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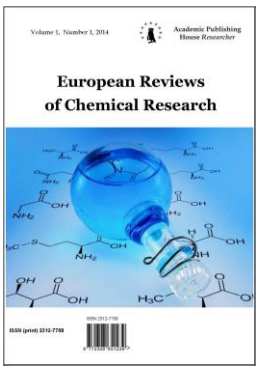

Articles and Statements

Origin of Life in Hot Mineral Water from Hydrothermal Springs and Ponds. Effects of Hydrogen and Nascent Hydrogen. Analyses with Spectral Methods, pH and ORP

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Abstract

The studies were performed of the composition of water, its temperature, pH and oxidation reduction potential (ORP) value in experiments with modelling of primary hydrosphere and possible conditions for origin of first organic forms of life in hot mineral water in hydrothermal springs and open ponds. Experiments with hot mineral and seawater from Bulgaria by IR-spectroscopy with DNES-method and Thermo Nicolet Avatar 360 Fourier-transform IR were conducted. Cactus juice of *Echinopsis pachanoi* and Jellyfish *Aurelia aurita* from Black Sea were used as model systems. The reactions of condensation and dehydration in alkaline aqueous solutions with pH = 9–11, resulting in synthesis of larger organic molecules as polymers and short polipeptides from separate molecules, were considered and scrutinized. It was shown that hot alkaline mineral water with temperature from +65 °C to +95 °C and pH value from 8.5 to 10 and ORP with negative value is more suitable for the origination of life and living matter than other analyzed water samples. The pH value of seawater on the contrary is limited to the range of 7,5 to 8,4 units. The research was connected with estimation of the common local extremums in hot mineral and sea water, cactus juice and jellyfish.

Keywords: origin of life, hot mineral water, hydrothermal conditions, IR-spectroscopy.

1. Introduction

Previous biological experiments with D_2O and structural-conformational studies with deuterated molecules, enable to modeling conditions under which the first living forms of life might be evolved (Ignatov, Mosin, 2013). The content of deuterium in hot mineral water may be increased due to the physical chemical processes of the deuterium accumulation. It can be presumed that primary water might contain more deuterium at early stages of evolution of first living structures, and deuterium was distributed non-uniformly in the hydrosphere and atmosphere (Ignatov, Mosin, 2012). The primary reductive atmosphere of the Earth consisted basically of gas mixture CO, H_2, N_2, NH_3, CH_4, lacked O_2–O_3 layer protecting the Earth surface from rigid short-wave solar radiation carrying huge energy capable to cause radiolysis and photolysis of water.

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The point regards the influence of temperature on the processes in living matter. Recent studies have shown that the most favorable for the origin of life and living matter seem to be hot alkaline mineral waters interacting with $CaCO_3$ (Ignatov, 2010; Ignatov, Mosin, 2013). According to the law for conservation of energy the process of self-organization of primary organic forms in water solutions may be supported by thermal energy of magma, volcanic activity and solar radiation. According to J. Szostak, the accumulation of organic compounds in open lakes is more possible compared to the ocean (Szostak, 2011). Probably the life has begun near a hydrothermal vent: an underwater spout of hot water. Geothermal activity gives more opportunities for the origination of life. In 2009 A. Mulkidjanian and M. Galperin demonstrate that the cell cytoplasm contains potassium (K), zinc (Zn), manganese (Mn) and phosphate ions (P), which are not particularly widespread in the sea aquatorium (Mulkidjanian, Galperin, 2009). Colín-García and co-authors also summarize a set of experiments proposed to test the role of hydrothermal vents in prebiotic synthesis (Colín-García et al., 2016). B. Damer and D. Deamer have come to the conclusion that cell membranes cannot be formed in salty seawater. Before the continents formed, the only dry land on Earth would be volcanic islands, where rainwater would form ponds where lipids could form the first stages towards cell membranes. Only when true cells had evolved they gradually would adapt to saltier environments and enter the ocean (Damer, Deamer, 2015). J. Trevors and G. Pollack were proposed that the first cells on the Earth assembled in a hydrogel environment (Trevors, Pollack, 2005). Gel environments are capable of retaining water, oily hydrocarbons, solutes, and gas bubbles, and are capable of carrying out many functions, even in the absence of a membrane. Hydrocarbons are an organic compounds consisting entirely of hydrogen (H) and carbon (C). The analyses show the possible scenario of the syntheses of periodically molecules of life (Colón Santos, 2019). The data presented in this paper show that the origination of living matter most probably occurred in hot mineral water. This occurred in hydrothermal springs and ponds with hot mineral water. There had been possible also in hydrothermal vents in seawater with hot mineral water. An indisputable proof of this is the presence of stromatolites fossils. They lived in warm and hot water in zones of volcanic activity, which could be heated by magma and seem to be more stable than other first sea organisms (Ignatov, 2012).

The purpose of the research was studying the conditions of primary hydrosphere (temperature, pH, ORP, isotopic composition) for possible processes for origin of life and living matter in hot mineral water. There was studied primary atmosphere and interaction with hydrosphere and with effects of gas discharge. Various samples of water from Bulgaria were studied within the frames of the research.

2. Material and methods

2.1. Objects of Studying

The research by the IR-spectrometry with DNES-method (Antonov, 1990; Antonov, Ignatov, 1998) was carried out with samples of water taken from various water sources:

1 – mineral water (Rupite, Bulgaria);

2 – seawater (Varna, Bulgaria);

Sediments from hot mineral spring and pond in Rupite, Bulgaria and sea salt from Black Sea were studied using the Thermo Nicolet Avatar 360 Fourier-transform IR;

Cactus juice of *Echinopsis pachanoi* and Jellyfish *Aurelia aurita* (Varna, Bulgaria, Black Sea) were used as two model systems which were both investigated by the IR-spectrometry with DNES method.

2.2. IR-Spectroscopy

IR-spectra of water samples were registered on Thermo Nicolet Avatar 360 Fourier-transform IR (K. Chakarova) and Differential Non-equilibrium Spectrum (DNES).

2.3. pH indicator and oxidation reduction potential (ORP)

The research of pH and ORP with Hanna instruments was performed.

3. Results and discussion

Research of various samples of mineral water from mineral springs and seawater from Bulgaria was carried out. The hot mineral spring and ponds of Rupite are located in eastern foot of

the extinct volcano Kozhuh (Figure 1).

Fig. 1. Hot mineral ponds of Rupite, Bulgaria, foot of the extinct volcano Kozhuh

For this DNES method was employed for research of cactus juice *Echinopsis pachanoi* (Table 1). The cactus was selected as a model system because this plant contains approximately 90 % of water. The closest to the spectrum of cactus juice was the spectrum of mineral water contacting with Ca^{2+} and HCO_3^- ions from Rupite, Bulgaria (Table 1).

DNES-spectra of cactus juice and mineral water from Rupite, Bulgaria with HCO_3^-(1320–1488 mg/L), Ca^{2+} (29–36 mg/L) t = 76 °C (source) and t = 52-54 °C (open ponds depending the season) have magnitudes of local extremums at –0.1112 (11.15); -0.1187 (10.45); -0.1262 (9.83); -0.1287 (9.64) and –0.1387 eV (9.85 μm). Similar local extremum in the DNES-spectrum between cactus juice and seawater were detected at -0,1362 eV (9.10 μm).

Table 1. Results with DNES spectral method and Thermo Nicolet Avatar 360 Fourier-transform IR of cactus juice, jelly fish, sea water and salt, sea water and salt, mineral water and sediments from Rupite, Bulgaria

-E (eV); λ (μm); k (cm^{-1}) DNES-method	-E (eV); λ (μm); k (cm^{-1}) DNES-method	λ (μm); k (cm^{-1}) Thermo Nicolet Avatar 360 Fourier-transform IR	-E (eV); λ (μm); k (cm^{-1}) DNES-method	λ (μm); k (cm^{-1}) Thermo Nicolet Avatar 360 Fourier-transform IR	-E (eV); λ (μm); k (cm^{-1}) DNES-method
Cactus juice	Mineral water Rupite (Bulgaria)	Mineral water Rupite (Bulgaria)	Sea Water Black Sea	Sea salt Black Sea	Jelly fish Black Sea
0.1112 (**11.15**; 897)	0.1112 (**11.15**; 897)	(**11.44**; 875)			
0.1187 (**10.45**; 957)	0.1187 (**10.45**; 957)	(**10.95**; 913)			0.1200; (**10.33**; 968)
0.1262 (**9.82**;1018)	0.1262 (**9.82**; 1018)				
0.1287 (**9.63**; 1038)	0.1287 (**9.63**; 1038)	(**9.78**; 1031)			
0.1362 (9.10;1099)	–	(**9.08**;1101)	0.1362 (**9.10**;1099)		(0.1375; **9.02**; 1109)
0.1387	0.1387 (**8.95**; 1117)	–		(**8.93**;1120)	

Note: *The function of the distribution of energies Δf was measured in reciprocal electron volts (eV^{-1}). It is shown at which values of the spectrum -E (eV) the biggest local maximums of this function are observed; λ – wave length; κ – wave number.

Common extremums in the IR-spectrum between cactus juice (DNES method) and minerals from the sediments (Thermo Nicolet Avatar 360 Fourier-transform IR) were detected at 11.44; 10.95; 9.78; 9.08; μm. Similar local extremum between cactus juice and sea salt was detected at 8.93 μm.

Study of the local extremums in DNES spectrum of jellyfish from Black Sea was performed (Table 1). There are two local extremums at 9.02 and 10.33 μm. The local extremum at 9.02 μm corresponds to the local extremums in sea salt (8.93 μm).

The local extremums of sediments from hot mineral spring in Rupite, Bulgaria were studied with Thermo Nicolet Avatar 360 Fourier-transform IR (Figure 2).

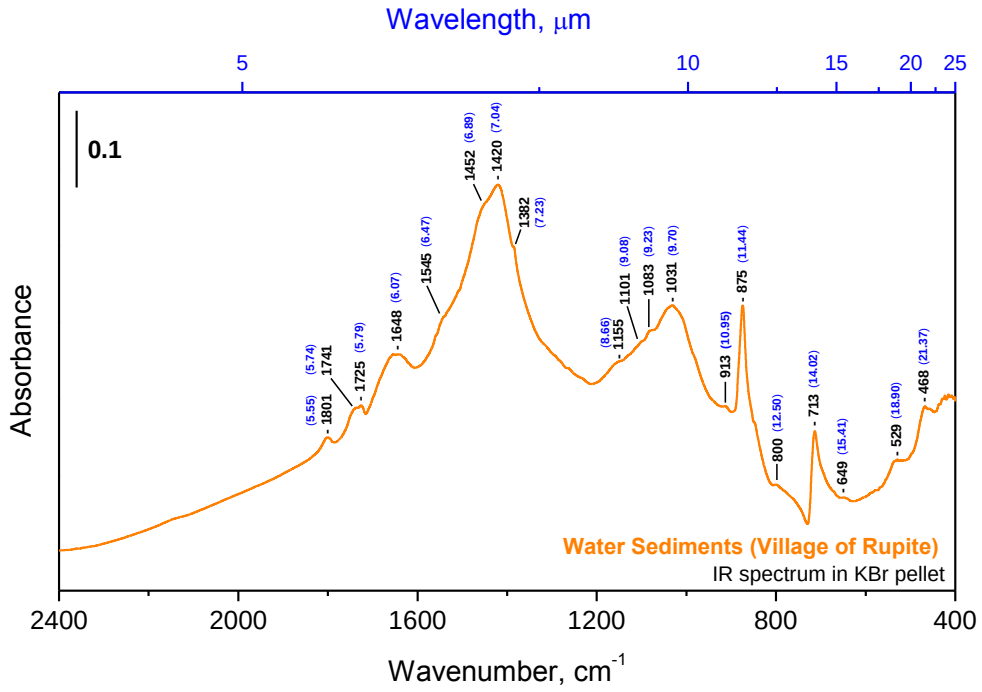


Fig. 2. IR-spectrum of water obtained from Rupite Village (Bulgaria)

The results with DNES method indicated that jellyfish *Aurelia aurita* had local exremums at 9.02 and 10.33 μm in IR-spectra (Table 1). Before measurements the jellyfish was kept in seawater for several days. For comparison seawater has a local extremum at 9.10 μm with DNES method and Sea salt 8.93 μm with Nicolet Avatar 360 Fourier-transform IR (Figure 3). Jellyfish contains approximately 97 (w/w) % of water and is the most unstable living organism compared to those ones that form stromatolites. The explanation for this is the smaller concentration of salts and, therefore, the smaller number of local extremums in the IR-spectrum in relation to seawater.

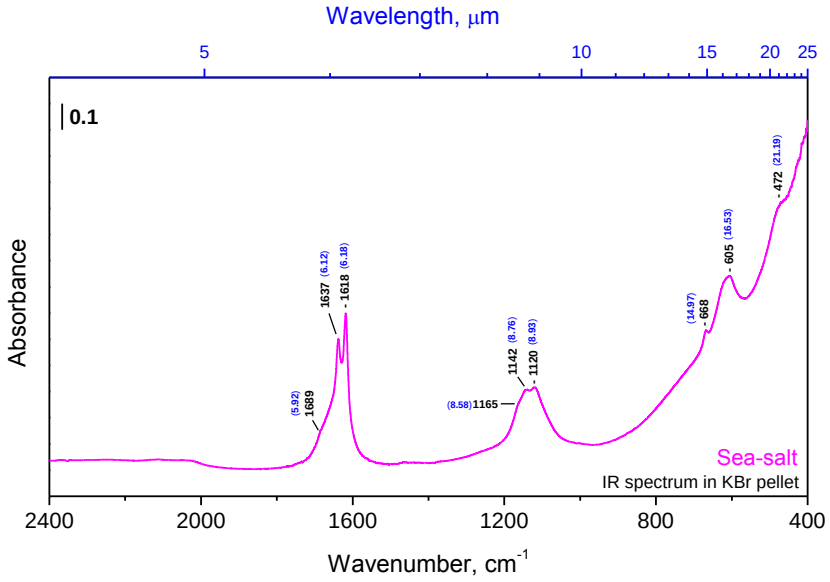


Fig. 3. IR-spectrum of seasalt obtained from Varna (Bulgaria)

Such a character of IR-spectrums and distribution of local extremums may prove that hot mineral alkaline water is preferable for origin and maintenance of life compared to other types of water analyzed by DNES and Nicolet Avatar 360 Fourier-transform IR. Thus, in hot mineral waters the local extremums in the IR-spectrums are more manifested compared to the local extremums obtained in IR-spectrum of the same water at a lower temperature (Ignatov, 2013). The difference in the local extremums in the interval from +20 °C to +95 °C at each 5 °C step is significant at $p < 0.05$ according to Student's t-criterion. These data indicate that the origination of life and living matter depends on the structure and physical chemical properties of water, as well as its temperature. The IR-spectrum of cactus juice is the closest to the IR- and DNES-spectrum of water, which contains bicarbonates and calcium ions typical for the formation of stromatolites. For this reason cactus juice was applied as a model system. The local extremums in IR-spectra of alkaline mineral water interacting with $CaCO_3$ and then seawater are the. The most closed closest to the local extremums in IR-spectrum of cactus juice.

In connection with these data the following reactions participating with $CaCO_3$ in aqueous solutions are important:

$$CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O, \quad (1)$$
$$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2, \quad (2)$$
$$CO_2 + OH^- = HCO_3^- \quad (3)$$
$$2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O \quad (4)$$

The equation (1) shows how some chemosynthetic bacteria use energy from the oxidation of H_2S and CO_2 to S and formaldehyde (CH_2O). The equation (2) is related to one of the most common processes in nature: in the presence of H_2O and CO_2, $CaCO_3$ transforms into $Ca(HCO_3)_2$. In the presence of hydroxyl OH^- ions, CO_2 transforms into HCO_3^- (equation (3)). Equation (4) is valid for the process of formation of the stromatolites – the dolomite layered acretionary structures formed in shallow seawater by colonies of cyanobacteria. In 2010 D. Ward described fossilized stromatolites in the Glacier National Park (USA) (Schirber, 2010). Stromatolites aged 3.5 billion years had lived in warm and hot water in zones of volcanic activity, which could be heated by magma. This suggests that the first living forms evidently evolved in hot geysers (Pons et al., 2011). It is known that water in geysers is rich in carbonates, while the temperature is ranged from +60 to +100 °C and more than +100 °C. In 2011 a team of Japanese scientists under the leadership of T. Sugawara showed that life originated in warm or, more likely, hot water (Kurihara et al., 2011). DNA and synthetic enzymes created proto cells from aqueous solution of organic molecules. For this the initial solution was heated to a temperature close to water's boiling point +95 °C. Then its temperature was lowered to +65 °C with formation of proto cells with primitive membrane. This laboratory experiment is an excellent confirmation of the possibility that life originated in hot water.

The above-mentioned data can predict a possible transition from synthesis of small organic molecules under high temperatures to more complex organic molecules as proteins. There are reactions of condensation-dehydration of amino acids into separate blocks of peptides that occur under alkaline conditions, with pH = 9–11.

A research is conducted of Oxidation Reduction Potential (ORP) of hot mineral water from Rupite, Bulgaria. With temperature increase ORP gets reduced with (-70 mV) from 50 to 25° C (Table 2). The measured pH is 7.70. The change of ORP shows that in hot mineral water are released electrons in alkaline medium.

When reviewing the processes of life origin it is necessary to consider the composition of primary atmosphere 3.5 billion years ago. It contains H_2, N_2, CO_2, CO. With the temperature rise of the water and boiling in the modern atmosphere, the bubbles contain oxygen and it gets acidified. The author suggests that the bubbles in the water in contact with the ancient atmosphere contain hydrogen, and the water gets more alkaline. Also ORP decreases and may result in negative values. An experiment is conducted with saturation of water from Rupite with hydrogen. The achieved average result is (-215 mV) with temperature 50°C.

Table 2. Results of temperature (°C) and Oxidation Reduction Potential (ORP) of mineral water from Rupite, Bulgaria

Temperature (° C)	Oxidation Reduction Potential (ORP) (mV)
25	57
30	45
35	37
40	30
45	18
50	-13

The difference is (-215 mV)-(-13 mV) = (-202 mV). This indicates that in the ancient atmosphere were gained more electrons in the water. In such a way are achieved more hydroxyl groups (OH^-) and bicarbonate ions (HCO_3^-).

The interaction with calcium ions (Ca^{2+}) during exchange of electric charges as per formulae makes the processes for structuring of stromatolites by formulae (1) and (2) more active.

$CO_2 + OH^- = HCO_3^-$ (3)

$2\ HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$ (4)

The following reaction (5) is valid in electrolysis. In the ancient atmosphere and hydrosphere there was increased gas discharge.

$2H_2O + 2\ e^- = H_2 + 2OH^-$ (5)

The same reaction contributed for the formation of stromatolites. Nowadays it is observed in elecrolyzer devices for waters catholyte and anolyte. In the ancient hydrosphere and land the charge had been negative and in the atmosphere positive. The conditions had been optimal for "nature" electrolysis. In these direction had been the experiments of Miller (Miller, 1953).

The reaction (4) is possible to structure nascent hydrogen H^* (Mehandjiev et al., 2019).

$H_2O + e^- \rightarrow H^* + OH^-$, (6)

The reaction (5) released in the gaseous form after recombination is formed:

$H^* + H^* \rightarrow H_2$ (7)

The nascent hydrogen is very active for chemical reactions in primary hydrosphere for origin of life and additional formation of H_2 makes alkalizing effect.

The allocation of H_2O molecule when a peptide chain is formed is important factor in reaction of condensation of two amino acid molecules into dipeptide. As reaction of polycondensation of amino acids is accompanied by dehydration, the H_2O removal from reaction mixture speeds up the reaction rate. This testifies that formation of early organic forms may have occured nearby active volcanoes, because at early periods of geological history volcanic activity occurred more actively than during subsequent geological times. However, dehydratation accompanies not only amino acid polymerization, but also association of other small blocks into larger organic molecules, and also polymerization of nucleotides into nucleic acids. Such association is connected with the reaction of condensation, at which from one block a proton is removed, and from another – a hydroxyl group with the formation of H_2O molecule (Ignatov, Mosin, 2012).

The results with ORP show that free electrons in water support for amino acid polymerization and small blocks into larger organic molecules (Ignatov, 2019).

In 1969 the possibility of existence of condensation-dehydration reactions under conditions of primary hydrosphere was proven by M. Calvin (Calvin, 1969). From most chemical substances hydrocyanic acid (HCN) and its derivatives – cyanoamid (CH_2N_2) and dicyanoamid ($HN(CN)_2$) possess dehydration ability and the ability to catalyze the process of linkage of H_2O from primary hydrosphere (Mathews, Moser, 1968). The presence of HCN in primary hydrosphere was proven by S. Miller's early experiments (Miller, 1953). Chemical reactions with HCN and its derivatives are complex with a chemical point of view; in the presence of HCN, CH_2N_2 and $HN(CN)_2$ the condensation of separate blocks of amino acids accompanied by dehydration can proceed at normal temperatures in strongly diluted H_2O-solutions. These reactions show the results of synthesis from separate smaller molecules to larger organic molecules of polymers, e.g. proteins,

polycarboxydrates, lipids, and ribonucleic acids (Figure 4).

Furthermore, polycondensation reactions catalyzed by HCN and its derivatives depend on acidity of water solutions in which they proceed (Abelson, 1966). In acid aqueous solutions with pH = 4–6 these reactions do not occur, whereas alkaline conditions with pH = 9–11 promote their course. There has not been unequivocal opinion, whether primary water was alkaline, but it is probable that such pH value possessed mineral waters adjoining with basalts, i.e. these reactions could occur at the contact of water with basalt rocks, that testifies this hypothesis (Ignatov, Mosin, 2012).

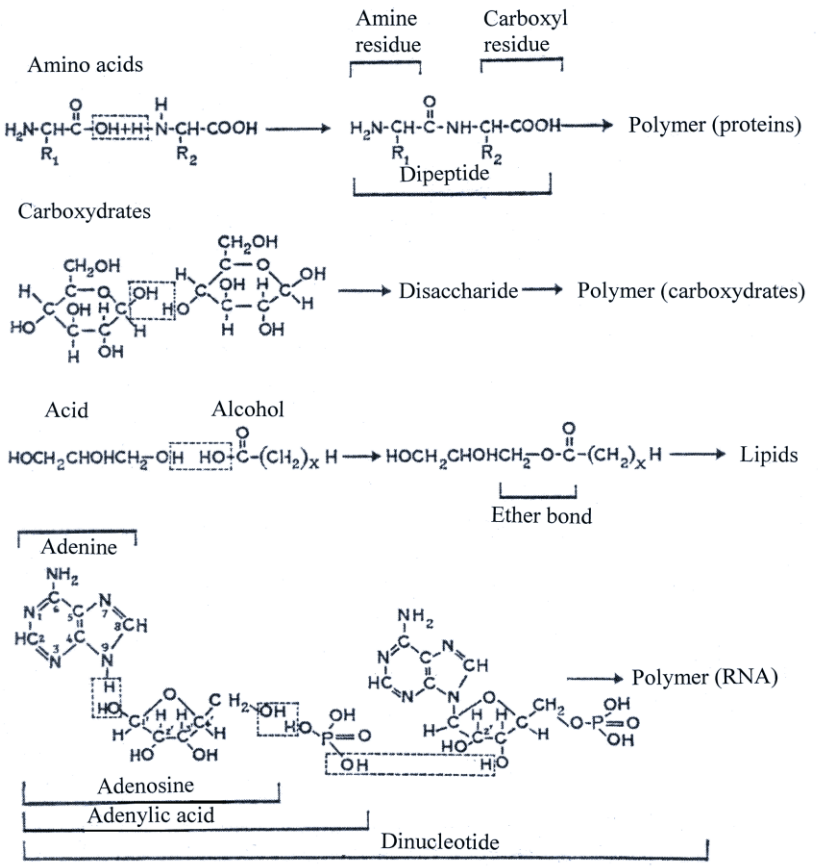
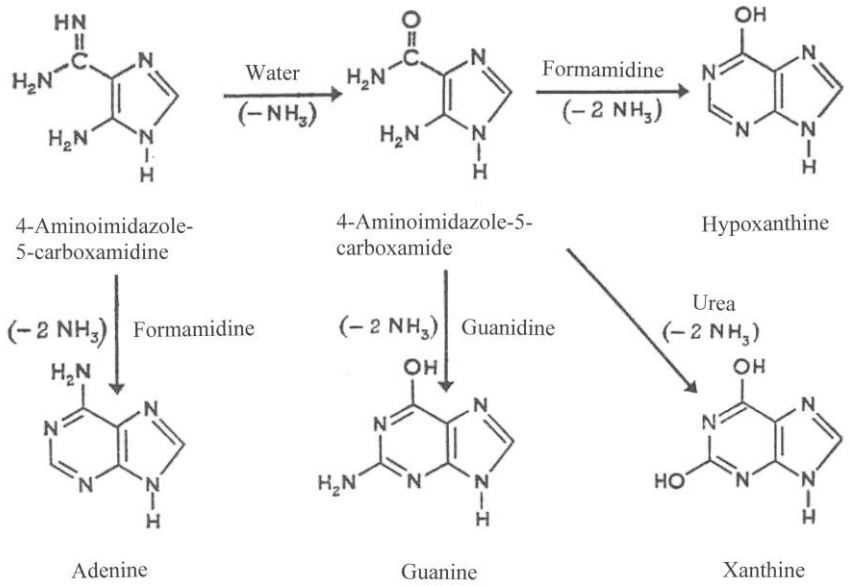


Fig. 4. Reactions of condensation and dehydration in alkaline conditions with pH = 9–11 catalyzed by HCN and its derivatives, resulting in synthesis from separate molecules larger organic molecules of polymers. The top three equations: condensation and the subsequent polymerization of amino acids in proteins; carbohydrates – in polycarboxydrates and acids and ethers – into lipids. The bottom equation – condensation of adenine with ribose and H_3PO_4, leading to formation of dinucleotide

It should be noted, that geothermal sources might be used for synthesis of various organic molecules. Thus, amino acids were detected in solutions of formaldehyde CH_2O with hydroxylamine NH_2OH, formaldehyde with hydrazine (N_2H_4) in water solutions with HCN, after heating of a reactionary mixture to +95 °C (Harada, Fox, 1964). In model experiments reaction products were polymerized into peptide chains that are the important stage towards inorganic synthesis of protein. Purines and pyrimidines were formed in a reactionary mixture of water solution with a HCN–NH_3 (Figure 5). In other experiments amino acid mixtures were subjected to influence of temperatures from +60 °C up to +170 °C with formation of short protein-like molecules resembling early evolutionary forms of proteins subsequently designated as thermal proteinoids (Ignatov, Mosin, 2012). They consisted of 18 amino acids usually occurring in protein hydrolyzates. The synthesized proteinoids are similar to natural proteins on a number of other important properties, e.g. on linkage by nucleobases and ability to cause the reactions similar to those catalyzed by enzymes in living organisms as decarboxylation, amination, deamination, and oxidoreduction. Proteinoids are capable to catalytically decompose glucose (Fox, Krampitz, 1964) and

to have an effect similar to the action of α-melanocyte-stimulating hormone (Fox, Wang, 1968). The best results on polycondensation were achieved with the mixes of amino acids containing aspartic and glutamic acids, which are essential amino acids occurring in all modern living organisms.



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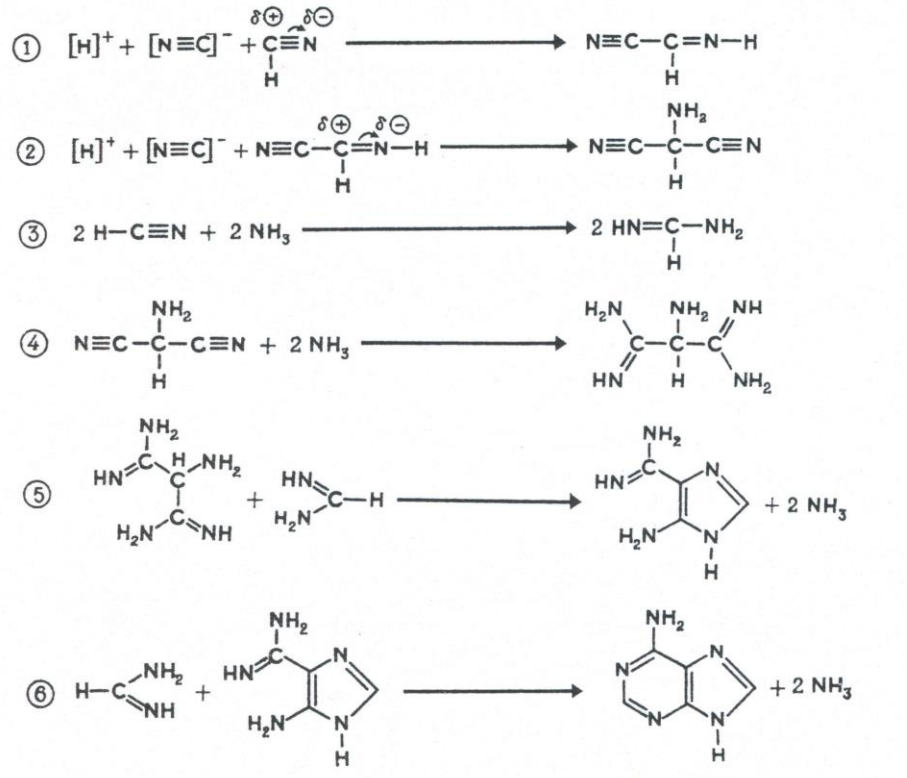

b)

Fig. 5. Prospective mechanisms of thermal (+95 °C) synthesis of purines in aqueous solutions: *a)* – synthesis of hypoxanthine, adenine, guanine and xanthine from 4-aminoimidazole-5-carboxamidine, 4-aminoimidazole-5-carboxamide, water, NH_3, formamidine and urea; *b)* – synthesis of adenine from NH_3 and HCN (total reaction: 5HCN = adenine)

In natural conditions water was heated by the magma. The structure formed from heated water was evidently a result of self-organization. Living organisms are complex self-organizing systems. They are open because they constantly exchange substances and energy with the environment and change the entropy (Ignatov, 2011). The changes in the open systems are relatively stable in time. The stable correlation between components in an open system is called a dissipative structure. According to I. Prigozhin, the formation of dissipative structures and the elaboration to living cells is related to changes in entropy (Nikolis, Prigozhin, 1979).

The initial stage of evolution, apparently, was connected with formation of the mixtures of amino acids and nitrogenous substances – analogues of nucleic acids at high temperature. Such synthesis is possible in aqueous solutions under thermal conditions in the presence of H_3PO_4. The next stage is polycondensation of amino acids into thermal proteinoids at temperatures 65–95 °C. After that membrane like structures were formed in a mix of proteinoids in hot water solutions. In 2011 T. Sugawara (Japan) created membrane like proto cells from aqueous solution of organic molecules, DNA and synthetic enzymes under temperature close to water's boiling point +95 °C (Sugawara, 2011).

4. Conclusion

The data obtained testify that origination of life and living matter depends on physical-chemical properties of water and external factors – temperatures, pH, ORP, electric discharges and isotopic composition. Hot mineral alkaline water interacting with $CaCO_3$ is closest to these conditions. Next in line with regard to quality is seawater. For chemical reaction of dehydration-condensation to occur in hot mineral water, water is required to be alkaline with pH range 9–11 and negative ORP. In warm and hot mineral waters the local extremums in IR-spectrums from 8 to 14 μm were more expressed in comparison with the local extremums measured in the same water samples with lower temperature. The new achievement is connected with chemical composition of ancient atmosphere and alkalization of the water from the hydrogen.

The research for origin of life and living matter in hot mineral water in mineral springs and open ponds (Ignatov, 2010; Szostak, 2011; Damer, Deamer, 2015) and in hydrothermal vents in the ocean (Mulkidjanian, Galperin, 2009) give the possibilities for life at the surface of the moons of the planets in Solar System. According to the law for conservation of energy the process of self-organization of primary organic forms in water solutions may be supported by thermal energy of magma (Ignatov, 2010). The candidates are – Europa (moon of Jupiter), Titan and Enceladus (moons of Saturn).

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References

Abdullah et al., 2012 – *Abdullah, A.M., Abdelsalam, E., Abdullah, B., Khaled, A.* (2012). Antioxidant Effects of Zamzam Water in Normal Rats and Those Under Induced-oxidant Stress. *Journal of Medicinal Plants Research*. 6(42): 5507-5512.

Abelson, 1966 – *Abelson, P.* (1966). Chemical Events on the "Primitive" Earth. *Proc. Natl. Acad. Sci. U.S.* 55: 1365-1372.

Antonov, 1995 – *Antonov, A.* (1995). Research of Nonequilibrium Processes in the Area of Allocated Systems, Diss. Thesis Doctor of Physical Sciences, 1-255.

Calvin, 1969 – *Calvin, M.* (1969). Chemical evolution. *Oxford: Clarendon*, 278.

Colin et al., 2016 – *Colin, G. et al.* (2016). Hydrothermal Vents and Prebiotic Chemistry: a Review. *Boletín de la Sociedad Geológica Mexicana*, 1-22.

Colon Santos, 2019 – *Colon Santos, S.M.* (2019). Exploring the Untargeted Synthesis of Prebiotically-plausible Molecules. PhD Thesis, University of Glasgow, 1-231.

Damer, Deamer, 2015 – *Damer, B., Deamer, D.* (2015). Coupled Phases and Combinatorial Selection in Fluctuating Hydrothermal Pools: A Scenario to Guide Experimental Approaches to the Origin of Cellular Life. *Life*, 5 (1): 872-887.

Fox, Krampitz, 1964 – *Fox, S.W., Krampitz, G.* (1964). Catalytic Decomposition of Glucose in Aqueous Solution by Thermal Proteinoids. *Nature*. 203: 1362-1364.

Fox, Wang, 1968 – F*ox, S.W., Wang, C.T.* (1968). Melanocytestimulating hormone: Activity in thermal polymers of alpha-ammo acids. *Science*. 160: 547-548.

Harada, Fox, 1964 – *Harada, I., Fox, S.W.* (1964). Thermal Synthesis of Natural Ammo-acids from a Postulated Primitive Terrestrial Atmosphere. *Nature*. 201: 335-336.

Ignatov et al., 1998 – *Ignatov, I., Antonov, A., Galabova, T.* (1998). Medical Biophysics – Biophysical Fields of Man, Gea Libris, Sofia.

Ignatov et al., 2014 – *Ignatov, I., Karadzhov, S., Atanasov, A., Ivanova, E., Mosin, O.V.* (2014). Electrochemical Aqueous Sodium Chloride Solution (Anolyte and Catholyte) as Types of Water, Mathematical Models, Study of Effects of Anolyte on the Virus of Classical Swine Fever Virus. *Journal of Health, Medicine and Nursing*. 8: 1-28.

Ignatov et al., 2015 – *Ignatov, I., Mosin, O.V., Gluhchev, G., Karadzhov, S., Miloshev, G., Ivanov, N.* (2015). The Evaluation of Mathematical Model of Interaction of Electrochemically Activated Water Solutions (Anolyte and Catholyte) with Water. *European Reviews of Chemical Research*. Vol. 2, No. 4, pp. 72-86.

Ignatov, 2010 – *Ignatov, I.* (2010). Which Water is Optimal for the Origin (Generation) of Life? *Euromedica, Hanover*. 34-37.

Ignatov, 2011 – *Ignatov, I.* (2011). Entropy and Time in Living Organisms. ArchivEuromedica, Hanover, 1st&2nd Edition, 74-75.

Ignatov, 2011a – *Ignatov, I.* (2011). Entropy and Time in Living Organisms. *Euromedica, Hanover*: 60-62.

Ignatov, Mosin, 2015 – *Ignatov, I. Mosin, O.V.* (2015). Studying the Properties of Hot Mineral Water to Sustain the Organic Forms of Life by IR, NES and DNES Methods. *Journal of Medicine, Physiology and Biophysics*. 18: 1-14.

Ignatov, Mosin, 2012 – *Ignatov, I. Mosin, O.V.* (2012). Hot Mineral Water with a High Deuterium Content in the Process of the Origin of Life and Living matter. *Everything about Water*: 1-26.

Ignatov, 2012 – *Ignatov, I.* (2012). Origin of Life and Living Matter in Hot Mineral Water. Conference on the Physics, Chemistry and Biology of Water, Vermont Photonics, USA.

Ignatov, Mosin, 2012 – *Ignatov, I., Mosin O.V.* (2012). Isotopic Composition of Water and its Temperature in Modeling primordial Hydrosphere Experiments. *VII Int. Conference Future Studies in Science and Technology, Veterinary Biological Science, Prague*. 15: 41-49.

Ignatov, Mosin, 2012a – *Ignatov, I., Mosin, O.V.* (2012). Hot Mineral Water with Deuterium Molecules for the Origin of Life and Living Matter. *Congress Science, Information, Consciousness, Saint-Petersburg Technical University*: 137-149.

Ignatov, Mosin, 2012b – *Ignatov, I., Mosin, O.V.* (2012). Isotopic Composition of Water and its Temperature in Modeling of Primordial Hydrosphere Experiments. *Euro-Eco, Hanover*, 62.

Ignatov, Mosin, 2013 – *Ignatov I., Mosin O.V.* (2013). Possible Processes for Origin of Life and Living Matter with modeling of Physiological Processes of Bacterium Bacillus Subtilis in Heavy Water as Model System. *Journal of Natural Sciences Research*. 3 (9): 65-76.

Ignatov, Mosin, 2013a – *Ignatov, I., Mosin, O.V.* (2013). Biological Effect of Deuterium on Prokaryotic and Eukaryotic Cells. *Astrahan Newspaper of Ecological Development*. 3: 124-138.

Ignatov, Mosin, 2013b – *Ignatov, I., Mosin, O.V.* (2013). Structure of Water for Origin of Life and Living Matter. *Acknowledge*. 15 (2): 1-16.

Ignatov, Mosin, 2013c – *Ignatov, I., Mosin, O.V.* (2013). Isotopic Composition of Water and Origin of Life. *School Biology*. 3: 5-16.

Ignatov, Mosin, 2013d – *Ignatov, I., Mosin, O.V.* (2013). Ideas about the Origin of Life in the Light of the Study of the Properties of the Natural Water. *Chemistry*. 10: 3-9.

Ignatov, Mosin, 2013e – *Ignatov, I., Mosin, O.V.* (2013). Isotopic Composition of Water and its Temperature in Modeling of Primordial Hydrosphere Experiments for Origin of Life and Living Matter. *Science Review*. 1: 17-27.

Ignatov, Mosin, 2013f – *Ignatov, I., Mosin, O.V.* (2013). Isotopic Composition of Water and its Temperature in Modeling of Primordial Hydrosphere Experiments for Origin of Life and Living Matter. *Acknowledge*. 14 (1): 1-16.

Ignatov, Mosin, 2013g – *Ignatov, I., Mosin, O.V.* (2013). Origin of Life and Living Matter in Hot Mineral Water. *Acknowledge*. 15 (2): 1-19.

Ignatov, Mosin, 2013h – *Ignatov, I., Mosin, O.V.* (2013). Isotopic Composition of Water and its Temperature in the Process of Evolutional Development of Life and Living Matter. *Astrahan Newspaper of Ecological Development*. 1: 113-127.

Ignatov, Mosin, 2013i – *Ignatov, I., Mosin, O.V.* (2013). Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium. *Journal of Environment and Earth Science*. 3 (14): 103-118.

Ignatov, Mosin, 2013j – *Ignatov, I., Mosin, O.V.* (2013). Isotopic Composition of Water and its Temperature in Modeling of Primordial Hydrosphere Experiments for Origin of Life and Living Matter. *Consciousness and Physical Reality, Natural Science*. 18 (4): 26-32.

Ignatov, Mosin, 2013k – *Ignatov, I., Mosin, O.V.* (2013). Origin of Life in Hot Mineral Water with Deuteriun. Everything about Water, Moscow, 1-15.

Ignatov, Mosin, 2014 – *Ignatov I., Mosin O.V.* (2014). Modeling of Conditions in Primary Hydrosphere in the Process of Origination of Organic Forms of Life in Hot Mineral Water, Nanoengineering, Moscow. 6, 37-46.

Ignatov, Mosin, 2014a – *Ignatov, I., Mosin, O.V.* (2014). Modeling of Possible Processes for Origin of Life and Living Matter in Sea and Hot Mineral Water. Process of Formation of Stromatolites. *Journal of Medicine, Physiology and Biophysics*. Vol. 5, pp. 23-46.

Ignatov, Mosin, 2014b – *Ignatov, I., Mosin, O.V.* (2014). Hot Mineral Water with More Deuterium for Origin of Live and Living Matter. Process of Formation of Stromatolites. *Journal of Health, Medicine and Nursing*. 6: 1-24.

Ignatov, Mosin, 2014c – *Ignatov, I., Mosin, O.V.* (2014). Origin of Life and Living Matter in Primary Atmosphere and Hydrosphere. Modeling of Non-equilibrium Electric Gas Discharge Conditions. *Journal of Health, Medicine and Nursing*. 6: 25-49.

Ignatov, Mosin, 2014d – *Ignatov, I., Mosin, O.V.* (2014). Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral Water. Research of Physiological Processes of Bacterium Bacillus Subtilis in Hot Heavy Water. *Journal of Medicine, Physiology and Biophysics*. 2: 53-70.

Ignatov, Mosin, 2014e – *Ignatov, I., Mosin,O.V.* (2014). Modeling of Possible Conditions For Origin of First Organic Forms in Hot Mineral Water. *Journal of Medicine, Physiology and Biophysics*. 3: 1-14.

Ignatov, Mosin, 2015 – *Ignatov, I. Mosin, O.V.* (2015). Studying the Hydrological Conditions for Origin of First Organic Forms of Life in Hot Mineral Water with HDO. *Journal of Medicine, Physiology and Biophysics*. 15: 20-41.

Ignatov, Mosin, 2015a – *Ignatov, I. Mosin, O.V.* (2015). Possible Processes for Origin of First Chemoheterotrophic Microorganisms with Modeling of Physiological Processes of Bacterium Bacillus subtilis as a Model System in 2H2O. *European Journal of Molecular Biotechnology*. 9 (3): 131-155.

Ignatov, Mosin, 2015b – *Ignatov, I., Mosin, O.V.* (2015). S. Miller's Experiments in Modelling of Non-Equilibrium Conditions with Gas Electric Discharge Simulating Primary Atmosphere. *Journal of Medicine, Physiology and Biophysics*. 15: 61-76.

Ignatov, Mosin, 2015c – *Ignatov, I., Mosin, O.V.* (2015). Origin of Life and Living Matter in Hot Mineral Water. *Advances in Physics Theories and Applications*. 39: 1-22.

Ignatov, Mosin, 2015d – *Ignatov, I., Mosin, O.V.* (2015). Non-equilibrium Gas Discharge Conditions for Origin of Life and Living Matter. Experiments of Miller. Modeling of the Conditions with Gas Coronal Discharge Simulating Primary Atmosphere. *Journal of Medicine, Physiology and Biophysics*. 9: 27-50.

Ignatov, Mosin, 2015e – *Ignatov, I., Mosin, O.V.* (2015). Hydrothermal Conditions for Origin of Life and Living Matter. *Journal of Health, Medicine and Nursing*. 10: 1-33.

Ignatov, Mosin, 2015f – *Ignatov, I., Mosin, O.V.* (2015). Possible Processes for Origin of First Chemoheterotrophic Microorganisms with Modeling of Physiological Processes of Bacterium Bacillus Subtilis as a Model System in 2H2O. *Journal of Medicine, Physiology and Biophysics*. 17: 53-75.

Ignatov, Mosin, 2016 – *Ignatov, I., Mosin, O.V.* (2016). Isotopic Composition, the Temperature and pH Value of Water in Experiments with Prognosis of Primary Hydrosphere and Possible Conditions for Origin of First Organic Forms in Hot Mineral Water with HDO. *Journal of Medicine, Physiology and Biophysics*. 24: 18-41.

Ignatov, Mosin, 2016a – *Ignatov, I., Mosin, O.V.* (2016) The Reactions of Condensation–

Dehydration Occurring in Aqueous Alkaline Solutions at pH = 9–11 and t = 65–95 °C in the Process of Modeling of Primary Hydrosphere. *Journal of Medicine, Physiology and Biophysics*. 24: 42-55.

Ignatov, Mosin, 2016b – *Ignatov, I., Mosin, O.V.* (2016). Studying the Process of Formation of Precambrian Period Limestone Dolomite Fossils of Stromatolites in Hot Mineral Water Interacting with $CaCO_3$. *Journal of Medicine, Physiology and Biophysics*. 25: 29-44.

Ignatov, Mosin, 2016c – *Ignatov, I., Mosin, O.V.* (2016). The Formation of Thermal Proteinoids in Hot Water. *Journal of Medicine, Physiology and Biophysics:* 26: 15-27.

Ignatov, Mosin, 2016d – *Ignatov, I., Mosin, O.V.* (2016). Deuterium, Heavy Water and Origin of Life. LAP LAMBERT Academic Publishing: 1-500.

Ignatov, Mosin, 2016e – *Ignatov, I., Mosin, O.V.* (2016). Water and Origin of Life. Altaspera Publishing & Literary Agency Inc.: 1-616.

Ignatov, Mosin, 2016f – *Ignatov, I., Mosin, O.V.* (2016). Can the First Organic Forms Of Life Originate in Hot Mineral Water with HDO? *Russian Journal of Biological Research*. (7)1: 4-19.

Ignatov, Mosin, 2016g – *Ignatov, I., Mosin, O.V.* (2016). Water and Origin of Life: Collection of Scientific Publications. Moscow, Berlin, Direct Media. pp. 1-658.

Ignatov, Tsvetkova, 2011 – *Ignatov, I., Tsvetkova, V.* (2011). Water for the Origin of Life and "Informationability" of Water, Kirlian (Electric Images) of Different Types of Water. *Euromedica, Hanover*: 62-65.

Kurihara et al., 2011 – *Kurihara, K., Tamura, M., Shohda, K.* (2011). Self-Reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nature Chemistry*. 4(10): 775-781.

Linsky, 2007 – *Linsky, J.L.* (2007). D/H and Nearby Interstellar Cloud Structures. Ed. J.I. Linsky. *Space Science Reviews, NY*: *Springer Science, Business Media*. 130. 1-367.

Mathews, Moser, 1968 – *Mathews, C.N., Moser, R.* (1968). Peptide Synthesis from Hydrogen-cyanide and Water. *Nature*. 215: 1230-1234.

Miller, 1953 – *Miller, S.L.* (1953). A Production of Amino Acids Under Possible Primitive Earth Conditions, *Science*. 117(3046): 528-529.

Mosin, Ignatov, 2011 – *Mosin, O.V., Ignatov, I.* (2011). Water – Substance of Life, Consciousness and Physical Reality, *Natural Sciences*. 17 (11): 9-21.

Mosin, Ignatov, 2015 – *Mosin, O.V., Ignatov, I.* (2015). Heavy Water as Medium for the Life of Organisms. *Journal of Health, Medicine and Nursing*. 9: 72-110.

Mulkidjanian, Galperin, 2009 – *Mulkidjanian, A.Y., Galperin, M.Y.* (2009). On the Origin of Life in the Zinc World. Validation of the Hypothesis on the Photosynthesizing Zinc Sulfide Edifices as Cradles of Life on Earth. *Biology Direct*, 4: 26-28.

Nakashima, 1987 – *Nakashima, T.* (1987). Metabolism of Proteinoid Microspheres. Ed. T. Nakashima. In: *Origins of Life and Evolution of Biospheres*. 20(3–4): 269-277.

Nikolis, Prigozhin, 1979 – *Nikolis, P., Prigozhin, I.* (1979). Self-Organization in Non-equilibrium Systems, *Moscow: Mir*. 1-512. [in Russian]

Pons et al., 2011 – *Pons, M.L., Quitte G.., Fujii, T. et al.* (2011). Early Archean Serpentine mud Volcanoes at Isua, Greenland, as a Niche for Early Life. *Proc. Natl. Acad. Sci. U.S.* 108: 17639-17643.

Schirber, 2010 – *Schirber, M.* (2010). First Fossil-makers in Hot water. *Astrobiology magazine*.

Sugawara, 2011 – *Sugawara, T.* (2011). Self-reproduction of Supramolecular Giant Vesicles Combined with the Amplification of Encapsulated DNA. *Nature Chemistry*. 1127: 775-780.

Szostak, 2011 – *Szostak, J.W.* (2011). An Optimal Degree of Physical and Chemical Heterogeneity for the Origin of Life? *Philos. Trans. Royal Soc. Lond. Biol. Sci.* 366(1580): 2894-901.

Trevors, Pollack, 2005 – *Trevors, J.I., Pollack, G.H.* (2005). Hypothesis: Origin of life in Hydrogel Environment. *Progress in Biophysics and Molecular Biology*. 89(1): 1-8.

Vassileva et al., 2019 – *Vassileva, P., Voykova, D., Ignatov, I., Karadzhov, S., Gluhchev, S., Ivanov, N., Mehandjiev, D.* (2019). Results from the Research of Water Catholyte with Nascent (Atomic) Hydrogen. *Journal of Medicine, Physiology and Biophysics*. 52: 7-11.

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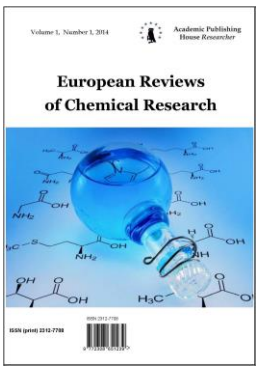

Results with IR Spectroscopy of CortiNon+ on the Development of Experimental *Graffi* Tumor on Hamsters

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Abstract

Studies were conducted with product CortiNon+. CortiNon+ is mix of Liquid Progesterone/DHEA. Progesterone is progestin, a female hormone, produced mostly in the ovaries.

DHEA is an endogenous hormone secreted by the adrenal gland of both males and females. The purpose of the research was to analyze the effects over blood serum of hamsters with *Graffi* tumor (Toshkova, 1995). The analyses have been conducted with Nonequilibrium Energy Spectrum (NES) and Differential Nonequilibrium Energy Spectrum (DNES) methods (Antonov, 1992; Ignatov, 1998). Experiments were carried out about the influence on tumor cells of mice in water. Reduction of DNES spectrum according to the control sample of cells in healthy animals was observed. (Antonov, 1992). Reduction has also been observed in DNES spectrum in blood serum of people having oncological diseases, compared to the one of healthy people (Ignatov, 2012). Such a reduction is most prevalent in (-0.1387 eV; 8.95 μm; 1117 cm^{-1}). The investigation of blood serum from hamsters injected with *Graffi* tumor cells and treated with CortiNon+ demonstrated a reduction in DNES spectrum in the range (-0.08 – -0.14 eV) (8.9 –15.5μm) (645–1129 cm^{-1}) (Toshkova et al., 2019).

Keywords: CortiNon+, *Graffi* tumor, NES, DNES.

1. Introduction

The research demonstrates the effects of CortiNon+ on the *Graffi* tumor of hamsters. The hamsters are separated into 5 groups noted as Gr. 1, Gr. 2, Gr. 3, Gr. 4 and Gr. 5. The first two groups Gr.1 and Gr.2 are for studying the effects of CortiNon+on the tumor development. Gr. 1 includes hamsters that started taking CortiNon+ once daily for 7 days before the injection of *Graffi* tumor cells and then continued taking daily it until the end of the experiment. Gr. 2 is from hamsters that were s.c. injected with the same amount of tumor cells and started taking CortiNon+on the same day as the tumor cell injection.The other groups are used as control. Gr. 3 is of non-treated tumor-bearing hamsters. Gr. 4 is of CortiNon+ treated healthy hamsters, and Gr. 5 is of healthynon-treated animals.

The research is conducted using spectral methods NES and DNES (Antonov, 1990; Antonov, Ignatov, 1998).

The spectrum analyses with methods NES and DNES are conducted on the 10th day after transplantation of *Graffi* tumor cells, which coincides with the appearance (formation) of

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subcutaneous tumor in the trial animals.

2. Materials and Methods

Experimental design. All the animals were divided into 5 groups as follows.

Gr. 1 – The hamsters from this group started taking CortiNon+ once daily for 7 days before the injection with 5x10⁴ *Graffi* tumor cells per hamster in the back area, and then continued taking it once daily until the end of the experiment.

Gr. 2 – These hamsters were s.c. injected with the same amount of tumor cells as Gr. 1 and started taking CortiNon+on the same day of the tumor injection.

Gr. 3 – Hamsters with *Graffi* tumor as a control untreated group.

Gr. 4 – Healthy hamsters takingCortiNon+as a control group.

Gr. 5 – Healthy hamstersas a control untreated group.

1. Experimental animals

Hamsters, breed "Golden Syrian", aged 2-4 months with weight around 90-100 g were used in the trials. The animals were grown in standard conditions in individual plastic cages with free access to food and water.

2.2. Experimental tumor model

Tumor cells (1-2.10⁶) from the experimental *Graffi* solid tumor are transplanted subcutaneously in the back of hamsters. Between days 7 and 15 after the transplantation tumor appears, grows progressively and the hamsters die around 30-35 days. In this tumor model 100 % tumor transplantation and 100 % mortality are observed. No spontaneous tumor's regression takes place (Toshkova, 1995).

2.3. Ethical aspects

All experiments were conducted in accordance with the European convention for protection of vertebrate animals, used for experimental and other scientific purposes (OJ L 222) and approved from the National Veterinary Medical Office.

2.4. NES and DNES Spectral Analyses

The device for DNES spectral analysis based on an optical principle was designed by A. Antonov. For this, a hermetic camera for evaporation of water drops under stable temperature (+22–24 °C) conditions was used. The water drops were placed on a water-proof transparent pad, which consisted 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° to 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 hamster serum blood.

3. Results and discussion

3.1. Parameters of NES and DNES spectrums of CortiNon+

The spectrum analysis is conducted on the 10th day after transplantation of *Graffi* tumor cells, which coincides with the appearance (formation) of subcutaneous tumor in the trial animals. 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 *Δf(E) = f (samples of water) – f (control sample of water)* – is called the "differential non-equilibrium energy spectrum of water" (DNES). The Figure 1 shows that on the X-axis are depicted three scales. The energies of hydrogen bonds among H_2O molecules are calculated in electronvolts (eV). On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in reciprocal electronvolts unit eV^{-1}. The local extremums of water samples are detected at E = -0.1112 eV, E = -0.1212 eV and E = -0.1387 eV. The value measured at E = -0.1212 eV is characteristic for anti-inflammatory effect (Ignatov et al., 2014) The value measured at E = -0.1112 eV is characteristic for the presence of Ca^{2+} ions in water

(Antonov, 1995). The value measured at E = -0.1387 eV is characteristic for inhibiting the growth of tumor cells (Ignatov, Mosin, 2012). Experiments conducted by Antonov with cancer cells of mice in water demonstrated a reduction of this local extremum to a negative value in DNES spectra.

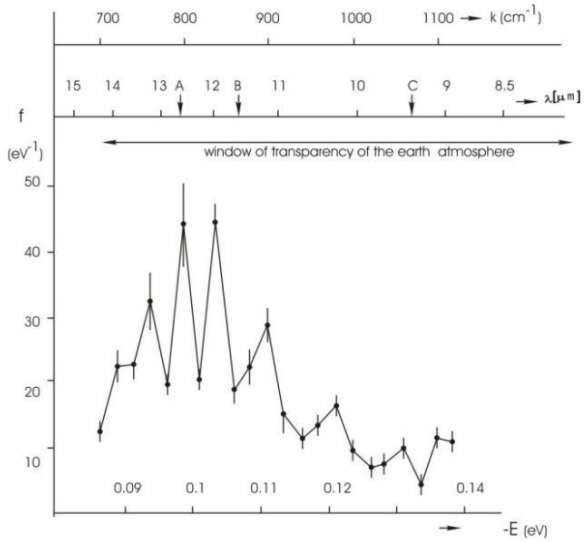

Fig. 1. The NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6,0–7,5; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H…O hydrogen bonds in the associates (– E (eV)); the vertical axis – the energy distribution function – f (eV^{-1}); *k* – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

The following results of the effects of CortiNon+ with NES and DNES methods are obtained:

3.1.1. The difference of DNES spectra between Gr. 1 and Gr. 3 is

ΔE = (-0.1194 eV)- (-0.1156 eV) = -3.8±1,1 meV. The difference is essential and shows effect of CortiNon+ on *Graffi* tumor of molecular and cell level

3.1.2. The difference of DNES spectra between Gr. 1 and Gr. 5 is

ΔE = (-0.1194 eV)-(-0.1179 eV) = -1.5±1,1 meV. This result is achieved for the products with anti tumor effect.

3.1.3. The difference of DNES spectra between Gr. 1 and Gr. 4 is

ΔE = (-0.1194 eV)-(-0.1177 eV) = -1.7±1,1 meV. This result is achieved for the products with anti tumor effect. For Gr. 1 CortiNon+ with implanted *Graffi* tumor after 7 days the result is higher according to healthy hamsters from Gr. 4 with CortiNon+.

3.1.4. The difference of DNES spectra between DNES spectrum of CortiNon+ and control sample of deionized water is ΔE = (-0.1230 eV)- (-0.1083 eV)= -14.7 ±1,1 meV. This is the maximal effect of the product for structuring of hydrogen bonds among water molecules.

3.2. The mathematical models of CortiNon+

The mathematical models of CortiNon+ of Gr.1, Gr.3 and Gr.5 give 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.

The average energy ($E_{H...O}$) of hydrogen H…O- bonds among H_2O molecules of the samples of blood serum of hamsters was measured for the following groups.

Gr. 1 – The result of NES for Gr.1 is E = - 0.1194 eV.

Gr. 2 – The result of NES for Gr. 2 is E = - 0.1168 eV.

Gr. 3 –The result of NES for Gr. 3 is E = - 0.1156 eV.

Gr. 4 – The result of NES for Gr. 4 is E = - 0.1177 eV.

Gr. 5 – The result of NES for Gr. 5 is E = - 0.1179 eV.

The Table 1 and Figure 2 show the mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5.

Table 1. Mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5

-E(eV) x-axis	1st group CortiNon+ 7 days before *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	3d group *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	5th group Healthy hamsters (%((-Evalue) */ (-Etotal value)**	-E(eV) x-axis	1st group CortiNon+ 7 days before *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	3d group *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	5th group Healthy hamsters (%((-Evalue) */ (-Etotal value)**
0.0937	0	0	0	0.1187	4.5	9.7	9.5
0.0962	0	0	0	0.1212	**13.6**	**6.1**	**0**
0.0987	0	5.1	4.7	0.1237	0	9.7	0
0.1012	9.1	5.1	4.7	0.1262	0	5.1	9.5
0.1037	9.1	9.7	9.5	0.1287	9.1	0	0
0.1062	9.1	9.7	19.4	0.1312	4.5	5.1	0
0.1087	0	5.1	4.7	0.1337	4.5	5.1	9.5
0.1112	**18.1**	**5.1**	**0**	0.1362	4.5	0	9.5
0.1137	0	9.7	0	0.1387	**13.9**	**0**	**9.5**
0.1162	0	9.7	9.5	–	–		–

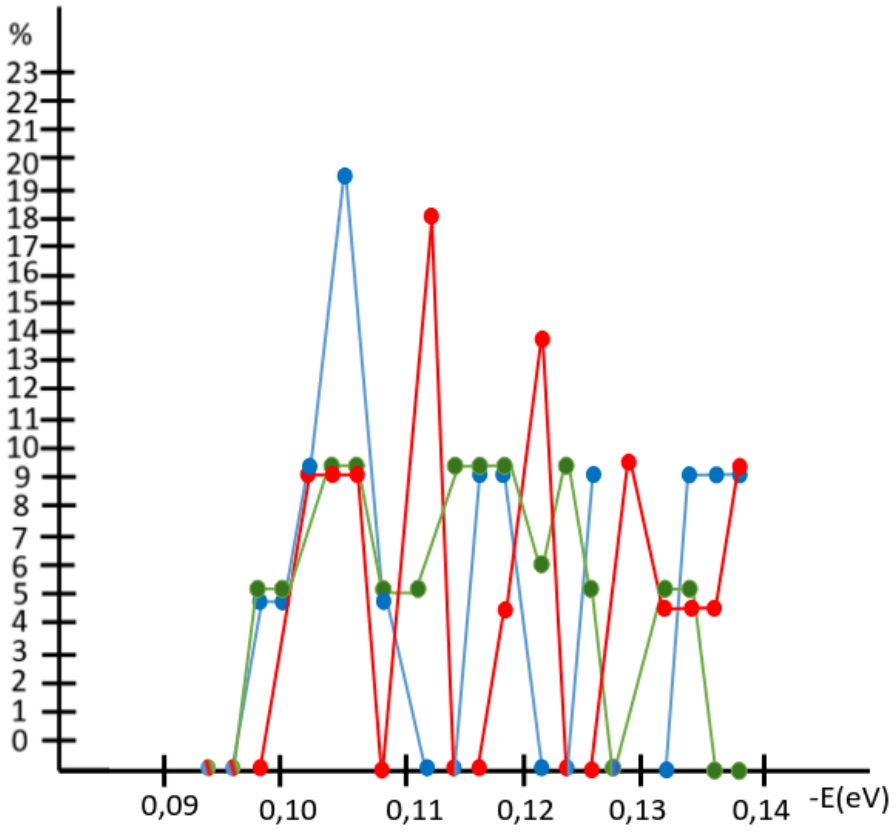

Fig. 2. Mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5

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.

* ($-E_{value}$) stands for the value of hydrogen bonds energy for one parameter of (-E).

** ($-E_{total\ value}$) stands for the total value of hydrogen bonds energy.

Figure 2 shows the distribution (%, ($-E_{value}$)/($-E_{total\ value}$) of H_2O molecules of CortiNon+ of Gr. 1, Gr. 3 and Gr. 5 respectively.

E = -0.1112 eV is the local extremum for stimulating effect on nervous system and improvement of nervous conductivity. The effect of CortiNon+ is 18.1 % for Gr.1, 5.1 % for Gr. 3 and 0 % for Gr. 5.

E = -0.1212 eV is the local extremum for anti inflammatory effect. The effect is 13.6 % for Gr. 1, 6.1 % for Gr. 3 and 0 % for Gr. 5.

E = -0.1387 eV is the local extremum for inhibition of development of tumor cells of molecular level. The effect is 13.9 % for Gr. 1, 0 % for Gr. 3 and 9.5 % for Gr. 5.

4. Conclusion

The influence of CortiNon+ on the development of *Graffi* tumor implanted in hamsters is assessed. The results show restructuring of water molecules in clusters with distribution that influences beneficially hamster (and human) health on molecular and cellular level.

The results from the application of NES and DENS spectrums and mathematical models confirm the effects of CortiNon+ on the nervous and endocrine systems, and anti-inflammatory and anti-tumor effects, as well. One interesting observation is that higher effect from the application of CortiNon+ on the tumor-bearing hamsters from Gr. 1 that have been treated 7 days before the injection is obtained relative to even the result of healthy hamsters from Gr. 4.

Product Name: CortiNon+

Inventor: Georgi D. Dinkov.

Trademark: Submitted to USPTO (USA) on December 10, 2017.

Product Distribution: IdeaLabs, LLC, 1200 18th Street NW #700, Washington, DC, USA.

References

Alberts et al., 1994 – *Alberts, B. et al.* (1994). Molecular Biology of the Cell 3rd ed.

Alexaki et al., 2018 – *Alexaki, V.I. et al.* (2018). DHEA Inhibits Acute MicrogliaMediated Inflammation through Activation of the TrkA-Akt1/2-CREB-Jmjd3 Pathway. *Mol. Psychiatry*. 23(6): 1410-1420.

Antonov, 1995 – *Antonov, A.* (1995). Research of the Non-equilibrium Processes in the Area in Allocated Systems. Dissertation thesis for degree "Doctor of Physical Sciences", Blagoevgrad, Sofia.

Antonov, Galabova, 1992 – *Antonov, A., Galabova, T.* (1992). Ext. Abstr. of *The 6th National Conference of Biomedical Physics and Engeneering*, 60.

Atif et al., 2015 – *Atif, F., Yousuf, S., Stein, D.G.* (2015). Anti-tumor Effects of Progesterone in Human Glioblastoma Multiforme: Role of PI3K/Akt/mTOR Signaling. *J Steroid Biochem Mol Biol*. Feb., 146: 62-73.

Azab et al., 2013 – *Azab B. et al.* (2013). Pretreatment Neurophil/lymphocyte Ratio is Superior to Platelet/lymphocyte Ratio as a Predictor of Long-term Mortality in Breast Cancer Patients. *Med. Oncol.* 30: 432.

Beilei Lei et al., 2014 – *Beilei Lei, Brian Mace, Hana N. Dawson, David S. Warner, Daniel T. Laskowitz, Michael L. James* (2014). Anti-Inflammatory Effects of Progesterone in Lipopolysaccharide-Stimulated BV-2 Microglia. *PLoS One*. 9(7): e103969.

Bosch et al., 2008 – *Bosch, E., Labarta, E., Crespo, J., Simon, C., Remohi, J., Jenkins, J. et al.* (2008). Circulating Progesterone Levels and Ongoing Pregnancy Rates in Controlled Ovarian Stimulation Cycles for in Vitro Fertilization: Analysis of Over 4000 Cycles. *Hum Reprod.* 23: 2346-51.

Calvin et al., 2012 – *Calvin, Yu. et al.* (2012). The Potential of Terahertz Imaging for Cancer Diagnosis. *A Review of Investigations to Date.*

Fahim et al., 2019 – *Fahim, A. et al.* (2019). Progesterone Treatment Attenuates Glycolytic Metabolism and Induces Senescence in Glioblastoma. *Scientific Reports 9 (Nature Journal)*. Article number: 988 (2019).

Gluhchev et al., 2015 – *Gluhchev, G., Ignatov, I., Karadzhov, S., Miloshev, G., Ivanov, N., Mosin, O.V.* (2015). Electrochemically Activited Water. Biophysical and Biological Effects of Anolyte and Catholyte as Types of Water. *Journal of Medicine, Physiology and Biophysics*. 10: 1-17.

Hoeijmakers, 2009 – *Hoeijmakers, J.H.* (2009). DNA Damage, Aging, and Cancer. *The New England Journal of Medicine*. 361(15): 1475-85.

Ignatov et al., 2014 – *Ignatov, I., Mosin, O. V., Velikov, B., Bauer, E. Tyminski, G.* (2014) Longevity Factors and Mountain Water as Factor. Research in Mountain and Fields Areas in Bulgaria. *Civil and Environmental Research*. 30 (4): 51-60.

Ignatov et al., 2019 – *Ignatov, I., Toshkova, R., Gluhchev, G., Drossinakis, Ch.* (2019). Results of Blood Serum from Cancer Treated Hamsters with Infrared Thermal Field and Electromagnetic Fields. *Journal of Health, Medicine and Nursing*. 58: 101-112.

Ignatov, 2011 – *Ignatov, I.* (2011). Entropy and Time in Living Organisms. *Euromedica, Hanover*. 60-62.

Ignatov, Mosin, 2013 – *Ignatov, I., Mosin, O.V.* (2013). Possible Processes for Origin of Life and Living Matter with modeling of Physiological Processes of Bacterium Bacillus Subtilis in Heavy Water as Model System. *Journal of Natural Sciences Research*. 3(9): 65-76.

Ignatov, Mosin, 2013a – *Ignatov, I., Mosin, O.V.* (2013). Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium. *Journal of Environment and Earth Science*. 3 (14): 103-118.

Ignatov, Mosin, 2013b – *Ignatov, I., Mosin, O.V.* (2013). Structural Mathematical Models Describing Water Clusters. *Journal of Mathematical Theory and Modeling*. 3(11): 72-87.

Ignatov, Mosin, 2013c – *Ignatov, I., Mosin, O.V.* (2013). Structural Mathematical Models Describing Water Clusters. *Journal of Mathematical Theory and Modeling*. 3 (11): 72-87.

Ortega-Calderon, Lopez-Marure, 2014 – *Ortega-Calderon, Y-N, Lopez-Marure, R.* (2014). Dehydroepiandrosterone Inhibits Proliferation and Suppresses Migration of Human Cervical Cancer Cell Lines. *Anticancer Research*. August, 34 (8): 4039-4044.

Philipp Lichte et al., 2014 – *Philipp Lichte et al.* (2014). Dehydroepiandrosterone Modulates the Inflammatory Response in a Bilateral Femoral Shaft Fracture Model. *Eur J Med Res*. 19(1): 27.

Shah et al., 2019 – *Shah, N.M., Lai, P.F., Imami, N., Johnson, M.R.* (2019). Progesterone-Related Immune Modulation of Pregnancy and Labor. *Front Endocrinol (Lausanne)*. 29(10): 198.

Shilkaitis et al., 2005 – *Shilkaitis, A., Green, A., Punj, V., Steele, V., Lubet, R., Christov, K.* (2005). Dehydroepiandrosterone Inhibits the Progression Phase of Mammary Carcinogenesis by Inducing Cellular Senescence via a p16-dependent but p53-independent Mechanism. *Breast Cancer Research*. Vol. 7, Article number: R1132.

Strauss et al., 2014 – *Strauss, S., Greve, T., Ernst, E., Fraidakis, M., Grudzinkas, J.G., Andersen, C.Y.* (2014). Administration of DHEA Augments Progesterone Production in a Woman with Low Ovarian Reserve Being Transplanted with Cryopreserved Ovarian Tissue. *J Assist Reprod Genet*. 31: 645-649.

Tang et al., 2018 – *Tang et al.* (2018). Detection of DNA Oligonucleotides with Base Mutations by Terahertz Spectroscopy and Microstructures. *PLoS ONE*. 13 (1): e0191515.

Toshkova et al., 2019 – *Toshkova, R., Ignatov, I., Zvetkova, E., Gluhchev, G.* (2019). Effects of Catholyte Water on the Development of Experimental Graffi Tumor on Hamsters. *Cells&Cellular Life Sciences Journal*. 4 (1): 000140.

Toshkova et al., 2019a – *Toshkova, R., Ignatov, I., Zvetkova, E.& Gluhchev, G.* (2019). Effects of Catholyte Water on the Development of Experimental Graffi Tumor on Hamsters. *European Journal of Medicine*. 7(1): 45-56.

Toshkova et al., 2019b – *Toshkova, R., Ignatov, I., Zvetkova, E., Gluhchev, G.* (2019). Effects of Catholyte Water on the Development of Experimental Graffi Tumor on Hamsters. *Cells&Cellular Life Sciences Journal*. 4(1): 000140.

Toshkova et al., 2019c – *Toshkova, R., Ignatov, I., Zvetkova, E., Gluhchev, G.* (2019). Bioinfluence with Infrared Thermal and Electromagnetic Fields as a Therapeutic Approach of Hamsters with Experimental *Graffi* Myeloid Tumor. *Journal of Natural Sciences Research*. 9(4): 1-11.

Toshkova, 1995 – *Toshkova, R.* (1995). Attempts for Immunomodulation in Hamsters with Transplanted Myeloid Tumor, Previously Induced by *Graffi* Virus, Bulgarian Academy of Sciences, PhD Dissertation, Sofia.

Weissman et al., 2011 – *Weissman, A., Horowitz, E., Ravhon, A., Golan, A., Levran, D.* (2011). Dehydroepiandrosterone Supplementation Increases Baseline Follicular Phase Progesterone Levels. *Gynecol Endocrinol.* 27: 1014-1017.

Zvetkova, 2006 – *Zvetkova, E.* (2006). F.140. Quantitative Reduction in the RNP-Contents of Peripheral Blood Lymphocytes in Cancer Patients. Conference: 6th Annual Meeting of the Federation-of-Clinical-Immunology-Societies Location: San Francisco, CA Date: JUN 07-11, *Clinical Immunology*. 119: S100.

Zvetkova, Fuchs, 2017 – *Zvetkova, E., Fuchs, D.* (2017). Medical Significance of Simultaneous Application of Red Blood Cell Distribution Width (RDW) and Neopterin as Diagnostic Biomarkers in Clinical Practice. *Pteridines*. 28(3-4): 133-140.

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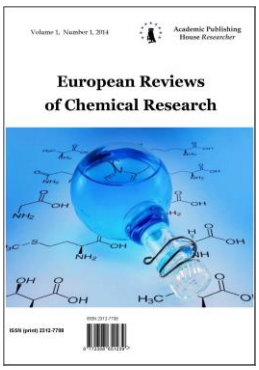

Physicochemical and Microbiological Characteristics of Thermal Healing Spring Waters in the District of Burgas

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Abstract

Defined are the physicochemical properties of healing thermal spring waters in the area of Burgas District. It is shown that according to 18 controlled parameters included in the research, the thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo, fulfill the required conditions for drinking water.

The spring waters from the given four water sources are characterized by microbiological indicators, as the pathogenic micro-organisms are defined by the membrane method. It is established that thermal healing spring water Burgas Mineral baths, thermal healing spring water village of Shivarovo, thermal healing spring water of village of Polyanovo, fulfill the standard requirements. The healing water of village Judge, District ofBurgas does not conform to the physicochemical indicators given for fluorides, and microbiological indicators with regards to coliform bacteria, *Escherichia coli* and enterococci.

Keywords: spring water, drinking water, physicochemical properties, microbiological indicators.

1. Introduction

In Bulgaria there are mineral and spring waters, which are not subjected to physicochemical and microbiological control by the Regional Health Inspectorate and microbiological control byt they are the most use springs for drinking from the population. Similar springs are located in the territory of Haskovo District, Stara Zagora District, Varna District (Valcheva et al., 2013).

Although water is an unfavorable environment for the development of microorganisms and for the development of microorganisms, studies by many authors including heir, own, that microorganisms with valuable properties (enzymes, antibiotics, thermopile can acidophilic strains) are in mineral and non – thermal spring waters. This was proved by the results obtained from the experimental work carried out to determinal the microflora of medicinal and spring waters in Haskovo, Stara Zagora, Plovdiv (Tumbarski et al., 2014) and Varna region (Valcheva, Ignatov, 2019).

Isolated bacteria from the healing and spring regions have been identified by *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Bacillus methylotrophicus, Aeromonashydrophila.*

The isolated bacteria from the healing and spring waters in the Plovdiv region have been identified by molecular genetic methods suchas *Aeromonassobria, Klebsiellaoxytoca, Bacillus*

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amyloliquefacienssubsp. plantarum, Bacillus thuringiensis, Bacillus cereus (Valcheva et al., 2013, 2014).

Strains with high proteolytic, lipolytic and amylolytic activity were selected (Valcheva et al., 2013, 2014).

Antimicrobial activity of the strains of *Bacillus* sp., against the saprophytic and pathogenic microorganisms was detected: *Penicillium sp., Fusariummoliniforme, Rhizopus sp., Aspergillusniger, Aspergillusoryzae, Aspergillusawamori, Mucorsp. Enterococcus faecalis*, in the process of development and growt of the four *Bacillus – Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis, Bacillus methylotrophicus*are the the most active strains – *Bacillus* methylotrophicus PY5, *Bacillus cereus LH1*, *Bacillus cereus WIF15* and *Bacillus thuringiensis B62* (Valcheva et al., 2013, 2014).

Pathogenic bacteria exhibit resistance and 4 retain their vitality in the process of develop pment and interaction between them and the strains of *Bacillus sp.* and at 37 C°.

A relatively low bactericidal effect was demonstrated against the (Gr+) bacterium *Enterococcus faecalis*. The isolated strains are likely to have a higher inhibitory ability agains (Gr -) bacteria compared to (Gr+) bacteria (Valcheva et al., 2013, 2014).

The yeasts used in the genus *Candida* exhibit a simulating effect of two of *Bacillus sp. – Bacillus methylotrophicus PY5*, and *Bacillus cereus LH1*. This indicates that synergism has occurred between these microorganisms (Valcheva et al., 2013, 2014).

Mineral springs Burgas city

Burgas is the second largest seaside town in Bulgaria, located on the Southern Black Sea coast. In addition to the beautiful sea and spa – pious beaches, Burgas offers great opportunities for balneological treatment with mineral water, characteristic sea mud and lye.

This is one of the oldest balneological centers in Bulgaria. Mineral water is suitable for the treatment and prevention of diseases of the musculoskeletal system, peripheral nervous system, chronic gastritis and pyelonephritis infertility, gout.

Water is also beneficial for strengthening the general state of the body.

Mineral spring District Sudievo

Sudievo is a village in southeastern Bulgaria. It is located in Aytos municipality. Water helps diseases of urinary system, disorders of locomotory system, endocrine diseases. It is suitable for daily use as drinking water. This water in Sudievo is hydrocarbonate, sodium, but contains fluoride. According to the requirements for drinking water, not mineral water, water should contains not more than 1.5 milligrams per liter of fluoride. The water in Sudievo contains much more in quantity than this chemical element.What those who consume this water need to know is that excessive ingestion of this fluoride per day can accordingly damage tooth enamel in young children. In the northern part of Aytosko Polje there are several mineral springs along the fault line: the "Smelly Fountain" near the village of Shivarovo and those near the villages of Cherry, Yabulchevo and Saedinenie. Geothermal water with a flow rate of 30 L/s and a temperature of 51 °C emerges from deep drilling in the village of Polyanovo, which flows freely without being used. Analyses show that the sources have extremely good healing properties.

Medicinal properties of water: in diseases of the locomotory system, gastrointestinal, liver-bile and renal diseases.

2. Materials and methods

In the work are used thermal healing waters from the district of Burgas – thermal healing spring Burgas Mineral baths with water temperature of 41°C, thermal healing spring village of Shivarovo with water temperature of 47°C, thermal healing spring village of Polyanovo with water temperature of 51°C, thermal healing spring village of Judge with water temperature of 51°C.

2.1. Nutrient media

Nutrientagar (MPA) with contents (in %) – meat water, peptone – 1 %, agar –agar – 2 %. Endo's Medium (for defining of *Escherichia coli*and coliform bacteria) with contents (g/dm^3) – peptone – 5,0; triptone – 5,0; lactose – 10,0; Na_2SO_3 – 1,4; K_2HPO_4 – 3,0; fuchsine – 0,14; agar – agar– 12,0, pH 7,5 – 7,7.

Nutrient gelatine (MPD) (for defining of *Pseudomonas aeruginosa*) with contents (in %) – Peptic digest of animal tissue; 25 % gelatin; pH = 7, 0-7, 2.

Medium for defining of enterococci (esculin – bile agar).

Medium for defining of sulphite reducing bacteria (Iron Sulfite Modified Agar).

Wilson-Bleer medium (for defining of sulphite reducing spore anaerobes (*Clostridium perfringens*) with contents(g/dm^3) – 3 % Nutrient agar; 100 cm^3 20 % solution Na_2SO_3 ; 50 cm^3 20 % glucose solution; 10 cm^3 8 % solution ofFe_2SO_4.

2.2. Methods for analysis

Methods for physicochemical analysis

Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;

Method for determination of smell at 20°C — method BDS 8451: 1977 technical device – glass mercury thermometer, conditions № 21;

Method for determination of turbidity – EN ISO 7027, technical device turbidimeter type TURB 355 IR ID № 200807088;

Method for determination of pH – BDS 3424: 1981, technical device pH meter type UB10 ID № UB10128148;

Method for determination of oxidisability – BDS 3413: 1981;

Method for determination of chlorides – BDS 3414: 1980;

Method for determination of nitrates – Validated Laboratory Method (VLM) – NO_3 – № 2, technical device photometer, "NOVA 60 A" ID № 08450505;

Method for determination of nitrites – VLM NO_3 –№ 3, technical device photometer "NOVA 60 A" ID № 08450505;

Method for determination of ammonium ions – VLM – NH_4 – № 1, technical device photometer "NOVA 60 A" ID № 08450505;

Method for determination of general hardness – BDS ISO 6058;

Method for determination of sulphates – VLM – SO_4 – № 4, technical device photometer, "NOVA 60 A" ID № 08450505;

Method for determination of calcium – BDS ISO 6058;

Method for determination of magnesium – BDS 7211: 1982;

Method for determination of phosphates – VLM - PO_4 –№ 5, technical device photometer, "NOVA 60 A" ID № 08450505;

Method for determination of manganese – VLM – Mn–№ 7, technical device photometer, "NOVA 60 A" ID № 08450505;

Method for determination of iron – VLM – Fe – № 6, technical device photometer "NOVA 60 A" ID № 08450505;

Method for determination of fluorides – VLM – F – № 8, technical device photometer "NOVA 60 A" ID № 08450505;

Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLabcond 720 ID № 11081137.

2.3. Methods for determination of microbiological indicators

Methods for evaluation of microbiological indicators according to Ordinance № 9/2001, Official State Gazette, issue 30, and decree № 178/23.07.2004 about the quality of water, intended for drinking purposes.

Method for determination of *Escherichia coli* and coliform bacteria –BDSEN ISO 9308 – 1: 2004;

Method for determination of enterococci – BDS EN ISO 7899 – 2;

Method for determination of sulphite reducing spore anaerobes – BDS EN 26461 – 2: 2004;

Method for determination of total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222: 2002;

Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266 : 2008.

Determination of coli – titre by fermentation method – Ginchev's method

Determination of coli – bacteria over Endo's medium – membrane method.

Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

3. Results and discussion

It is done a comparative physicochemical analysis of mineral spring waters at the territory of Burgas District by the main indicators (colour according to Rublyovska Scale, smell at 20°C, turbidity, pH, oxidisability, chlorides, nitrates, nitrites, ammonium ions, general hardness, sulphates, calcium, magnesium, phosphates, manganese, iron, fluorides, electrical conductivity). The results from these examinations are given in Table 1.

The trial data reveal that thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo swimming pool are in compliance with the controlled parameters set out in Ordinance №9/2001, Official State Gazette, issue 30, and decree № 178/23.07.2004 about the quality of water, intended for drinking purposes(RZI (Regional Health Inspection) – Burgas).

Table 1. Comparison of the examined spring waters in Burgas District by physicochemical properties

Controlled parameter	Measuring unit	Maximum Limit Value	Result Burgas Mineral baths	Result Shivarovo	Result Polyanovo	Result Judge
1. Color according to Rublyovska Scale	Chromaticity Values	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers
2. Smell at 20°C	Rating	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers
3. Turbidity	NTU	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers	Acceptable to consumers
4. pH indicator	pH values	≥ 6,5 и ≤ 9,5	9,95	9	9,11	9,1
5. Oxidisability	mgO_2/dm^3	5,0	0,50	0,4	0,5	0,5
6. Chlorides	mg/ dm^3	250	30,7	26,3	26,3	26,0
7. Nitrates	mg/ dm^3	50	0,2	2,10	0,1	0,15
8. Nitrites	mg/ dm^3	0,50	0,007	0,00	0,006	0,005
9. Ammonium ions	mg/ dm^3	0,50	0,111	0,150	0,154	0,158
10. General hardness	$mgekv/ dm^3$	12	0,4	0,4	0,4	0,4
11. Sulphates	mg/ dm^3	250	37	34	35	36
12. Calcium	mg/ dm^3	150	120	118	116	117
13. Magnesium	mg/ dm^3	80	68	66	67	66
14. Phosphates	mg/ dm^3	0,5	0,015	0,016	0,016	1,018
15. Manganese	mg/ dm^3	50	0,0005	0,0008	0,0007	0,0009
16. Iron	$\mu g/ dm^3$	200	0,0016	0,0020	0,0022	0,0037
17. Fluorides	mg/ dm^3	1,5	7,73	0,4	1,48	5,5
18. Electrical conductivity	$\mu S/ dm^3$	2000	633	620	612	615

For the same spring waters are determined their microbiological indicators by the membrane method. In Table 2 are shown the experimental studies from the determination of total number of mesophilic aerobic and facultative anaerobic bacteria.

Table 2. Determination of total number of mesophilic aerobic and facultative anaerobic bacteria

Examined water source	Indicator, cfu/cm^3
1. Thermal healing spring Burgas Mineral baths with water temperature of 41°C	6 ± 1
2. Thermal Healing Spring village of Shivarovowith water temperature of 41 °C	11 – 17
3. Thermal Healing Springvillage of Polyanovowith water temperature of 51°C	5 – 8
4. Thermal Healing Springvillage of Judge with water temperature of 51 °C	120 – 150

According to the standard requirements from the examined water samples from the four springs, the water is clean.

The presence of coliforms and *Escherichia coli* is determined by the membrane method, and according to Ginchev's method. The trial results (Table 3 and Table 4) reveal that thermal healing spring Burgas Mineral baths with water temperature of 41°C, thermal healing spring village of Shivarovo with water temperature of 41°C, thermal healing spring village of Polyanovo with water temperature of 51°C swimming pool, are in compliance with the requirements for presence of coli bacteria. Thermal healing spring village of Polyanovo does not comply with the requirements for presence of coliform bacteria and enterococci. The given results are also confirmed by the analyses via the membrane method (Table 4). All the remaining indicators are determined by the membrane method.

Table 3. Coli – titre of thermal healing spring waters

Name of water source	Coli – titre	Culture volumes 50cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³
1. Thermal healing spring Burgas Mineral baths with water temperature of 41°C	> 100	–	–	–	–	–	–
2. Thermal Healing Spring village of Shivarovo with water temperature of 41 °C	> 100	–	–	–	–	–	–
3. Thermal Healing Springvillage of Polyanovo with water temperature of 51°C	> 100	–	–	–	–	–	–
4. Thermal Healing Spring village of Judge with water temperature of 51 °C	80	+ Acid	+ Acid	+ Acid and gas	+ Acid and gas	+ Acid and gas	–

Table 4. Microbiological indicators of spring waters in Burgas District

Indicators	Measuring unit	Thermal healing spring Burgas Mineral baths with water temperature of 41°C	Thermal healing springvillage of Shivarovo with water temperature of 41 °C	Thermal healing spring village of Polyanovo with water temperature of 51 °C	Thermal healing springvillage of Judge with water temperature of 51 °C
Coliforms	cfu/cm³	0/100	0/100	0/100	10/100
Escherichiacoli	cfu/cm³	0/100	0/100	0/100	10/100
Enterococci	cfu/cm³	0/100	8/100	0/100	8/100

Sulphite reducing anaerobic bacteria(*Clostridium perfringens)*	cfu/cm3	0/100	0/100	0/100	0/100
Pseudomonas aeruginosa	cfu/cm3	0/250	0/250	0/250	0/250

Based on the conducted physicochemical and microbiological evaluations it is established that from the four examined springs at the territory of BurgasDistrictby physicochemical parameters only thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo swimming pool correspond to all controlled parameters according to Ordinance № 9/2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes. With regards to microbiological parameters thermal healing water Burgas Mineral baths, thermal healing spring village of Shivarovo, thermal healing spring water village of Polyanovo swimming pool are in compliance with the requirements for drinking water.

4. Conclusion

The research shows the effects of hot mineral water from Burgas region, Bulgaria.

There are the results with

- Comparison of the examined spring waters in Burgas District by physicochemical properties;
- Determination of total number of mesophilic aerobic and facultative anaerobic bacteria;
- Coli – titre of thermal healing spring waters;
- Microbiological indicators of spring waters in Burgas District.

References

Gluhchev et al., 2015 – *Gluhchev, G., Ignatov, I., Karadzhov, S., Miloshev, G., Ivanov, N., Mosin, O.V.* (2015). Electrochemically Activited Water. Biophysical and Biological Effects of Anolyte and Catholyte as Types of Water. *Journal of Medicine, Physiology and Biophysics*. 10, 1-17.

Ignatov et al., 2014 – *Ignatov, I., Mosin, O.V., Velikov, B., Bauer, E. Tyminski, G.* (2014). Longevity Factors and Mountain Water as Factor. Research in Mountain and Fields Areas in Bulgaria. *Civil and Environmental Research*. 30 (4): 51-60.

Ignatov et al., 2019 – *Ignatov, I., Toshkova, R., Gluhchev, G., Drossinakis, Ch.* (2019). Results of Blood Serum from Cancer Treated Hamsters with Infrared Thermal Field and Electromagnetic Fields. *Journal of Health, Medicine and Nursing*. 58: 101-112.

Ignatov, 2011 – *Ignatov, I.* (2011). Entropy and Time in Living Organisms. *Euromedica, Hanover*. 60-62.

Ignatov, Mosin, 2013 – *Ignatov I., Mosin O.V.* (2013). Possible Processes for Origin of Life and Living Matter with modeling of Physiological Processes of Bacterium Bacillus Subtilis in Heavy Water as Model System. *Journal of Natural Sciences Research*. 3(9): 65-76.

Ignatov, Mosin, 2013 – Ignatov, I., Mosin, O.V. (2013) Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium, *Journal of Environment and Earth Science*, 3 (14): 103-118.

Ignatov, Mosin, 2013a – *Ignatov, I., Mosin, O.V.* (2013). Structural Mathematical Models Describing Water Clusters. *Journal of Mathematical Theory and Modeling*. 3 (11): 72-87.

Ignatov, Mosin, 2013b – *Ignatov, I., Mosin, O.V.* (2013). Structural Mathematical Models Describing Water Clusters. *Journal of Mathematical Theory and Modeling*. 3 (11): 72-87.

Tumbarski et al., 2014 – *Tumbarski, T., Valcheva, N., Denkova, Z., Koleva, I.* (2014). Antimicrobial Activity against Some Saprophytic and Pathogenic Microorganisms of Bacillus species Strains Isolated from Natural Sping Waters in Bulgaria. *British Microbiology Research Journal*. 4(12): 1353-1369.

Valcheva et al., 2013 – *Valcheva, N., Denkova, Z. Nikolova, Denkova, R.* (2013). Physiological – Biochemical and Molecular – Genetic Characteristics of Bacterial Strains Isolated from Spring and Healing Waters in the Haskovo Region. N.T. at UCT, Volume LX.

Valcheva et al., 2013 – *Valcheva, N., Denkova, Z., Denkova, R.* (2013). Physicochemical and microbiological characteristics of spring waters in Haskovo. J*ournal of Food and Packaging Science Technique and Technologies*. № 2: 21-25.

Valcheva et al., 2014 – *Valcheva, N., Denkova, Z., Denkova, R., Nikolova, R.* (2014). Characterization of bacterial strains isolated from a thermal spring in Pavel Banya, Stara Zagora Region. N.T. at UCT, Volume LXI.

Valcheva et al., 2014 – *Valcheva, N., Denkova, Z., Nikolova, R., Denkova, R.* (2014). Physiological, Biochemical, and Molecular – Genetic Characterization of Bacterial Strains Isolated From Sping and Healing Waters in Region of Haskovo. *Food, Sciense, Engineering and technologiest, Plovdiv*. *LX*: 940-946.

Valcheva, 2014 – *Valcheva, N.* (2014). The Microflora of Medicinal and Spring Waters in Haskovo and Stara Zagora Region, *Dissertation, University of Food Technology*. 1-142.

Valcheva, Ignatov, 2019 – *Valcheva, N., Ignatov, I.* (2019). Physicochemical and Microbiological Characteristics of Thermal Healing Spring Waters in the District of Varna. *Journal of Medicine, Physilogy and Biophysics*. 59: 10-16.

Standards

Ordinance №9/2001, Official State Gazette, issue 30.

Decree № 178/23.07.2004 about the quality of water, intended for drinking purposes.

BDS8451: 1977 – defining of colour according to Rublyovska Scale, determination of smell at 20 °C.

EN ISO 7027 – determination of turbidity.

BDS3424: 1981 – determination ofpH.

BDS3413: 1981 – determination of oxidisability.

BDS3414: 1980 – determination of chlorides.

BDS ISO 6058 – determination of calcium, determination of general hardness.

BDS EN 27888 – determination of electrical conductivity.

VLM – NH_4 –№ 1 – determination of ammonium ions.

VLM – NO_3 – № 2 – determination of nitrates.

VLM – NO_2 – № 3 – determination of nitrites.

VLM – SO_4 – № 4 – determination of sulphates.

VLM – PO_4 – № 5 – determination of phosphates.

VLM – Fe – № 6 – determination of iron.

VLM – Mn – № 7 – determination of manganese.

VLM – F – № 8 – determination of fluorides.

BDS 7211: 1982 – determination of magnesium.

BDSEN ISO 7899 – 2 – determination of nitrates.

BDSEN ISO 9308 – 1: 2004 – determination of *Escherichia coli*andcoliformbacteria.

BDSEN26461 – 2: 2004 – determination of sulphite reducing anaerobic bacteria*(Clostridiumperfringens)*.

BDSEN ISO 16266 – determination of *Pseudomonas aeruginosa*.

BDSEN ISO 7899 – 2 – determination of eneterococci.

BDS EN ISO 6222: 2002 – determination of total number of aerobic and facultative anaerobic bacteria.