

Biophysical and Biochemical Results of the Product from Bulgaria Gift from Garabito[®]

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Abstract

I studied the mathematical model of interaction with water of Gift from Garabito[®] (Bulgaria). In this report are submitted data about the interaction of Gift from Garabito[®] with water, obtained by non-equilibrium (NES) and differential-equilibrium energy spectrum (DNES) of water. The average energy ($\Delta E_{H...O}$) of hydrogen H...O-bonds among individual molecules H₂O after treatment of Gift from Garabito[®] with water measured by NES- and DNES-methods is $\Delta E = -0.0136 \pm 0.0011$ eV. These results suggest the restructuring of $\Delta E_{H...O}$ values among H₂O molecules with a statistically reliable increase of local extremums in DNES-spectra. The research is performed for Gift from Garabito[®], with study of pH and oxidation reduction potential (ORP). There is review of the effects of the chemical composition of Gift from Garabito[®] – anti-inflammatory, antioxidant effects, inhibition development of tumor cells. As results of these effects Gift from Garabito[®] have detoxification and anti aging influence. The base of this influence is anti-inflammatory effect. There are effects on nervous system and muscle tissues. This article deals with the review of the basic biophysical-biochemical and biological processes underlying the Gift from Garabito[®]. The author is studying their physical-chemical properties and biophysical and biological effects on human organism.

Keywords: Gift from Garabito[®], anti-inflammatory, antioxidant, anti-aging, mathematical model, NES, DNES.

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, 2013). These factors, the structure and composition of water are of great importance in many biophysical studies. The peculiarities of the chemical structure of the H₂O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H₂O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H...O) with neighboring H₂O molecules, binding them into complex intermolecular associates which composition represented by general formula (H₂O)_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 H₂O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H₂O molecule.

The product Gift from Garabito[®]. The research is with methods NES and DNES. There is research of ORP and pH and there are executing the conclusions from electrochemically activated waters – anolyte and catholyte for anti-inflammatory effects (Ignatov *et al.*, 2014).

The aim of this research is to show the usefully of Gift from Garabito[®] on the base of the following results and conclusions. The author also performed the research of 1% (v/v) solution of Gift from Garabito[®] on the distribution of H₂O 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.

2. Materials and Methods

2.1. 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 °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 $\lambda = 580 \pm 7$ 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 $\lambda = 8.9$ –13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{\text{H...O}}$) of hydrogen O...H-bonds between H₂O molecules in water samples and human blood serum.

2.2. Product Gift from Garabito[®]

The product Gift from Garabito consists honey, apple vinegar, garlic and ginger.

2.3. 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.

2.4. 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.

2.5. 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. 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 & Yuskesseliyeva, 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 H₂O molecules according to the value of $f(\theta)$. 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 H₂O-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:

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

where $b = 14.33 \text{ eV}^{-1}$

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H₂O molecules is calculated by the formula:

$$\theta = \arcsin(-1 - 14.33E) \quad (2)$$

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 $\Delta f(E) = f(E_{\text{samples of water}}) - f(E_{\text{control sample of water}})$ – 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 H₂O molecules in water samples according to the energy is a statistical process of dynamics.

Figure 1 shows the average NES-spectrum of deionised water. On the X-axis are depicted three scales. The energies of hydrogen bonds among H₂O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H₂O molecules according to energies f(E), measured in reciprocal unit eV⁻¹.

Arrow A designates the energy of hydrogen bonds among H₂O molecules, which is accepted as most reliable in spectroscopy.

Arrow B designates the energy of hydrogen bonds among H₂O molecules the value of which is calculated as:

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

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 °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.

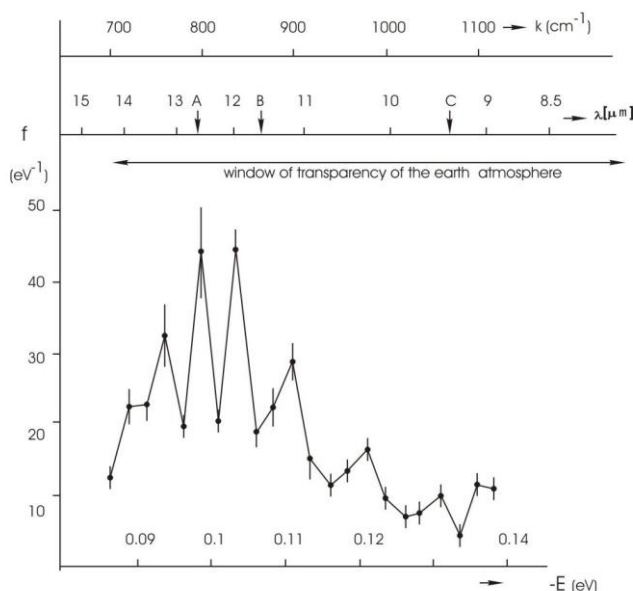


Figure 1: The NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV); the vertical axis – the energy distribution function – 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 (ΔE_{H...O}) between H₂O 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 (ΔE_{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 $\lambda = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35$ and $9.76 \mu\text{m}$ (Krasnov, Gordetsov, 2009). The resulting peak at $\lambda = 8.95 \mu\text{m}$ in the IR-spectrum (Ignatov, 2012) approaching the peak at $\lambda = 8.85 \mu\text{m}$ was monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function $f(E)$ at $\lambda = 8.95 \mu\text{m}$ compiles $E = 75.3 \text{ eV}$, and in a group of people in critical condition – $E = 24.1 \text{ eV}$. The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of $f(E) \sim 24.1 \text{ eV}^{-1}$. 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 $E = -5.5 \pm 1.1 \text{ meV}$; 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 $\lambda = 8.95 \mu\text{m}$, 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 $E = -0.11 \text{ eV}$ and $E = -0.1387 \text{ eV}$. The value measured at $E = -0.11 \text{ eV}$ is characteristic for the presence of Ca^{2+} in water. The value measured at $E = -0.1387 \text{ eV}$ 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 sorbent –zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local extremum at $E = -0.1387 \text{ eV}$ for shungite and $E = -0.11 \text{ eV}$ 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).

3.2. Results of 1% (v/v) solution in deionized water of Gift of Garabito[®]

The research with the NES method of water drops is received with 1% solution Gift from Garabito[®], and deionized water as control sample. The mathematical models of 1% (v/v) solution Gift from Garabito[®] gives the valuable information for the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies (Table 1 and Fig. 2). These distributions are basically connected with the restructuring of H_2O molecules having the same energies.

Table 1: The distribution (% , $(-E_{\text{value}})/(-E_{\text{total value}})$) of H_2O molecules in 1% water solution of Gift from Garabito[®] and control deionized water

-E(eV) x-axis	1% water solution Gift from Garabito [®] y-axis (% $(-E_{\text{value}})$ */ ($-E_{\text{total value}}$)**	Control Sample Deionized water y-axis (% $(-E_{\text{value}})$ */ ($-E_{\text{total value}}$)**	-E(eV) x-axis	1% water solution Gift from Garabito [®] y-axis (% $(-E_{\text{value}})$ */ ($-E_{\text{total value}}$)**	Control Sample Deionized water y-axis (% $(-E_{\text{value}})$ */ ($-E_{\text{total value}}$)**
0.0937	0	0	0.1187	0	12.6
0.0962	0	0	0.1212	23.5²	0
0.0987	0	6.2	0.1237	0	0
0.1012	0	12.6	0.1262	0	0
0.1037	0	6.2	0.1287	0	6.2
0.1062	0	0	0.1312	4.3	6.2
0.1087	11.7	6.2	0.1337	4.3	0
0.1112	28.4¹	6.2	0.1362	0	6.2
0.1137	0	12.6	0.1387	23.5³	6.2
0.1162	4.3	12.6	–	–	–

$E = -0.1112$ eV is the local extremum for effect on nervous system
 $E = -0.1212$ eV is the local extremum for anti-inflammatory effect
 $E = -0.1387$ eV is the local extremum for inhibition of development of tumor cells of molecular level

Notes:

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

** The result ($-E_{\text{value}}$) is the total result of hydrogen bonds energy

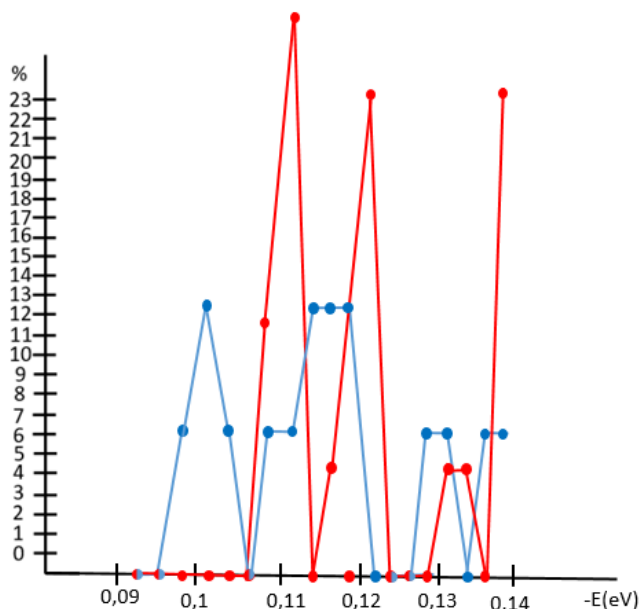


Figure 2: Mathematical model (Ignatov, Mosin, 2013) of 1% water solution of Gift from GarabitoV

Notes:

$E = -0.1112$ eV is the local extremum for stimulating effect on nervous system and improvement of nervous conductivity

$E = -0.1212$ eV is the local extremum for anti-inflammatory effect

$E = -0.1387$ eV is the local extremum for inhibition of development of tumor cells of molecular level

The experimental data obtained testified the following conclusions from the mathematical model of in 1% water solution of Gift of GarabitoV[®] and control deionized water. The distribution ($\%$, $(-E_{\text{value}})/(-E_{\text{total value}})$) of water molecules in mathematical model of in 1% water solution of Gift of GarabitoV[®] and control deionized water. The distribution ($\%$, $(-E_{\text{value}})/(-E_{\text{total value}})$) of water molecules in Gift of GarabitoV[®] according control sample is different. However, for the value $E = -0.1387$ eV or $\lambda = 8.95$ μm there is the bigger local extremum (23.5 ($\%$, $(-E_{\text{value}})/(-E_{\text{total value}})$) corresponding to the re-structuring of hydrogen bonds among H_2O molecules for inhibition of development of tumor cells of molecular level. This difference may indicate on the different number of hydrogen bonds in water samples, as well as their physical parameters (pH, ORP), resulting in different distribution of H_2O molecules and different values of H_2O molecules with ratios of $(-E_{\text{value}})/(-E_{\text{total value}})$. 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 stipulates the restructuring of H_2O molecules on molecular level and may be used for the prophylaxis against of development of tumor cells. For the value $E = -0.1212$ eV or $\lambda = 10.23$ μm there is the biggest local extremum (23.5 ($\%$, $(-E_{\text{value}})/(-E_{\text{total value}})$) corresponding to the re-structuring of hydrogen bonds among H_2O molecules for anti inflammatory effect.

The value for $E = -0.1112$ eV or $\lambda = 11.15$ μm is with biggest in local extremums or (28.4 ($\%$, $(-E_{\text{value}})/(-E_{\text{total value}})$). This value is corresponding to the re-structuring of hydrogen bonds among H_2O molecules for stimulating effect on the nervous system and improvement of nervous conductivity. The high values of local extremum at $E = -0.1112$ eV is connected with effects for stimulation of muscle tissue.

The experimental data for Gift of GarabitoV[®] may prove that stipulates the restructuring of H_2O molecules on molecular level and the biophysical effects are:

The local extremum at $E = -0.1212$ eV is indicator for anti inflammatory effect

The local extremum at $E = -0.1387$ eV is indicator for inhibition of development of tumor cells of molecular level. As a result of different energies of hydrogen bonds, the surface tension of 1% solution of water sample with Gift of Garabito[®] is increasing. The increasing of surface tension is regarding the control sample. This effect is connected with increasing and preservation of the energy in human body as result of biochemical process among water molecules and bio molecules.

3.3. Research of acidity and basicity (pH) and the oxidative redox potential (ORP) of 1% water solution of Gift of Garabito[®]

There are valid the following results of pH as indicator for acid alkaline medium of 1% solution of Gift of Garabito[®]. The results are for 1% of water solutions of Gift of Garabito[®]. This research is performed with Georgi Gluhchev from Bulgarian Academy of Science. Table 2 shows the results of pH and ORP.

Table 2. Results of 1% water solution of Gift of Garabito[®]

Продукт	pH	ORP (mV)	Coordinates Fig.3
1% разтвор на Gift of Garabito [®]	3.61 ± 0.02	+160 ± 0.20	Point 1 (3.61;160)

Figure 3 shows the dependence between the acidity and basicity (pH) of electrochemically activated solutions and the oxidation-reduction potential (ORP). The pH value within the interval from 3 to 10 units and the ORP within the interval from -400 mV to +900 mV characterize the area of the biosphere of microorganisms. Outside these ranges of pH and ORP the microorganisms will hardly survive.

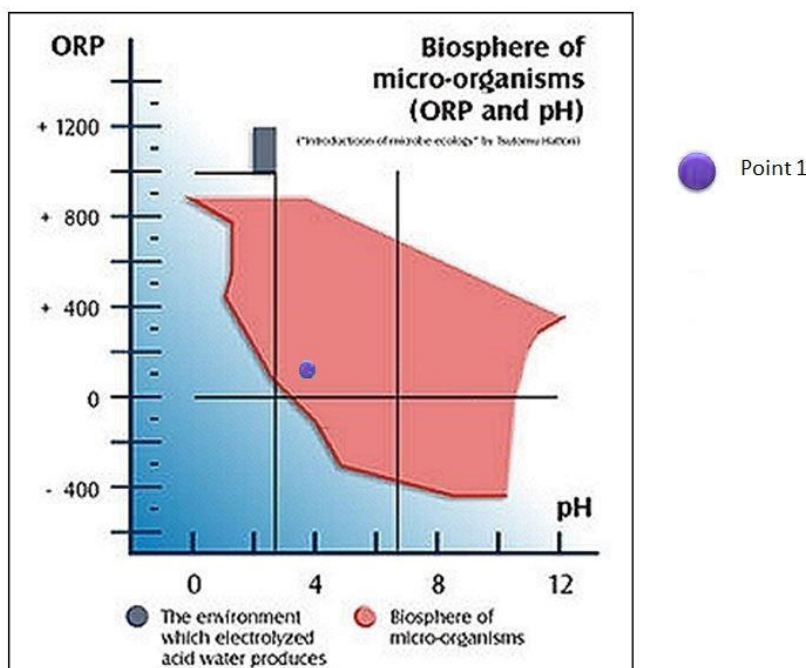


Figure 3. The dependence between acidity and basicity (pH) of solutions and the ORP on the biosphere of micro-organisms (point 1; Gift of Garabito[®])

The results from the Figure 3 show that the product Gift of Garabito[®] is useful as medium for microorganisms, which are with positive effects on human health.

4. Conclusion

The interaction of Gift from Garabito[®] with water is quiet complex and results the restructuring of energy values among H₂O molecules with a statistically reliable increase of local extremums in DNES-spectra after treatment of Gift from Garabito[®] with water. The result with NES is $E = -0.1291$ eV for Gift from Garabito[®]. The result for control sample (deionized water) is $E = -0.1155$ eV. The results with NES method were

recalculated by the DNES method. The result of Gift from Garabito[®] with DNES method is $\Delta E = -0.0136 \pm 0.0011$ eV. From the NES and DNES spectrum and mathematical model of 1% solution of Gift from Garabito[®] and deionized water as control sample are valid the following conclusions for biophysical effects for Gift from Garabito[®].

- Stimulating effect on nervous system and improvement of nervous conductivity
- anti-inflammatory effect;
- inhibition of development of tumor cells of molecular level;

The value for $E = -0.1112$ eV or $\lambda = 11.15$ μm is with biggest in local extremums. This value is corresponding to the re-structuring of hydrogen bonds among H_2O molecules for stimulating effect on the nervous system and improvement of nervous conductivity. The high values of local extremum at $E = -0.1112$ eV is connected with effects for stimulation of muscle tissue.

The dependence between acidity and basicity (pH) of solutions and the oxidation-reduction potential (ORP) on the biosphere of micro-organisms (point 1; Gift from Garabito[®]) on Figure 3 show the anti-inflammatory effects and that the two products are medium for microorganisms, which are with positive effects on human health.

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