Conductivity measurements as a possible means to measure the degree of water ordering

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Abstract. In our extended experimental work with aqueous solutions of NaHCO₃ we noticed higher electrical conductivity in aged solutions in comparison to the conductivity of chemically analogous fresh (one day old) solutions. The phenomenon was found in solutions left undisturbed for longer time (~ 1 year). Most probably the dissolved ions as well as the contact with hydrophilic surfaces play the essential role. We found that higher conductivity is proportional to higher surface vs. volume ratio.

1 Introduction

Water is a simple compound with many peculiar properties. Some of them are well known, while the others are still debated within the scientific community. According to water researchers the extraordinary properties of water represent the reason for the establishment and maintenance of life (Henry 2005, 2009). One of such properties is the so-called water ordering (ordered water, structured water etc.). While it is clear that such phenomenon exists very close to polar (or hydrophilic) surfaces it is not yet resolved whether the order in water can extend considerably far from such surfaces or if it can exist even without such hydrophilic surfaces. Even though the hydrogen bonds that connect water molecules are considered to be responsible for water ordering (Fisenko and Malomuzh 2008) the principles of this ordering are not yet finally settled neither accepted within scientific community.

There are two main streams of concepts that try to model the dynamic order of water. One stems from the quantum field theory and sees the water medium organized in dynamic coherent domains. Namely, an ensemble of molecules interacting with the radiative electromagnetic (EM) field acquires according to Del Giudice above a density threshold and below a critical temperature a new non-trivial minimum energy state, different from the usual one where the oscillations of the molecules are uncorrelated and the EM field is vanishing. The new minimum energy state implies a configuration of the system where all molecules enclosed within an extended region (coherence domain, CD) oscillate in unison and in tune with an EM field trapped within the CD (Montagnier et al. 2010, Del Giudice et al. 2010). The CDs according to Del Giudice characterize every liquid, even the non-polar ones. What could be peculiar in water is that the coherent oscillations are proposed to occur just between the ground and excited state, at 12.06 eV, which is just below the ionization threshold (12.60 eV). In the case of water the dimensions of CDs in normal conditions are proposed to be around 100 nm and they may include an ensemble of almost free electrons that can further accept free energy and convert it to coherent excitations (Del Giudice et al. 2010).
The second stream of concepts trying to explain water ordering seeks various possible cluster formations that would endure more than some ps, perhaps extending into ordinary human time-frame (Chaplin 2004, 2006, Martin Chaplin’s homepage: http://www.lsbu.ac.uk/water/). In any case, it seems that the water ordering is not an all or none phenomenon, but on the contrary, it can have many levels and nobody knows where and what is the supreme level – as far as water still remains a liquid, of course (Stanley et al. 1999).

Regarding its role in maintenance of life, water is mostly treated as a relatively simple, passive solvent for the life’s sustaining molecules, either the small ones (ions, sugars etc.) or the larger ones like proteins, DNA or RNA macromolecules. On the other hand, an increasing number of researchers are coming to the conclusion that water is much more an active ingredient of life as seen conventionally. For instance, Bizzarri and Cannistraro (2002) found the dynamics of the protein and solvent so strongly coupled that they “should be considered as a single entity with a unique rough energy landscape”. Therefore, let us look more closely on the influence of hydrophilic and hydrophobic surfaces on the water’s hydrogen bonded network.

Conventionally it is considered that the impact of surfaces on water’s hydrogen bonded network extends to a distance of no more than a few molecular layers. Yet, Zheng and Pollack (2003) found that colloidal and molecular solutes are excluded from the vicinity of various hydrophilic surfaces. The width of these solute-free exclusion zones (EZ) may amount to few hundred μm, EZs are physically distinct, less mobile and coexist infinitely with the bulk (Zheng et al. 2006).

These large zones of ordered water are similar to findings of Elia and co-workers that served as a foundation of our empirical work (Verdel et al. Submitted to J. Sol. Chem., Verdel et al.2011). Elia and co-workers explored the physico-chemical properties of extremely dilute aqueous solutions prepared by iterations of dilution and succussion (vigorous shaking). They repeated these two processes until the chemical composition of the final solution was identical to that of the solvent (NaHCO₃ (aq.)). They measured electrical conductivities of aged, extremely dilute solutions, and compared the results with electrical conductivities of one day old untreated, chemically analogous solutions and found excess conductivity i.e. higher conductivity values (e.g. Elia et al. 2004, 2005, 2007, 2008, 2009 and 2010). They ascribed their unexpected results to water’s self-organizing abilities that are triggered by the input of kinetic energy during succussion. According to this view, the organised, larger water clusters should accelerate the Grotthuss mechanism of the proton transfer (Agmon 1995) that predominates in the aqueous hydrogen carbonate solutions. Through this mechanism an ‘excess’ proton or protonic defect diffuses through the network of hydrogen-bonded water molecules through the formation and cleavage of hydrogen and covalent bonds (see Figure 1).

![Figure 1](http://en.wikipedia.org/wiki/Grotthuss_mechanism)

In harmony with Elia’s observations, excess conductivities were found only when solutions were left to stand undisturbed for some time (Verdel et al. Submitted to J. Sol. Chem.). However, we found no differences in excess conductivities between diluted and succussed solutions, on the one, and the untreated aged controls, on the other hand. This reminded us on the observations of Vybiřal and Voráček (2007), who noticed that distilled water left to stand undisturbed for some time in closed
vessels spontaneously developed viscous properties which they ascribed to self-organising abilities of water. The phenomenon was named autothixotropy. Thixotropy is a property of certain gels and liquids that are viscous under normal conditions, but flow (or become less viscous) when shaken or otherwise stressed. In water’s autothixotropic properties, ions, as well as the contact with hydrophilic surfaces could play an essential role. We propose autothixotropy to be the reason for faster proton transfer along the lines of the Grotthuss mechanism found in aged aqueous NaHCO₃ solutions in comparison to the freshly prepared solutions with an otherwise identical chemical composition (Verdel et al. submitted to J. Sol. Chem.).

2 The summary of our experiments

Our extended experiments are extensively described in Verdel et al. (submitted to J. Sol. Chem.). We measured the conductivity of aqueous solutions under various conditions and found interesting results wherefrom some important findings can be inferred.

We measured the conductivity of highly dilute fresh (one day old) KCl 10cH and for 310 and 370 days aged KCl 10cH in 0.05 mmol/L NaHCO₃. In this, 0.05 mmol/L NaHCO₃ was used as starting point solution, solvent. To prepare KCl 1cH, a 1% mass solution of KCl was prepared in 0.05 mmol/L NaHCO₃ and succussed for 45 seconds with vigorous mixing. To prepare KCl 2cH, 1 g of KCl 1cH was added to 99 g of 0.05 mmol/L NaHCO₃ and succussed. Hence, to prepare KCl 10cH the two steps, dilution 1:100 and succussion were iterated ten times. The final concentration of KCl in KCl 10cH was identical to the concentration of KCl in the starting point solution. Hence, the final solution (KCl 10cH) was chemically identical to the solvent (0.05 mmol/L NaHCO₃).

The actual ionic concentration of aged solutions was determined by ICP-MS. The determination of impurities of aged solutions was namely important because Na⁺ ions are constantly leaching from the glass and in aqueous solutions sodium ions react with dissolved CO₂ to form NaHCO₃ (Elia et al. 2008).

No differences in conductivity of fresh mechanically treated (succussed and diluted) and untreated solutions were found. Basically 2 groups of aged solutions were prepared: in the A group water was aged for 310 days by 2 mL in 2.5-mL flasks and in the B group for 370 days having 2 mL, 5 mL and 10 mL solutions in 20-mL flasks. The solutions in the A group were kept under 3 different conditions (see Table 1).

Table 1. Ageing conditions.

|       | F         | S                  | D                  |
|-------|-----------|--------------------|--------------------|
| exposed to low temperatures at -20 °C | protected from daylight | exposed to daylight |

All solutions in the B group were aged exposed to daylight and other environmental EM disturbances (condition D). The conductivity was measured at 120, 1000, 10,000 and 100,000 Hz 25 and 5 °C. When the frequencies were not in focus conductivity at 1000 Hz was used. For comparison coefficients between measured and theoretical conductivity, σ/σ₀, were used (for detailed information of the method see Verdel et al. submitted to J. Sol. Chem.). From σ/σ₀ excess conductivities were deduced by the following equation:

\[
\sigma/\sigma_0 = \sigma / (\sigma - \sigma^d)
\] … (1)

Conductivity values measured at 1000 Hz were highly reproducible. However, no differences between aged mechanically treated with iteration of dilution and succussion, on the one hand, and aged untreated solutions, on the other hand, were found. Therefore the aged treated and untreated solutions were combined. In Figure 2 the conductivity values of fresh and 370 days aged solutions of the B group are shown.
Figure 2. Conductivity of fresh solutions, $\sigma_{CC}$, (CC – calibration curve at 25 °C and 1000 Hz) and 370 days aged solutions, $\sigma$ (the B group), 2 mL (blue), 5 mL (red) and 10 mL (green), as a function of $c_{\text{NaHCO}_3}$ – all solutions measured at 25 °C and 1000 Hz. Repetitions of 2 mL: 8, 5 mL: 10, 10 mL: 10. Conductivity above CC line is excess.

We can see that with lesser volume the conductivity values are more excess (see Fig. 2). Further calculations uncovered that the excess conductivities are proportional to the ratio of glass surface and solution volume, $S/V$. With increasing $S/V$, the ratio $\sigma/\sigma_{CC}$, increases according to the following equation 2 with $R^2 = 0.928$:

$$\frac{\sigma}{\sigma_{CC}} = 19 \frac{S}{V} + 100$$  \hspace{1cm} \text{(2)}

The interesting point was also the condition of ageing (the group A). The frozen samples (condition F) demonstrated even a little subnormal conductivity that we interpret as roughly equal to the theoretical conductivity (see Table 2 and Fig. 3). Hence, no excess conductivity values were found in samples aged frozen. However, the solutions aged under condition S (daylight protected) and D (exposed to daylight) clearly showed excess conductivities. The highest excess conductivities were achieved under the condition S and amounted to 43 % of the measured conductivity $\sigma$, the $\sigma^E$ under condition D amounted to 41.5 % $\sigma$, whereas under F $\sigma^E$ were -17 % $\sigma$.

Table 2. Influence of ageing condition on $\sigma/\sigma_{CC}$ when frequencies, temperatures and treatments are combined; with standard errors (SE).

| condition | D | S | F |
|-----------|---|---|---|
| $\sigma/\sigma_{CC}/\%$ | 168.6 | 180.0 | 88.9 |
| SE/\%     | 2.1 | 2.5 | 2.5 |
Figure 3. Influence of ageing conditions PR (blue), ST (red) and MD (green) on $\sigma/\sigma_{CC}$ with standard error (SE) intervals (treatments, frequencies and temperatures combined). Repetition of D: 28, S: 20, F: 20.

$\sigma/\sigma_{CC}$ of solutions aged under condition S were significantly higher than D ($p = 0.003$), and at the same time $\sigma/\sigma_{CC}$ of solutions aged under both conditions (D and S) were significantly higher ($p = 0.000$) than under F (see Fig. 3).

In general, the results demonstrated anomalies in conductivity of aged solutions in comparison to the chemically analogues freshly prepared controls (CC) except for those samples aged frozen (see excess conductivities in Figs. 2 and 3).

Very interesting, although rather sophisticated, effects were uncovered by experiments concerning temperature and the $\sigma/\sigma_{CC}$. Namely, as shown in Figure 4 with higher frequencies (starting above 1000 Hz and below 10,000 Hz) higher $\sigma/\sigma_{CC}$ at lower (5 °C) than at higher (25 °C) temperature are found. The difference is statistically significant at 10,000 Hz and even more at 100,000 Hz.
The most important inferences and further implications

Excess conductivity values ($\sigma^E$) in aged aqueous solutions may indicate a highly ordered water system that forms spontaneously with time. Namely the long range and relatively long lasting water structure that should enable a faster transfer of protons—the most mobile ions in aqueous solutions. $\sigma^E$—correctly measured and determined—could be regarded as an indicator of water structuring. On its basis we could potentially develop a system of (at least partial) measuring of water ordering. Of course, it is only an idea that should be further thoroughly elaborated and developed in its theoretical as well as empirical aspects.

The second important area of further research following our line of study concerns the living process, especially bioenergetics, mostly founded on the transport of protons.

Let us first examine how some of the most conspicuous results of our research should help in the first task.

3.1. Time effect

Through time of resting the $\sigma^E$ value in aqueous solutions arises therefore we may conclude that resting time works in favour of spontaneous water structuring. These findings are in harmony with the findings of Vybiral (2006) as well as Vybíral and Voráček (2007) who found the so-called autothixotropic properties of water. The observed phenomenon is relatively weak and appears only on a macroscopic scale, if water is standing still for some time. It causes a force of mechanic resistance against an immersed body, when it should change its position. In their research static and dynamic gravimetric methods were used. The explanation for autothixotropy of water was based on a supposition of cluster formation by H$_2$O molecules in the aged water. Since the phenomenon does not appear in deionized water, a conclusion was preliminary drawn that the phenomenon is determined by the presence of ions.

Similar time related changes in water characteristics were noticed by Lobyshev and co-workers (1999). They found that distilled water possesses weak luminescence in the near UV and visible regions of the EM spectrum. Its emission spectra possess wide bands at 360 and 410 nm that depend...
on unusual properties of water. The intensity of luminescence depends on the duration of water storage in a closed vessel. After two to four months of holding the samples in the dark the intensity of the emission spectrum bands increased twofold. Further storage of samples did not change the intensity appreciably.

3.2. **Effect of ions**
Through raising the concentration of ions $\sigma^E$ values arise (see Fig. 2). Hence, what is the part that ions play in spontaneous structuring of water? Turton et al. (2008) separated rotational and translational motions of the water molecules with the ultra-fast Kerr effect and dielectric relaxation spectroscopic measurements. The results of these two methods showed that salt solutions behave like a supercooled liquid approaching a glass transition, where rotational and translational motions become decoupled. At the glass transition concentration the density of clusters of water molecules and ions is so high that they effectively turn the electrolyte solution into glass.

3.3. **Surface effect**
Higher surface to volume ratio, $S/V$ ratio, yields higher $\sigma/\sigma_{CC}$, hence higher $\sigma^E$ (see Equation 2). Most probably a higher $S/V$ ratio promotes higher ordering of water clusters. As already mentioned, the hydrophilic surfaces may have rather thick exclusion zones – zones of highly ordered water (Zheng et al. 2006).

3.4. **Frequency and temperature effect**
As can be seen on Figure 4 at 1000 Hz there is no significant difference between $\sigma/\sigma_{CC}$ measured at two different temperatures, 5 and 25 °C. However, at higher frequencies (starting at 10,000 Hz and much more conspicuously at 100,000 Hz) we can clearly see a significantly higher $\sigma/\sigma_{CC}$ at lower temperature. It seems that even if there is lesser protonic mobility at lower temperature, this is counteracted by higher frequency that somewhat positively interacts with water clusters. And since the clusters are more stable and better organised at lower temperature, a sufficiently high frequency can uncover this order in terms of higher conductivity. It seems that oscillating electric field promotes coherent orientation of water molecules thereby enhancing the formation of ordered chains or Zundel cations (Han et al. 2006).

3.5. **Ageing condition**
We learned that the partially protected flasks under condition S have the highest $\sigma/\sigma_{CC}$, hence the highest $\sigma^E$, while no $\sigma^E$ was found in frozen samples (see Fig. 3). According to our hypothesis ordered water demands liquid phase, the capacity to move that is normally seen as a striking contrast to order. At the same time solutions aged under the condition S (daylight protected) have higher $\sigma^E$ values than those aged under condition D (exposed to daylight).

As far as the biological implication goes, we know that biological energy supplying electricity is based on proton currents, mostly via mitochondrial membranes. In water the proton current is the quickest and can be raised significantly through water’s self-ordering (Han et al. 2006).

If we look on our main results, they indicate the following:

3.6.1. **Aging**
At our measured scale (months) the results would have little biological meaning since biological water is only exceptionally at rest for longer time. But the effect could still have importance on a very different scale, perhaps the one of μs. Further experiments should be devoted to shorter time scales.

3.6.2. **Surface**
This factor should have a profound significance on the ordering of water in biological systems, since the biological (mostly intracellular) surfaces are very large vs. volume. Further experiments should be devoted to explore the $\sigma^E$ of very small volumes of aqueous solutions in vicinity of very large surfaces.
3.6.3. Ionic composition
As already pointed out by the findings of Turton et al. (2008) as well as the ones of Vybíral and Voráček (2007), the ionic composition of water is very important. In further researches concerning the biological role of enhanced electrical conductivity various ionic solutions should be tested, above all those expected in living tissues.

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