Advances in Physics Theories and Applications
ISSN 2224-719X (Paper) ISSN 2225-0638 (Online)
Vol. 51, 2016

Mathematical Model of Kangen Water®. Biophysical and Biochemical Effects of Catholyte

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Abstract

This article deals with the review of the basic physical-chemical processes underlying the electrolysis of water and preparation of electrochemically activated water solution catholyte by Kangen Water® Ionizer and studying their physical-chemical properties and biophysical effects on human organism. Additionally, by using IR, NES, and DNES methods were investigated various samples of water from Bulgarian water springs: the melt water from Glacier Rosenlaui, Swiss Alps, as well as the human blood serum of people with excellent health and cancer patients between 50 and 70 years old. Other experiments were performed on a Kangen Water® Ionizer (Enagic USA Inc.) for improvement of quality of tap water by electrochemically activation. As an estimation factor in NES and DNES was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules in water samples, as well as a local extremums in the NES and DNES-spectra of various samples of water and the human blood serum at E = -0.1387 eV and λ = 8.95 μm. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in IR-, DNES-spectra were shifted to lower energies relative to the control healthy group. Further we applied this method for calculation of percent distribution of H_2O molecules in all studied water samples according to energies of hydrogen bonds ranged from (-0.08 to -0.1387 eV). It was shown that mountain water is among the most important factors for human longevity and human health. The variety of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}), the chemical-physical parameters (pH, electroconductivity) and the decreased content of deuterium in studied water samples renders beneficial effects of these types of water on human health. Also our research has confirmed the efficiency of water electrical treatment with Kangen Water® Ionizer. In frames of the research was carried out the computer calculation of elemental polyhedral water nanoclusters with a formula $(H_2O)_n$, where n = 3–20. Based on this data some important physical characteristics of water were obtained, e.g. the average energy of hydrogen bonding between H_2O molecules in the process of cluster formation was measured by the DNES method compiles -0.1067 ± 0.0011 eV.

Key words: longevity, mountain water, IR, NES, DNES, Glacier Rosenlaui, Kangen Water® Ionizer.

1. Introduction

Water is the main substance of life. The human body of an adult person is composed from 50 to 55% of water. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality and its amount in organism is an essential factor for the research (Pocock *et al.*, 1981; Howard & Hopps, 1986). Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water also is the main factor for metabolic processes and aging (Ignatov, 2012). Earlier studies conducted by us have demonstrated the role of water, its structure, the isotopic composition and physical-chemical properties (pH, temperature) on the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content (Mosin & Ignatov, 2012; Ignatov & Mosin, 2013a; Ignatov & Mosin, 2013b). These factors, the structure and composition of water are of great importance in many biophysical studies. The peculiarities of the chemical structure of the H_2O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H_2O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H…O) with neighboring H_2O molecules, binding them into complex intermolecular associates which composition represented by general formula $(H_2O)_n$, where n can vary from 3 to 50 (Keutsch & Saykally, 2011). The hydrogen bond is a form of association between the electronegative O-atom and a H-atom, covalently bound to another electronegative O-atom, is of vital importance in the chemistry of intermolecular interactions, based on weak electrostatic forces and donor-acceptor interactions with charge-transfer (Pauling, 1960). It results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H_2O molecule.

The electrochemical treatment of water with using the electric current is a promising modern approach in the water treatment technique, resulting in obtaining the electrochemically activated water solutions (catholyte/anolyte) carrying new physical-chemical properties stipulated by the changing of the electrochemical characteristics of water as ORP, E_h and pH (Ignatov et al., 2014). The process of electrochemical water treatment includes several electrochemical processes associated with the transfer in a constant electric field the electrons, ions and other charged particles (electrolysis, electrophoresis, electroflotation, electrocoagulation), the main of which is the electrolysis of water.

The aim of this research was studying the various samples of water from Bulgarian water springs: the melt water from Glacier Rosenlaui, Swiss Alps, as well as human blood serum of people with excellent health and cancer patients between 50 and 70 years old. In frames of this research on the water quality were investigated 415 people living in the municipalities of Teteven, Yablanitza. Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Plovdiv district (Bulgaria), where is lived the most of long lived people and their siblings, were studied.

The authors also performed the research of electrochemically activated Kangen Water® on the distribution of H_2O molecules according to the energies of hydrogen bonds, as well as studies of the NES and DNES spectrum and the biophysical effect of this type of water on human body. Particularly there was obtained and studied the electrochemically activated Kangen alkaline water catholyte with pH = 8.5.

2. Materials and Methods

2.1. Preparation of Water Samples with Varying Deuterium Content

For preparation of water samples with varying deuterium content we used D_2O (99.9 atom%) received from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D_2O and dried in vacuum before using. D_2O distilled over $KMnO_4$ with the subsequent control of deuterium content in water by ^{1}H-NMR-spectroscopy on Brucker WM-250 device ("Brucker", Germany) (working frequency – 70 MHz, internal standard – Me_4Si) and on Brucker Vertex ("Brucker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}).

2.2. NES and DNES Spectral Analysis

The device for DNES spectral analysis was made by A. Antonov on an optical principle. For this was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 ^{0}C) conditions. The water drops were placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light was monochromatic with filter for yellow color with wavelength at λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72.3^0 to 0^0. The DNES-spectrum was measured in the range of -0.08– -0.1387 eV or λ = 8.9–13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water samples and human blood serum.

2.3. Kangen Water® Ionizer

The experiments for improvement of quality of tap water were carried out by electrochemically activation with a Kangen Water® Ionizer (Enagic USA Inc.) with LeveLuk ionizer. The experiments were performed with water samples from tap water from Plovdiv and Sofia, Bulgaria. Kangen Water® was prepared from the tap water from Plovdiv and Sofia with electrochemically activation and purifying. The pH of Kangen Water® in experiments was 8.5. Kangen Water® Ionizer also makes the pH value to 8.0 and 9.0. Kangen Water® Ionizer is equipped with the big LCD screen and voice commands available in 5 languages. LeveLuk SD 501 Platinum has up to 7 electrodes made of pure titanium which is fully coated with platinum (99.97%). **The devise is equipped with an adsorption filter which eliminates chlorine, lead, lime scale and improves the taste of water.** The filter consists:

- 3-way 1 μm filter (sediment/GAC/KDF);
- GAC (Granular Activated Carbon Granular activated carbon) removes organic pollutants;
- KDF (Kinetic Degraduation Fluxion) removes chlorine and heavy metals;
- **The filter capacity:** 5 700 liters of water (~6 months).

2.4. Studying the Bulgarian Long Living People and Centenarians

Interviews have been conducted with 415 Bulgarian centenarians and long living people and their siblings. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using DNES method was performed a spectral analysis of 15 mountain water springs located in municipalities Teteven and Kuklen (Bulgaria). The composition of water samples was studied in the laboratory of "Eurotest Control" (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria.

2.5. Studying the Human Blood Serum

1% (v/v) solution of human blood serum was studied with the methods of IR-spectroscopy, non-equilibrium (NES) and differential non-equilibrium (DNES) spectral analysis. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases.

2.6. IR-spectroscopy

IR-spectra were registered on Brucker Vertex ("Brucker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; the accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}) and on Thermo Nicolet Avatar 360 Fourier-transform IR.

2.7. Statistical Processing of Experimental Data

Statistical processing of experimental data was performed using the statistical package STATISTISA 6.0 using the Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Electrolysis of water

The main element of the apparatus for obtaining the electrochemical activated solutions of water is electrolyzer consisting of one or more electrolysis cells (Figure 1). The electrolysis cell is formed by two electrodes – a positively charged anode and a negatively charged cathode connected to different poles to a DC source (Stoner, 1982). The cathodes are made of metals that require high electrical voltage (lead, cadmium), allow to generate the reactive free radicals as Cl*, O*, OH*, HO_2*, which react chemically with other radicals and ions. The typical apparatus for electrochemical treatment of water usually comprises the water preparation unit (1), the electrolyzer (2) and the processing unit after the electrochemical treatment of water (3) (Figure 1).

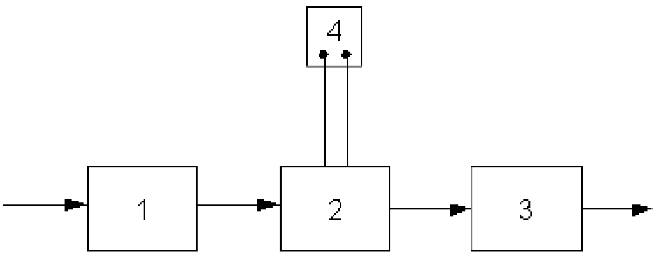


Figure 1: The apparatus for electrochemical water treatment: 1 – water treatment unit; 2 – electrolyzer; 3 – the block of post-treatment; 4 – rectifier of the electric current.

An interelectrode space is filled up with water, which is an electrolyte capable of conducting the electric current. As a result it is transferred electric charges through the water – electrophoresis, i.e. migration of the polar particle charge carriers – ions transferring to the electrode having an opposite sign. Wherein the negatively charged anions are moved toward the anode, whereas the positively charged cations are moved toward the cathode (Figure 2). At electrodes the charged ions lose charge and become depolarized, turning into the decay products. In addition to these charged ions, in the electrophoresis participate the polar particles with different particle sizes, including solid particles (emulsified particles, gas bubbles, etc.), but the main role in the transfer of electrochemical charges plays the ions possessing the greatest mobility.

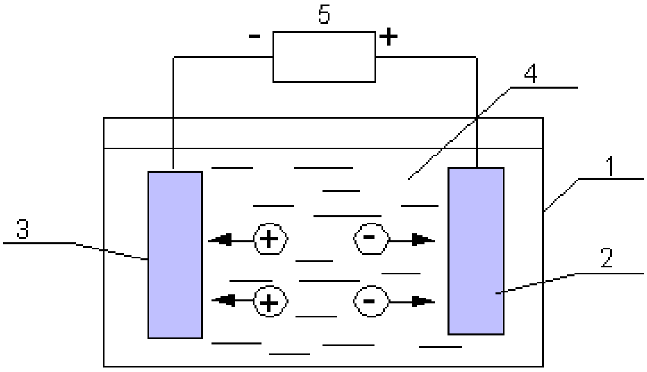


Figure 2: Scheme of the electrolysis cell: 1 – the case; 2 – anode; 3 – cathode; 4 – interelectrode space; 5 – DC power source.

The products of electrode reactions are the neutralized aqueous admixtures, gaseous hydrogen and oxygen generated during the electrolytic destruction of H_2O molecules, metal cations (Al^{3+}, Fe^{2+}, Fe^{3+}) in the case of metal anodes made of aluminum and steel, and the molecular chlorine. Wherein at the cathode is generated the gaseous hydrogen, and at the anode – oxygen. Water also contains a certain amount of hydronium ions (H_3O^+) depolarizing at the cathode with formation of the atomic hydrogen:

$$H_3O^+ + e^- \rightarrow H + H_2O, \qquad (1)$$

In an alkaline environment there occurs the disruption of H_2O molecules, accompanied by formation of the atomic hydrogen and hydroxide ion (OH^-):

$$H_2O + e^- \rightarrow H + OH^-, \qquad (2)$$

The reactive hydrogen atoms are adsorbed on the surfaces of the cathode, and after recombination are formed the molecular hydrogen H_2, released in the gaseous form:

$$H + H \rightarrow H_2, \qquad (3)$$

At the same time at the anode is released the atomic oxygen. In an acidic environment, this process is accompanied by the destruction of H_2O molecules:

$$2H_2O - 4e^- \rightarrow O_2 + 4H^+, \qquad (4)$$

In an alkaline environment, the source of oxygen source are OH^- ions, moving under the electrophoresis from the cathode to the anode:

$$4OH^- \rightarrow O_2 + 2H_2O + 4e^-, \qquad (5)$$

The normal redox potentials of these reactions compiles +1,23 V and +0,403 V, respectively, but the process takes place under certain conditions of the electric overload.
The anolyte usually has pH = 3.5–5.0 and ORP = +1050 mV; the active components – HClO, Cl_2, HCl, HO_2*.
The catholyte usually has pH = 8.5–9.5 and ORP = -300–-500 mV; the active components – O_2, HO_2^-, HO_2*, H_2O_2, OH^-.
Figure 3 shows Kangen Water® Ionizer in working. The device is set up with electrodes and adsorption filter. At the level 1 the filter removes chlorine, lead, sediments and microbes. At the level 2 there is electrolysis cell which hydrolyses water into alkaline and acidic water.

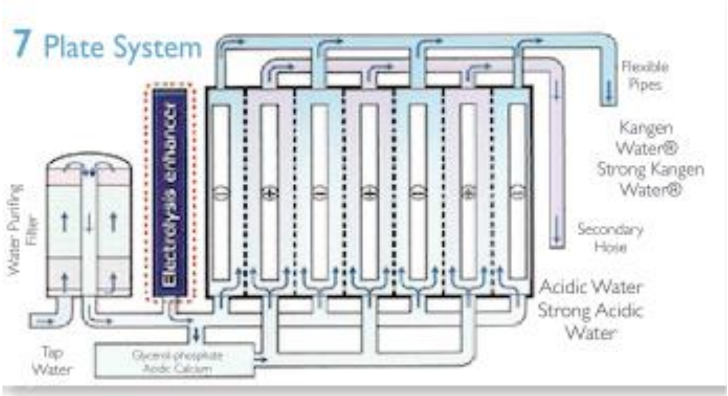


Figure 3: The scheme representation of electrochemical processes of water treatment in the Kangen ionizer

3.2. Comparative analysis between longevity of long living people, centenarians and their siblings and the quality of water

In frames of the research on the water quality 121 long living people from Bulgaria over 90 years of age have been studied together with their 294 siblings. The average lifespan of long lived people and centenarians in mountain areas is 94.1 years. For the average lifespan of long lived people in plain areas the result is 90.6 years. The most adult person from mountain areas is 104 years old and for plain areas is 97 years old. For the brothers and sisters of long live people from mountain areas the average lifespan is 88.5 years. For the brothers and sisters of long live people from plain areas the average lifespan is 86.4 years. The difference in life expectancy of the two groups of people is reliable and it corresponds to the Student's t-criteria at $p < 0.05$ with a confidence level of $t = 2.36$. There are distances of no more than 50–70 km between these places and the only difference is the mountain water and air.

There have been 21519 residents in Teteven and 142 of them were born before 1924. Figure 4 demonstrates the interrelation between the year of birth (1912–1924) of long living people (age) and their number (Teteven municipality, Bulgaria).

Figure 4: Interrelation between the year of birth of long living people (age) and their number in Teteven municipality, Bulgaria.

From the standpoint of genetics, the process of aging is associated with disruption of the genetic program of the organism and gradual accumulation of errors during the process of DNA replication. Aging may be associated with the accumulation of somatic mutations in the genome and be influenced by free radicals (mainly oxygen and primary products of oxidative metabolism) and ionizing radiation on DNA molecules as well (Woodhead, 1984; Adelman *et al*., 1988). Such mutations can reduce the ability of cells to the normal growth and division and be a cause of a large number of various cell responses: inhibition of replication and transcription, impaired cell cycle division, transcriptional mutagenesis, cell aging that finally result in cell death. Cells taken from the elderly people show a reduction in transcription when transferring information from DNA to RNA.

From the standpoint of dynamics, aging is a non-linear biological process, which increases over time. Accordingly, the rate of aging increases with time. The accumulation of errors in the human genome increases exponentially with time and reaches a certain stationary maximum at the end of life. L. Orgel shows that, for this reason, the probability of cancer occurrence increases with age (Orgel, 1963). Figure 5 shows L. Orgel's results on the interrelation between age and the number of cancer cases. The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian centenarians in the mountainous municipality of Teteven and their age is close to exponential.

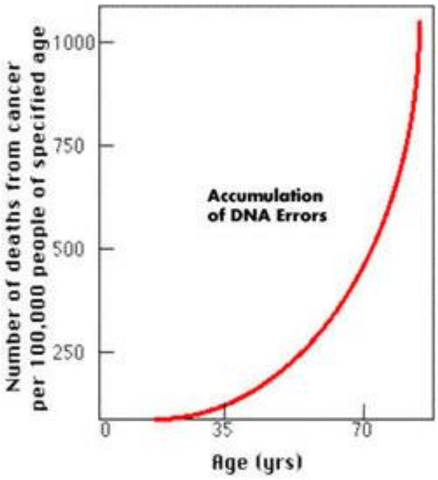


Figure 5: Interrelation between age and the number of cancer patients (Orgel, 1963).

Here are submitted the data for Bulgaria:
1) Varna district – 44 centenarians per 1 million of inhabitants, plain and sea regions;
2) Pleven district – 78 centenarians per 1 million of inhabitants, plain regions;
3) Teteven district – 279 centenarians per 1 million of inhabitants, hills and mountainous regions;
4) Bulgaria – 47 centenarians per 1 million of inhabitants.

The analogous situation is observed in the Russian North. According to G. Berdishev (Berdishev, 1989), people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink the mountain water obtained after the melting of ice. Altai and Buryat as well as Caucasus water sources in Russia are known as moderately warm, with temperatures of +8–10 ^{0}C; the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with an ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. The melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and thought to have a beneficial effect on metabolism (Goncharuk *et al*., 2013). In the world are also popular the water sources containing the melt water from Canada, Norway, Island and Alaska.

The analyses of water from various sources of Russia and Bulgaria show that the mountain water contains on average ~2–5% less deuterium in form of HDO, than river water and sea water. In natural waters, the deuterium content is distributed irregularly: from 0.02–0.03 mol.% for river and sea water, to 0.015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing deuterium in 1.5 times less than that of seawater. According to the international SMOW standard the isotopic shifts for D and ^{18}O in sea water: D/H = (155.76±0.05)$\cdot 10^{-6}$ (155.76 ppm) and $^{18}O/^{16}O$ = (2005.20±0.45)$\cdot 10^{-6}$ (2005 ppm) (Lis *et al*., 2008). For the SLAP standard the isotopic shifts for D and ^{18}O in seawater: D/H = 89$\cdot 10^{-6}$ (89 ppm) and for a pair of $^{18}O/^{16}O$ = 1894$\cdot 10^{-6}$ (1894 ppm). In surface waters, the ratio D/H = ~(1.32–1.51)$\cdot 10^{-4}$, while in the coastal seawater – ~(1.55–1.56)$\cdot 10^{-4}$. Waters of other underground and surface water sources contain varied amounts of deuterium (isotopic shifts) – from δ = +5,0 D,%, SMOW (Mediterranean Sea) to δ = -105 D,%, SMOW (Volga River). The natural waters of CIS countries are characterized by negative deviations from SMOW standard to (1.0–1.5)$\cdot 10^{-5}$, in some places up to (6.0–6.7)$\cdot 10^{-5}$, but there are observed positive deviations at 2.0$\cdot 10^{-5}$. The content of the lightest isotopomer – $H_2{}^{16}O$ in water corresponding to SMOW standard is 997.0325 g/kg (99.73 mol.%), and for SLAP standard – 997.3179 g/kg (99.76 mol.%).

The thawed snow and glacial water in the mountains and some other regions of the Earth also contain less deuterium than ordinary drinking water. On average, 1 ton of river water contains 150–200 g deuterium. The average ratio of H/D in nature makes up approximately 1:5700. According to the calculations, the human body throughout life receives about 80 tons of water containing in its composition 10–12 kg of deuterium and associated amount of heavy isotope ^{18}O. Such a considerable amount of heavy isotopes in the composition of drinking water is capable to cause the genetic damage, lead to the development of cancer, and to initiate aging. According to our study, a high concentration of heavy water is toxic to the body; chemical reactions in the environment, it is slow in comparison with ordinary water, the hydrogen bonds involving deuterium conventional somewhat stronger hydrogen bonds due to the kinetic isotope effect deuterium (Ignatov & Mosin, 2014a; Ignatov & Mosin, 2014b; Ignatov & Mosin, 2014c). According to our studies the animal cells can withstand up to 25-30% $D_2{}^{16}O$, plants – up to 60% $D_2{}^{16}O$, while protozoa and the cells are able to exist on 90% $D_2{}^{16}O$. Once being in the body, $D_2{}^{16}O$ can cause metabolic disorders, kidney and hormonal regulation. At high concentrations in the body $D_2{}^{16}O$ inhibited the enzymatic reactions, cell growth, carbohydrate metabolism and synthesis of nucleic acids. The effects of $D_2{}^{16}O$ are particularly susceptible to the systems that are most sensitive to the substitution of H^+ with D^+, which use high speed formation and rupture of the hydrogen bonds. Such cell systems are the unit of biosynthesis of macromolecules and the respiratory chain. The last fact allows us to consider the biological effects $D_2{}^{16}O$, as a complex negative effect, acting simultaneously on the functional state of the large number of systems: metabolism, biosynthetic processes, cell transport, the structure and function of deuterated macromolecules and cellular membranes. This results in inhibition of cell growth followed by cell death in $D_2{}^{16}O$. This occurs even when using solutions of $D_2{}^{16}O$ shaped with $HD^{16}O$. That is why it seems so important to purify water from heavy isotopes of D and ^{18}O.

3.3. Clinical studies with human blood serum testing

A convenient method for studying of liquids is non-equilibrium differential spectrum. It was established experimentally that the process of evaporation of water drops, the wetting angle θ decreases discreetly to zero, and the diameter of the water drop basis is only slightly altered, that is a new physical effect (Antonov, 1995; Antonov & Yuskesselieva, 1983). Based on this effect, by means of the measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of f(θ). The distribution function is denoted as the energy spectrum of the water state. The theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules (Antonov, 1995).

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33\, f(\theta)}{[1-(1+bE)^2]^2} \qquad (1)$$

where b = 14.33 eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = arcos\,(-1 - 14.33E) \qquad (2)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference $\Delta f(E) = f\,(E samples\ of\ water) - f\,(E control\ sample\ of\ water)$ – is called the “differential non-equilibrium energy spectrum of water” (DNES).

Thus, the DNES spectrum is an indicator of structural changes in water, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different waters have different structures and composition and various intermolecular interactions – various associative elements etc (Ignatov et al, 2014; Ignatov et al., 2015). The redistribution of H_2O molecules in water samples according to the energy is a statistical process of dynamics.

Figure 6 shows the average NES-spectrum of deionised water. On the X-axis are depicted three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in reciprocal unit eV^{-1}.

Arrow A designates the energy of hydrogen bonds among H_2O molecules, which is accepted as most reliable in spectroscopy.

Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated as:

$$\bar{E} = -\,0.1067 \pm 0.0011 \text{ eV} \qquad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature +36.6 ^{0}C, is at its maximum.

A horizontal arrow designates the window of transparency of the Earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding space. It can be seen that the atmosphere window of transparency almost covers the NES-spectrum of water.

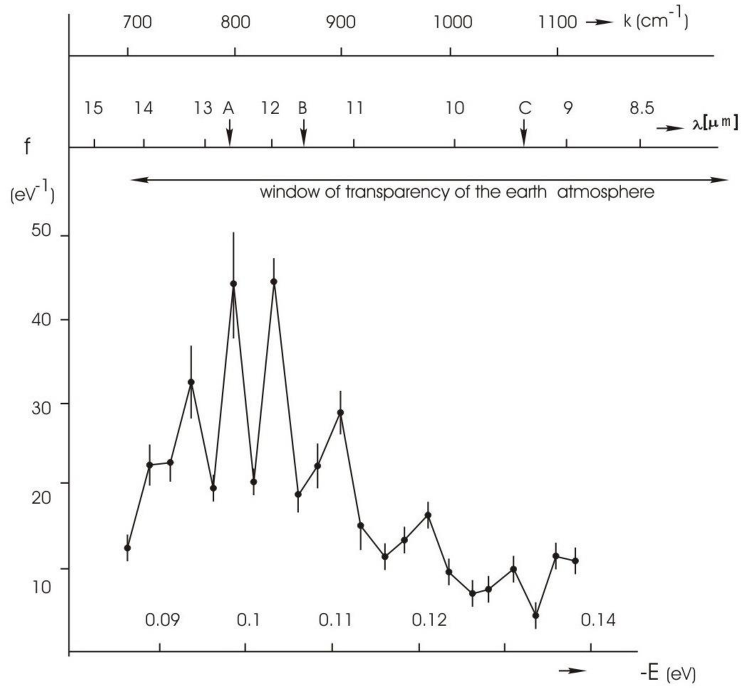


Figure 6: The NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV); the vertical axis – the energy distribution function – f (eV^{-1}); *k* – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

We have conducted studies of 1% (v/v) solution of human blood serum taken from two groups of people between 50 and 70 years of age by IR, NES and DNES spectral analysis. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. The average energy of hydrogen bonds ($\Delta E_{H...O}$) between H_2O molecules in the blood serum was investigated as the main biophysical parameter. The result was registered as a difference between the NES-spectrum of 1% solution of human blood serum and the NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference Δf(E) = f (samples of water) – f (control sample of water). The DNES-spectrum obtained from the first group has a local extremum energy ($\Delta E_{H...O}$) at E = -9.1±1.1 meV and from the second group at E = -1.6±1.1 meV. The results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local maximum in the DNES-spectrum was detected at E = -0.1387 eV, or at a wavelength λ = 8.95 μm. For the group of people in a critical health state and the patients with malignant tumors, the analogous values of the largest local maximums of the DNES-spectrum shifted to lower energies compared with the control group of people. For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected 8 local maxima at λ = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm (Krasnov, Gordetsov, 2009). The resulting peak at λ = 8.95 μm in the IR-spectrum (Ignatov, 2012) approaching the peak at λ = 8.85 μm was monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function f(E) at λ = 8.95 μm compiles E = 75.3 eV, and in a group of people in critical condition – E = 24.1 eV. The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of f(E) ~24.1 eV^{-1}. The level of reliability of the results is $p< 0.05$ according to the Student's t-test. In 1995 were performed DNES-experiments with an impact on tumor mice cells in water solutions containing Ca^{2+} (Antonov, 1995). There was a decrease in the DNES-spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the DNES-spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by the DNES-method at E = -5.5±1.1 meV; the difference in age was of 20–25 years in relation to the control group. It should be noted that many of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters the similar to the DNES-spectrum of blood serum of healthy people at λ = 8.95 μm, was the DNES-spectrum of water in the Rhodopes. The mountain water from Teteven, Boyana and other Bulgarian provinces has similar parameters. Tables 1, 2 and 3 show the composition of mountain water springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water samples. The local extremums is water samples were detected at E = -0.11 eV and E = -0.1387 eV. The value measured at E = -0.11 eV is characteristic for the presence of Ca^{2+} in water. The value measured at E = -0.1387 eV is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice in water with Ca^{2+} demonstrated a reduction of this local extremum to a negative value in spectra. Analysis by the DNES-method of aqueous solutions of natural mineral sorbents – shungite (carbonaceous mineral from Zazhoginskoe deposit in Karelia, Russia) and zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local extremum at E = -0.1387 eV for shungite and E = -0.11 eV for zeolite (Mosin & Ignatov, 2013, Ignatov & Mosin, 2014a). It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbers effectively removing from water organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating water with micro-and macro-elements until the physiological levels (Mosin & Ignatov, 2013). It is worth to note that in Bulgaria the main mineral deposits of Bulgarian zeolites are located in the Rhodope Mountains, whereat has lived the greatest number of Bulgarian centenarians. It is believed that water in these areas is cleared out in a natural way by mineral zeolite.

3.4. Composition of water in the mountain area in Teteven municipality, in Stara Planina Mountain, Kuklen municipality, Rhodopes Mountain (Bulgaria), and Glacier Rosenlaui, Swiss Alps

The statistical data demonstrated that the difference between the age of long lived people in mountain and plain areas is reliable at the Student's *t*-criteria ($p < 0.05$) with a confidence level of $t = 2.36$. The analyses of water samples obtained from various water sources show the differences regarding the chemical composition, hardness, local extrema in NES-spectra of water eV^{-1} at (-0.1362–-0.1387 eV), and the isotopic shifts of D/H in water. Tables 1, 2, 3 and 4 show the chemical composition of mountain water springs in Teteven, Kuklen (Bulgaria) and Glacier, Rosenlaui, Swiss Alps and local extremums in NES-spectra of water samples. A new parameter is entered into Tables 1, 2 and 3 – a local extremum of energy at (-0.1362– -0.1387 eV). This value was determined by the NES-spectrum as the function of distribution of individual H_2O molecules according to energy f(E). The function of distribution of H_2O molecules according to energy f(E) for tap water in Teteven is 11.8±0.6 eV^{-1} (Table 1, 2, 3).

Table 1: The composition of mountain water springs in Zlatishko-Tetevenska Mountain (Teteven municipality, Bulgaria) and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na^+)	0.96	< 200
Calcium (Ca^{2+})	100.4	< 150
Magnesium (Mg^{2+})	12.65	< 80
Iron (Fe)	0.016	<0.2
Manganese (Mn^{2+})	0.0018	<0.2
Zinc (Zn^{2+})	0.18	<4.0
Sulfates (SO_4^{2-})	81.8	< 250
Chlorides (Cl^-)	3.96	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	184.0	–
Other values	Results	
Active reaction (pH)	7.9 alkaline	6.5–9.5
Electroconductivity	536.8 µS/cm	< 2000
Hardness of water	16.5 dH Hard	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	36.9	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 2: The composition of mountain water springs in Vasiliovska Mountain (Teteven municipality, Bulgaria) and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na^+)	4.5	< 200
Calcium (Ca^{2+})	55.5	< 150
Magnesium (Mg^{2+})	2.28	< 80
Iron (Fe)	0.0127	<0.2
Manganese (Mn^{2+})	0.0014	<0.2
Zinc (Zn^{2+})	0.006	<4.0
Sulfates (SO_4^{2-})	16.9	< 250
Chlorides (Cl^-)	3.4	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	118.0	–
Other values	Results	
Active reaction (pH)	7.4 alkaline	6.5–9.5
Electroconductivity	285.0 μS/cm	< 2000
Hardness of water	7.9 dH slightly hard	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	40.1	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 3: The composition of mountain water spring Eco Hotel Zdravetz, Rhodopes Mountain (Kuklen municipality, Bulgaria) and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na^+)	7.6	< 200
Calcium (Ca^{2+})	3.5	< 150
Magnesium (Mg^{2+})	0.63	< 80
Iron (Fe)	0.007	<0.2
Manganese (Mn^{2+})	0.002	<0.2
Zinc (Zn^{2+})	0.007	<4.0
Sulfates (SO_4^{2-})	26.8	< 250
Chlorides (Cl^-)	3.00	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	21.3	–
Other values	Results	
Active reaction (pH)	5.93 Normal	6.5–9.5
Electroconductivity	536.8 μS/cm	< 2000
Hardness of water	1.4 dH Soft	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	59.3	>24.1

*Function of distribution of H_2O molecules according to energy f(E).

Table 4: The composition of mountain melt spring water Glacier Rosenlaui, Swiss Alps and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na^{2+})	0.53	< 200
Calcium (Ca^{2+})	8.7	< 150
Magnesium (Mg^{2+})	0.6	< 80
Iron (Fe)	0.106	<0.2
Manganese (Mn^{2+})	0.0023	<0.2
Zinc (Zn^{2+})	0.009	<4.0
Sulfates (SO_4^{2-})	8.4	< 250
Chlorides (Cl^-)	< 1.0	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	36.0	–
Other values	Results	
Active reaction (pH)	7.3 alkaline	6.5–9.5
Electroconductivity	82.3 µS/cm	< 2000
Hardness of water	<1.4 dH Soft	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	70.1	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 5 shows the results on water composition in field area of Dolni Dabnik. The maximum extremum in NES-spectra of Dolni Dabnik water (eV^{-1}) is detected at (-0.1362–-0.1387 eV), in water from Danubian Plain is detected at 23.2 eV^{-1}, and in water from Thracian Valley – at 21.3 eV^{-1}. Additionally, in water samples from Danubian Plain and Thracian Valley there are data for presence of nitrites (NO_2^-), nitrates (NO_3^-), ammonia (NH_4^+), phosphates (HPO_4^{2-}) more than the norm.

Table 5: The composition of artesian spring Sadovetz, Dolni Dabnik municipality and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na^+)	14.2	< 200
Calcium (Ca^{2+})	103.3	< 150
Sulfates (SO_4^{2-})	19.2	< 250
Magnesium (Mg^{2+})	64.0	< 80
Chlorides (Cl^-)	9.2	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	184.4	–
Other values	Results	
Active reaction (pH)	7.3 alkaline	6.5–9.5
Hardness of water	29.1 dH Very hard	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	23.2	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 6 shows the optimal chemical composition of water, hardness, the local extremum (eV^{-1}) at (-0.1362–-0.1387 eV), and total mineralization of water as the middle result of different studies. The water samples were taken from areas between 600 m and 1300 m attitude in Bulgaria and from Caucasus region, Russia. It is worth to note that these areas are populated by long living people.

Table 6: The chemical composition of water, hardness, local extremum (eV^{-1}) at (-0.1362–-0.1387 eV) and total mineralization of water

Indicators	Results of melt and mountain water (Bulgaria) (mg/dm^3)	Results of melt water (Russia) (mg/dm^3)
Sodium(Na^+) + Potassium (K^+)	6.1	< 30
Calcium (Ca^{2+})	29.5	< 50
Magnesium (Mg^{2+})	1.5	< 10
Iron (Fe)	0.083	–
Manganese (Mn^{2+})	0.0017	–
Zinc (Zn^{2+})	0.007	–
Sulfates (SO_4^{2-})	21.9	< 100
Chlorides (Cl^-)	3.2	< 70
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	69.7	< 100
Other values	Results	
Active reaction (pH)	6.7 normal	6.5–7.0
Electroconductivity	410.9 μS/cm	< 2000
Hardness of water	4.65 dH Moderately soft	<33.7
Total mineralization (g/l)	0.132	< 0.3
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	49.7	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

3.5. Effects of Ca^{2+}, Mg^{2+}, $Zn^{2+}$and Mn^{2+} in water on biophysical and biochemical processes in the human body

The research of distribution of local extremums (eV^{-1}) in spectra of various water samples as a function of distribution of H_2O molecules according to energy f(E) at λ = 8.95 μm shows the analogue extremum at analogous values of f(E), E and λ, which was detected in water with Ca^{2+} ions earlier demonstrated inhibiting the growth of cancer cells. Magnesium (Mg^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) ions dissolved in water have influence on enzymes, which are antioxidants (Ignatov & Mosin, 2015a). The research of China team was categorized three groups of elements from the rice and drinking water according to their effect on longevity: Sr, Ca, Al, Mo, and Se, which were positively correlated with longevity: Fe, Mn, Zn, Cr, P, Mg, and K, which had a weak effect on local longevity, and Cu and Ba, which had a negative effect on longevity (Lv et al., 2011). There was a positive correlation between the eSOD activity and the age and a negative correlation between the eSOD activity and concentration of Zn^{2+} in plasma. An inverse correlation was also found between the content of Zn^{2+} ions in plasma relative to the age. The prevalence of Zn^{2+} deficiency is increased with age; with normal Zn^{2+} levels it is observed in about 80% of adult people and only in 37 % of the non-agenarians. Aging is an inevitable biological process that is associated with gradual and spontaneous biochemical and physiological changes and the increased susceptibility to diseases. Because the nutritional factors are involved in improving the immune functions, metabolic balance, and antioxidant defense, some nutritional factors, such as Zn, may modify susceptibility to disease and promote healthy aging. *In vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old or young mice fed with low zinc dietary intake) studies revealed that zinc is important for immune efficiency (innate and adaptive), antioxidant activity (superoxide dismutase), and cell differentiation *via* clusterin/apolipoprotein J. The intracellular Zn homeostasis is regulated by metallothioneins (MT) *via* an ion release through the reduction of thiol groups in the MT molecule (Mocchegiani, 2007). Zinc in composition of water improves the antioxidative enzymes in red blood cells (Malhotra & Dhawan, 2008).

The magnesium deficiency and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. The link between these two factors is unclear in humans although, in experimental animals, severe Mg^{2+} deficiency has been shown to lead to the increased oxidative stress (Begona et al, 2000). The antioxidants against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, Mn) and proteins such as ceruloplasmin (Co). The extent of the tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system (Machlin & Bendich, 1988). There was reported the antioxidant effects of water on rats (Abdullah, 2012). The norm in water for Zn^{2+} and Mg^{2+} according to the World Health Organization (WHO) should be less than 20 μg. For the Na^{+} content the norm according to the WHO is less than 20 mg.

The interesting results on the concentration of Ca^{2+} in water were obtained in USA and Canada. According to the statistical information the most number of centenarians in Canada per 1 million of population is observed in Nova Scotia (210 of centenarians per 1 million). In the water from Nova Scotia the Ca^{2+} content makes up 6.8 mg/l. N. Druzhyak, Russia showed that in the places wherein live the most number of centenarians the Ca^{2+} content in water was 8–20 mg/l. The only risk factor regarding the increased Ca^{2+} content in water is cardiovascular diseases.

The following reactions occur in water if there are high concentrations of Ca^{2+} and Mg^{2+} ions: the reaction of limestone ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) with water to separate the calcium (Ca^{2+}), carbonates (CO_3^{2-}) and sulfate (SO_4^{2-}) ions. By increasing the mineralization of water the content of Ca^{2+} ions decreases. During the concentration of the solutions Ca^{2+} ions are precipitated. With the increase of carbon dioxide (CO_2) in water and decreasing of the pH value the content of Ca^{2+} increases. The reaction of interaction of dolomite ($CaCO_3 \cdot MgCO_3$) with water makes the formation of Mg^{2+} ions. Hydrocarbonates (HCO_3^{-}) and carbonates (CO_3^{2-}) ions are formed by reaction of interaction of karst rocks, CO_2 and water. For example, in Zamzam water there is Ca^{2+} – 299.7 mg/l; Mg^{2+} – 18.9 mg/l; Zn^{2+} – 0.001 mg/l.

3.6. Results on Kangen Water®. A resume for Kangen Water® Ionizer type of preparation

The main results of the research are demonstrated in Table 7 which presents the local extremums in NES spectra of water samples of different origin.

Table 7: The local extremums in NES spectra of water samples of different origin

Sources and Types of Waters	Local extremum at (-0.1112…-0.1137 eV)[1]	Local Extremum at (-0.1362…-0.1387 eV)[2]	[%,($-E_{value}$)*/($-E_{total\ value}$) **] -0.1112 eV	[%,($-E_{value}$)*/($-E_{total\ value}$)**] -0.1137 eV	[%,($-E_{value}$) */($-E_{total\ value}$) **] -0.1362 eV	[%,($-E_{value}$) */($-E_{total\ value}$) **] -0.1387 eV
	eV^{-1} norm (>24.1)	eV^{-1} norm (>24.1)				
1.Zlatishko-Tetevenska Mountain (Bulgaria)	46.1±2.3	36.9±1.9	4.1	7.0	4.0	7.4
2.Vasiliovska Mountain (Bulgaria)	12.0±0.6	44.9±2.2	1	2	5	6.2
3.Rhodops Mountain (Eco hotel Zdravetz) (Bulgaria)	53.3±1.6	59.3±3.0	11.0	3.6	5.5	13.0
4. Danubian Plain, Sadovetz (Bulgaria)	25±1.25	23.2±1.7	0	6.3	0	3.1

5. Tap Water Teteven (Bulgaria)	16.0±0.8	16.0±0.8	4.0	0	0	4.0
6. Glacier Rosenlaui (Switzerland)	129.0±6.3	70.1±3.5	22.6	9.7	6.5	12.9
7. Deionized Water	38.7±1.9	32.3±1.6	3.2	6.4	4.8	3.2
8. Tap Water, Plovdiv	22.2±1.1	44.4±2.2	5.6	0	5.6	5.6
9. Kangen Water® from Tap Water, Plovdiv	44.4±2.2	111.0±5.6	5.6	5.6	5.6	22.0
10. Tap Water, Sofia	0	34.8±1.7	0	0	4.3	8.7
11. Kangen Water® from Tap Water, Sofia	32.0±1.6	96.0±4.8	8.0	0	8.0	16.0

Notes:
[1]The values (E = -0.1112…-0.1137 eV) correspond to the wavelengths (λ = 10.91–11.91μm).
[2]The values (E = -0.1362…-0.1387 eV) correspond to the wavelengths (λ = 8.95– 9.10 μm).
* The result ($-E_{value}$) is the result of hydrogen bonds energy for one value of (-E)
** The result ($-E_{value}$) is the total result of hydrogen bonds energy

There are two conclusions from analyzing the NES spectra of Kangen® Water obtained after the electro-chemical treatment of various water samples by Kangen Water® Ionizer. Thus, Kangen Water®, which was prepared from tap water from Plovdiv has the energy at E = -0.1387 eV; there was the local extremum with f(E) value measured at 88.8±4.4 eV^{-1}. Kangen Water®, which was prepared from tap water from Sofia has the energy at E = -0.1387 eV; there was the local extremum with f(E) value measured at 64.0±3.2 eV^{-1}. In 1992 A. Antonov demonstrated that in aqueous solutions of tumor cells at E = -0.1387 eV there was detected a decrease of local extremum corresponding to a statistical error. NES-spectra of aqueous solution containing Ca^{2+} (67 mg/l) had a local extremum of energy at -0,11 eV in NES-spectra. In 1992 it was established by A. Antonov that the differential spectra of karst water and water solutions of calcium carbonate ($CaCO_3$) with the same content of calcium (Ca^{2+}) ions were similar at statistical level to the Student *t*-criterion at $p < 0.05$. For Kangen Water®, which was prepared from tap water from Plovdiv the local extremum was detected at 22.2±1.1 eV^{-1}. For Kangen Water®, which was prepared from tap water from Sofia the local extremum was detected at 32.0±1.6 eV^{-1}. The result of tap water from Plovdiv was observed within a statistical error of f(E) at 22.2±1.1 eV^{-1} for E = -0,11 eV and E = -0.1387 eV. The result of tap water from Sofia was observed within a statistical error or 0 eV^{-1} for -0,11 eV and 17.4±0.9 eV^{-1} for -0.1387 eV. The measurement of the DNES spectrum for Kangen Water®, which was prepared from tap water from Plovdiv was 22.2±1.1 eV^{-1} in the range (-0.1112– -0.1137 eV). The result in the range (-0.1362–-0.1387 eV) of the DNES spectrum was 66.7±3.3 eV^{-1}. The measurement of the DNES spectrum for Kangen Water®, which was prepared from tap water from Sofia was 32.0±1.6 eV^{-1} in the range (-0.1112– -0.1137 eV). The result in the range (-0.1362–-0.1387 eV) of the DNES spectrum was 61.2±3.1 eV^{-1}. **These data may indicate that on the molecular level Kangen Water® supposedly is more structurally organized than tap water and other analyzed water samples.**

In 2012 the research of 1% solution of human blood serum was made by I. Ignatov and O. Mosin with the collaboration of K. Naneva from Municipality hospital, Teteven, Bulgaria (Ignatov et al., 2012). In the control group of healthy people, the function of distribution of individual H_2O molecules according to energy f(E) at λ = 8.95 μm was detected at an average value of f(E) 75.3±3.8 eV^{-1}. In the group of people in critical condition this value was 24.1±1.2 eV^{-1}. The confidence level of the obtained results according to the Student *t*-criterion was at $p < 0.05$. The result for Kangen Water® compiles 88.8±4.4 eV^{-1} and 64.0±3.2 eV^{-1}. On the molecular level there was reported a possibility for decreasing the number of tumor cells in various water samples with Ca^{2+} ions. However, this depends on the health status and other factors – heredity, the quality of water and medical prophylactics.

The distribution [%, $(-E_{value})/(-E_{total\ value}]$ of H_2O molecules in Kangen Water® according to energies of hydrogen bonds of H_2O molecules and local extremums in NES and DNES spectra of mountain and melt water are shown in Table 7 in the ranges of (-0.1112…-0.1137 eV) and (-0.1362…-0.1387 eV). The average energy ($E_{H...O}$) of hydrogen H…O-bonds among individual H_2O molecules in Kangen Water®, which was prepared from Plovdiv tap water was measured at E = -0.1259 eV. The result for the control sample (tap water from Plovdiv) was E = -0.1142 eV. The results obtained with the NES method were recalculated with the DNES method as a difference of the NES (Kangen Water®) minus the NES (control sample from tap water) equalled the DNES spectrum of Kangen water®. Thus, the result for Kangen Water® recalculated with the DNES method was ΔE = 0.0117±0.0011 eV. The results showed the increasing the values of the energy of hydrogen bonds in Kangen Water® regarding the tap water from Plovdiv. The result for the control sample (tap water from Plovdiv) was E = -0.1142 eV.

The average energy ($E_{H...O}$) of hydrogen H…O-bonds among individual H_2O molecules in Kangen Water®, which was prepared from Sofia tap water was measured at E = -0.1228 eV. The result for the control sample (tap water from Plovdiv) was E = -0.1148 eV. The results obtained with the NES method were recalculated with the DNES method as a difference of the NES (Kangen Water®) minus the NES (control sample from tap water) equalled the DNES spectrum of Kangen Water®. Thus, the result for Kangen Water® recalculated with the DNES method was ΔE = 0.008±0.0011 eV. The results showed the increasing of the values of the energy of hydrogen bonds in Kangen Water® regarding the tap water from Sofia. The result for the control sample (tap water from Sofia) was E = -0.1148 eV.

Further it was carried out the research of melt water from Glacier Rosenlaui, Alps. The local extremum at (-0.1362…-0.1387 eV) in Glacier Rosenlaui water, Alps was detected at f(E) = 70.1±3.5 eV^{-1} and Kangen Water® at f(E) = 111.1±5.6 eV^{-1} and 96.0±4.8 eV^{-1}. The results obtained with the NES method were recalculated with the DNES method as a difference of the NES (Glacier Rosenlaui) minus the NES (the control sample deionized water) equalled the DNES spectrum of melt water from Glacier Rosenlaui. Thus, the result for water from Glacier Rosenlaui measured with the DNES method was ΔE = -0.0093±0.0011 eV.

Another important physical parameter was calculated with using the NES method – the average energy ($E_{H...O}$) of H…O-bonds between H_2O compiled -0.1067±0.0011 eV.

According to the analysis of various water samples by the NES and DNES methods can be drown the main conclusions:

- The energy of hydrogen bonds of water in the samples was differed because of the different number of hydrogen bonds in the water samples, which may result from the fact that different waters have a different structure and various intermolecular interactions – various associative elements with different structure, clusters of formula $(H_2O)_n$ with different n, connected into the molecular associates;
- As a result of different energies of hydrogen bonds, the surface tension of water samples was increasing or decreasing. For Kangen Water® and melt water from Glacier Rosenlaui there was the increasing of surface tension regarding the control samples;
- The redistribution of H_2O molecules in water samples according to the energy (statistical process of dynamics);
- The hydrogen bond network may be stabilized with metal cations and anions, contained in water. In ice the hydrogen bonds have the energy of 4.6 kcal/mol or 0.1995 eV. For liquid water the energy of hydrogen bonds makes up 1–3 kcal/mol or 0.043–0.13 eV.

The further empirical results obtained under the project of “Water, Ecology and Longevity” on the research of the water quality, DNES-spectra of human blood serum and long living people in Bulgaria from 2012 till 2015 (Ignatov & Mosin, 2015a), showed the difference in the age of the people who lived in mountainous and plain areas in Bulgaria that was statistically proven by the Student *t*-criterion at a confidence level $p < 0.05$. The most adult person in mountain was 104 and in plain areas 97 years old. The difference between mountainous and plain areas is 50–70 km. For the average lifespan of long living people in plain areas the result was 90.6 years. For the brothers and sisters of long living people from mountain areas the average lifespan is 88.5 years. The analyses show the difference in the water quality derived from different water springs and the differences in DNES-spectra of the human blood serum of tested people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules in DNES-spectra of human blood serum according to energies f(E) at -0.1387 eV is decreasing. In this group of tested people the result obtained by the DNES method was – ΔE= -5.5±1.1 meV; the difference in age was of 20–25 years in relation to the control group.

3.7. Results of purifying of tap water from Plovdiv with Kangen Water® Ionizer

There are valid the following results from Bulgarian laboratories for the purifying the tap water with Kangen Water® Ionizer (Table 8). Table 8 shows that the amount of nitrates (NO_3), nitrites (NO_2) and bicarbonates (HCO_3) after electro-processing of water with Kangen Water® Ionizer is significantly reduced.

Table 8: Results on purifying of tap water with Kangen Water® Ionizer

Chemical compound	Result before purifying (mg/dm^3)	Result after purifying (mg/dm^3)	Reliability of the results (mg/dm^3)
Nitrates NO_3	131.61	5.36	50
Nitrites NO_2	0.012	0.002	0.5
Carbonates and Bicarbonates CO_3+HCO_3	45.3	38.1	7.1

3.8. Mathematical model of Kangen Water®

The research with the NES method of water drops was received with tap water from Plovdiv and Kangen Water®, which was prepared from Plovdiv tap water. The second sample of Kangen Water® was prepared from Sofia tap water. The mathematical model of Kangen Water® gives the valuable information on the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies (Table 9) and (Table 10). These distributions are basically connected with the restructuring of H_2O molecules having the same energies.

Table 9: The distribution (%, ($-E_{value}$)/($-E_{total\ value}$) of H_2O molecules in tap water from Plovdiv and Kangen Water®, which was made from tap water of Plovdiv

-E(eV) x-axis	Kangen Water Plovdiv (%(($-E_{value}$)*/ ($-E_{total\ value}$)** y-axis	Tap Water® from Plovdiv (%(($-E_{value}$)*/ ($-E_{total\ value}$) ** y-axis	-E(eV) x-axis	Kangen Water® from Plovdiv (%(($-E_{value}$)*/ ($-E_{total\ value}$)** y-axis	Tap Water® from Plovdiv (%(($-E_{value}$)*/ ($-E_{total\ value}$)** y-axis
0.0937	0	0	0.1187	0	19.2
0.0962	0	11.0	0.1212	5.6	5.6
0.0987	0	0	0.1237	5.6	0
0.1012	11.1	0	0.1262	11.1	2.8
0.1037	0	0	0.1287	5.6	5.6
0.1062	0	11.1	0.1312	11.1	5.6
0.1087	0	5.6	0.1337	11.1	5.6
0.1112	5.6	5.6	0.1362	5.6	5.6
0.1137	5.6	0	0.1387	22.0	5.6
0.1162	0	11.1	–	–	–

Notes:
* The result ($-E_{value}$) is the total result of hydrogen bonds energy for one parameter of (-E)
** The result ($-E_{total\ value}$) is the total result of hydrogen bonds energy

Table 10: The distribution (%, ($-E_{value}$)/($-E_{total\ value}$) of H_2O molecules in tap water from Sofia and Kangen Water®, which was made from tap water of Sofia

-E(eV) x-axis	Kangen Water® from Sofia (%((-E_{value})*/ (-$E_{total\ value}$) ** y-axis	Tap Water® from Sofia (%((-E_{value})*/ (-$E_{total\ value}$) ** y-axis	-E(eV) x-axis	KangenWater® from Sofia (%((-E_{value})*/ (-$E_{total\ value}$) ** y-axis	Tap Water® from Sofia (%((-E_{value})*/ (-$E_{total\ value}$)** y-axis
0.0937	0	8.7	0.1187	12.0	13.3
0.0962	8.0	4.3	0.1212	0	0
0.0987	0	4.3	0.1237	0	4.3
0.1012	8.0	0	0.1262	4.0	8.7
0.1037	4.0	4.3	0.1287	12.0	4.3
0.1062	0	13.2	0.1312	4.0	4.3
0.1087	0	8.7	0.1337	12.0	4.3
0.1112	8.0	0	0.1362	8.0	4.3
0.1137	0	0	0.1387	16.0	4.3
0.1162	4.0	8.7	–	–	–

Notes:
* The result (-E_{value}) is the result of hydrogen bonds energy for one parameter of (-E)
** The result (-$E_{total\ value}$) is the total result of hydrogen bonds energy

The experimental data obtained testified the following conclusion from the mathematical models of Kangen Water® according to the mathematical model of tap water. The distribution (%, (-E_{value})/(-$E_{total\ value}$) of water molecules in tap water form Plovdiv and Kangen water®, which was made from tap water Plovdiv was different. However, for the value E = -0.1387 eV or λ = 8.95 µm there is the biggest local maximum corresponding to the re-structuring of hydrogen bonds among H_2O molecules. This difference may indicate on the different number of hydrogen bonds in water samples, as well as their physical parameters (pH, ORP, E_h), resulting in different distribution of H_2O molecules and different values of H_2O molecules with ratios of (-E_{value})/(-$E_{total\ value}$). Particularly it was observed the statistical re-structuring of H_2O molecules in water samples according to the energies. **The experimental data may prove that Kangen Water® (electrochemical method) stipulates the restructuring of H_2O molecules on molecular level and may be used for the prophylaxis of development of cancer cells.**

3.9. Studying in water the water associates $(H_2O)_n$, where n = 3–50.

The peculiarities of chemical structure of H_2O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H_2O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H…O) with neighboring H_2O molecules, binding them into complex intermolecular associates which composition represented by general formula $(H_2O)_n$, where n can vary from 3 to 50 (Keutsch & Saykally, 2011). The hydrogen bond is a form of association between the electronegative O-atom and H-atom, covalently bound to another electronegative O-atom, is of vital importance in the chemistry of intermolecular interactions, based on weak electrostatic forces and donor-acceptor interactions with charge-transfer (Pauling, 1960). It results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H_2O molecule; the structure of hydrogen bonding, therefore may be defined as $O…H^{\delta+}–O^{\delta-}$. As the result, the electron of the H-atom due to its relatively weak bond with the proton easily shifts to the electronegative O-atom. The O-atom with increased electron density becomes partly negatively charged – δ^-, while the H-atom on the opposite side of the molecule becomes positively charged – δ^+ that leads to the polarization of $O^{\delta-}$ – $H^{\delta+}$ covalent bond. In this process the proton becomes almost bared, and due to the electrostatic attraction forces are provided good conditions for convergence of O…O or O…H atoms, leading to the chemical exchange of a proton in the reaction O–H...O ↔ O...H–O. Although this interaction is essentially compensated by mutual repulsion of the molecules' nuclei and electrons, the effect of the electrostatic forces and donor-acceptor interactions for H_2O molecule compiles 5–10 kcal per 1 mole of substance. It is explained by a negligible small atomic radius of hydrogen and shortage of inner electron shells, which enables the neighboring H_2O molecule to approach the hydrogen atom of another molecule at very close distance without experiencing any strong electrostatic repulsion. The H_2O molecule has four sites of hydrogen bonding – two uncompensated positive charges at H-atoms and two negative charges at the O-atom. Their mutual disposition is characterized by direction from the centre of regular tetrahedron (nucleus of O-atom) towards its vertexes. This allows to one H_2O molecule in condensed state to form up to 4 classical hydrogen bonds, two of which are donor bonds and the other two – acceptor ones (taking into consideration the bifurcate ("two-forked") hydrogen bond – 5) (Pasichnyk *et al.*, 2008).

A hydrogen bond according to Bernal–Fowler rules (Bernal & Fowler, 1933) is characterized by the following parameters:

a) O-atom of each H_2O molecule is bound with four neighboring H-atoms: by covalent bonding with two own H-atoms, and by hydrogen bonding – with two neighboring H-atoms (as in the crystalline structure of ice); each H-atom in its turn is bound with O-atom of neighbouring H_2O molecule.

b) On the line of O-atom – there can be disposed only one proton H^+;

c) The proton, which takes part in hydrogen bonding situated between two O-atoms, therefore has two equilibrium positions: it can be located near its O-atom at approximate distance of 1 Å, and near the neighboring O-atom at the distance of 1.7 Å as well, hence both a usual dimmer HO–H...OH_2 and an ion pair HO...H–OH_2 may be formed during the hydrogen bonding, i.e. the hydrogen bond is partly electrostatic (~90%) and partly (~10%) covalent (Isaacs et al., 2000). The state of "a proton near the neighboring oxygen" is typical for the interphase boundary, i.e. near water–solid body or water–gas surfaces.

d) The hydrogen bonding of a triad O–H...O possess direction of the shorter O–H ($\rightarrow$) covalent bond; the donor hydrogen bond tends to point directly at the acceptor electron pair (this direction means that the H-atom being donated to the H-atom acceptor on another H_2O molecule).

The most remarkable peculiarity of hydrogen bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond (Pimentel & McClellan, 1960). In respect of energy the hydrogen bond has an intermediate position between covalent bonds and intermolecular van der Waals forces, based on dipole-dipole interactions, holding the neutral molecules together in gasses or liquefied or solidified gasses. Hydrogen bonding produces interatomic distances shorter than the sum of van der Waals radii, and usually involves a limited number of interaction partners. These characteristics become more substantial when acceptors bind H-atoms from more electronegative donors. Hydrogen bonds hold H_2O molecules on 15% closer than if water was a simple liquid with van der Waals interactions. The hydrogen bond energy compiles 5–10 kcal/mole, while the energy of O–H covalent bonds in H_2O molecule – 109 kcal/mole (Arunan *et al.*, 2011). The values of the average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds between H_2O molecules make up 0.1067 ± 0.0011 eV (Antonov & Galabova, 1992). With fluctuations of water temperature the average energy of hydrogen H...O-bonds in of water molecule associates changes. That is why hydrogen bonds in liquid state are relatively weak and unstable: it is thought that they can easily form and disappear as the result of temperature fluctuations (Ignatov & Mosin, 2013).

Another key feature of hydrogen bond consists in its cooperatively coupling. Hydrogen bonding leads to the formation of the next hydrogen bond and redistribution of electrons, which in its turn promotes the formation of the following hydrogen bond, which length increasing with distance. Cooperative hydrogen bonding increases the O–H bond length, at the same time causing a reduction in the H…O and O…O distances (Goryainov, 2012). The protons held by individual H_2O molecules may switch partners in an ordered manner within hydrogen networks (Bartha *et al*., 2003). As the result, aqueous solutions may undergo autoprotolysis, i.e. the H^+ proton is released from H_2O molecule and then transferred and accepted by the neighboring H_2O molecule resulting in formation of hydronium ions as H_3O^+, $H_5O_2^+$, $H_7O_3^+$, $H_9O_4^+$, etc. This leads to the fact, that water should be considered as associated liquid composed from a set of individual H_2O molecules, linked together by hydrogen bonds and weak intermolecular van der Waals forces (Liu *et al.*, 1996). The simplest example of such associate is a dimmer of water: $(H_2O)_2 = H_2O\cdots HOH$.

The energy of the hydrogen bonding in the water dimmer is 0.2 eV (~5 kcal/mol), which is larger than the energy of thermal motion of the molecules at the temperature of 300 K. Hydrogen bonds are easily disintegrated and re-formed through an interval of time, which makes the water structure quite unstable and changeable (George, 1997). This process leads to structural inhomogeneity of water characterizing it as an associated heterogeneous two-phase liquid with short-range ordering, i.e. with regularity in mutual positioning of atoms and molecules, which reoccurs only at distances comparable to distances between initial atoms, i.e. the first H_2O layer. As it is known, a liquid in contrast to a solid body is a dynamic system: its atoms, ions or molecules, keeping short-range order in mutual disposition, participate in thermal motion, the character of which is much more complicated than that of crystals. For example H_2O molecules in liquid state under normal conditions (1 atm, +22 ^{0}C) are quiet mobile and can oscillate around their rotation axes, as well as to perform the random and directed shifts. This enabled for some individual molecules due to cooperative interactions to "jump up" from one place to another in an elementary volume of water. The random motion of molecules in liquids causes continuous changes in the distances between them. The statistical character of ordered arrangement of molecules in liquids results in fluctuations – continuously occurring deviations not only from average density, but from

average orientation as well, because molecules in liquids are capable to form groups, in which a particular orientation prevails. Thus, the smaller these deviations are, the more frequently they occur in liquids.
In 2005 R. Saykally (USA) calculated the possible number of hydrogen bonds and the stability of water clusters depending on the number of H_2O molecules (Figure 7) (Saykally, 2005). It was also estimated the possible number of hydrogen bonds (100) depending on the number of H_2O molecules (250) in clusters (Sykes, 2007). O. Loboda and O.V. Goncharuk provided data about the existence of icosahedral water clusters consisting of 280 H_2O molecules with the average size up to 3 nm (Loboda & Goncharuk, 2010). The ordering of H_2O molecules into associates corresponds to a decrease in the entropy (randomness), or decrease in the overall Gibbs energy ($G = \Delta H - T\Delta S$). This means that the change in enthalpy ΔH minus the change in entropy ΔS (multiplied by the absolute temperature T) is a negative value. These results are consistent with our research of the DNES spectrum of water on which it may make conclusion about the number of H_2O molecules in elemental water clusters (Ignatov & Mosin, 2015b). The DNES spectrum of water has energy ranges from -0.08 to -0.14 eV. The spectral range lies in the middle infrared range from 8 to 14 μm ("window" of the atmosphere transparency to electromagnetic radiation). Under these conditions, the relative stability of water clusters depends on external factors, primarily on the temperature. We demonstrated that H_2O molecules change their position in clusters depending on the energy of intermolecular H…O hydrogen bonds. The values of the average energy ($E_{H...O}$) of hydrogen bonds between H_2O molecules in the formation of cluster associates with the formula $(H_2O)_n$ compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H_2O molecules increases up to -0.14 eV, the cluster formation of water becomes “restructuring”. In this case, the redistribution of energies among the individual H_2O molecules occurs.

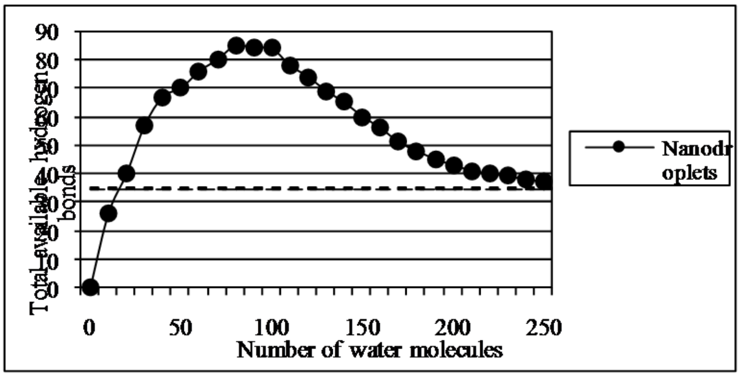
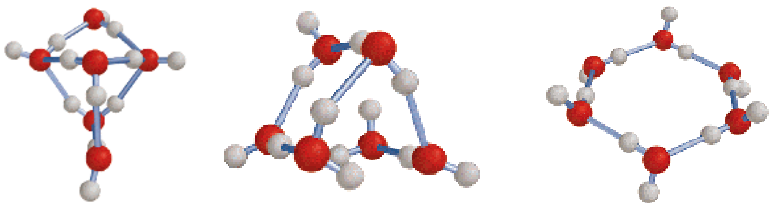


Figure 7: The total number of hydrogen bonds depending on the number of H_2O molecules in clusters.

All these experimental data including our data indicate that water is a complex associated non-equilibrium liquid consisting of associative groups containing according to the present data, from 3 to 20 individual H_2O molecules (Tokmachev *et al*., 2010). The associates can be perceived as unstable groups (dimmers, trimmers, tetramers, pentamers, hexamers etc.) in which H_2O molecules are linked by van der Waals forces, dipole-dipole and other charge-transfer interactions, including hydrogen bonding. At room temperature, the degree of association of H_2O molecules may vary from 2 to 6. In 2000 it was deciphered the structure of the water trimmer, and in 2003 – tetramer, pentamer and the hexamer (Wang & Jordan, 2003). In 1993 K. Jordan (USA) (Tsai & Jordan, 1993) calculated the possible structural modifications of small water clusters consisting of six H_2O molecules (Figure 8a–c). Subsequently, it was shown that H_2O molecules capable of hydrogen bonding by forming the structures representing topological 1D rings and 2D chains composed from numerous H_2O molecules. Interpreting the experimental data, they are considered as pretty stable elements of the structure. According to computer simulations, elemental clusters are able to interact with each other through the exposed protons on the outer surfaces of hydrogen bonds to form more complicated clusters of more complex structure.



a) *b*) *c*)

Figure 8: Computer calculations of small water cluster structures (*a*–*c*) with general formula $(H_2O)_n$, where n = 6 (Tsai & Jordan, 1993).

The quantum-chemical calculations of middle size clusters with the general formula $(H_2O)_n$, where n = 6–20, have shown that the most stable structures are formed by the interaction of tetrameric and pentameric structures (Maheshwary *et al.,* 2001; Choi & Jordan, 2010). Thus the structures of $(H_2O)_n$, where n = 8, 12, 16, and 20 are cubic, and structures $(H_2O)_n$ where n = 10 and 15 – pentagons. Other structures with n = 9, 11, 13, 14, 17, 18 and 19 evidently have a mixed composition. Large tetrahedron clusters as $(H_2O)_{196}$, $(H_2O)_{224}$, $(H_2O)_{252}$ (Figure 10) composed from the smaller ones formed a vertex of a $(H_2O)_{14}$ tetrahedron are also described (Chaplin, 2011).
It is reasonable that the structure of liquid water should be related to the structure of hexagonal ice, formed from H_2O tetrahedrons, which exist under atmospheric pressure. In the computer simulation H_2O tetrahedrons grouped together, to form a variety of 3D-spatial and 1D, 2D-planar structures, the most common of which is hexagonal structure where 6 H_2O molecules (tetrahedrons) are combined into a ring. A similar type of structure is typical for ice I_h crystals. When ice melts, its hexagonal structure is destroyed, and a mixture of clusters consisting of tri-, tetra-, penta-, and hexamers of water and free H_2O molecules is formed. Structural studies of these clusters are significantly impeded, since the water is perceived as a mixture of different clusters that are in dynamic equilibrium with each other.
In ice I_h the carcasses of hydrogen bonds allocate H_2O molecules in form of a spatial hexagon network with internal hollow hexagonal channels. In the nodes of this network O-atoms are orderly organized (crystalline state), forming regular hexagons, while H-atoms have various positions along the bonds (amorphous state). When ice melts, its network structure is destroyed: H_2O molecules begin to fall down into the network hollows, resulting in a denser structure of the liquid – this explains why water is heavier than ice. The hydrogen bonding explains other anomalies of water (abnormality of temperature, pressure, density, viscosity, fluidity etc. According to theoretical calculations, at the melting of the ice breaks about 15% of all hydrogen bonds (Mosin & Ignatov, 2012a); by further heating up to +40 ^{0}C breaks down about half of hydrogen bonds in water associates.
The clusters formed of D_2O are some more stable and resistant than those ones from H_2O due to isotopic effects of deuterium caused by 2-fold increasing nuclear mass of deuterium (molecular mass of D_2O is more by 11% than that of H_2O). The structure of D_2O molecule is the same, as that of H_2O, with small distinction in values of lengths of covalent bonds. D_2O crystals have the same structure as a conventional ice I_h, the difference in unit cell size is very insignificant (0.1%). But they are heavy (0.982 g/cm^3 at 0^{0}C over 0.917 g/cm^3 for conventional ice). D_2O boils at +101.44 ^{0}C, freezes at +3.82 ^{0}C, has density at +20 ^{0}C 1.105 g/cm^3, and the maximum density occurs not at +3.89 ^{0}C, as for H_2O, but at +11.2 ^{0}C (1.106 g/cm^3). The mobility of D_3O^+ ion on 28.5% lower than that of H_3O^+ ion and OD^- ion – 39.8% lower than that of OH^- ion, the constant of ionization of D_2O is less than the constant of ionization of H_2O, which means that D_2O has a bit more hydrophobic properties than H_2O. All these effects lead that the hydrogen bonds formed by deuterium atoms differ in strength and energy from ordinary hydrogen bonds (O–H length 1.01 Å, O–D length 0.98 Å, D–O–D angle 106^0). Commonly used molecular models use O–H lengths ~0.955 Å and 1.00 Å and H–O–H angles from ~105.5^0 to ~109.4^0. The substitution of H with D atom affects the stability and geometry of hydrogen bonds in apparently rather complex way and may, through the changes in the hydrogen bond zero-point vibrational energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of associates. In general, isotopic effects stabilize hydrogen bond with participation of deuterium, resulting in somewhat greater stability of associates (clusters) formed from D_2O molecules (Mosin & Ignatov, 2012b, Ignatov, Mosin, 2015b).
The mathematical model of Kangen Water® (Tables 9 and 10) gives the valuable information on the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies. These distributions are basically connected with the restructuring of H_2O molecules having the same energies in water clusters. The values of the average energy ($E_{H...O}$) of hydrogen bonds between H_2O molecules in the formation of the elemental clusters compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H_2O molecules increases up to -0.14 eV, the cluster formation of water becomes “restructuring”.

4. Conclusion

The composition of various samples of water from Bulgarian water springs: the melt water from Glacier Rosenlaui, Swiss Alps, Kangen Water® as well as human blood serum of people with excellent health and cancer patients was studied by IR, NES and DNES-methods. In frames of the research 415 people living in the municipalities of Teteven, Yablanitza, Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Pleven district (Bulgaria), where is lived the largest number of long lived people and their siblings, were also investigated regarding the water consumption. The research conducted by us shows that the natural mountain water and melt water with unique chemical composition, the pH value and less amount of deuterium seems to be one of the most important factors for human health. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at λ = 8.95 μm. The similar spectral characteristics possess mountain water from Teteven and other Bulgarian sources. Studying the human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H_2O molecules and the distribution function of H_2O molecules on energies it is possible to show a vital status of a person and associated life expectancy. Our data indicates that water in the human body has the IR-spectrum resembling the IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water also exerts an influence the presence of deuterium in water samples. In the research there is the optimal composition of mountain and melt water from areas where are lived the long live people and centenarians. The decreased content of deuterium in studied water samples with residual deuterium content of 60-100 ppm, the variety of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}), and chemical-physical parameters (pH, electroconductivity) of studied water samples renders beneficial effects of this type of water on human health. We have also obtained new proofs for biophysical and biochemical effects of Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} in composition of water on human organism and DNES-spectra of water. There are obtained new results of chemical composition of water from Glacier Rosenlaui, Swiss Alps.

According to the analysis of various water samples by the NES and DNES methods can be drown the main conclusions:

- The energy of hydrogen bonds of water in the samples was differed because of the different number of hydrogen bonds in the water samples, which may result from the fact that different waters have a different structure and various intermolecular interactions – various associative elements with different structure, clusters of formula $(H_2O)_n$ with different n, connected into the molecular associates;
- As a result of different energies of hydrogen bonds, the surface tension of water samples was increasing or decreasing. For Kangen Water® and melt water from Glacier Rosenlaui there was the increasing of surface tension regarding the control samples;

-The experimental data obtained with Kangen Water® testified the following conclusion from the mathematical models of Kangen Water® according mathematical models of tap water. For the value E = -0.1387 eV or λ = 8.95 μm there is local the biggest structuring of hydrogen bonds. This fact may be a proof that Kangen Water® (electrochemical method) stipulates the restructuring of H_2O molecules and on molecular level and is very useful for the prophylaxis of development of cancer cells.

- The redistribution of H_2O molecules in water samples according to the energy (statistical process of dynamics);
- The hydrogen bond network may be stabilized with metal cations and anions, contained in water. In ice the hydrogen bonds have the energy of 4.6 kcal/mol or -0.1995 eV. For liquid water the energy of hydrogen bonds makes up 1–3 kcal/mol or -0.043– -0.13 eV.

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