations,  $n$ , and specific electrical conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ) of samples after filtrations using 450 nm porosity filters

450 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$
0	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
20	2.4	2.5	4.5	14.8	8.8	5.9	8.5	11.9	8.3
40	3.6	9.5	5.5	15.4	8.9	6.4	9.9	12.4	8.7
60	12.4	10.9	7.7	16.0	9.5	6.9	10.6	13.0	9.5
80	20.0	11.3	8.1	16.9	9.9	7.3	11.5	13.6	10.1
100	25.0	11.8	8.6	17.2	10.3	7.6	12.1	14.1	10.7
120			9.6			8.4	15.0		
140			10.5			9.0	15.5		
160			11.9			10.0	17.1		
180			13.6			10.3	19.3		
200			14.9			11.5	19.8		
220			17.8			14.3			
240			21.4			14.8			
260			22.1			15.4			
280			23.6			16.2			
300			25.4			23.2			
320						24.0			

At  $n = 100$ , the volume was reduced to 2 mL, and the iterations were continued to  $n = 300$  or  $320$  for the 10 and 5 mL samples, respectively

**Table 2** Volume of MilliQ water filtered,  $V$  (mL), number of iterations,  $n$ , and specific electrical conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ) of samples after filtrations using 200 nm porosity filters

200 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$
0	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
20	3.7	2.8	3.2	9.2	10.8	6.7	11.3	14.3	13.3
40	4.9	2.9	3.6	9.2	11.3	6.7	15.6	15.8	13.7
60	5.4	3.1	4.0	13.0	11.9	7.1	23.8	16.5	16.3
80	5.8	3.3	5.6	17.5	12.4	7.5	25.0	20.8	18.3
100	7.2	3.6	7.0	20.6	12.9	8.0	26.4	22.1	24.8
120			11.1						
140			17.5						
160			22.0						
180			25.5						
200			27.3						
220			30.1						
240			31.8						
260			36.6						
280			43.0						

At  $n = 100$ , the volume was reduced to 2 mL, and the iterations were continued to  $n = 280$  for the 10 mL samples

**Table 3** Volume of MilliQ water filtered,  $V$  (mL), number of iterations,  $n$ , and specific electrical conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ) of samples after filtrations using 100 nm porosity filters

100 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	10 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	5 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$	2 mL $\chi/\mu\text{S cm}^{-1}$
0	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
10	1.7	7.7	3.4	6.1	5.9	7.9	10.9	8.3
20	2.1	8.5	4.0	7.1	7.5	9.0	12.4	9.5
30	2.6	9.0	4.5	7.7	8.4	10.6	14.2	10.3
40	2.9	9.4	5.1	8.5	11.5	11.3	16.6	11.7
50	3.2	10.3	5.9	10.6	16.6	13.2	18.7	13.6
60		11.2	6.5	11.8	21.0	14.3		17.1
70		12.7	8.9	13.0		15.6		
80		13.2		14.1		17.5		
100		14.4		14.8		20.2		
120		15.5				23.1		
140		16.8						
160		18.5						
180		21.2						
200		28.7						

At  $n = 70$  or  $80$ , the volume was reduced to 2 mL, and the iterations were continued to  $n = 120$  or  $200$  for the 10 and 5 mL samples

of excess heat and excess conductivity turn out to be correlated, pointing at a presumably single cause of the exhibited behavior. Plotting the excess conductivity versus the excess heat of mixing with NaOH  $0.01 \text{ mol kg}^{-1}$  (Fig. 1), a linear relationship can be noticed between these two independent chemical parameters.

Table 7 shows the electrical conductivity  $\chi$  ( $\mu\text{S cm}^{-1}$ ) and density  $(d - d_0) \times 10^5$  ( $\text{g cm}^{-1}$ ) for glass and Millipore filters, irrespective of the diameters of the pores.

We also examined 29 different samples of IFW obtained with Millipore filters, regardless of the porosity or the number of filtrations, following the variation of the electrical conductivity of the samples up to 44 days after preparation. The samples were stored in Eppendorf containers which guarantee an almost null release of electrolyte that could increase the electrical conductivity. For all samples were obtained increments of  $\chi$  variables from 3 to 300 % compared to the initial value. The mean increment is

**Table 4** Volume of MilliQ water filtered,  $V$  (mL), number of iterations,  $n$ , and specific electrical conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ) of samples after filtrations using 25 nm porosity filters

25 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	$n$	2 mL $\chi/\mu\text{S cm}^{-1}$	$n$	1.5 mL $\chi/\mu\text{S cm}^{-1}$	$n$	1 mL $\chi/\mu\text{S cm}^{-1}$	$n$	<1 mL $\chi/\mu\text{S cm}^{-1}$
0	1.2	12	11.8	20	12.7	25	14.7	31	16.3
1	2.0	13	12.1	21	13.3	26	15.1	32	17.5
2	4.1	14	12.2	22	14.7	27	15.3		
3	7.4	15	12.7	23	14.1	28	15.5		
4	7.6	16	12.4	24	14.3	29	15.6		
5	8.0	17	12.3	25	14.7	30	16.7		
6	8.5	18	12.5			31	16.3		
7	8.4	19	12.7						
8	8.0	20	12.7						
9	8.1								
10	8.3								
11	13.6								
12	11.8								

At  $n = 12$ , the volume was reduced to 2 mL. At  $n = 21$ , the volume was reduced to 1.5 mL. At  $n = 26$ , the volume was reduced to 1 mL

**Table 5** Iterative procedure using 450 and 200 nm Millipore filters

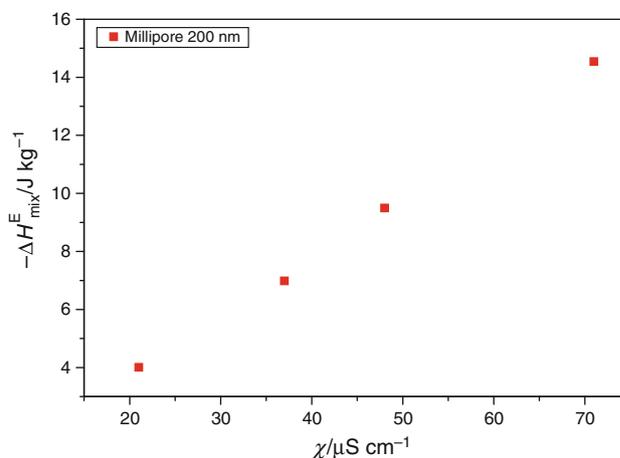
450 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	$n$	7 mL $\chi/\mu\text{S cm}^{-1}$	$n$	3 mL $\chi/\mu\text{S cm}^{-1}$
0	2.0	160	13.5	160	13.5
10	3.2	180	14.2	180	14.9
20	4.3	200	15.0	200	17.2
40	5.8	220	16.5	220	18.6
60	7.3	240	17.1	240	25.5
80	8.7	260	18.6	260	27.0
100	9.2	290	21.4	290	34.7
120	12.7				
140	13.2				
160	13.5				

200 nm $n$	10 mL $\chi/\mu\text{S cm}^{-1}$	$n$	7 mL $\chi/\mu\text{S cm}^{-1}$	$n$	3 mL $\chi/\mu\text{S cm}^{-1}$
0	2.0	100	12.4	100	12.4
10	3.2	120	12.3	120	12.7
20	5.8	140	12.6	140	14.2
40	8.7	160	14.3	160	19.2
60	9.3	180	14.6	180	22.2
80	10.2	200	15.6	200	27.0
100	12.4	220	16.2	220	26.9
				240	29.8

**Table 6** Initial conductivity,  $\chi^i/\mu\text{S cm}^{-1}$ , and excess heats of mixing,  $\Delta H^E/\text{J kg}^{-1}$ , of the IFW with NaOH 0.01 mol  $\text{kg}^{-1}$

System/nm	$\chi^i/\mu\text{S cm}^{-1}$	$\Delta H^E/\text{J kg}^{-1}$
IFW <sub>1</sub> Millipore 200	21	4.0
IFW <sub>2</sub> Millipore 200	37	7.0
IFW <sub>3</sub> Millipore 200	48	9.5
IFW <sub>4</sub> Millipore 200	71	14.5



**Fig. 1** Excess heat of mixing  $\Delta H^E/\text{J kg}^{-1}$ , versus excess conductivity,  $\chi^i/\mu\text{S cm}^{-1}$

71 %. It must be emphasized that this characteristic dependence on the age of the samples is typical of far from equilibrium open systems.

For 30 different samples of IFW, we measured the electrical conductivity  $\chi$  ( $\mu\text{S cm}^{-1}$ ) and the pH after the

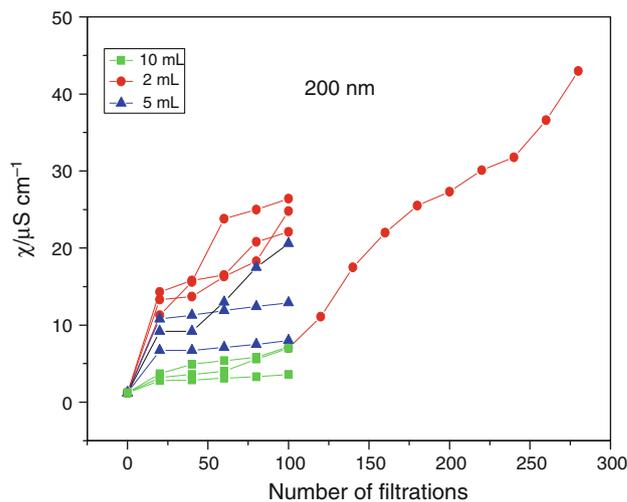
filtrations using filters of different porosities (100, 200, 450 nm). No significant correlations exist between pH and conductivity or with the porosity of the filters. The only information obtained is that pH is slight alkaline as for glass filters IFW [22, 23] or EDS [13, 20]. In the presented

**Table 7** Specific electrical conductivity,  $\chi$  ( $\mu\text{S cm}^{-1}$ ), and density,  $(d - d_0) \times 10^5$  ( $\text{g cm}^{-3}$ ), for glass and Millipore filters irrespective of the number of filtrations or the filter porosity

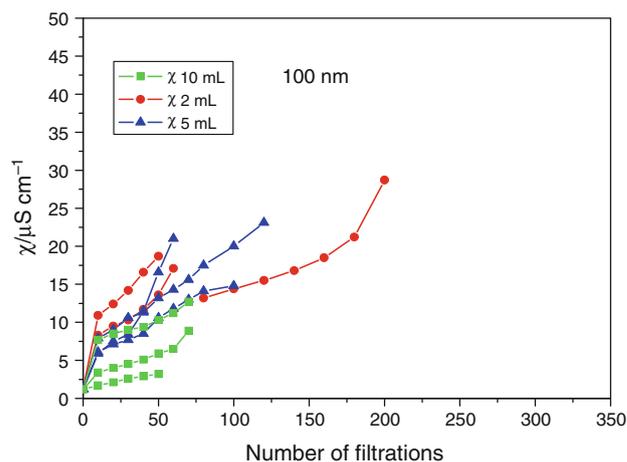
Glass filters		Millipore filters	
$\chi/\mu\text{S cm}^{-1}$	$(d - d_0) \times 10^5$	$\chi/\mu\text{S cm}^{-1}$	$(d - d_0) \times 10^5$
1.2	0.0	1.2	0.0
115	8.0	27.3	1.7
153	11.8	25.4	1.5
82	5.5	30.5	1.3
83	5.3	27.8	1.5
39	4.2	15.7	0.7
45	4.8	14.8	0.7
46	5.0	8.9	0.5
43	2.4	9.2	0.3
139	13.3	8.8	0.4
146	14.7	4.5	0.3
139	14.1	50.1	2.4
65	4.9	11.1	0.9
59	4.6	18.2	1.2
60	4.9	11.6	0.7
85	6.5	24.7	1.5
61	5.0	9.9	0.8
214	14.6	20.4	1.2
178	13.2	9.2	0.5
229	17.5	7.6	0.3
292	22.4	28.8	1.5
394	31.7	13.7	0.9
		31.3	1.7
		16.2	0.8
		20.2	1.1
		9.1	0.8
		14.3	0.8
		17.5	1.1
		24.1	1.7
		29.5	1.9

case, we cannot invoke the presence of alkaline impurities because of the chemical nature of the Millipore filters.

As the number of iterations increases, the specific electrical conductivity, pH, and density always increase. The conductivity and density of the filtered samples always increase with the passage of time as happens for the EDS [14, 16, 17]. There is also a dependency on pore size. The phenomenon is repeatable but not reproducible, i.e., the numeric outcome varies with the experiment. Furthermore, all the filter types exhibit a significant “volume effect” [16, 21], whereby the increase in specific conductivity for each iteration step is higher for small volume samples (see Figs. 2, 3, 4, 6). In fact, the roughly linear trends of  $\chi$  as a function of the number of iterations for each volume of



**Fig. 2** Specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) versus number of iterations,  $n$ . Porosity of the filter: 200 nm. The *square* symbols refer to a volume of 10 mL of water. The *triangle* symbols refer to experiments using 5 mL of water and *circles* to 2 mL samples. See Table 2

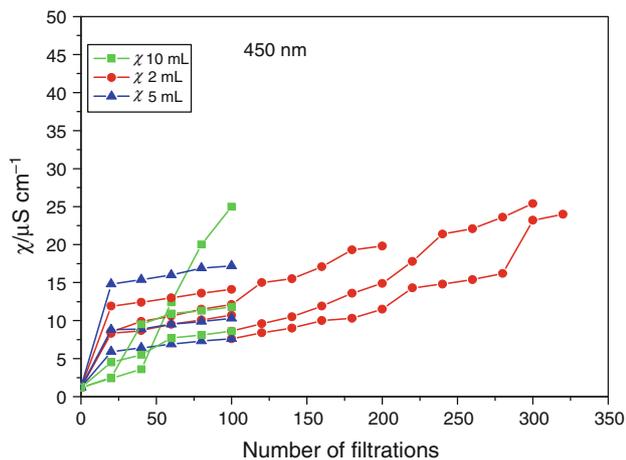


**Fig. 3** Specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) versus number of iterations,  $n$ . Porosity of the filter: 100 nm. The *square* symbols refer to a volume of 10 mL of water. The *triangle* symbols refer to experiments using 5 mL of water and *circles* to 2 mL samples. See Table 3

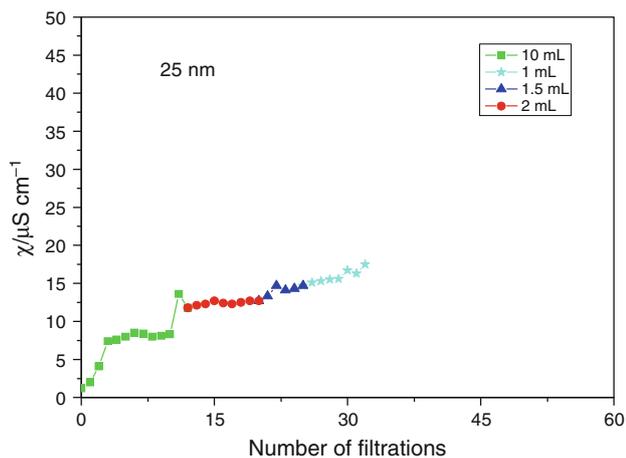
filtered water exhibit very different slopes, which are higher for smaller volumes.

A different phenomenology is observed for the 25 nm Millipore filter which does not show any significant volume effect (see Fig. 5), probably because the very small diameter of the pores prevents the largest water molecules aggregates from reaching the filtered liquid, thus removing the largest contribution to conductivity.

The dependence of the results on the sample volumes emerges very clearly from the experiments carried out according to one of the following protocols: starting with a



**Fig. 4** Specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) versus number of iterations,  $n$ . Porosity of the filter: 450 nm. The *square* symbols refer to a volume of 10 mL of water. The *triangle* symbols refer to experiments using 5 mL of water and *circles* to 2 mL samples. See Table 1



**Fig. 5** Specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) versus number of iterations,  $n$ . Porosity of the filter: 25 nm. The *square* symbols refer to a volume of 10 mL of water. The *triangle* symbols refer to experiments using 5 mL of water and *circles* to 2 mL samples. See Table 4

10–2 mL sample volume, repeated filtrations are performed to produce a roughly linear trend of  $\chi$  as a function of  $n$ . Then, the sample is partitioned. One part is used, for example, for chemical analysis, and the other is used to continue the previous process, with  $n'$  filtrations, yielding a new linear trend for  $\chi$ , but this time with a smaller sample volume. The volume partitioning is repeated several times, and each new linear trend of  $\chi$  as a function of filtration steps exhibits a steeper slope than that of the larger volume of the previous step. For small volumes ( $<3$  mL), the slope increases conspicuously (Tables 2, 3, 4; Figs. 2, 3, 6) as happens also for IFW using glass filters. Through this

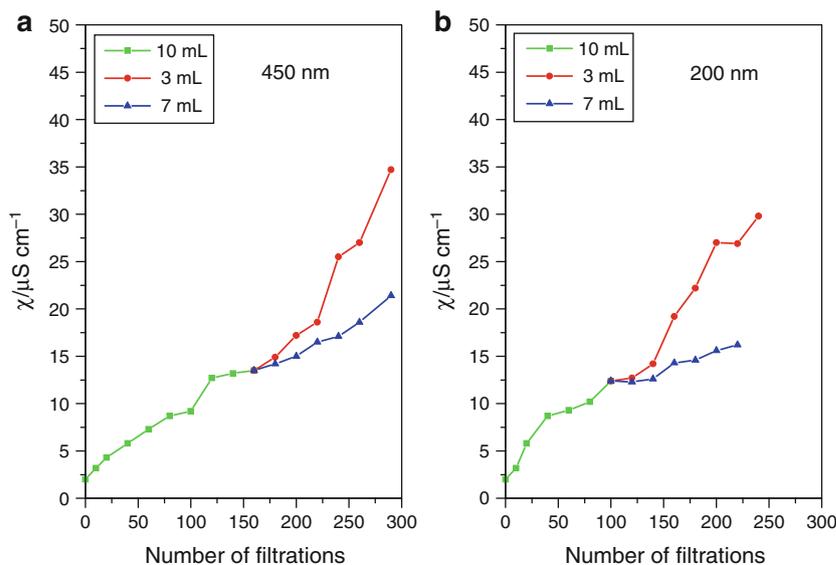
procedure, we can observe how the response of a single filter varies when changing a single parameter: the filtered volume. From the figures, it is possible to note that the developments of the electrical conductivity as a function of the number of iterations, statistically group the experimental data in three areas of the graph. The lowest zone contains the data relating to the largest volume (10 mL). The central zone contains the data relating to the intermediate volume (5 mL). The upper zone contains the data relating to the smallest volume (2 mL). Of course, given the non-reproducibility of the results, but only the repeatability of them, there are some significant exceptions to the behavior described. However, there is a dependency, quite clear from the volume. The dependence from the volume is most noticeable when, after 100 iterations, the iteration is continued reducing the initial volume to 2 mL. In all cases examined, the slope of the performance is increased.

A very simple method to illustrate the “volume effect” is shown in Fig. 6; Table 5, for the iterative procedure using 450 and 200 nm Millipore filters. Starting with a 10 mL sample, 160 or 100 iterations are performed using the entire volume of the sample. At this point, the sample is divided into two parts, of approximately 3 and 7 mL, respectively. The iterations are then continued using the 3 mL sample to reach 290 or 240 iterations, respectively, obtaining the expected volume effect of an increasing slope of the linear trend. Then the smaller volume is set aside, and the filter is used to perform the iterative procedure on the 7 mL samples, to reach 290 or 220 iterations starting from 160 or 100 iterations, respectively (see Fig. 6a, b). It is easy to see that, whereas the latter procedure is aligned with the preceding 160 or 100 iterations, the first one (3 mL) has a much steeper slope. In this manner, it is possible to compare the behavior of the same filter used to iterate different volumes (3 and 7 mL).

## Discussion

A large number of thermodynamic and transport measurements were conducted on MilliQ water samples subjected to iterated filtrations. The increases in conductivity and density after filtrations as well as due to time passing are a new phenomenon, that appears to clash with the thermodynamics of near-equilibrium systems but is in agreement with physico-chemical results on EDS or IFW using glass filters [14, 16, 17, 22–25] and, as such, expected. The discovery of a “volume effect” is a peculiar feature of the systems under study, arising from the interactions of mechanical disturbances or electromagnetic energy in a small-scale open system (exchange of energy and matter) [16, 21]. The picture provided by the experimental data suggests that the procedure of iterative filtrations alters the physico-chemical properties of water.

**Fig. 6 a, b** Specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) versus number of iterations,  $n$ . Porosity of the filters: 450 and 200 nm. The *square* symbols refer to a volume of 10 mL of water. The *triangle* symbols refer to experiments using 5 mL of water and *circles* to 2 mL samples. See Table 5



The washing protocol, described in the Experimental Section, yielded results that were much more numerically significant and reproducible, confirming the null contribution of impurities to the physico-chemical parameters.

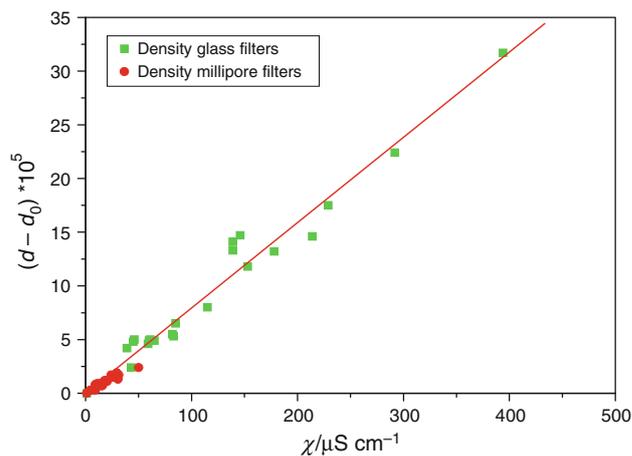
A working hypothesis is to consider water filtration as being akin to water passing through thin capillaries. The iterative procedure is thus associated with a flux of energy and material in an open system. The energy flux is partially dissipated as heat, enabling the formation of “dissipative structures”. Such a perturbation appears to trigger the formation of aggregates of water molecules in the liquid (water clusters, aqueous nanostructures, molecular aggregates of water), which increase the effects of the proton hopping mechanism [32, 33]. The presence of molecular aggregates of water (aqueous nanostructures) could cause higher conductivity through the Grotthus hopping mechanism. Such structures consequently lead to an increase in  $\chi$  ( $\mu\text{S cm}^{-1}$ ) as well as to a higher density resulting from a denser bundling of the water molecules. The higher the value of electrical conductivity, the higher the density: this suggests that the increase in density is caused by the same phenomenon responsible for the increase in conductivity [22].

The results of heat of mixing with NaOH are in agreement with the proposed mechanism. For the interaction with the basic solution, an exothermic heat was found, increasing with increasing  $\chi$ . The increase was about one order of magnitude. For this reason, it has been hypothesized a favorable interaction between the aggregates of water molecules and  $\text{OH}^-$  ions from the probe. The calorimetric results are in accordance with the data obtained for the filtered samples on glass [22].

It is interesting to note that also the density results show common linear trend of  $(d - d_0) \times 10^5$  as a function of the

experimentally measured conductivity for the two series of experiments using the glass and the Millipore filters (see Fig. 7). The result worth noting here is that the linear trends are fitted by the same equation, irrespective of the nature of the filter used (chemical or physical), pointing to a common genesis of the molecular aggregates of water molecules—namely the formation of dissipative structures, due to the passage through very thin capillaries (see Table 7; Fig. 7).

The calorimetric results and density underline a single cause for the phenomenon that is independent of the chemical nature of the filters and of the chemical nature of the impurities. The single cause is probably the hypothesized presence of aqueous nanostructures. Another interesting observation concerns the change in conductivity



**Fig. 7** Density  $(d - d_0) \times 10^5 \text{ g cm}^{-3}$ , versus specific electrical conductivity ( $\mu\text{S cm}^{-1}$ ) for glass and Millipore filters, irrespective of the number of filtration or the filter porosity. See Table 7. *Square* symbols: glass filters. *Circle* symbols: Millipore filters

induced by the number of iterations, which is lower for the Millipore filters compared to that of sintered glass or ceramic filters. Assuming that the filter can be modeled as a set of tiny capillaries, an important role is, of course, played by the length of the capillaries which, along with the diameter, determines the amount of energy dissipated. The Millipore filters used in this study can be described as being composed of capillaries shorter than those of the analog glass filters, and with a smaller diameter. Glass filters: average diameter 120–2.5  $\mu\text{m}$ , length 2–5 mm. Millipore filters: average diameter 0.450–0.025  $\mu\text{m}$ , length about 50  $\mu\text{m}$ . The pore size (nm) of the Millipore filters used can also be supposed to select the maximum size of the aqueous structures generated by the iterative filtration process. This provides indirect information about the effect of the average size of aggregates on the electrical conductivity of the perturbed water. Furthermore, with the 100–25 nm filters, the perturbed water obtained was sterile. This observation allows us to rule out that the changes in the physico-chemical characteristics of the system are attributable to the presence of mold or bacteria. The outcome of the iterative filtration procedure (IFW) also exhibits a path dependence relating to the filter. Obviously we are not yet in a position to speculate about the nature of the dissipated energy, or of the water structures that are formed; however, the experimental data point to a very significant variation of some physico-chemical parameters. That said, it is easy to see from both the tables and figures that the variations in conductivity are approximately one to two orders of magnitude higher than the initial values. It should be underlined that the increase in the measured parameters is permanent, and often continues to increase with time even though the samples are aged in Eppendorf containers as happens for EDS and IFW on glass filters. This last-mentioned behavior is very strong evidence of a system that is not in equilibrium, i.e., which is in a far-from-equilibrium state. See Prigogine's "The End of Certainty" [28] for a discussion of the peculiarities of far-from-equilibrium systems.

## Conclusions

The experimental data show a significant variation in certain physico-chemical parameters of the MilliQ water after repeated filtrations. The conductivity values after filtration were up to one order of magnitude higher than the initial values. It should be underlined that the observed increases in water conductivity and density are permanent and those parameters often continue to increase with time.

The complex phenomenology that emerges from these different experimental conditions and results definitively

dispels the naive assumption that such changes can be attributed to the problem of impurities.

The systems under study are non-equilibrium systems in which irreversible processes occur. This new field of research on water opens up a completely new scenario, whereby the supramolecular structure of liquid water can be modified through mechanical perturbations, aging, and particularly through aging in small volumes. The latter characteristic might be linked to the very different properties of water contained in biological cells.

At the present time, we are not yet able to clarify the nature of the dissipated energy or the kinds of water structures that are formed. We have preliminary indications that dissipated energies are of environmental nature, because the variation of conductivity related to years seasons [14, 16, 17]. Work is in progress using microscopic and spectroscopic methodologies to improve our knowledge about the nature of the hypothesized aqueous nanostructures.

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