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SPECTROSCOPY OF LIGHT SCATTERING

Bimodal Dependence of Light Scattering/Fluctuations on the Concentration of Aqueous Solutions

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Abstract—Two concentration ranges (from 10^{-5} to 10^{-9} and from 10^{-13} to 10^{-18} M) corresponding to enhanced fluctuations of Rayleigh and Raman scattering of second-harmonic (527 nm) pulses of YVO_4:Nd^{3+} laser are found for aqueous solutions of antioxidant potassium phenosan. A correlation is revealed between the rise in elastic Rayleigh scattering intensity and its fluctuations and the shift of the center of OH Raman band of water toward the ice component characteristic frequency (3200 cm^{-1}). The development of phase-equilibrium instabilities is analyzed based on the model of fluctuations of the number of hydrogen bonds on the assumption of formation/destruction of ordered hydration layer of phenosan molecules in water.

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1. INTRODUCTION

Brownian motion and collisions of water molecules are known to be accompanied by fluctuations of the number of hydrogen bonds [1] and formation/destruction of hydrogen-bonded complexes of different configurations [2] at order–disorder transitions. The fluctuation amplitude increases significantly in the vicinity of critical water temperature points, e.g., 4°C [1], which manifests itself in broadening/deformation [3, 4] of the OH Raman band envelope at critical points (4, 19, and 36.6°C). It is of importance that the centroid of the OH Raman band, introduced by us in [3–5] as an indicator of entropy temperature evolution in water [4], undergoes a sharp shift by $\sim$150 cm^{-1} toward the high-frequency wing of the OH band at the ice–water phase transition [3, 4]. This high sensitivity of the shift of OH band centroid to a variation in the fraction of ice-like complexes in water (i.e., to the number of hydrogen bonds) was used by us for remote measurements of water temperature by a Raman lidar [6, 7]. In addition, processing Raman spectra in this way made it possible to substantiate the reversion of convection [8] upon heating or cooling water in the vicinity of the temperature point corresponding to maximum density (4°C) due to the thermally induced transition between the phases of denser and less dense water with ice-like frameworks, which is accompanied by fluctuation of the number of hydrogen bonds [1].

Note that the hexagonal ice structure is most likely among the large variety of configurations of hydrogen-bonded complexes in water [2]. We drew this conclusion based on the analysis of the experimental data obtained by different physical methods in several laboratories. In particular, small-angle X-ray scattering [9, 10] showed simultaneous existence of amorphous complexes, framework ice-like structures, and H_2O monomers in water. Atomic-force microscopy revealed an ordered structure with a period of I_h ice lattice in a water layer at room temperature [11]. Further study of the spontaneous ice growth (which occurred in only three modifications: amorphous, cubic, and hexagonal) from H_2O vapor on the surface of liquid helium and subsequent thermally induced phase transformations of these modifications upon heating showed [12] that the dominant ice modification at temperatures above 200 K is hexagonal ice I_h, the characteristic component of which ($\sim$3200 cm^{-1}) is always observed in the

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spectra of water [13] up to the boiling temperature [14]. It is of importance that this component is pronounced in the spectra of large clusters formed in beams of H_2O molecules [15, 16]. Moreover, specifically this component made it possible to reveal for the first time [15] the size effect in the spontaneous transition from an amorphous close packing of H_2O molecules to ordered ice-like frameworks. This effect is induced by thermal fluctuations of the number of hydrogen bonds in molecular-beam clusters. The occurrence of a low-frequency shoulder was observed in the OH band envelope at the characteristic ice component ($3200\,\mathrm{cm}^{-1}$) when, as was suggested in [2], the number of molecules per cluster exceeded 275, and the pressure in the cluster reduced because of the decrease in the surface curvature. The decrease in pressure is accompanied by an increase in the amplitude of intermolecular-distance fluctuations to the hexagonal-ice lattice period and formation of framework hydrogen bonds. The above facts justify the existence of ice-like structures with varied sizes (numbers of hydrogen bonds) in water; these variations manifest themselves in Raman spectra as anomalously large fluctuations of the OH-band envelope and centroid [17].

At the same time, it is known that elastic Rayleigh scattering in aqueous solutions is rather sensitive to variations in the concentration of material during its hydration. In particular, an analysis of the physics of aqueous solutions [18] showed the existence of concentration ranges characterized by a multiple increase in the elastic light scattering amplitude. This increase is a manifestation of hydration processes during the formation of large molecular clusters as a result of phase transitions, which are accompanied by the formation of an interface (spinodal) between two liquids [19, 20]. It is of importance that this anomaly is observed in solutions having unlimited solubility, for example, alcohols [18]. Moreover, according to the recent four-wave mixing spectroscopy data [21], there is a hysteresis in the concentration dependence of the Rayleigh linewidth in water–ethanol and ethanol–water binary solutions, which is indicative of nonreciprocity between mixing with the solvent and hydration of solute molecules.

A very interesting fact is the increase in the Brillouin shift in the spectrum of aqueous protein solution with an increase in concentration, which was found by four-wave mixing [22]. The cubic concentration dependence of the Brillouin shift toward to the values characteristic of bulk ice was interpreted by us as the increase in the solution elasticity because of the increased number of ice-like frameworks in hydrate protein layers, like in the case of spontaneous crystallization. This conclusion is consistent with the correlation (established in [23]) between the structural quality of lysozyme protein crystals (according to the X-ray diffraction data) containing up to 70% water and the increase in the contribution of the I_h ice component ($3200\,\mathrm{cm}^{-1}$) to the OH Raman band envelope.

Naturally, one would expect the formation of ice-like frameworks with an effective diameter of several hundreds of nanometers [24–26]. The size of frameworks (hydration shells) is comparable with the laser spectrometer wavelength (527 nm) and may have a soft [19, 20] interface or spinodal during hydration of molecules in solutions. Then it should be manifested in a change in the elastic scattering amplitude at unshifted probe frequency [18], a fluctuation of this amplitude, and a shift of the OH Raman band center [3–6], simultaneously. However, no such measurements have been performed previously. To fill in this gap, we carried out corresponding experiments with solutions of potassium phenosan (PP); the properties of this synthesized antioxidant had been investigated in a number of studies.

2. EXPERIMENTAL

Potassium phenosan. Aqueous solutions of many biologically active substances (BASs) are known to exhibit biological effects (change in various properties of biological systems treated by BAS aqueous solutions) both at conventional (physiological, therapeutic) solute concentrations (from 10^{-2} to 10^{-7} M) and at very low (from 10^{-10} to 10^{-20} M) concentrations [27]. One of these agents is synthetic antioxidant 3-(3,5-di-tret-butyl-4-hydroxyphenyl) potassium propionate, or PP (Fig. 1); it was synthesized and characterized at Professor Ershov's laboratory (Institute of Biochemical Physics, Russian Academy of Sciences) [28].

Due to the presence of hydroxyl group (bound to the phenol ring), PP can easily provide a hydrogen atom to reduce free radicals and thus interrupt chain oxidative reactions in lipids. The presence of tret-butyl groups in the 3,5-positions of the PP phenol ring makes the perpendicular orientation of the hydroxyl group more likely and thus facilitates its break.

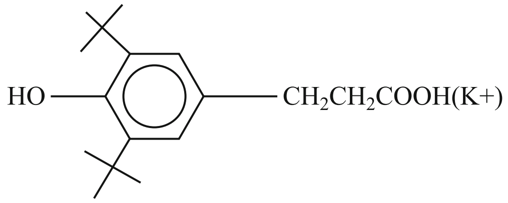

Fig. 1. Structural formula of synthetic antioxidant 3-(3,5-di-tret-butyl-4-hydroxyphenyl) potassium propionate.

These groups are also involved in delocalization of spin density of phenoxyl radical [28], thus making this radical more stable. Undoubtedly, the main physicochemical property of PP is its antioxidative activity, which was revealed both in purely chemical models and in the lipid peroxide oxidation (LPO) occurring in biological membranes; in the latter case, the effect was observed even at ultralow doses (ULDs) [29]. Along with the ability to LPO inhibition, PP was found to exhibit activity at ULDs in a series of samples, both in vitro and in vivo. Despite the variety of experiments and measured values, the PP effect was most pronounced in the concentration ranges from 10^{-4} to 10^{-6} M and from 10^{-14} to 10^{-15} M; it manifested itself in the activity of membrane-bound enzymes of acetylcholinesterase, glutathione peroxidase and glutathione reductase of mitochondria, microsomal and cytoplasmic forms of aldolase and lactate dehydrogenase, and protein kinase C [29]. A change in the physicochemical properties of the lipid component leads to modification of the structural and functional state of membrane-bound proteins, which is confirmed by experiments on the effect of PP ULDs on the thermodynamic properties of proteins (change in the enthalpy and transition temperature) [30].

Thus, the processes occurring in a biomembrane under PP effect in a wide concentration range can currently be considered as well-established. However, the processes causing structural transformations in aqueous solutions remain unclear. It is important that, on the whole, the data obtained by us suggest definitely that specifically water is responsible for the BAS effect at ULDs (it was experimentally shown that the BAS effect is absent in the ULD range when BAS is dissolved in vaseline oil rather than in an aqueous medium) [31]. It was also found that the IR spectra of low-concentration aqueous solutions characterized by biological effect differ significantly from standard spectra [32]. The study of aqueous solutions by elastic and Raman scattering was continued based on the experience gained in the analysis of PP solutions [29–32] and the data of [26] and other studies.

Phenosan potassium solutions for our experiments were prepared at the Institute of Biochemical Physics, Russian Academy of Sciences, by successive hundred-fold dilutions of the initial solution with as-prepared double-distilled water (having a conductivity of 1.5 μS). Two series of PP solutions were prepared with a month's interval. The concentration of solutions decreased with a step of 10^{-2} M, from 10^{-4} to 10^{-20} M in the first series and from 10^{-3} to 10^{-21} M in the second series. The solutions were poured into identical plastic test tubes (15 mm in diameter and 100 mm high) and closed with a plug. The solution column height (90 mm) was the same in all test tubes. The test tubes filled with solution were stored for 1 or 2 days in a refrigerator at about 8°C. All measurements on solutions were performed at room temperature.

Spectrometer. The Raman–Rayleigh spectrometer (see [33]) was based on a pulsed $YVO_4{:}Nd^{3+}$ LCM-DTL-329QT diode-pumped laser (pulse duration 7 ns, power up to 200 μJ) (www.laser-compact.ru) and a frequency doubler (527 nm). Test tubes with solution were installed in a holder, which provided their identical position in each session of measurements. The test tubes were inclined at a fixed angle with respect to vertical (~10° to 15°) in order to exclude the uncontrolled contribution of the reflective glare from the solution surface to the backscattering signal. The laser beam was directed along the test tube axis to make the same angle with the normal to the solution surface (which was open). Under these conditions, the surface reflective glare did not enter the aperture of the spectrometer lens, which served to focus the signal onto the spectrometer input slit. The spectrometer made it possible to record simultaneously a Raman signal at the water OH vibrational frequency and a Rayleigh (elastic scattering) signal at the pump frequency. A 2-mm-thick OR-13 glass filter was installed before the input slit to attenuate the elastic scattering signal in the solution.

3. RESULTS AND DISCUSSION

The scattering spectrum of each pulse, arriving with a repetition rate of 12 Hz, was automatically summed with the spectra of subsequent pulses (a sample of 100 spectra was set by operator) and recorded in the data file. Ten files were recorded for each sample at identical positions of test tubes. Typical scattering spectra of PP solutions with concentrations of 10^{-11} and 10^{-17} M (lines *1* and *2*, respectively), obtained by summation over 10 samples (1000 spectra) and subtraction of the baseline, are presented in Fig. 2. The frequency scale (X axis) was chosen in wave number units (cm^{-1}). The spectra demonstrate a shift of the OH band by ~3500 cm^{-1} from the pump frequency toward the Stokes spectral region. One can clearly see that the amplitude of the elastic scattering line (0 cm^{-1}) changes significantly with the solution concentration, which is accompanied by a decrease in the OH band amplitude with an increase in the pump loss in the solution at elastic backscattering.

The concentration dependence of the integrated elastic scattering line was obtained from experimental spectra after subtracting the background baseline. The scattering line was integrated (i.e., the area under curve was determined) by the trapezoidal method, and the first and second moments of measured values

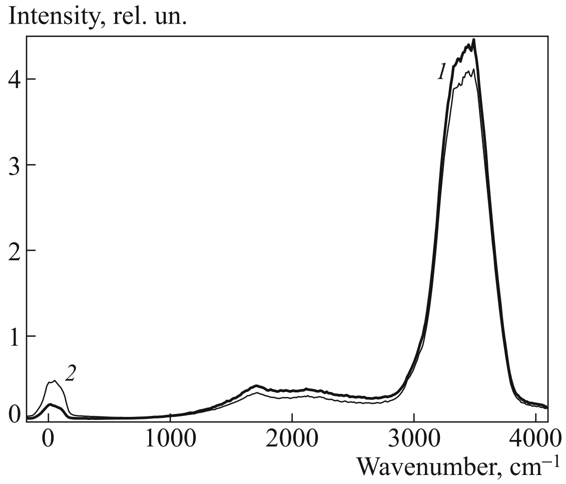

Fig. 2. Typical scattering spectrum of laser pulses in PP solutions with concentrations of 10^{-11} (curve *1*) and 10^{-17} M (curve *2*).

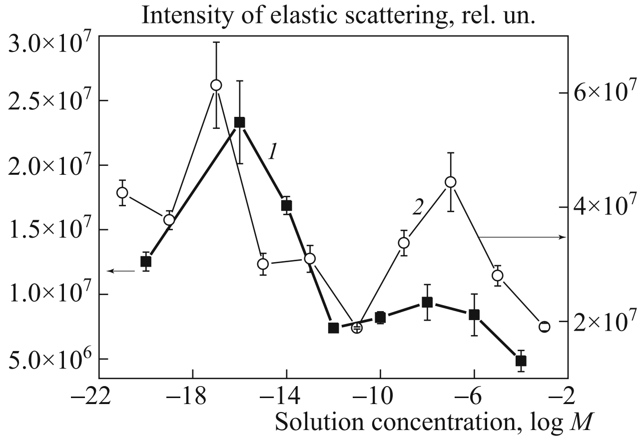

Fig. 3. Nonmonotonic concentration dependence of integrated elastic scattering profile of 527-nm laser pulses in PP aqueous solutions with even (curve *1*, left ordinate) and odd (curve *2*, right ordinate) degree of concentration.

were calculated. For each concentration, characterized by integrated elastic scattering intensity

$$I_k = \overline{I}_k \pm \Delta I_k, \tag{1}$$

where k is the concentration index, we calculated the mean integral of the elastic scattering line, $\overline{I}_k$, averaged over 10 spectra,

$$\overline{I}_k = \frac{1}{N}\sum_{i=1}^{N} I_{ki}, \tag{2}$$

and the standard deviation $\Delta\overline{I}_k$ from the mean or the error in measuring the line profile over $N = 10$ spectra:

$$\Delta I_k = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(I_{ki} - \overline{I}_k)^2}. \tag{3}$$

The results of the analysis of the fluctuation value as a function of concentration in the solution of synthesized antioxidant PP are presented in Fig. 3 for two series of measurements. For convenience of analysis, the plots were aligned in the vicinity of a concentration of 10^{-11} M. It can be seen that both dependences are simbatic, with a characteristic minimum in the concentration range from 10^{-10} to 10^{-12} M. With a decrease in the solution concentration, the backscattering coefficient twice reaches a maximum in the ranges of 10^{-5} to 10^{-8} and 10^{-14} to 10^{-18} M. This maximum is indicative of second-order phase transition during structure transformation (change in the number of hydrogen bonds [1]) of solutions, as was established previously in [18–20]. The observed increase in elastic scattering fluctuations (see Fig. 3) with a simultaneous increase in their amplitude (see Fig. 2) is an additional manifestation of phase transitions in the solutions under study [18–20].

Note also that the concentration ranges characterized by the strongest elastic scattering correlate well with the increase in the effective diameter of nanoassociates [25, 26] and with the extrema of other parameters of ULD solutions: conductivity [25] and bioactivity (exhibiting a maximum) [26]. The correlation obtained by us and the bimodality of the concentration dependence of elastic scattering coefficient expands the instrumental base for studying this phenomenon and increases the reliability of previous results. The observed nonmonotonicity and asymmetry (Figs. 3 and 4) of the bimodal dependence (the amplitude of Rayleigh backscattering and its fluctuations are larger by a factor of 1.5 to 2 in the low-concentration range) indicate a change in the dominant mechanism the nature of which is

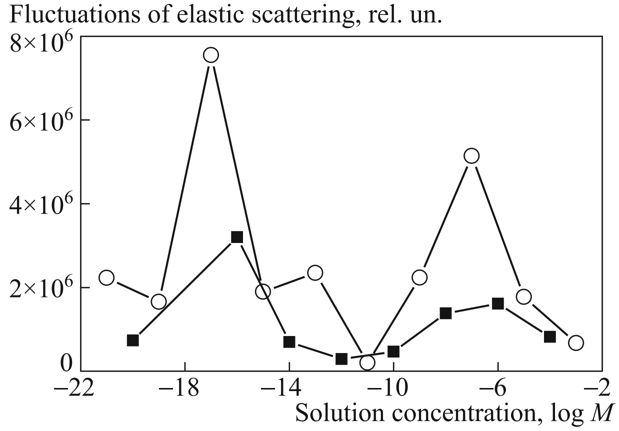

Fig. 4. Dependence of the fluctuation of elastic-scattering coefficient in a PP aqueous solution on its concentration at even (○) and odd (■) degree of dilution.

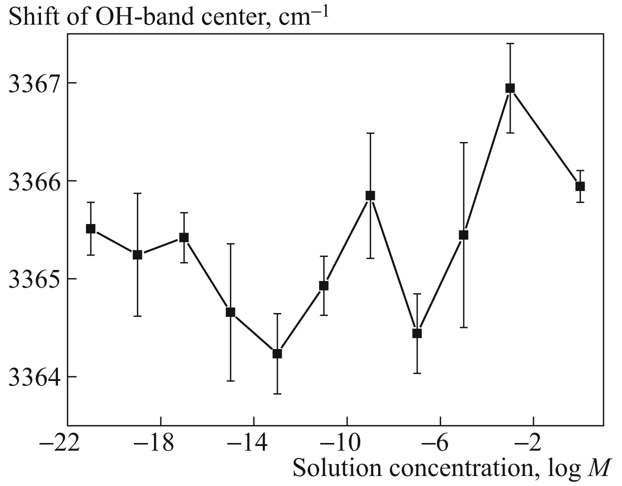

Fig. 5. Nonmonotonic concentration dependence of the shift of OH-band center in the Raman spectra of PP aqueous solutions exposed to 527-nm laser pulses.

still unknown [26]. The difference in the formation mechanisms of bimodal dependence is also evidenced by the smoothed response in the low-concentration range which is observed when samples are put in a permalloy container to shield magnetic fields [34].

In accordance with the proposed model, we allow for the formation of hydrate layers (increase in diameter [26]) with a hexagonal ice structure [22, 23] around PP molecules (as crystallization centers, especially in the low-concentration range, characterized by a large intermolecular distance). The existence of this structure is evidenced, for example, by the increased coefficient of elasticity of aqueous solutions, which was observed by us previously for hydration of α-chymotrypsin [22], as well as the correlation between the structural quality of ice-like structures of hydrated lysozyme molecules and the increase in the 3200-cm^{-1} component of hexagonal ice in the OH-band envelope [23]. These facts give grounds to expect asymmetric deformation of the OH band or shift of its center in our case.

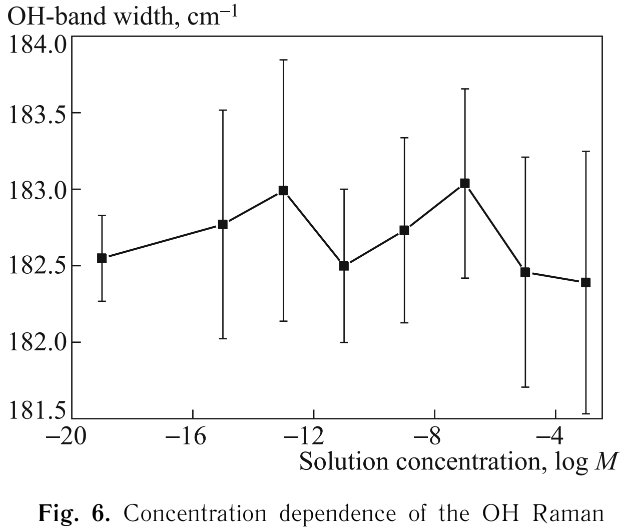

Fig. 6. Concentration dependence of the OH Raman band width for PP aqueous solutions.

The measured values of the shift of the OH band center in the Raman spectra recorded with 527-nm laser pulses and the OH band width as an indicator of the number of OH oscillators (number of hydrogen bonds [1]) in PP aqueous solutions are presented in Figs. 5 and 6, respectively. Indeed, one can see a bimodal dependence of the redshift of the OH-band center (ice component at 3200 cm^{-1}) in the concentration ranges from 10^{-5} to 10^{-8} M and from 10^{-14} to 10^{-18} M and the increase in the bandwidth. The large spread in the values of the center shift (see Fig. 4) and band width (see Fig. 5) can be explained by the occurrence of intrinsic spontaneous thermally induced fluctuations of the number of hydrogen bonds [1] and structures [10–16, 23] in water, as well as the nonoptimal size (100) of the averaging sample of spectra in measurements.

4. CONCLUSIONS

Thus, we revealed a bimodal concentration dependence of laser pulse scattering in potassium phenosan aqueous solutions. In particular, we found that the elastic-scattering coefficient increases (see Fig. 3), and the center of the OH Raman band (see Fig. 5) shifts toward the low-frequency wing with simultaneous band broadening (see Fig. 6) in the concentration ranges from 10^{-5} to 10^{-9} M and from 10^{-13} to 10^{-18} M. In addition, the fluctuations of measured values were found to increase at the aforementioned concentrations; this increase was multiple for the elastic-scattering coefficient (see Fig. 4).

Based on the set of measured data, with regard to the increase in OH band fluctuations in IR spectra (the imaginary part of permittivity) of aqueous solutions in the same concentration ranges [32, 35], one can conclude that the observed features are indicative of a second-order phase transition [18–20] to ordered structures during hydration of potassium phenosan molecules in aqueous solutions. Note that multiple changes in the elastic scattering intensity (see Fig. 3) and, especially, its fluctuations (see Fig. 4) are more sensitive (in comparison with Raman spectra) to the formation of large hydrogen-bonded complexes. Undoubtedly, the development of the physics of soft media, to which aqueous solutions belong, with application of surface tension fluctuations at the interface (bimodal, spinodal) between two liquids [19, 20] and self-organization in polymer systems with microphase layering, expands the applicability of the model of fluctuation of the hydrogen bond number [1] at the boundary of structured hydration layers [22] of molecules that manifests itself in an increase in the amplitude and fluctuation of elastic scattering intensity.

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REFERENCES

1. A.F. Bunkin, G.A. Lyakhov, S.M. Pershin, and N.V. Suyazov, "Sequence of Water Thermodynamic Singularities in Raman Spectra," J. Raman Spectrosc. **31**, 857 (2000).
2. M. Chaplin, "Water Structure and Science," www.btinternet.com/martin.chaplin/phase.html
3. S.M. Pershin and A.F. Bunkin, "A Jump" in the Position and Width of the Raman Band Envelope of O–H Valence Vibrations upon Phase Transitions of the First and Second Kinds in Water," Opt. Spectrosc. **85**(2), 190 (1998).
4. S.M. Pershin and A.F. Bunkin, "Direct Observation of the Configurational Energy Evolution and Anomalies Near Some Temperature Points in Water," Phys. Vibr. **7**(4), 217 (1999).
5. A.F. Bunkin and S.M. Pershin, RF Patent No. 98103249 (1998).
6. S.M. Pershin, A.F. Bunkin, V.K. Klinkov, V.N. Lednev, D. Lushnikov, E.G. Morozov, and R.N. Yul'metov, "Remote Sensing of Arctic Fjords by Raman Lidar: Heat Transfer Screening by Layer of Glacier's Relict Water," Phys. Wave Phenom. **20**(3), 212 (2012) [DOI: 10.3103/S1541308X12030090].
7. A.F. Bunkin, V.K. Klinkov, V.N. Lednev, D.L. Lushnikov, A.V. Marchenko, E.G. Morozov, S.M. Pershin, and R.N. Yulmetov, "Remote Sensing of Sea Water and Drifting Ice in Svalbard Fjords by Compact Raman LIDAR," Appl. Opt. **51**(22), 5477 (2012).
8. S.M. Pershin, L.M. Krutyansky, and V.A. Luk'yanchenko, "On the Revealing of Nonequilibrium Phase Transitions in Water," JETP Lett. **94**(2), 131 (2011).
9. C. Huang, K.T. Wikfeldt, T. Tokushima, D. Nordlund, Y. Harada, U. Bergmann, M. Niebuhr, T.M. Weiss, Y. Horikawa, M. Leetmaa, M.P. Ljungberg, O. Takahashi, A. Lenz, L. Ojamäe, A.P. Lyubartsev, S. Shin, L.G.M. Pettersson, and A. Nilsson, "The Inhomogeneous Structure of Water at Ambient Conditions," PNAS. **106**(36), 15214 (2009) [DOI: 10.1073/pnas.0904743106].
10. A. Nilsson, and L.G.M. Pettersson, "Perspective on the Structure of Liquid Water," Chem. Phys. **389**, 1 (2011).
11. K.B. Linesh and J.W.M. Frenken, "Experimental Evidence for Ice Formation at Room Temperature," Appl. Phys. Lett. **101**, 036101 (2008).
12. V.B. Efimov, A.N. Izotov, A.A. Levchenko, L.P. Mezhov-Deglin, and S.S. Khasanov, "Structural Transitions in Ice Samples at Low Temperatures and Pressures," JETP Lett. **94**(8), 621 (2011).
13. S.M. Pershin, "Two Liquid Water," Phys. Wave Phenom. **13**(4), 192 (2005).
14. S.M. Pershin, A.F. Bunkin, and V.A. Luk'yanchenko, "Evolution of the Spectral Component of Ice in the OH Band of Water at Temperatures from 13 to 99°C," Quantum Electron. **40**(12), 1146 (2010).
15. Ch.C. Pradzinski, R.M. Forck, T. Zeuch, P. Slavicek, and U. Buck, "A Fully Size-Resolved Perspective on the Crystallization of Water Clusters," Science. **337**, 1529 (2012).
16. C. Perez, M.T. Muckle, D.P. Zaleski, N.A. Seifert, B. Temelso, G.C. Shields, Z. Kisiel, and B.H. Pate, "Structures of Cage, Prism, and Book Isomers of Water Hexamer from Broadband Rotational Spectroscopy," Science. **336**, 897 (2012).
17. S. Pershin, "Effect of Quantum Differences of Ortho and Para H_2O Spin-Isomers on Water Properties: Biophysical Aspects," Biophysics. **58**(5), 910 (2013).
18. F.M. Vuks, *Light Scattering in Gases, Liquids, and Solutions* (Izd-vo LGU, Leningrad, 1977) [in Russian].
19. I.I. Potemkin, K.B. Zeldovich, and A.R. Khokhlov, "Statistical Physics of Associating Polyelectrolyte Solutions," Polymer Sci. Ser. C. Selected Topics. **42**(2), 154 (2000).
20. A.A. Aerov, A.R. Khokhlov, and I.I. Potemkin, "Clusters in a Mixture of an "Amphiphilic" Ionic Liquid and a Nonionic Liquid: Theoretical Study," J. Chem. Phys. **136**, 014504 (2012).
21. A.F. Bunkin, M.A. Davydov, and S.M. Pershin, "Four-Wave Mixing Rayleigh Scattering Spectroscopy of Binary Solutions," Phys. Wave Phenom. **22**(2), 91 (2014) [DOI: 10.3103/S1541308X1402002222].
22. A.F. Bunkin and S.M. Pershin, "Low-Frequency Four-Wave Mixing Spectroscopy of Biomolecules in Aqueous Solutions," Quantum Electron. **40**(12), 1098 (2010).
23. A.B. Kudryavtsev, G. Christopher, C.D. Smith, S.B. Mirov, W.M. Rosenblum, and L.J. DeLucas, "The Effect of Ordering of Internal Water in Thaumatin and Lysozyme Crystals As Revealed by Raman Method," J. Cryst. Growth. **219**, 102 (2000).
24. N.P. Pal'mina, E.L. Mal'tseva, and E.B. Burlakova, "Protein Kinase C, a Peroxylipid-Dependent Enzyme," Khim. Fiz. **14**(11), 47 (1995) [in Russian].
25. I.S. Ryzhkina, Yu.V. Kiseleva, L.I. Murtazina, and A.I. Konovalov, "Effect of Ultralow Concentrations and Electromagnetic Fields," Dokl. Phys. Chem. **446**(1), 153 (2012).

26. A.I. Konovalov and I.S. Ryzhkina, "Formation of Nanoassociates As a Key to Understanding of Physicochemical and Biological Properties of Highly Dilute Aqueous Solutions," Russ. Chem. Bull. **63**(1), 1 (2014).
27. E.B. Burlakova, A.A. Konradov, and E.L. Mal'tseva, "Extremely Small Actions of Chemical Compounds and Physical Factors on Biological Systems," Biophysics. **49**(3), 522 (2004).
28. I.S. Belostotskaya and V.V. Ershov, "Synthesis of 4-Aminoalkyl-2, 6-Di-Tert-Butylphenols," Bull. Acad. Sci. USSR, Div. Chem. Sci. **13**(4), 715 (1964).
29. E.L. Mal'tseva, N.P. Pal'mina, and E.B. Burlakova, "Natural (α-Tocopherol) and Synthetic (Phenozan Potassium Salt) Antioxidants Regulate the Protein Kinase C Activity in a Broad Concentration Range (10^{-4}–10^{-20} M)," Membrane Cell Biol. **15**(2), 251 (1998).
30. S.S. Kozlov, T.E. Chasovskaya, M.G. Semenova, and N.P. Palmina, "Effects of Low Concentrations of Synthetic Antioxidant Phenosan Potassium Salt on the Thermoinduced Structural Transitions in the Protein Component of Plasma Membranes," Dokl. Biochem. Biophys. **459**, 190 (2014).
31. V.V. Belov, E.L. Mal'tseva, N.P. Pal'mina, and E.B. Burlakova, "The Role of Solvent Polarity in the Mechanism of Action of Biologically Active Compounds at Ultralow Concentrations," Dokl. Biochem. Biophys. **399**, 362 (2004).
32. V.V. Belov, I.A. Belyaeva, G.P. Shmatov, G.M. Zubareva, and N.P. Palmina, "IR Spectroscopy of Thin Water Layers and the Mechanism of Action α-Tocopherol in Ultra Low Concentrations," Dokl. Phys. Chem. **439**(1), 123 (2011).
33. S.M. Pershin, "Konovalov Effect in Low-Concentration Aqueous Solutions: The Role of Ortho/Para Spin Isomers of Water," Dokl. Phys. Chem. **455**(1), 37 (2014).
34. A. Konovalov, I. Ryzhkina, E. Maltzeva, L. Murtazina, Yu. Kiseleva, V. Kasparov, and N. Palmina, "Nanoassociate Formation in Highly Diluted Water Solutions of Potassium Phenosan with and without Permalloy Shielding," Electromagn. Biol. Med. **34**(2), 141 (2015).
35. G.M. Zubareva, A.V. Kargapolov, and L.S. Yaguzhinskii, "Fluctuations of Pass Band Coefficients of Water and Water Salt Solutions in the Spectral Infrared Region," Biofizika. **48**(2), 197 (2003) [in Russian].