

The possible role of active oxygen in the Memory of Water

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Phenomena of long-term ‘memory of water’ imply that aqueous systems possessing it remain for a long period after the initial perturbation in an out-of equilibrium state without a constant supply of energy from the environment. It is argued here that various initial perturbations initiate development of a set of chain reactions of active oxygen species in water. Energy, in particular high grade energy of electronic excitation, released in such reactions can support non-equilibrium state of an aqueous system. In principle, such reactions can continue indefinitely due to specific local structuring of water with even minute ‘impurities’ that are always present in it and by continuous supply of oxygen amounts due to water splitting. Specific properties of several real aqueous systems, in particular, homeopathic potencies in which such processes could proceed, are discussed. The role of coherent domains in water in maintenance of active oxygen reactions and in emergence of oscillatory modes in their course is considered. *Homeopathy* (2007) **96, 196–201.**

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Introduction

‘Memory of water’ is a popular idiom meaning long-term effects of various physical factors upon physical–chemical properties and biological activity of aqueous systems. The phenomenon of ‘water memory’ is on area of heated debate. The particular case of ‘water memory’ controversy is homeopathy. Its assertion that a homeopathic preparation can hardly contain a single molecule of an initial biologically active material but retain biological activity cannot be explained in the frame of the current biochemical and pharmacological paradigm. According to the latter a medicine exerts its action due to local interactions of active substantial principles present in a medication with appropriate biomolecules (enzymes, receptors, etc.). Specificity of these interactions is due to complementarities of electronic landscapes of interacting due to species. Specific binding of an appropriate

ligand to the particular ‘receptor’ induces its conformational change, necessary for the development of a downstream chain of reactions.

It is generally considered that there is no problem in energy supply and transformation for performance of this chemical work. Conformational change in the receptor is supposed to be provided by energy released as a drug binds to it. Chemical work associated with all the downstream reactions of a cell is supported by energy supplied by metabolism. This reasoning tacitly implies the initial non-equilibrium state of the whole system: drug + a target cell.

From this perspective homeopathy seems improbable for several reasons. One of them is bewilderment about how a preparation not containing a single biologically active molecule from its original solution or tincture exerts any specific biological effect (the problem of specificity). Claims that the original molecular principle somehow leaves its imprint in water contradicts the textbook model of water, according to which water cannot retain any ‘memory’ after a perturbation due to fast relaxation to an equilibrium state for the given ambient conditions.

Thus, one of the key questions related to the problem of ‘water memory’ is the question whether

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water is a substance that after a perturbation does not easily relax to the original state and under special circumstances can even further move away from the equilibrium state? If so, what mechanisms provide for its stable non-equilibrium state? If one can answer these questions, the question of the specificity of homeopathic preparations may be solved more easily.

Here the hypothesis is presented that due to water's capability to transform low grade energy (eg, mechanical) into high grade energy of electronic excitation and due to its dual oxidant–reductant nature, water may remain in a non-equilibrium (dissipative) state for a very long time.

However, before we go further clarity about the word 'water' should be introduced. 'Water' is never pure H₂O. Real water always contains impurities: products of its ionization (H⁺ and OH[−]), ions, dissolved gases, and traces of other substances. Even ultra-pure water is kept in a vessel. Water properties in the vicinity of its walls (interfacial water) may significantly differ from those in 'bulk' water and from those at a water/gas (air) interface. Unlike common belief that effects of solid surfaces with which water is in contact vanish on a nanometre scale, new evidence shows that they may propagate at distances of tens and hundreds of microns.¹

Long-term effects of physical factors upon the properties of water

Currently there is no shortage in evidence of long-term effects of physical factors, such as static and oscillating magnetic and electromagnetic fields, mechanical stirring and vibrations, sonication, etc. upon the properties of water. Here we will refer to only few of these studies that are seriously substantiated and relevant for further discussion of the role of active oxygen species in water memory.

In more than a decade of study of the effects of vigorous succussion and extreme serial dilutions in bi-distilled water or different aqueous solutions. Elia and co-workers found that already the third centesimal dilutions prepared with vigorous succussion demonstrated significant excess in heat release upon mixing with dilute alkali or acid and significant increase in electrical conductivity over unsuccussed solvent or dilutions.² Even more important was that these differences did not attenuate or vanish with time, but rather magnified in all the samples during several weeks of storage. Differences did not disappear even after several years of storage, and the smaller the volume of stored samples, the larger was the deviation.³ Elia *et al* establish that "these extremely diluted solutions (EDS), after strong agitation (succussion), enter a far from equilibrium state and remain there or get even farther by dissipating energy in the form and amount necessary to stay in a far from equilibrium state. What is the source of dissipating energy that does not exhaust for several years?

Elia *et al* acknowledge that in the process of vigorous succussion of aqueous samples traces of substances may be released by the glass of the containers, and these traces are able to 'activate' the EDS. Strong support for the suggestion that a long-term perturbation of aqueous systems treated by a physical factor depends on nano-'impurities' is provided by the recent seminal paper of Katsir and co-authors.⁴ They demonstrated that radio-frequency treatment (in the megahertz frequency range) of aqueous solutions can dramatically change their properties expressed, in particular, in peculiar patterns of electrochemical deposition of zinc sulphate solutions. Again, it takes some time after irradiation for the aqueous system to change its properties. The effects of radio-frequency treatment of solutions lasted for hours. If ultra-pure water used as a solvent was doped under radio-frequency treatment with barium titanate nanoparticles (diameter range 10–100 nm) special properties of zinc sulphate solutions prepared on such water were amplified and lasted for months. Saturation concentration for nanoparticles in irradiated water did not exceed 10^{−12} M; at higher concentrations they clump and sediment. As it takes many hours for the emergence of special properties of nanoparticle-doped water (NPD) the authors assume that water goes through a self-organization process.

The findings of Katsir and co-authors have much in common with the research into physical–chemical properties and biological activity of aqueous dispersions of Fullerene C₆₀. Since the discovery of fullerenes a lot of surprising biomedical effects both *in vivo* and *in vitro* were reported. Those include antiviral (in particular, anti-HIV), anti-bacterial, anti-tumour, anti-oxidant, and anti-apoptosis effects among others.⁵ In most cases hydrophilic C₆₀ derivatives were used because pristine fullerenes C₆₀ are considered to be water-insoluble. Andrievsky *et al* developed a procedure for preparing molecular–colloidal solution of pristine C₆₀, with the help of ultrasonic treatment of fullerene water suspension. It contains both single fullerene molecules and small clusters.⁶ In such 'fullerene–water-systems' (FWS) single C₆₀ molecules and their clusters do not precipitate because they are covered with water shells in which water molecules are absorbed so strongly that water is not completely lost even in vacuum of 10^{−3} Pa. FWS do not have toxic effects and possess strong biological activity even in dilutions down to 10^{−9} M.⁷ Andrievsky ascribes the wide spectrum of beneficial biological effects of FWS to their strong 'anti-oxidant' activity that is also ascribed to aqueous solutions of hydrophilic fullerenes⁵ (we will discuss below what 'antioxidant activity' really means).

Water systems described above: 'EDS' of Elia *et al*, 'NPD' of Katsir *et al*, and 'FWS' of Andrievsky *et al* have much in common, though they are prepared using quite different procedures and have completely different chemical composition. On the one hand, in the course of their

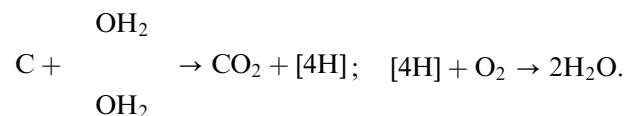
preparation basically the same procedure is used—physical treatment of water causing cavitation in it ('cavitation' is the emergence of gas-filled cavities and bubbles in a liquid and vigorous change of their volume and behaviour depending upon local pressure changes). Katzir *et al*, ascribe the "anomalous effects of radio-frequency treatments of water and aqueous solution to the formation of pliable network of gas nanobubbles that has special hierarchical organization effect". They suggest that much more long-term changes in the properties of NPD than in irradiated water or simple aqueous solutions is explained by replacement of less stable nanobubbles with stable barium titanate nanoparticles. Succussion used for the preparation of EDS and ultrasound treatment of water used for the preparation of FWS also produce cavitation in water. In all three systems water becomes 'doped' with nanoparticles. In the case of EDS they are supposedly represented by silica oxide, in NPD—with barium titanate, and in the third case—with fullerenes. At least in the last two cases it has been demonstrated that nanoparticles serve 'kernels' around which water shells with properties very different from those characteristic for usual 'bulk' water originate. And for FWS so-called 'anti-oxidant' properties were demonstrated.

Water forming shells around nanoparticles is 'gel-like' and the shell may extend up to a micron in range (at least in the case of NPD). Thus, it is difficult to explain physical-chemical ('anti-oxidant') and biological activity of all these aqueous systems by chemical properties of 'impurities'—nanoparticles that are so chemically different and rather inert. It is much more plausible that this activity is based on a specific structuring of interfacial water. But how can such 'gel-like' (or 'ice-like') water structures provide for stable non-equilibrium, energy dissipative properties of aqueous systems?

Water, a two-faced Janus: Pro- and anti-oxidant activity of water

Until recently water was considered just as a solvent in which biochemical processes go on and as a fluid used to transport different substances throughout the body. Though 'anomalous' properties of water, its role in base-acid equilibrium, its direct participation in the reactions of hydrolysis and photosynthesis is generally acknowledged, the much deeper fundamental role of water in practically *all* chemical reactions is neglected. Yet the discovery that *water is the catalyst* of at least oxidative reactions was made as long ago as in 18th century. In 1794 a British researcher, Elizabeth Fulhame published in London a book entitled 'An Essay on Combustion'. Based on her own studies she stated that "hydrogen of water is the only substance, that restores oxygenated bodies to their combustible state; and that water is the only source of the oxygen, which oxygenates combustible bodies" (cited after [8]). For example, to explain the combustion of charcoal she suggested that

"the carbon attracts the oxygen of the water, and forms carbonic acid, while the hydrogen of the water unites with oxygen of the vital air, and forms a new quantity of water equal to that decomposed":



Thus, water according to Fulhame is both pro-oxidant (it oxidizes a fuel) and anti-oxidant (it reduces oxygen).

Though the discovery of Fulhame was soon forgotten, chemists of the 19th century acknowledged that water is necessary for oxidation (oxygenation) even of easily combustible bodies. They knew that metallic sodium and potassium do not lose their metallic luster in an atmosphere of dry oxygen, and that carbon, sulphur, and phosphorus burn under very dry conditions at much higher temperatures than in humid air.⁹ However, until the beginning of the 21st century, when it was rediscovered that water can 'burn'—be oxidized by singlet oxygen¹⁰ this 'mysterious' property of water was neglected. It was also proved by quantum chemical modelling that water oxygenation is *catalysed by water*.¹¹

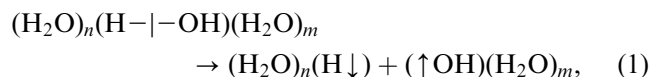
Water participation in chain reactions

How are catalytic and red/ox properties of water related to the phenomenon of water memory? Above it has been argued that water forming shells around nanoparticles is 'gel-like', so it has features of a polymeric substance. It is well known that polymers can undergo chemical transformations under the action of mechanical impacts, freezing-thawing and fast temperature variations, action of audible sound and ultrasound, and of other low density energy forces too weak to induce chemical reactions in monomers or short oligomers. Polymers may accumulate and concentrate mechanical energy to densities that comprise energy quanta sufficient to excite and break down their internal covalent bonds. Unpairing of electrons and appearance of a pair of free radicals results in the development of new reactions.¹²

Based on the presumption that liquid water contains quasi-polymeric structures Domrachev *et al* investigated the effect of low density energy physical factors on homolytic water dissociation ($\text{H}-\text{O}-\text{H} \rightarrow \text{HO}\cdot + \cdot\text{H}$, cf. ionic water dissociation: $\text{H}-\text{O}-\text{H} \rightarrow \text{H}^+ + \text{OH}^-$). It was shown that water freezing-thawing, evaporation-condensation, sonication even with audible sound, filtration through narrow capillaries resulted in an increase of H_2O_2 even in ultra-pure and carefully degassed water. Efficiency of water splitting resulting from water filtration through narrow capillaries (where a significant part of it forms interfacial water) was more than 100 times greater than photodissociation with far UV-light.¹³ Yield of H_2O_2 in water containing ions and dissolved oxygen

was much higher, and notably, H_2O_2 concentration continued to grow in water containing dissolved oxygen for some time after the completion of any treatment, as if it 'remembered' it.

In the case of a single water molecule in a mechanically excited polymeric entity being split:

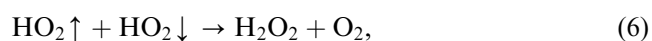


the initial products of water splitting are free radicals $\text{H}\downarrow$ and $\uparrow\text{OH}$ (here we symbolize a given electron as \uparrow or \downarrow to stress their alternative spin states). In most cases this singlet pair of radicals recombines back to water:



However, even in such a case this is not just a reverse, equilibrium reaction because water splitting has been achieved under the action of mechanical forces while back recombination of radicals gains an energy quantum of 5.2 eV. In condensed and organized media (such as water), long-range energy transfer of electronic and vibrational excitation has been demonstrated already in 1930s–1950s by J. Perrin, S. Vavilov, Th. Foerster, A. Szent-Giorgyi, and others. This phenomenon was recently confirmed with new techniques.¹⁴

The probability of radicals moving away from each other significantly increases when dissolved gases and other molecules and particles are present in water, especially in cases when multiple layers of water are organized by surfaces which it hydrates and when these layers move relative to each other at different rates (consider a vortex as an example). Here, a rich set of reactions may proceed, for example:



Besides these more or less stable products exotic metastable substances may appear, for example: HOOOH , H_2O_4 , HOO-HOOO , HOOH-OOO , etc. Reactions 6 and 7 in which oxygen molecules are released are notable as they provide evidence that oxygen may abiogenically arise from water under very mild conditions. What is also important is that this 'newborn' oxygen arises in an activated, singlet state.

It should be reminded that O_2 is unique among molecules because in its ground state its two electrons are unpaired [$\text{O}_2(\uparrow\downarrow)_2\uparrow\uparrow$ or $\text{O}_2(\uparrow\downarrow)_2\downarrow\downarrow$] (besides, an oxygen atom also has two unpaired electrons). Thus, oxygen molecule is a bi-radical (in fact it is a tetra-radical) and it represents a vast store of energy. But the laws of quantum physics forbid direct reactions of bi-

radicals (they are also called particles in a triplet state) with molecules in which all electrons are paired (singlet state particles). That is why oxygen needs to be activated to release its energy reserve.

There are a few ways for O_2 to be activated. It may be excited by an appropriate energy quantum (≥ 1 eV) and turn into a highly reactive singlet oxygen ($\text{O}_2(\uparrow\downarrow)$, also denoted, $^1\text{O}_2$). A peculiar feature of $^1\text{O}_2$ is that this electronically excited species may relax only to triplet state because oxygen, unlike other substances does not have ground singlet state. Since singlet–triplet transition is 'forbidden' by quantum physics laws, the lifetime of excited singlet oxygen is usually much longer than that of any other molecule in an excited singlet state. Probably that is why the reaction of singlet oxygen with water goes with sufficiently high probability— $^1\text{O}_2$ is long-living enough to find an appropriate catalytic environment for water oxidation.

On the other hand, triplet oxygen easily reacts with free radicals—atoms and molecular particles with an odd number of electrons. In these reactions oxygen gains an electron, turns into a mono-radical which can easily take new electrons releasing large quanta of energy at each consecutive step of one-electron reduction.

The principal property of free radical reactions in which O_2 participates is that they may easily turn into a branching (or run-away) process.¹⁵ Several specific features distinguish branching chain reactions (BCRs) from 'normal' chemical reactions.¹⁶

First, the quantum yield (the ratio of the quantity of reaction events to the quantity of quanta that initiated the initial reaction events) is extremely high.

Second, BCR often start to develop after an induction period, long after the completion of the initiating stimulus impact. (Development of BCR is expressed in exponential growth of reaction centres represented usually by free radicals, until the rates of their production and annihilation equalize).

Third, the reaction proceeds at a very low rate below and above threshold values of critical parameters: temperature, volume of the reaction mixture or ratio of the reaction mixture volume to the surface of the reaction vessel, concentrations of reagents, etc.

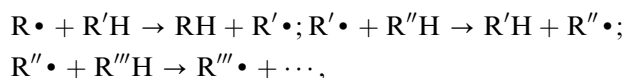
The fourth specific feature of BCR is a very strong accelerating or rate-retarding effect of certain minute admixtures in the reaction mixture.

Fifth, large deviation of kinetics of BCR from classical laws of chemical kinetics—Arrhenius temperature law and the law of mass action—is observed at certain stages of BCR development.

Finally, as long as a BCR proceeds it serves as a source of high density energy—energy of electronic excitation, equivalent to quanta of visible or UV light, because free radical recombination events (recombination of unpaired electrons) are highly exergonic. That is why the reaction systems in which such reactions occur are often chemiluminescent.

In the gaseous phase BCRs usually develop as explosions. However, in condensed phases a lot of red/

ox-reactions with O_2 participation meet many criteria of the BCRs though they develop and proceed without termination for an extremely long time. Semyonov¹⁶ suggested that these reactions go on as linear chain reactions in which chains do not branch:



where $R''\cdot$ is a free radical with an unpaired electron, and $R'''H$ is a molecule which it oxidizes.

But if a free radical is in turn oxidized with a bi-radical molecule oxygen, a peroxide radical, $ROO\cdot$, is produced. When it oxidizes a certain molecule, a metastable and energy-rich peroxide ($ROOH$) is produced in addition to a new radical, which provides for chain propagation. Usually low energy of activation is needed for decomposition of peroxides at which *two* new active centres, $RO\cdot$ and $\cdot OH$ emerge. Thus, even if a 'parent' chain is eliminated, the system in which peroxides appear stays 'charged' and new chains arise in it sometimes after a very slight perturbation. Such reactions are named "chain reactions with delayed branching" (CRDB). Systems in which CRDB go on are intrinsically non-equilibrium, though at a first sight may seem to be at rest.

Evidence is accumulating that very slow CRDB may under 'appropriate conditions' readily develop in water. As mentioned above, quantum chemical calculations show that if water is organized in a favourable way (water molecules are arranged in space, in particular, in relation to singlet oxygen and to each other), the energy of activation for oxidation of a water molecule with singlet oxygen diminishes to reasonable values. The immediate products of water oxidation are exotic and highly energy-rich peroxides such as $HOOOH$, $HOOOOH$, $HOO-HOOO$.¹¹ All these peroxides are typical active oxygen species. They easily decompose giving birth to new free radicals, initiating propagation of new chains, or to ozone, generating new singlet oxygen molecules. Stationary levels of all these active oxygen species are extremely low due to their instability, but since water is never devoid of molecular oxygen (recall that any perturbation of water gives birth to oxygen and hydrogen at a non-zero probability), high energy quanta-generating processes never completely fade out.

We observed that in the course of CRDB of slow oxidation of amino acids in aqueous solutions initiated with H_2O_2 addition or with low intensity UV-irradiation, concentration of H_2O_2 increases to levels that can be explained only by water oxidation with O_2 .¹⁷ Recently, it has been shown that in water containing carbonates and phosphates¹⁸ or in water bubbled with noble gases, such as argon,¹⁹ concentration of H_2O_2 spontaneously increases and its augmentation goes on faster if water is stirred. Using chemiluminescent methods we also found that such processes spontaneously develop and proceed for an indefinitely long

time in aerated mineral waters from natural sources.²⁰ Also as it was mentioned above H_2O_2 yield in pure water equilibrated with air under the conditions favourable for its splitting¹³ occurs faster, continues longer after initial perturbation, and reaches higher levels than in degassed water.

Oscillatory nature of reactions with active oxygen participation

At the beginning of this essay a question was asked: is water a special case of a substance that can stay after a perturbation for a sufficiently long time out of equilibrium with the environment required by the second law of thermodynamics, and if it can, what mechanisms provide its stable non-equilibrium state? According to the theory developed by Del Giudice *et al* based on the principles of quantum electro-dynamics coherent domains of sub-micron ('nano') dimensions spontaneously emerge in water and coexist in it together with non-coherent dense water 'gas'.²¹ According to this theory water particles within a coherent domain oscillate coherently between two states belonging to individual spectrum of these states. Calculations show that two relevant levels involved in a coherent oscillation are separated by an energy of 12.06 eV whereas the ionization threshold of the water molecule is 12.6 eV, that is only 0.54 eV below ionization threshold.²² Del Giudice pointed my attention to the fact that, provided that this threshold is overcome, coherent domains (CD) may tunnel 'hot' electrons in the non-coherent surroundings where they stick to oxygen molecules thus initiating chain reactions described above.²³

Turning back to the three examples of stable non-equilibrium aqueous system mentioned earlier: 'extremely diluted solutions', 'nanoparticle doped water', and 'FWS', one may suggest that water shells surrounding nanoparticles present in these systems represent stable coherent domains that may supply electrons to oxygen. Energy of electronic excitation released due to oxygen reduction and free radical reactions may serve as activation energy for additional release of electrons from a CD. When too many electrons are extracted from a CD, it dissipates. Chain reactions terminate though metastable products of CRDB stay in a system. During this period water shells begin to build up around nanoparticles and as soon as they turn into CDs the latter again start to supply electrons to oxygen. This hypothetical scenario shows how complex oscillations of energy of electronic excitation generation, of red/ox potentials, and of other properties of aqueous systems may originate in systems where coherent water domains reduce oxygen, and its active species are present. The frequency range of oscillations varies from the optical region, characteristic for electronic excitation, to extremely low frequencies of oscillations of other parameters of the

system. Thus, aqueous systems in which chain reactions with the participation of active oxygen proceed may serve as emitters and receivers of oscillatory signals in an extremely wide frequency range.²⁴ This to our mind is a necessary condition for homeopathic potencies to exert its action on living systems.

Conclusion

It is now evident that a substantial part of oxygen consumed by all aerobic organisms is one-electron reduced, and that all the processes in which active oxygen species participate described above in connection with inanimate aqueous systems in principle take place in living systems. The indispensable role of active oxygen species in regulation of practically all physiological processes is no longer disputed. According to our point of view their ubiquitous regulatory role is paradoxically provided by extremely fast elimination of active oxygen by multiple 'anti-oxidant' systems as soon as they emerge. As we reasoned elsewhere²⁵ evidence is accumulating that the energy of electronic excitation generated by unpaired electrons pairing may be utilized as energy of activation of particular biochemical reactions, as regulatory signals, and in special cases as the major source of energy for performing physiological functions. Since oscillatory patterns are characteristic for all processes in which active oxygen participate, both insufficient production of active oxygen and distortions in its use may result in derangement of oscillatory patterns of biochemical and physiological processes and their malfunction. External resonators such as homeopathic medicines may restore normal patterns of deranged processes. However, the problem of high specificity of particular homeopathic medicines needs further reflection.

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