HIGH-DENSITY GREEN PHOTONS EFFECTS ON NaCl SOLUTIONS DETECTED BY RED BLOOD CELLS MEMBRANES

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This paper presents a new technique for investigating the modifications induced by high-density green light [GL] on water in NaCl solutions. Solutions of 0,45 g% and 0,9 g%, irradiated with green light (λ=527 nm, intensity $3 \cdot 10^5$ Lx) were used. As a receptor for measuring the irradiation effect we used red blood cell (RBC) permeability in hypotonic media, the so-called osmotic shock. After RBC lysis in hypotonic medium, the released hemoglobin was spectrophotometrically determined at λ=550 nm. The values of the osmotic shock for the samples obtained with GL-irradiated NaCl solutions were significantly lower than the values obtained with non-irradiated controls. This may indicate that the penetration of water inside the membranes canaliculi is hindered. This new type of result was complemented by chronoamperometry and impedance spectroscopy determinations. The current density of the irradiated solution decreases from the value of 29.5 $\mu A \cdot cm^{-2}$ in the control to 17.74 $\mu A \cdot cm^{-2}$ in the irradiated sample with a corresponding decrease of ionic mobility. The impedance value of the GL irradiated NaCl solutions were significantly lower than the control values, thus correlating well with the data recorded by chronoamperometry. All these data may indicate large water cluster formation through GL irradiation which are beyond the cellular aquaporine channels capacity. A similar process is identified by using red light, as well as blue light, though with a much smaller output.

1. Introduction

The interactions between electromagnetic field and biological systems were extensively investigated, with remarkable results and advanced technologies. Nevertheless, the visible domain of the spectrum has been rather neglected, since classic physics did not allow electronic transitions induced by visible light.

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Starting with the '70 decade a research group centered around the Society of Quantum Biology (San Francisco) advanced the idea of possible "molecular activations", different from the "electronic excitations" level. Phenomenological models have been published, based on the molecular coherence at macroscopic scale with the ability of maintaining long-lived coherence, (see the pioneering paper of Fröhlich [1] on long range coherence in the enzyme activity). Within this group, Comorosan [2-4] published the first experimental evidence for these possible "molecular activations" performing experiments on enzymes and revealing kinetics changes through low level irradiations of their substrates. A string of papers on this new phenomenology appeared in the international journals of that time period (for reviews, see Comorosan [5]). The field expanded with the advent of new theoretical developments and of new type of technologies, in areas of free-space molecular diffraction, magnetic biomolecules, quantum optics and Josephson devices (see the review of Leggett [6]). A large new type of evidence appeared from the nanotechnology and from the biological field. Molecular films were designed displaying a surprising molecular coherence with space and time stability. The modern domain of quantum computing provided a wealth of data on biological quantum coherence and molecular superposition states. (see the review of Mavromatos and Powel [7]). An important contribution has been put forward by Penrose [8] in connection with molecular coherence in biological structures.

Penrose considers *quantum coherence* the circumstances when large numbers of particles can collectively cooperate in a *single* quantum state which remains essentially *unentangled* with its environment. This large scale quantum coherence is found in Bose-Einstein condensation (which occurs in laser action) where the entire system behaves as a whole, and in superconductors, when electrons have a way of "pairing up" in Cooper pairs, which behave as individual bosons. Theoretical and experimental developments by the Comorosan group [9-10] renewed the interest of visible light interaction with biological structures. The recently discovered variety of biological effects induced by irradiations in cellular systems [11] suggested the investigation of water, as a common element of cellular structures. Within this context, we developed a special geometrical experimental set-up to explore visible light, in particular green light induced effects on water from a biological as well as physical viewpoint.

2. Experimental

2.1 Osmotic Shock

When cells are placed in hypotonic medium water enters inside, (a Donnan-type of physical effect) [12] to readjust the internal osmotic pressure, and finally disrupt the cell. This is called the osmotic shock [13]. When red blood cells (RBC), are placed in such a medium, water enters through the membrane's tiny canaliculi and breaks the structure, releasing hemoglobin.

In this study we used RBC from blood samples obtained from patients at different clinical departments and Fundeni Clinical Institute, previously screened for normal biochemical parameters. Samples which displayed at a preliminary checking an osmotic shock value between 0.220-0.320 $AU_{550\ nm}$ are used for the experiments with GL-irradiated NaCl solutions. Physiological solutions (0.9%) of NaCl represent internal controls, 0.45% NaCl hypotonic solutions Control (C) and GL-irradiated (I^*) samples. GL-irradiations are performed in air with a two Cree LED (100 Lumens) device, emitting λ=527 nm, $3 \cdot 10^5$ Lx, on 3.0 ml NaCl solutions, in small glass dishes (2.5 cm diameter). RBC-are washed in phosphate buffer solution [PBS]. 10 μl of cells are placed in the (C) and (I^*) solutions, for 1 min., centrifuged for another 1 min. at 5000 rpm and the supernatant checked spectrophotometrically at λ=550 nm. Sets of 20 experiments are realized, with irradiations times of 15, 30, 45 and 60 min. Experiments were performed at room temperature.

Variations of temperature (<0.3^{o}C) and evaporation of solutions (<0.015g) are rigorously controlled and included in all the results.

In this study, to complement the osmotic shock investigation (which includes only a biological segment) with a physical determination, we used two electrochemical techniques: 1-Chronoamperometry, through which one may compute ionic mobility, hence a direct evaluation of

"clusters" formation. 2- High resolution impedance spectroscopy, which links three important electrochemical parameters: resistance, capacity and inductance.

2.2 Chronoamperometry

Chronoaperometry was recorded with a high-level performance electrochemical combine (accuracy ±0.2% of range), VoltaLab 40, Radiometer Analytical, with Platinum Plate Electrode, Saturated Calomel Electrode, and Cell with external thermostatic jacket. Solutions of 0.9% and 0.45% NaCl were used as electrolyte. A rigorously measured volume of 3.5 ml is placed on two identical electrochemical cells (Control & Irradiated). Irradiations were performed through a hole in a Faraday-type cage, in dark, with the irradiation device fixed outside, on top of it. Two LED`s of 100 Lumens each on a ventilated copper radiator, emitting a $\lambda=527$ nm at $3 \cdot 10^5$ Lx were used. The electrochemical technique, (current density vs time at a fixed potential) determines the current density j, through which ions mobility $(u^+ + u^-)$ may be computed. Ten samples were measured, for the control and irradiated probes. In order to evaluate the conductivity and ion mobility, the galvanostatic technique at an applied potential of 50 mV was performed.

The recorded current density is the number of charges that cross in unit time and the unit area, due to the migration of ions:

$$j = z_i Fc(u^+ + u^-)E$$

where: j–current density; z_i-coulombian charge (1 in the case of Na^+ and Cl^-), F- Faraday constant$=9.65*10^4$ C/mol; $u^+ + u^-$–ionic mobilities for Na^+ and Cl^- ; c–molar concentration; E–applied electric field.

2.3 Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a powerful method to characterize many of the electrical properties of solution and interfaces with electronically conducting electrodes.

Impedance Spectroscopy has been recorded using a Zahner IM6 Khronach (Germany) electrochemical unit with <0.0025% frequency stability. A specially designed miniaturized electrochemical cell (50 μl) with two platinum bands electrodes (1 mm width, 0.1 mm thickness) is used. A volume of 1 ml NaCl 0.9% is GL irradiated for 45 min. in a small Petri dish (ø=30mm), with two LED$_s$ of 100 Lumens each on a ventilated copper radiator, emitting a $\lambda=527$ nm at $3 \cdot 10^5$ Lx. Blue light irradiations are performed in air with one Cree LED (75 Lumens) device, emitting at $\lambda=455$ nm, $3 \cdot 10^5$ Lx, on 1 ml NaCl solutions, in small Petri dish (ø=30mm). Red light irradiations are performed in air with one Cree LED (118 Lumens) device, emitting $\lambda=625$ nm, $3 \cdot 10^5$ Lx, on 1 ml NaCl solutions, in small Petri dish (ø=30mm). Volumes of 50 μl from the control and from the irradiated samples are then introduced into the electrochemical cell for recording. Impedance spectrum was measured in the 1-10 kHz frequency domain. High frequency impedance measurements in the frequency range of 10^3 -10^4 Hz, were employed to eliminate the double layer impedance on platinum /electrolyte surfaces, thereby allowing correct conductive behavior to be observed.

The measurements gave us immediately the complex impedance, $Z^* = Z' - iZ''$, and complex conductivity $\sigma^* = \sigma' + i\sigma''$.

3. Theory/calculation

In order to detail the mechanism, that takes place in water molecules, we have performed our quantum mechanical modeling on a well defined chemical structure, using Gaussian 03 software. The molecular geometry was optimized using Kohn-Sharm theory. The Hartree-Fock functional and the 6-31g* polarized functions were used as basic sets. A constant electric field of 0,05 a.u. has been applied along the axis between two hydrogen atoms in order to simulate the green light action.

4. Results

4.1 Osmotic Shock

The main result obtained with this biological type of technique is a clear effect of green light on hypotonic NaCl solutions.

As seen from table 1 the values of the osmotic shock for the samples obtained with GL-irradiated NaCl solutions are significantly lower than the values obtained with non-irradiated NaCl solutions of the controls. This may indicate that the penetration of water inside the membranes canaliculi is hindered. A possible interpretation of this result may be the generation of large water aggregates (clusters), under the GL-irradiation, which "slide" less efficiently along membranes canaliculi.

Table 1. Irradiation time dependence of the GL effect on hypotonic saline solutions

Irradiation times (min.)	Controls, $AU_{550\ nm}$ $\pm$ SD	GL-Irradiated[*], $AU_{550\ nm}$ $\pm$ SD
15	0.225 ± 0.040	0.181 ± 0.031
30	0.228 ± 0.021	0.170 ± 0.039
45	0.321 ± 0.052	0.246 ± 0.021
60	0.246 ± 0.035	0.158 ± 0.039

4.2 Chronoamperometry

We represent in table 2 the effect of GL-irradiation on ionic mobility in a 0.9% NaCl solutions irradiated 60 min. by GL. As seen from the table, the current density of the irradiated solution decreases from the value of 29.5 $\mu A.cm^{-2}$ in the control to 17.74 $\mu A.cm^{-2}$ in the irradiated sample with a corresponding decrease of ionic mobility, from $6.804 \cdot 10^{-10}$ $(m.s^{-1}/V.m^{-1})$ to $4.085 \cdot 10^{-10}$ $(m.s^{-1}/V.m^{-1})$. This result correlates well with the osmotic shock values, suggesting formation of large molecular aggregates of cluster type, which may decrease ionic mobility.

We represent in fig.1 the direct instrument recording of an experiment of 60 min. GL-irradiated 0.45% NaCl solution as compared with the control and fig. 2 the representation of a 60 min. GL-irradiated 0.9% NaCl solution as compared with the control.

The values represent results of chronoamperometry at the fixed potential of 50 mV vs Saturated Calomel Electrode.

Table 2. Effect of 60 min GL-irradiation on current density & ionic mobility in NaCl solution

sample	c [g/100 ml]	j_{max} ($\mu A*cm^{-2}$)	$\mu*10^{-8}$ ($m^2/V*s$)
control	0.45	20	5.38
GL	0.45	11.5	3.09
control	0.9	40	5.38
GL	0.9	23	3.09
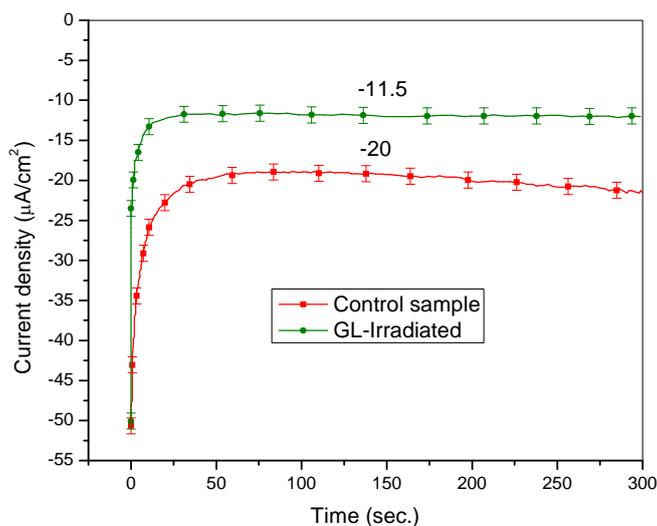
Fig.1: Current density recording (µA·cm⁻²) of a 0.45% NaCl solution, 60 min. GL-irradiation, as compared with the non-irradiated control. Values represented at 300 sec. of recording.
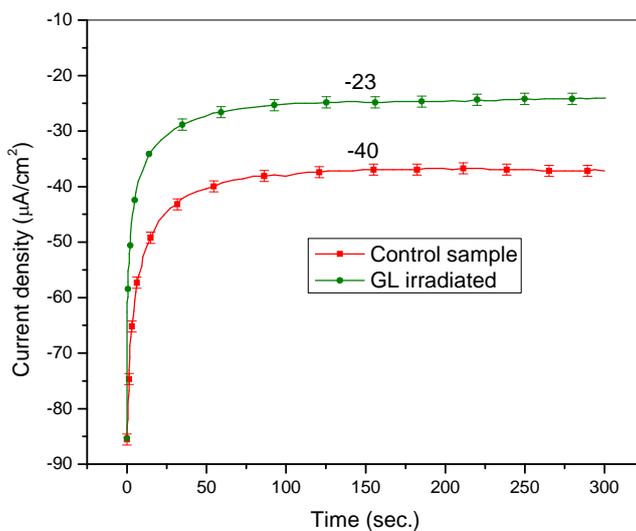
Fig.2: Current density recording (µA·cm⁻²) of a 0.9% NaCl solution, 60 min. GL-irradiation, as compared with the non-irradiated control. Values represented at 300 sec. of recording.

4.3 Impedance Spectroscopy

Since the impedance spectroscopy may reveal ionic transport properties, our results may indicate large molecular aggregates generation which may alter the ionic mobility. As seen from fig.3 the impedance value of the GL-irradiated 0.9% NaCl solution is significantly lower than the control, thus correlating well with the data recorded by chronoamperometry. The experiments performed by chronoamperometry and impedance spectroscopy on ionic mobility all correlate well with the results obtained on the osmotic shock, suggesting water clusters formation in the respective GL-irradiated water solutions. The impedance values obtained with the blue and red

EM-visible bands present intermediate values, thus indicating the green band as the most efficient one.
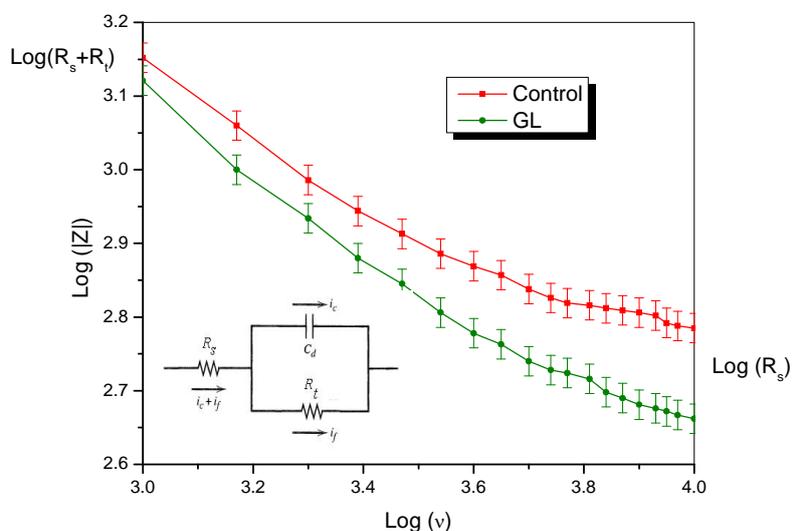
Fig. 3: Impedance spectroscopy recording of a 0.9% NaCl solution, irradiated 45 min. in the green, blue and red visible light band and of the nonirradiated control. C_d-double layer capacity, R_s-water resistance, R_t-charge transfer resistance

Impedance spectroscopy results were interpreted by using ZView software and indicate a slightly increasing of the charge transfer resistance in the irradiated sample, supporting the idea of water clustering.

4.4 Computational simulations

High green density photons action on the water molecules is equivalent with the applying of an electric field. Simulation of different orientations for this electric field along all three axis, suggests better results when is applied between the two hydrogen atoms. A complete image of the increasing for the electric dipole when the light is acting is shown in the figure 4.
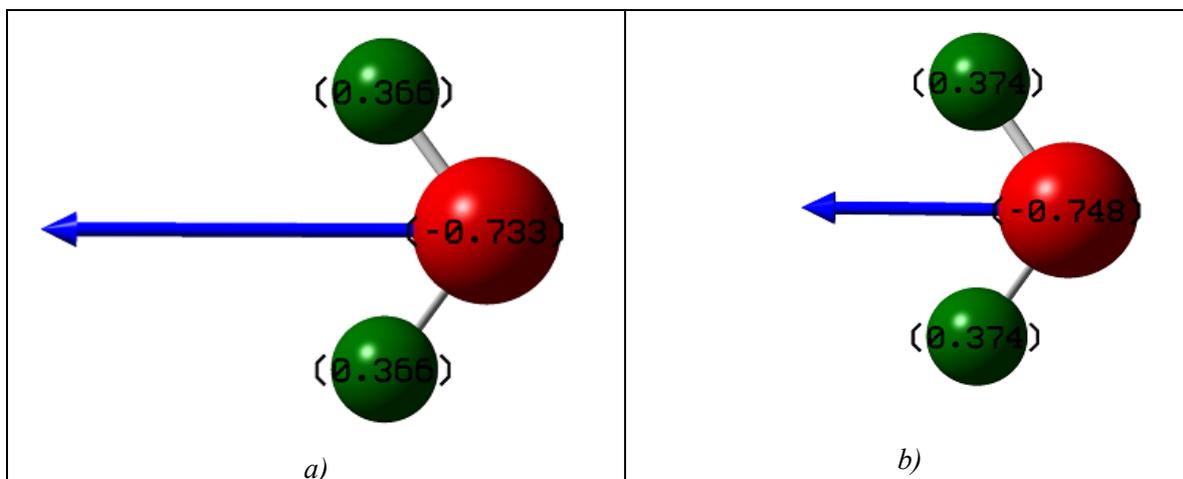
Fig. 4 Charge distribution and dipole lengths: a) before, b) after irradiation

The results of the computational simulation are given in the table below:

Sample	Dipole length	Angle
Control	2.3871 D	105.45
Irradiated	1.4937 D	110.52

5. Discussions

The elusive and highly dynamical water structure has generated numerous investigations as well as discussions and controversies. In biology a long interest in the cell-associated water is recorded, originating with the Drost-Hansen concept of "vicinal" water [14]. The large, more or less empirical evidence has been recently complemented by the advent of new domains with new technologies: molecular dynamics simulations and computer generated models. The concept of "polywater" as the most adequate possible configuration is presently prevailing [15].

The association of water molecules to form aggregates ("clusters") has a solid chemical ground. Water is a polar molecule, forming layers of clusters by attraction, mainly by hydrogen bridges (binding energy of ~20 kJ/mol), and, to a smaller extent, by dipolar and van der Waals forces. In solution, the structure of the first hydration layer around a molecule is strongly dependent on the surrounding molecular field configuration.

The recent techniques of solvation dynamics and quantum mechanics methods of computer simulation have succeeded in generating complex water cluster structures (see for review M.Chaplin: http://www.lsbu.ac.uk/water/clusters.html, 2006). The basic arrangement seems to be the tetrahedral unit with 14-water molecules.

It is thought that a series of substructures would finally generate the complex cluster of 14 tetrahedral units in a icosahedral structure of 280 water molecules. Let us point that this type of structures has been determined also by physical methods. Coutinho [16] measured a cluster of 142 water molecules with long range effects up to 10 Å from the center. Let us observe at this point that the icosahedral symmetry (I_h) is one of the highest symmetries possible in nature; at present time it is the most probable candidate for favored clusters in water [17]. Let us also observe that this domain is still in a rather hypothetical stage and in this context our comments should be considered, intended only as a framework for a possible interpretation of our experimental results.

A suggested mechanism. We assume that the green photons may react with the water aggregates found in normal cellular compartments, and modify their cluster structures. This hypothesis seems to be supported by the cooperativity effects observed in water that have been reported recently [18].

The molecular dynamics and computer simulation studies have determined that different hydrogen bonding configurations may induce cluster stability changes that result in collapse or expansion of the cluster's network, with fluctuations between the expanded (ES) and collapsed (CS) forms. The expanded (ES)-structure is formed when stronger hydrogen bonds are present, as a result of structuring solutes and surface interactions.

We suggest that GL may change the [ES] ⇄ [CS] equilibrium, favoring the [ES] configuration, with large cavities, that may allow occupancy by suitable components (a property suggestive for cells biochemistry) and may thus accommodate the UV-induced hydroxyl radical (H:O·) and fix it in the network [9] – hence the free radical scavenger GL-effect, observed on biological systems. Similar effects were observed with other bands of the visible light spectrum, though with a much smaller output.

Let us observe in this context that the [ES] configuration may be induced through the electromagnetic field of the green photons, by extending the H-O-H angle of the water molecule, (105.45°) thus realizing stronger hydrogen bonds, which in turn change the density of the structure, to 0.94 g·cm^{-3} from the 1.00 g·cm^{-3}density of the collapsed configuration.

This fact is supported by the computational simulations. In the control sample, the H-O-H angle is 105.45° and the electric dipole is 2.3871 D. By applying the electric field along the dipole moment in the opposite direction, a reduction of the dipole length up to 1.4937 D, together with an increasing of the H-O-H angle up to 110.52°, is recorded.

Our hypothesis may acquire a particular significance in the context of the osmotic shock results. In the RBC, hydrophobic interactions are a driving force that generates well known lipid bilayers structures. At the peripheral level a series of proteins are bound to membrane surfaces by electrostatic and hydrogen-bond interactions, involved in ionic transport and communications. The discovery by Benga [19] of a red blood cells transport protein, denoted *aquaporin,* [20] involved in water permeability, opened a new field of investigations. The protein has an extremely high permeation rate $\sim 10^9$ H_2O molecules.sec^{-1} per channel. The architecture of the aquaporin channel allows water molecules to pass only in single file and moreover, the electrostatic tuning of the channel interior controls aquaporin selectivity against charged species such as salts and hydroxyl ions. These are the membrane properties that prevent the newly, GL-induced, larger water clusters from entering the cell.

Thirteen homologs of aquaporins have been identified by now. They appear to have significant clinical implications [21]. Although aquaporin expression is not required for structural integrity of RBC membrane, the physiology of the membrane is dependent on it. Preliminary clinical studies have indicated such a possible link with pathology [22].

The physics of the green light effect, as revealed in our experiments, is a much more complex phenomenon. When interacting with matter it generates excited states that may react chemically. The green photon, as part of the visible spectra, is not absorbed by transparent materials. We have discussed in detail the physics of GL as revealed in our previous studies [10], within the context of non-linear Raman-type transitions, through a simultaneously multiphotons absorption mechanism [23]. The light interaction with a system is determined by the density of dielectric polarization, which defines the interaction of light with the electrons. In homogenous media, the polarization is proportional with the electric field E, expanded in Taylor series as follows:

$$P_i \Big/ \varepsilon_0 = \sum_j \chi_{ij}^{(1)} E_j + \sum_k \chi_{ijk}^{(2)} E_j E_k + \sum_l \chi_{ijkl}^{(3)} E_j E_k E_l + \ldots$$

where P_i- polarization density (dipole moment per unit volume) for the *i*-species, ε_0-vacuum dielectric constant, χ-high order nonlinear terms describing medium polarizability which are dependent of the normal coordinate of the vibrations and *E*- applied electric field. The third term describes the Raman processes, and, more important, multi-photon absorption/emission. This term is proportional with E^3 and the efficiency is 10^{-3} from the power of incident light. To obtain such an effect an intensive light is needed, which is exactly the set-up of our experimental models, using powerful LED's.

This may lead to polarization phenomena resulting in long range type of interactions, which finally may generate large molecular aggregates (water clusters), connected with the present phenomenology. The long range interactions as presented in the above theoretical discussion may account for our suggested mechanism for the [ES] $\rightleftarrows$ [CS] equilibrium through a process of molecular aggregates formation. Let us emphasize the significance of experimental water cluster demonstration, for a highlight field, in which direct experimental observations of this phenomenon are scarce. As a final point: the novelty of our experimental set-up rests on the method to detect physical phenomena, using biological receptors. Due to their complex topological structure, their resolution power surpasses by far the most sophisticated physical instruments. This might be a concept of biological spectroscopy suggested long ago by Comorosan [24].

References

[1] H. Frőhlich, Nature, **228**, 1093 (1970).

[2] S. Comorosan, Nature **227**, 64 (1970).

[3] S. Comorosan, Vieru S., Sandru D., Int. J. Radiat. Biol., **17**, 105 (1970).

[4] S. Comorosan, S.Vieru, P. Murgoci, Biochim. Biophys. Acta **268 (3),** 620-1 (1972).

[5] S. Comorosan, J. Biol. Physics, **17**, 105 (1990).

[6] A.J. Leggett, J. Phys. Condens. Matter, **14** R415, (2002).

[7] N. Mavromatos, K. Powel, On the possibility of Quantum Coherence in Biological Systems in Decoherence & Entropy in Complex Systems, Elze H.T. Ed. Springer (2000).

[8] R. Penrose, Quantum theory and the brain in Shadows of the mind, Oxford University Press (1994).

[9] S. Comorosan, W. Kappel, I. Constantinescu, M. Gheorghe, J.Biol.Physics**, 35(3),** 265 (2009).

[10] S. Comorosan, S. Polosan, I. Popescu, E. Ionescu, Eur Biophys J, **39(11),** 1483 (2010).

[11] S. Apostol, C. Stihi, C. Oros, J. Optoelectron. Adv. Mater. **9(9)**, 2921 (2007).

[12] Royal Society of Chemistry, IUPAC Compendium of Chemical Terminology, Second Edition, Blackwell Science, (1997).

[13] R.P. Rand, V.A. Parsegian, D.C. Rau, Cell Mol Life Sci. **57(7),** 1018-32, (2000).

[14] W. Drost-Hansen, Biophysics of Water (eds. F. Franks and S. Mathias). pp. 163-169. New-York, Wiley J and Sons Inc. (1982).

[15] R.M. Stratt, M. Maroncelli, J. Phys. Chem. **100**, 1016 (1996).

[16] K. Coutinho, S. Canuto, J.Chem.Phys., **113**, 9132 (2000).

[17] H. Tanaka, J. Chem. Phys. **112**, 799 (2000).

[18] W.A.P. Luck, J. Mol. Struct. **448**, 131 (1998).

[19] Gh. Benga, O. Popescu, V. Borza, V.I. Pop, Eur J Cell Biol **41 (2),** 252 (1986).

[20] P. Agre, L. S King, M. Yasui, W. B Guggino, O. P. Ottersen, Y. Fujiyoshi, A. Engel, S. Nielsen, J. Physiol. **542**, 3 (2002).

[21] P. Agre, Biol.& Cell. **97**, 355 (2005).

[22] S.C. Liu , J. Palek, S.J. Yi , P.E. Nichols, L.H. Derick , S.S. Chiou , D. Amato , J.D. Corbett , M.R. Cho , D.E. Golan , J .Hematol. **86(1)**, 349 (1995).

[23] S. Comorosan, S. Polosan, S. Jipa, I. Popescu, G. Marton, E. Ionescu, L. Cristache, D. Badila, R. Mitrica, J. Photochem. Photobiol. B*:* **102(1)**, 39 (2011).

[24] S. Comorosan, Biological observables in Progress Theoretical Biology, Academic Press, New York, pp 161-203. (1976).