THE RELATIONSHIP OF PHYSICO-CHEMICAL PROPERTIES AND ACTIONS ON BIOLOGICAL OBJECTS OF ARTESIAN WATER WITH GAS NANOBUBBLES (Ar, O_2)

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Abstract

It is established that the physico-chemical properties of gas saturated artesian water in the electromagnetic field change non-monotonic with increasing saturation time. It is shown that in areas with minimal coefficients of expansion and extremums of the viscosity the highest biological activity of water containing nanopositive phase of argon and oxygen is occur. It is suggested that the cause of the biological activity of the treated water is the effect based on atomic and structural features of water molecules, and nanobubbles electric double layer formed in artesian water in the experiment.

Key words: artesian water, nanobubbles, phisico-chemical properties, biological activity.

1. Introduction

In recent years, micro- and nanobubble technologies have drawn great attention due to their wide applications in many fields of science and technology, such as aquaculture, biomedical engineering, and agriculture (Ashutosh Agarwal et al., 2011; Fan et al., 2010; Calgaroto et al., 2014; Oshita et al., 2013; Anup Gurung et al., 2016; Micro-and nanobubbles, 2015). Very indicative in this respect is the report presented at the conference (Akimi Serizawa et al., 2017). The problems of water containing nanobubble gas phase has involved the representatives of fundamental science later than the practitioners, so at present the physico-chemical mechanisms of action of such water on different type of biological objects are at the stage of nomination, justification and discussion of hypotheses.

This paper presents the results of experimental studies of artesian water with nanobubbles gas phase (argon, oxygen) and discusses the possible mechanisms on biological action of such water.

2. Experimental

The artesian water with a mineralization degree of 0.8 g/l was the object of the study. Saturation of water with gases (oxygen and argon) was performed by the method of bubbling in the electromagnetic field. The oxygen content in the water was determined by the method of direct current voltammetry with the help of polarograph PU-1 and interface unit Graphite-2, with the following shooting modes for voltammograms: the initial sweep voltage of 0.0 V, the amplitude of scan of 1.5 V, scan rate of potential of 0.2 V/c. Measurements of water density were performed with an Ostwald pycnometer with a volume of 0.934 ml. On the basis of the density values, measured at different temperatures, the thermal coefficients of expansion were calculated. Viscosity of water was measured with a capillary viscometer (d = 0.34 mm). The study of the microelectrophoretic cells motility was performed using the complex "Cytoexpert" (Russia).

3. Results and discussion

3.1. Physico-chemical properties of gas-saturated artesian water

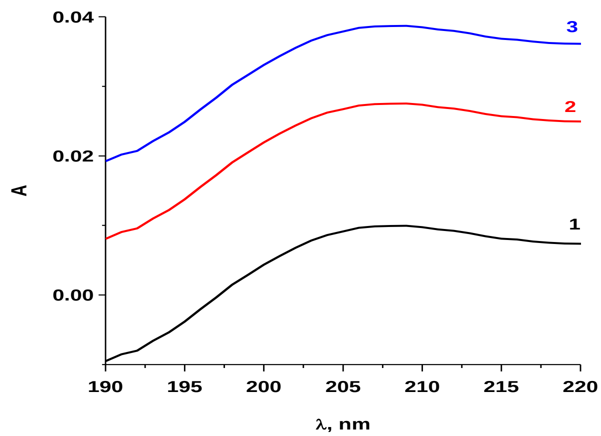
Saturation of water with argon or oxygen in the processing of the electromagnetic field leads to a slight decrease in the concentration of dissolved ions (Table 1) and the change of pH from 7.7 to 8.3.

TABLE 1: Composition of the initial and barbotage treated water (mg/l)

Ion	Gas nanobubble nature		
	Initial water	Ar barbotage	O_2 barbotage
K^+	4.2	4.2	4.2
Ca^{2+}	63.0	63.0	60.0
Mg^{2+}	71.0	71.0	70.0
NO_3^-	107.0	107.0	103.0
Cl^-	39.5	39.0	39.0
SO_4^{2-}	45.0	36.0	19.0

Studies conducted in the Prokhorov General Physics Institute of RAS **(**Moscow) under the leadership of prof. N.F. Bunkin have shown that barbotage of the artesian water results in nanobubble gas phase formation with a double electric layer. The size of the gas nanobubbles and the ζ- potentials are the same for oxygen and argon nanobubbles. Nanobubbles sizes are of the order of 270-300 nm, and ζ-potentials – of 10 – 40 mV (Butolin et al., 2016).

Supersaturation of water with oxygen in comparison with the natural saturation threshold of its content in the water is over 2-3.5 times and depends on the duration of the burbotage. The oxygen content affects the intensity of the UV- spectra of the treated water: with increasing oxygen content the intensity of the spectrum decreases, although in absolute terms these differences are small. With increasing the duration of nanobubble gas phase formation the intensity of the spectrum changes nonmonotonic with the minimum intensity corresponds to the highest gas concentration. It was found that the oxygen content in water increases with increasing the time of barbotage, and then a decrease in its content is observed. This effect reflected on the change of the intensity of respective UV- spectra, as well as with argon barbotage results in non-monotonic change of the intensity of UV-spectra (Fig.1). As follows from the Fig.1 at barbotage time of 20s-40s the low intensity of UV- spectra is observed, and in the same conditions according to the dynamic laser light scattering the clusters of two or three gas nanobubbles are formed.



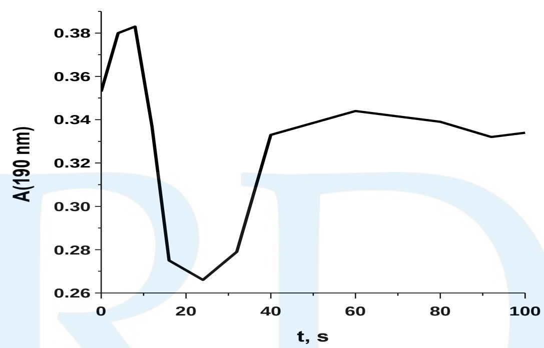


Fig.1. The change of intensity of UV- spectra depending on the content of gases:
a – the intensity of the spectra of artesian water with oxygen content of 3mmol/l (1); 5 mmol/l (2); 7 mmol/l (3); b – change of the UV-spectrum intensity of water depending on the time of argon barbotage

The change of the viscosity and thermal coefficients of volumetric expansion of water were interpreted on the basis of data on the molecular content of dissolved oxygen and the nano-bubble phase structure. It was established that the formation of clusters of several nanobubbles leads to increase in liquid viscosity at 25° C and decrease the coefficient of volume expansion in the area of 25º- 35º C. With increasing temperature and degree of water degassing the values of its viscosity and the expansion coefficient decreases sharply. In Fig.2 the artesian water kinematic viscosity and thermal coefficient of volume expansion variations depending on the argon barbotage time are presented.

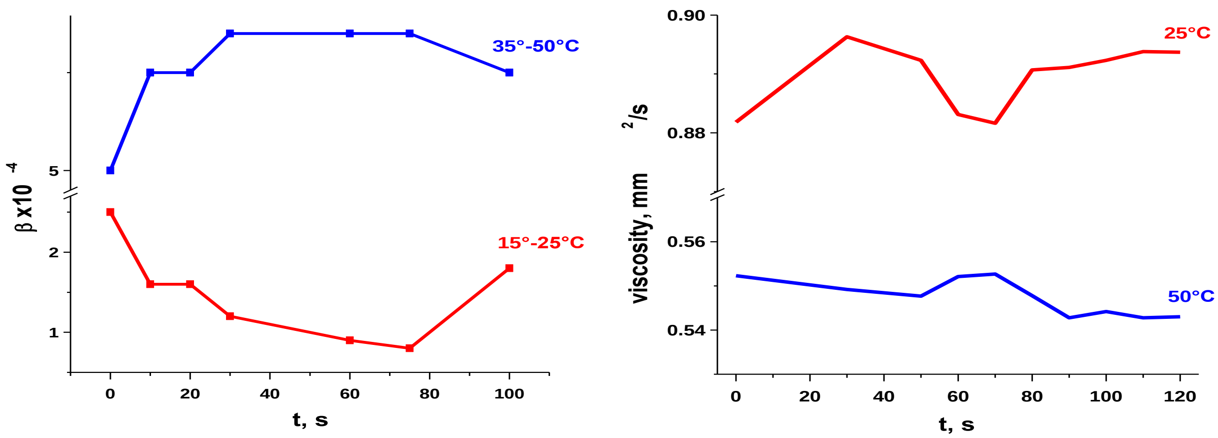


Fig.2. The change in kinematic viscosity and thermal coefficient of volume expansion of artesian water depending on the time of argon barbotage.

3.2. Biological properties of artesian water with gas nanobubbles

It is known that the magnitude of the electrophoretic mobility of red blood cells and buccal epithelial cells is considered as one of the important indicators of the immunological status of the organism. Increased activity of red blood cells indicates on the favorable environment impact.

Erythrocytes are the highly specialized nuclear-free blood cells that are shaped like a biconcave disk. On average, their diameter is of the order of 7.5 µm, the thickness at the periphery is of 2.5 µm, wich increases the surface of red blood cells for diffusion of gases. If it enters the red blood cells in the unfavorable environment the effect of hemolysis is observed: under the influence of the stimulus the red blood cell grows in sizes, sheath cell is not able to stretch, it collapses and the contents of the red blood cell comes out. Osmotic hemolysis occurs when the red blood cells get to the medium with an osmotic pressure lower than that of blood. Water enters the red blood cells, they swell and burst. Chemical hemolysis occurs when the sheath disintegrates under the action of a substance.

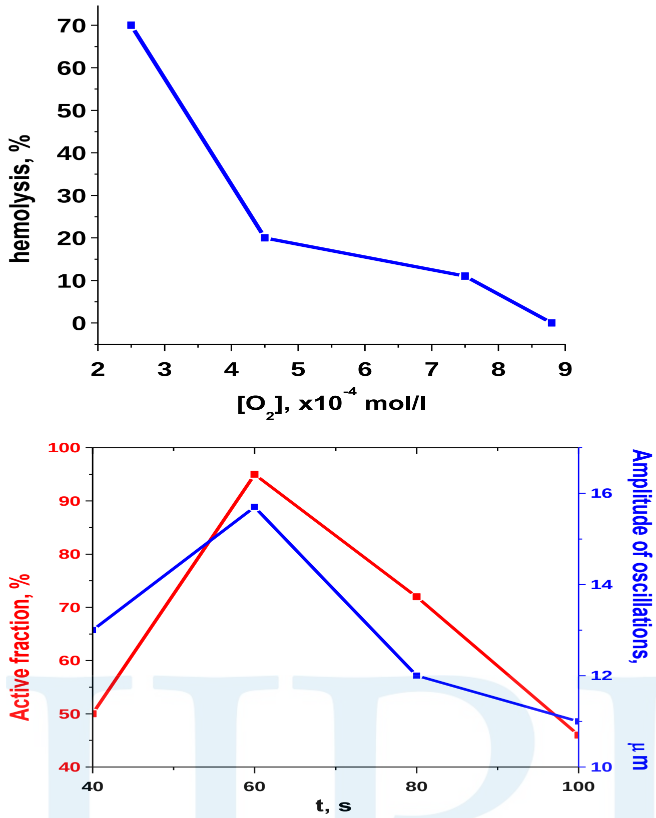


Fig.3. The condition of erythrocytes in artesian water with oxygen and argon nanobubbles a - the proportion of red blood cells destroyed by osmotic hemolysis, depending on the content of oxygen in water; b – the proportion of active red blood cells and the amplitude of their oscillations in the water depending on the argon barbotage time (in microelectrophoretic box)

In the initial artesian water almost 100% osmotic hemolysis is observed. In water with nanobubble gas phase the degree of osmotic and chemical hemolysis is reduced. In Fig.3 the dependence of the degree of osmotic hemolysis of red blood cells depending on the oxygen content in the artesian water is shown. A similar effect is also observed for red blood cells placed in water with argon nanobubbles. Consequently the nature of gas in this case is not a reason for the increase in membrane resistance of erythrocytes against osmotic hemolysis. From Fig.3 it is evident too that the largest proportion of active cells and the largest amplitude of erythrocytes are observed in the region of argon nanobubbles clusters formation. In water with nanobubbles gas phase chemical hemolysis – the destruction of the erythrocyte membrane in the presence of insulin is suppressed too (Table 2).

TABLE 2: Hemolysis of erythrocytes in presence of insulin

Argon barbotage time, s	Hemolysis, %
0	94.5±1.6
10	0
20	0
60	0
75	0
80	8.4±1.6
100	64.7±2.4

Epithelial cells, unlike red blood cells, are the nuclear cells. The study of functional condition of epithelial cells of the mucous membranes is a rapid method of obtaining diagnostic and prognostic information on the influence of various factors and conditions on health. Studies have shown that the proportion of active cells and the amplitude of their oscillations in microelectrophoretic box increases in water with nanobubble gas phase (Fig. 4). The greatest effect, as well as for erythrocytes, observed in the presence of nanobubble phase formed during the middle time of the bubbling (55s – 65s).

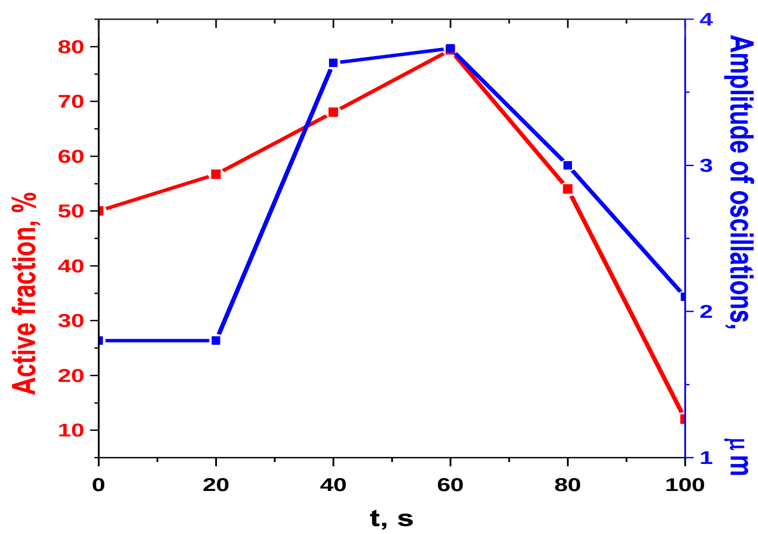


Fig.4. The fraction of active epithelial cells and the amplitude of their oscillations depending on the time of argon barbotage (microelectrophoretic box).

To assess the causes of observed effects, it is useful to start from the atomic and structural features of water molecules and the structural-phase state of gas-saturated aqueous solution.

Recognizing the existence of different spin states of hydrogen atoms, it is necessary to recognize the same opportunities in water molecules. The spins of the two protons in the water molecule can be oriented in the same or in opposite directions. The water molecules of the first type are called ortho-molecules, and the molecules of the second type - para-molecules. In (Tichonov et al., 2002) it was shown that these isomers can be distinguished by the infrared and NMR- spectra. It was found that para- water molecules without the moment of momentum have a lot of adsorption activity on some types of surfaces than the ortho-molecules.

For two types of molecules, differing in the number of hydrogen bonds with its neighbors (Pershin, 2005; Pershin, 2006), statistically correspond two kinds of structures found in (Ashutosh Agarwal et al., 2011). Molecules with a tetrahedral coordination is preferably presented in the ice-like clusters, while the structure with "broken" connections built primarily of molecules with one donor and one acceptor hydrogen bonds. Metastable ortho- and para-modifications long time existence ability as an independent substances has shown: the authors (Bunkin et al., 2006) have found experimentally ortho- and para-molecule in liquid water. The differences of physico-chemical properties of the spin isomers of water can occur across a broad range of phenomena and processes with participation of water molecules, both in gas and liquid phase. So the spin-selective interaction of para-molecules with proteins and DNA molecules dissolved in water (Bunkin et al., 2007) was established.

In (Artmann et al., 1998) 0-100% leap "fluidity" of human erythrocytes in the capillary, while sampling by pipette with a bore diameter of 1.3±0.2 μm, providing a pressure drop of 2.3 kPa, was discovered. Leap "fluidity" was observed in a narrow temperature range: 36.4 ± 0.30^{0}C.

The diameter of the erythrocyte (about 7μm) significantly larger than the diameter of the capillary. It was found that at temperatures below the leap, cell partially drawn into the capillary losing up to 20 % of the water and have stayed at the end of the capillary as an elastic ball. At temperatures above the leap erythrocyte compressed, drawn into the capillary and slipped it with great speed in just a few seconds, the volume of the erythrocyte in the capillary has decreased more than twice. This decrease was accompanied by the loss of the water up to 55% through water channels aquaporin protein in the erythrocyte membrane (Artmann et al., 1998). In addition, it was found that the viscosity of aqueous solution of hemoglobin extracted from red blood cells, is reduced by almost an order of magnitude in the vicinity of this temperature, and its concentration in solution increases of 1.7 times.

In (Pershin, 2009) the quantum nature and the mechanism of the effect of the leap in the permeability of erythrocytes through the capillary, which is based on the quantum properties of water molecules, differing in the mutual orientation of the spin of the proton is justified. In accordance with this mechanism in the cytoplasm of the erythrocyte develops a self-sustaining sequence of processes of a type of chain reaction at a temperature of 36.6^{0}C: (1) approximately at 36.6^{0}C increases the probability of conversion of para - H_2O to ortho-H_2O in the hydration layer close to the protein of hemoglobin; (2) the hydration layer around the hemoglobin becomes thinner, partially destroyed it litopterna structure which prevented adhesion of the molecules of hemoglobin; (3)-hemoglobin molecules become closer to each other; (4) the volume of the erythrocyte is reduced by almost 55% due to the release of the liberated in the conversion of ortho-isomers of H_2O from the sheath; (5) concentration of catalysts (iron and oxygen in hemoglobin) in a unit volume is increased; (6) the para-ortho conversion of water molecules is accelerated (Limbach et al., 2006; Buntkowsky et al., 2008; Salihov, 2000; Zel'dovich et al., 1988; Michout et al., 2004), forming an avalanche-shaped leap: 1-2-3-4-5-6-1. The same mechanism is manifested in the anomalous (almost an order of magnitude) increase in the fluidity of the aqueous solution of the molecules of hemoglobin with increasing concentrations of 1.7 times. In contrast to the above process chain, in this case molecules of ortho-H_2O remain in solution and reduce its viscosity since in free state they are always revolving (Rothman et al., 2005).

In the above-proposed mechanism, thermal fluctuations and collisions provide and support the spin conversion of ortho-para H_2O in the magnetic field of the paramagnetic O_2 as a catalyst (Limbach et al., 2006; Buntkowsky et al., 2008; Michout et al., 2004), so removing oxygen from the water will reduce the probability of ortho-para conversion. Perhaps this factor was shown in (Pal et al., 2002; Peon et al., 2002; Zheng et al., 2003) as the insensitivity of the water without oxygen to a magnetic effect. On the contrary it is known that increasing the concentration of triplet oxygen leads to increase of the rate of ortho-para H_2O conversion which has been recently experimentally proved in (Valiev et al., 2017).

We can assume that the electric double layer of gas nanobubbles (not only oxygen) influences the ratio of ortho-para water molecules and the reason for the increase of erythrocytes membrane resistance to osmotic and chemical hemolysis in water with nanobubbles is the change of this ratio.

It is shown that the ability of single-celled animals to live in the treated water is also activated: the oscillation amplitude of the ciliates shoes increases (Table 3), the multiplication of the ciliates - Stylonychia increases in 3-4 times. In contrast to the ciliates and cells many bacteria are additionally covered over a top of membrane by a thick and strong cell wall. Inhibition of the synthesis of a component of the cell wall leads to the cessation of bacterial growth. This effect is observed for Staphylococcus aureus and Escherichia coli.

TABLE 3: Microelectrophoretic activity of ciliate-shoes in water with Ar and O_2 nano-bubbles

Water	Fraction of active ciliate-shoes, %	Fluctuations amplitude of ciliate-shoes, µm
inutial	29	3
NB Ar	93	14
NB O_2	55	5

Unicellular algae spirulina, although it has a cell wall, but unlike bacteria, in water with nanobubbles feels well enough: the share of active spirulin in water with nanobubbles increased by 20% compared to initial water; the oscillation amplitude increases from 3 µm in the initial water to 4.5 µm and 6 µm in water with argon and oxygen nanobubbles, respectively.

The reason of different behavior of bacteria and spirulina in water with nanobubbles may be explained by different mechanisms of its reproduction. Bacteria multiply by dividing, and the cell with disrupted cell wall can not divide. Spirullina has a vegetative method of reproduction, and therefore spirullina with a cell wall could give rise to a new life.

Earlier we have investigated the influence of drinking water with nanobubbles on the bio-chemical parameters of blood of rats with experimental diabetes mellitus. On rats with diabetes water has an effect similar to drugs–antioxidants which are used in the treatment of diabetes: without glucose-lowering therapy the decline of glycolytically hemoglobin is observed (Butolin et al., 2016). Antioxidant properties of water with nanobubbles of inert gas may be connected with the redox properties of its electrical double layer too.

4. Conclusion

The physico-chemical properties of the artesian water with gas nanobubbles has been investigated, the relationship of these properties with the biological activity of water, its influence on hemolysis of red blood cells, the functional state of epithelial cells and single-celled animals has been estimated, the cause of metastable state of supersaturated gas solution in water consisting in the presence of nanobubbles with high internal pressure has been stated. It has been suggested that the cause of the detected effects can be the ratio of ortho- and para-water changing, occurring under the influence of the electric double layer of gas nanobubbles that determines the formation of water cluster structure, the number of monomers and the ability of water molecules to adsorption and formation of hydrogen bonds.

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