Journal of Russian Laser Research, Volume 26, Number 1, 2005

SECONDARY RADIATION IN AQUEOUS MEDIA WITH EXCITATION BY A PULSE-PERIODIC ULTRAVIOLET LASER

V. S. Gorelik and P. P. Sverbil'

P. N. Lebedev Physical Institute, Russian Academy of Sciences
Leninskii Pr. 53, Moscow 119991, Russia
e-mail: sverbil@sci.lebedev.ru

Abstract

Spectra of secondary radiation (Raman scattering and photoluminescence) of water, aqueous solutions, and suspensions under excitation by a pulse-periodic ultraviolet laser are studied. Both bands associated with intramolecular and intermolecular vibrations of water and the characteristic photoluminescence bands of the corresponding chromophore groups are found to manifest themselves in the secondary radiation spectra under excitation of aqueous solutions of various aromatic compounds by the second harmonics of a copper-vapor-laser radiation. It was shown that traces of aromatic compounds present in water as well as microorganisms in it can be revealed from the form of the secondary radiation spectra. When using delay, the photoluminescence spectrum takes a number of characteristic maxima. This circumstance can be used for identification of the type of microorganism studied.

Keywords: spectrum, secondary radiation, ultraviolet excitation, aqueous medium, heavy water, microorganism.

1. Introduction

The molecular structure of water is due to both the structure of its molecules and the formation of the so-called long-range order. The latter results in the formation of microclusters and a spatially organized "network." The molecular structure of water can be strongly modified by introduction of various objects in the form of dissolved components or suspended particles into the water.

The techniques of laser molecular spectroscopy are quite efficient in the analysis of the molecular structure and composition of water. These techniques include photoluminescence (PL) and Raman scattering [1]. The characteristic feature of water is its transparence within a broad spectral range including both the visible spectrum and a large portion of the ultraviolet one. In this connection, ultraviolet light sources as exciting sources of PL and Raman spectra show significant promise when studying water and more complex molecular objects formed on its basis. In the present paper, we report the results of studies of water, aqueous solutions of some organic compounds, and suspended microorganisms using ultraviolet laser excitation.

Translated from a manuscript submitted September 20, 2004.

 1071-2836/05/2601-0042

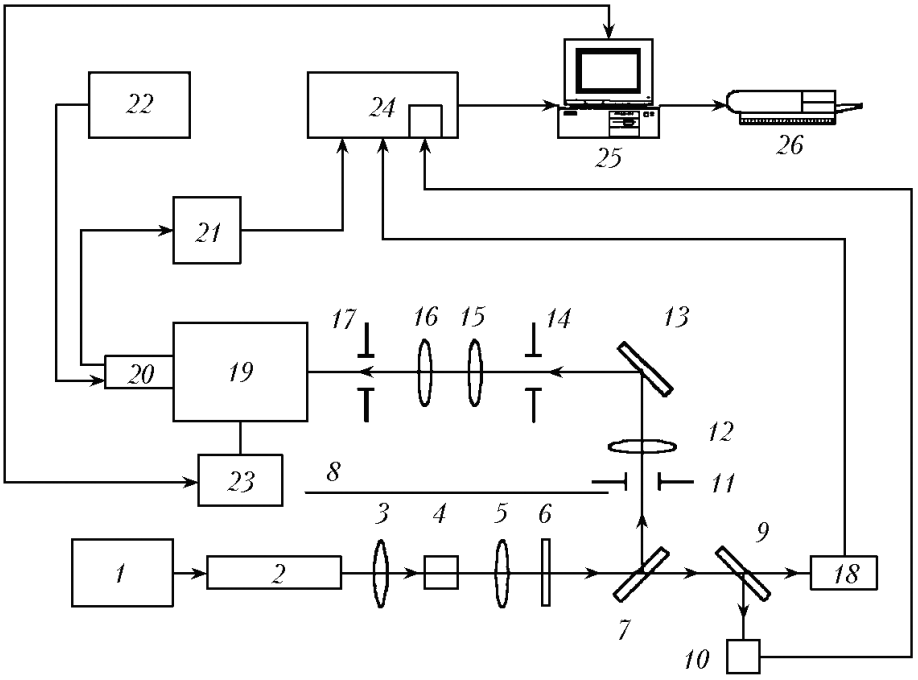

Fig. 1. Schematic diagram of the experimental set-up for observation of the secondary radiation in liquids: 1) power supply of the laser; 2) active element of the laser; 3), 5), 12), 15), and 16) quartz lenses; 4) nonlinear optical crystal; 6), 7), and 9) dielectric mirrors; 8) screen; 10) strobe-pulse shaping unit; 11), 14), and 17) diaphragms; 13) cell with the substance under study; 18) photodiode; 19) monochromator; 20) photomultiplier; 21) amplifier; 22) power supply of the photomultiplier; 23) stepping motor; 24) recording system; 25) computer; and 26) printer.

2. Experimental Technique

We use a recently developed copper vapor laser to solve the problem posed. This laser is characterized by a relatively high efficiency [2]. The copper vapor laser generates radiation in the visible spectral range with wavelengths of 510.6 and 572.8 nm. The mean radiating power for such devices can be as high as 10 W. The copper vapor laser generates in the pulse-periodic mode with a high repetition rate (10^4 Hz) of short (20 ns) lasing pulses having a peak power of 10^5 W. The high peak power of laser radiation allows one to perform efficient doubling and summation of frequencies of the laser emission lines using nonlinear-optical crystals (BaB_2O_4). Thus, ultraviolet emission with wavelengths of 255.3, 271.2, and 289.1 nm was obtained at the output of the laser set-up. A selective element placed in the laser cavity allowed us to separate a single excitation line. The second optical harmonic of the green emission line (510.6 nm) corresponding to the emission wavelength $\lambda = 255.3$ nm proved to be the most efficient for solution of the problem posed. Radiation with such a wavelength is strongly absorbed by all aromatic compounds as well as by microorganisms that include the chromoform groups in the form of heteroaromatic amino acids (tryptophan, tyrosine, phenylalanine) and nucleotide bases (adenine, guanine, cytosine, thymine, and uracyl).

A schematic diagram of the experimental set-up used is presented in Fig. 1. Radiation of the active element 2 of the copper vapor laser operating in the unstable-resonator mode is focussed by a long-focus

lens into a nonlinear-optical crystal 4. BaB_2O_4 crystal was cut in such a way as to comply with the synchronism condition for the frequency doubling of the initial laser radiation. By this means ultraviolet radiation with wavelengths of 255.3, 271.2, and 289.1 nm arises as it leaves the crystal. Visible laser radiation was returned to the cavity by a dielectric mirror 6 placed immediately after the nonlinear-optical crystal. The conversion factor of the visible radiation into the ultraviolet one was about 1%. Thus, the exciting ultraviolet radiation was characterized by a mean power of 10 mW and a peak power of 100 W. Ultraviolet radiation was focussed into a cylindrical quartz cell with plane-parallel mirrors and transparent walls.

Secondary radiation arising in a cell 13 represents PL of aromatic and other chromophores as well as Raman scattering. The latter is due to inelastic processes in the water molecules and in other molecules present in water in the form of foreign components. Secondary radiation was collected by a quartz condenser at an angle of 90° to the direction of the exciting beam. The slit of a MSD-2 spectrometer 19 was in the focal plane of a second quartz condenser (see Fig. 1). Radiation was detected using an FEU-106 photomultiplier 20 operating in the photon counting mode. A strobe pulse with a duration of 30 ns was formed in the unit 10 by a small portion of the exciting radiation split off from the main beam. This pulse was entered into the recording system for time scanning. The strobe pulse allowed one to detect the spectra of secondary radiation with various time delays within the range 0–300 ns. The diffraction grating of the spectrometer was turned by a computer-controlled stepping motor. The acquisition time of the signal with the fixed position of the diffraction grating was varied within the range 0.1–100 s depending on the intensity of the signal detected. Information from the recording system was accumulated in the computer.

3. Specific Features of Raman and PL Spectra of Various Water Samples Excited by the Ultraviolet Laser Source

It is well known [3] that the bands corresponding to the three fundamental types of vibrations of the H_2O molecule are present in the Raman spectrum of water. They are the A_1 type, $\nu_1 = 3450$ cm^{-1} (the stretching totally symmetrical vibration), the A_1 type, $\nu_2 = 1645$ cm^{-1} (the deformation totally symmetrical vibration), and the B_1 type, $\nu_3 = 3830$ cm^{-1} (the stretching nontotally symmetrical vibration).

Various water samples were prepared for the Raman studies by purification of the water using industrially fabricated filtration systems. The PL spectra of water samples purified using these commercial filters were analyzed to compare their efficiencies. Figure 2 illustrates the results of analysis of the PL spectra of these water samples for various filters. At the left of Fig. 2 is the exciting line ($\lambda = 255.3$ nm). The strong peak (shown by the arrow) corresponds to Raman scattering by the totally symmetrical vibration $\nu_1 = 3450$ cm^{-1} in water. The PL bands corresponding to the presence of aromatic chromophores in the water samples manifest themselves at large wavelengths. As is seen from these figures, curve 1 (the "Aquafor Modern" filter) corresponds to the lowest concentration of the aromatic components. Samples with various contents of the aromatic component (fluorene) were prepared for the qualitative analysis.

In Fig. 3, the PL spectra of a water sample passed through the "Aquafor Modern" filter (curve 4), a water solution of fluorene with a concentration of 1 μg/liter (curve 5), and other water samples (curves 1, 2, and 3) taken from the natural sources are compared. From this figure it follows that the concentration of the aromatic components in the sample corresponding to curve 4 is at the lowest detectable level (1 ng/liter). The concentration level for sample 1 exceeds the maximum permissible concentration

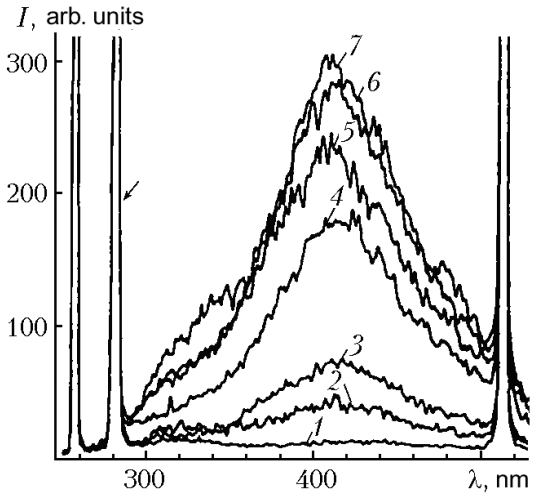

Fig. 2. Raman and PL spectra of various water samples: 1) "Aquafor Modern" filter, 2) "Bar'er" filter, 3) "Spring" filter, 4) "Paragon" filter, 5) "Briz" filter, 6) "Geizer" filter, and 7) initial water sample from the water-supply system of Moscow City.

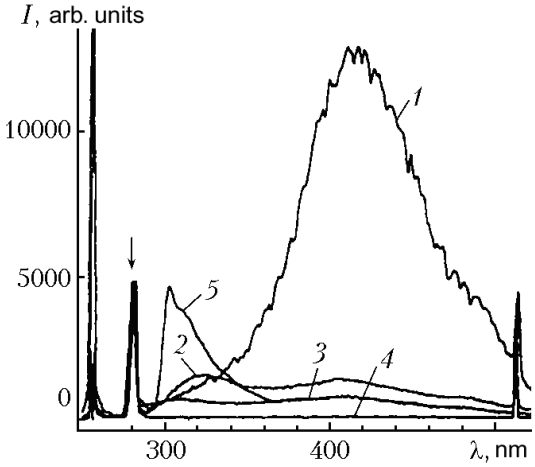

Fig. 3. Raman and PL spectra of the water samples: 1) and 2) water from the water-supply system of Moscow City, 3) "pure" water from the districts near Moscow, 4) water purified with the "Aquafor Modern" filter, and 5) water solution of fluorene with a concentration of 1 μg/liter. Spectra were excited by the 255.3 nm laser line. The arrow indicates the position of the Raman line of the totally symmetrical vibration $\nu_1(A_1)$ of the water molecule.

(1 μg/liter). The concentration of the aromatic components in samples 2 and 3 is below the maximum permissible concentration.

Figure 4 illustrates the Raman spectra of two water samples. Curve 2 corresponds to water treated with the "Aquafor Modern" filter, while curve 1 corresponds to a water sample taken from the water-supply system of Moscow City. The region of the stretching vibration $\nu_1(A_1)$ can be seen in the inset to Fig. 4. The main part of this figure illustrates the low-frequency region of the Raman spectrum as well

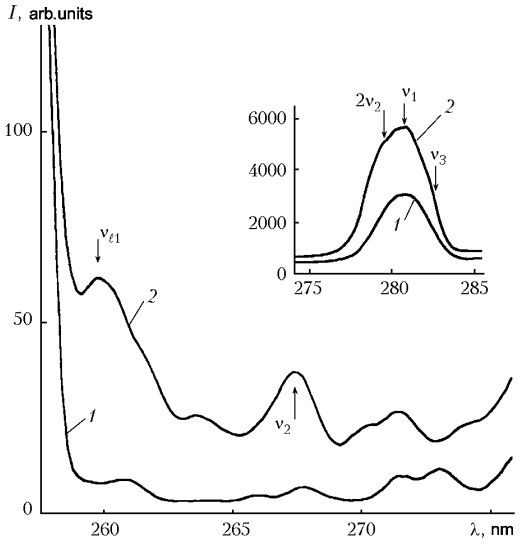

Fig. 4. Comparison of the Raman spectra of water from the Moscow water-supply system (1) and that purified by the "Aquafor Modern" filter. The spectra were excited by the 255.3 nm laser line.

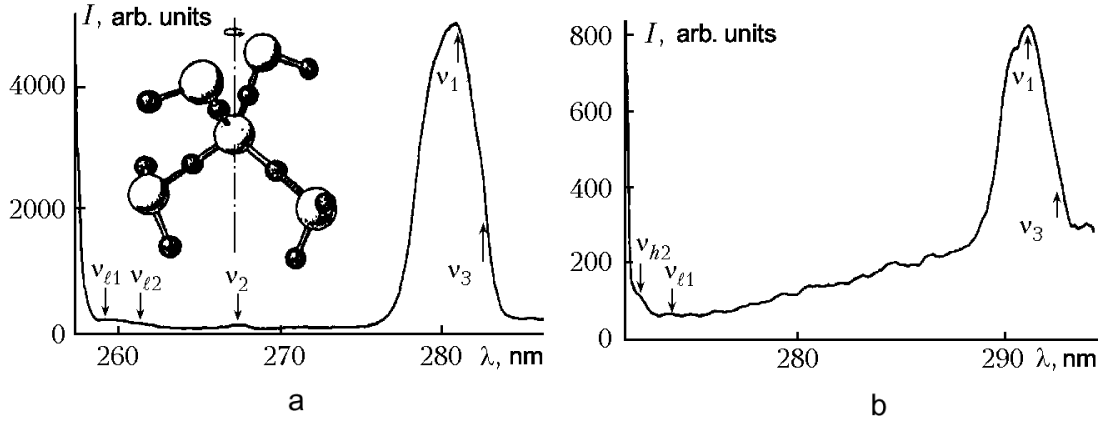

Fig. 5. Raman spectra of water: H_2O, excitation by the 255.3 nm laser line, (a) and D_2O, excitation by the 271.2 nm laser line, (b). Water samples H_2O were purified by the "Aquafor Modern" filter.

as the region corresponding to the deformation vibration $\nu_2(A_1)$. As is seen from this figure, the Raman spectrum corresponding to "pure" water (curve 2, sample passed through the "Aquafor Modern" filter) is characterized by strong lines of the fundamental vibrations ν_1 and ν_2. A rather strong peak ν_{l1} can also be found in the frequency region corresponding to the libration motions of the H_2O molecule in the intermolecular cluster. One can see a number of extra peaks corresponding to the manifestation of the Raman bands of the aromatic components in the spectrum 1 for untreated water.

Figures 5 and 6 present a comparison of the spectra of water passed through the "Aquafor Modern" filter, heavy water D_2O, and of a mixture of heavy and light water. The presence of a continuous

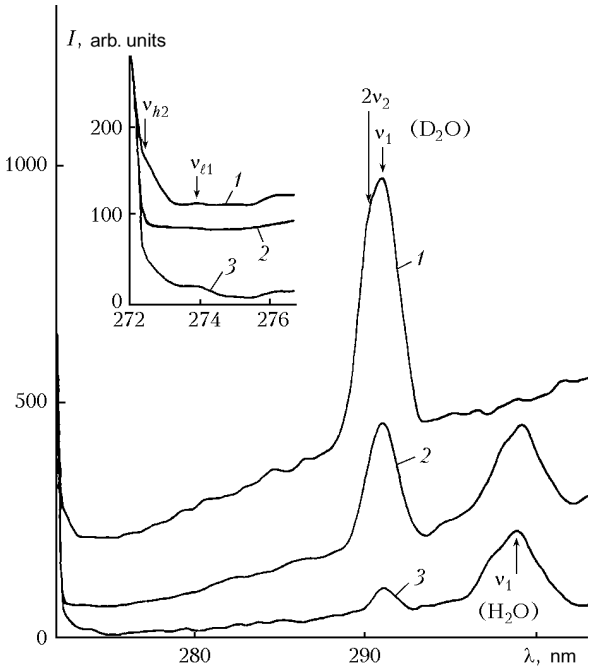

Fig. 6. A comparison of the Raman spectra of heavy water (D_2O, curve 1) and of mixtures of heavy and light water with a D_2O content of 50% (curve 2) and 5% (curve 3). The spectra were excited by the 271.2 nm laser line.

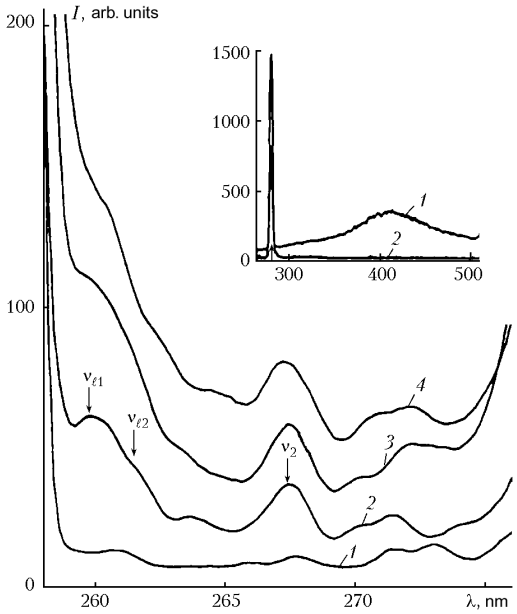

Fig. 7. Low-frequency Raman spectra for various water samples. The corresponding spectra in the long-wavelength region are shown in the inset. The maximum in the curve 1 is associated with the PL. The arrow in the inset shows the stretching vibration $\nu_1(A_1)$. The spectra were excited by the 255.3 nm laser line.

TABLE 1. Frequencies of Intramolecular and Intermolecular Vibrations of Water Molecules Appearing in the Raman Spectra

Type of vibration	Frequency, cm^{-1}			
		H_2O		
ν_{l1}	475 [3]	510 [4]	450 [1]	470 [*]
ν_{l2}	758 [3]	780 [4]	∼780 [1]	∼760 [*]
$\nu_2(A_1)$	-	-	1645 [1]	1650 [*]
$\nu_1(A_1)$	-	-	3450 [1]	3450 [*]
$\nu_3(A_1)$	-	-	3630 [1]	3630 [*]
		D_2O		
ν_{h2}	176 [3]	170 [4]	175 [1]	170 [*]
ν_{l1}	-	350 [5]	∼375 [1]	380 [*]
$\nu_1(A_1)$	-	-	2520 [6]	2520 [*]
$\nu_3(A_1)$	-	-	2680 [6]	2670 [*]
* Results of this paper.				

background in the region of intramolecular frequencies is characteristic of heavy water (Fig. 5b). In this case, the vibration $\nu_2(A_1)$ is virtually absent in the Raman spectrum. Intense bands stemming from the stretching vibrations $\nu_1(A_1)$ manifest themselves in the spectra of mixtures of heavy and light water (see Fig. 6). The intensity of the libration modes in the region of intermolecular modes of the Raman spectrum for the mixture drastically decreases (see the inset to Fig. 6).

The presence of intermolecular modes of the libration and translation types in the Raman spectra of pure water (see Table 1 and Figs. 4–6) is indicative of the formation of a quasi-crystal network of molecular clusters. Thus, the appearance of these modes characterizes the structuring degree of water. The structuring of water is an important factor associated with the efficiency of water assimilation by living cells. Therefore, the intensity of the low-frequency Raman bands resulting from the intermolecular cluster modes can serve as a further criterion of water quality.

In Fig. 7, the Raman and PL (in the inset) spectra of water samples taken from various sources are presented. Spectra 1 and 4 correspond to water samples taken from the Moscow water-supply system, spectrum 2 is for water purified with the "Aquafor Modern" filter, and spectrum 3 is for water from the spring in the district near Moscow. As is seen from the comparison of these spectra, the structuring degrees of water in the samples under discussion are different. This is evident from the form of the bands ν_{l1} and ν_{l2} of the intermolecular vibrations of the cluster complexes. The best structuring is for water treated with the "Aquafor Modern" filter. The presence of foreign components results in the appearance of a broad PL band (the inset to Fig. 7) and additional maxima in the Raman spectrum (see spectra 1, 3, and 4).

The spectra of secondary radiation shown in Figs. 2–7 correspond to the case where the strobe pulse

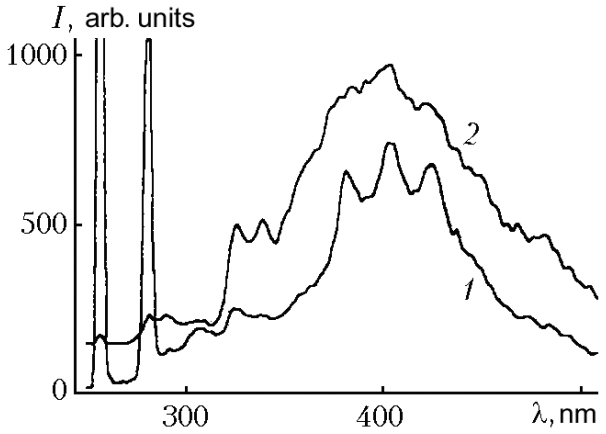

Fig. 8. Spectra of secondary radiation of an aqueous solution of anthracene obtained without delay (spectrum 1) and with a delay $\tau = 50$ ns (spectrum 2). Spectrum 2 is multiplied by a factor of 7. The spectra were excited by the 255.3 nm laser line.

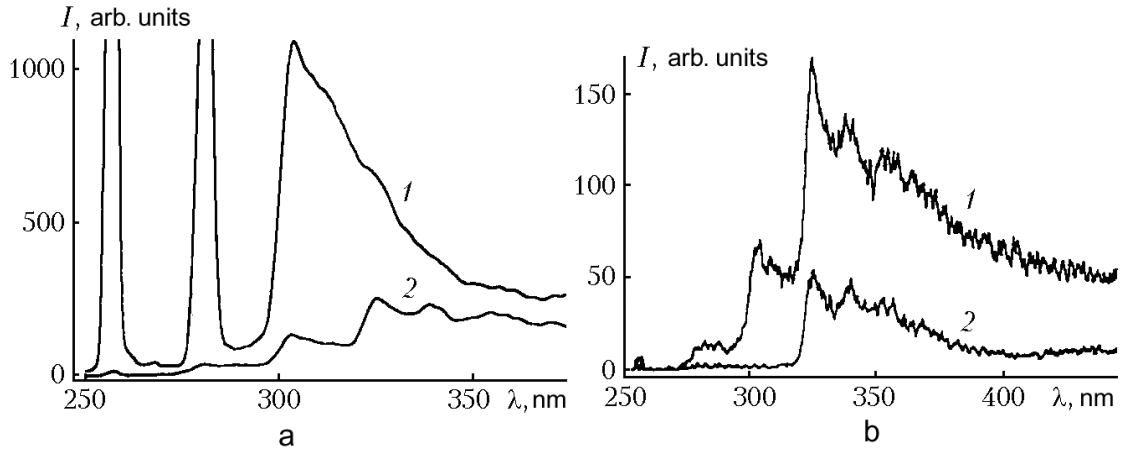

Fig. 9. Spectra of secondary radiation of an aqueous solution of fluorene: $\tau = 0$ (spectrum 1) and $\tau = 50$ ns (spectrum 2 at the same scale) (a); $\tau = 50$ ns (spectrum 1) and $\tau = 70$ ns (spectrum 2) (b). The spectra were excited by the 255.3 nm laser line.

in the recording system is synchronized with the signal of secondary radiation. The possibility of time shift of this strobe pulse allows one to study the spectra of secondary radiation delayed with respect to the exciting pulse.

Figure 8 demonstrates the spectra of an aqueous solution of anthracene with a concentration of 1 μg/liter. Note that the spectrum of the "delayed" PL (spectrum 2 in Fig. 8) significantly differs from the "synchronous" spectrum. Indeed, the peaks due to the exciting radiation ($\lambda = 255.3$ nm) and Raman scattering, which takes place virtually in synchronism with the exciting pulse, are drastically attenuated. Moreover, some maxima in the "delayed" spectrum become more pronounced.

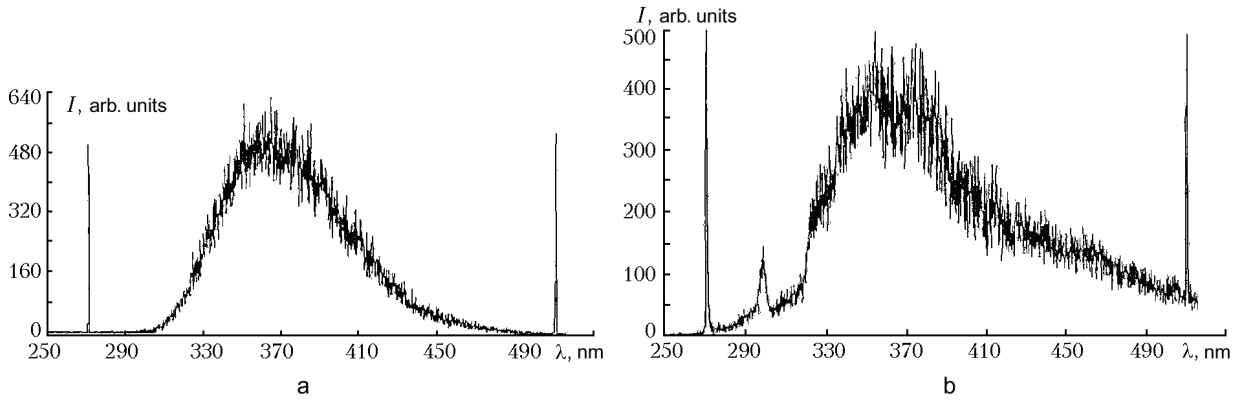

Fig. 10. PL spectra of an aqueous solution of L-tryptophan (taken without delay) at concentrations of 10 mg/liter (a) and 10 μg/liter (b).

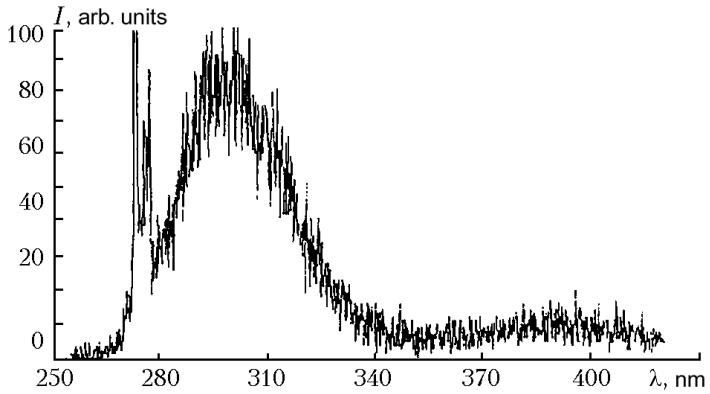

Fig. 11. Spectra of resonance Raman scattering and PL of guanosine triphosphate excited by the line $\lambda = 271.2$ nm (at the left).

The effect of delay on the spectra of the secondary radiation of the aqueous solution of fluorene (1 μg/liter) is illustrated in Fig. 9.

Thus, analysis of the spectra of secondary radiation of aqueous solutions provides further information on the types and concentration of the foreign components present in water. In this case, one can separate two types of secondary radiation (Raman scattering in synchronism with the exciting pulse and PL "delayed" in time).

4. Secondary Radiation of Microorganisms Present in Aqueous Media

Both unicellular and multicellular organisms can be the sources of electromagnetic radiation arising as a result of the processes of their vital activity. In particular, it is well known that living organisms are characterized by the visible PL. Microorganisms contain chromophore groups associated with the presence of heteroaromatic compounds (amino acids in proteins and nucleotide bases in desoxyribonucleic and

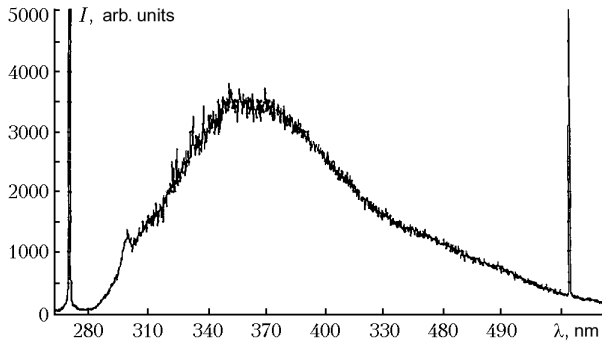

Fig. 12. PL spectrum of the bacterium *Bacillus thuringiensis* with a concentration of 10^3 ml^{-1} in the aqueous suspension taken *in vivo*. The spectrum was excited by the 271.2 nm laser line.

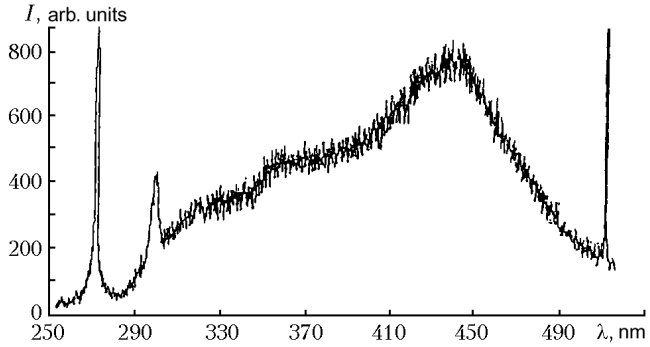

Fig. 13. PL spectrum of the bacterium *Bacillus thuringiensis* with a concentration of 10^3 ml^{-1} taken after exposure to hard ultraviolet radiation during 30 min. The spectrum was excited by the 271.2 nm laser line.

ribonucleic acids). The PL spectrum of these groups corresponds to the mid-ultraviolet range. However, observation of this PL was hampered up to now because of its strong quenching. In the present paper, the spectra of secondary radiation of aqueous solutions of tryptophan, suspensions of nucleotide compounds, and two types of microorganism were obtained on the basis of the technique of ultraviolet pulse-periodic excitation of secondary radiation in condensed media developed by us.

In Fig. 10 are shown the PL spectra of aqueous solutions of *L*-tryptophan (the main chromophore of proteins) recorded. The spectra were excited by the 271.2 nm line (the composite tone of two emission lines of a copper-vapor laser). At high concentration of *L*-tryptophan the PL spectrum is structureless (see Fig. 10a). The fine structure of the spectrum manifests itself as the concentration of *L*-tryptophan decreases down to 10 μg/liter (Fig. 10b).

The PL spectrum of guanosine triphosphate excited by the line $\lambda = 271.2$ nm without delay ($\tau = 0$) is presented in Fig. 11. As is seen from this figure, the peaks of resonance Raman scattering characteristic of this compound manifest themselves in the immediate vicinity of the exciting line. One can also see the PL band with a maximum at about 300 nm in the long-wavelength region. It should be noted that (as is evident from the comparison of Figs. 10 and 11) the maximum in the Raman spectrum of guanosine triphosphate is shifted to short wavelengths with respect to the maximum for *L*-tryptophan.

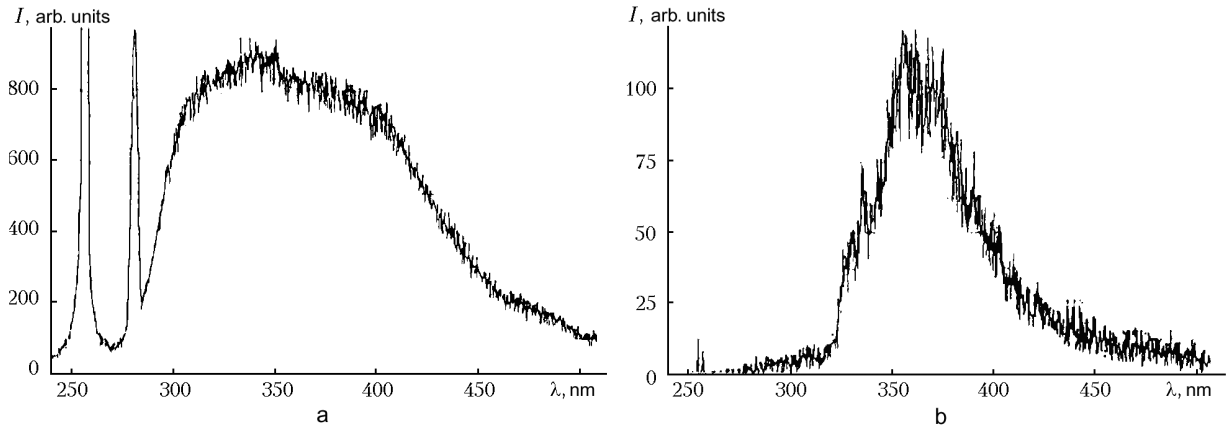

Fig. 14. PL spectra of the microorganism *Bacillus thuringiensis* with a concentration of 10^5 ml^{-1} in water taken with zero delay $\tau = 0$ (a) and with a delay $\tau = 50$ ns (b). Spectra were excited by the 255.3 nm laser line.

The bacterium *Bacillus thuringiensis* in the spore state in physiological (0.9%) aqueous solution was chosen as one of the objects. The microorganism concentration was varied in the range of 10–10^6 ml^{-1}. Figures 12 and 13 illustrate the PL spectra of an aqueous suspension of such a bacterium with a concentration of 10^3 ml^{-1} obtained *in vivo* (Fig. 12) and after exposure to hard ultraviolet radiation of a xenon lamp for 30 min. One can see from a comparison of Figs. 12 and 13 that the exposure of bacteria to hard ultraviolet radiation strongly affects the form of the PL spectrum. In this case, the spectrum maximum shifts to the long-wavelength region up to 450 nm. This effect can be explained as being due to the destruction of microorganisms with transformation of long chains of proteins and desoxyribonucleic acid into short chains of polypeptides.

Figure 14 shows the modification of the PL spectrum of the microorganism *Bacillus thuringiensis* when passing from synchronous recording with the exciting pulse ($\tau = 0$, Fig. 14a) to recording with a delay $\tau = 50$ ns (Fig. 14b). One can see that the PL spectrum recorded with the delay takes a number of characteristic maxima, which can be used for identification of the type of the microorganism analyzed.

The PL spectra of an aqueous suspension of another microorganism, the bacterium *Bacillus subtilis*, with a concentration of 10^2 ml^{-1} are presented in Figs. 15 and 16. Figure 16 corresponds to the bacterium suspension, which was preliminarily boiled for 30 min. As is seen from these spectra, boiling results in partial destruction of the microorganisms. This is manifested by the long-wavelength shift of the maximum of the PL band of the microorganisms.

5. Conclusions

Thus, the technique of pulsed ultraviolet excitation of the secondary radiation of water, aqueous solutions, and suspensions developed allows one to analyze the molecular structure of water and foreign components present in aqueous media. The threshold sensitivity of analysis for aromatic compounds is 1 ng/liter. This is far below the maximum permissible concentration of the typical aromatic compounds in water and allows one to identify traces of extremely dangerous toxic agents of the type of benzpyrene, dioxin, potassium cyanide, and others. The application of the delay technique in the detection of the

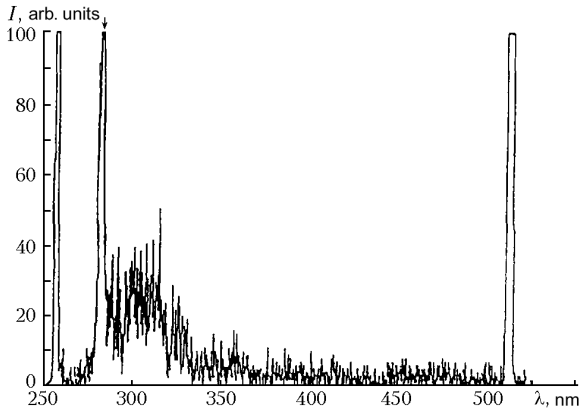

Fig. 15. PL spectrum of an aqueous suspension of *Bacillus subtilis* bacteria with a concentration of 10^2 ml^{-1} (in vivo) excited by the 255.3 nm laser line. The Raman line of water is shown by the arrow.

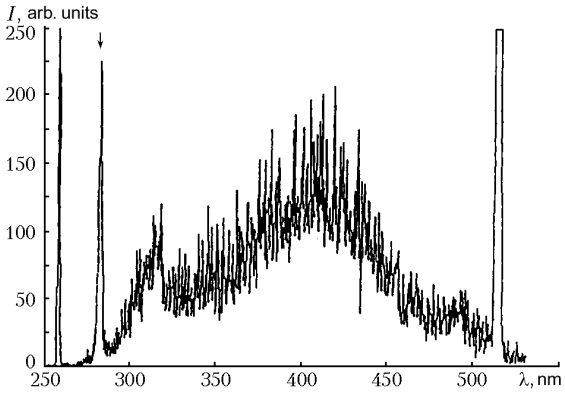

Fig. 16. PL spectrum of an aqueous suspension of *Bacillus subtilis* bacteria with a concentration of 10^2 ml^{-1} boiled for 30 min. The spectrum was excited by the 255.3 nm laser line. Arrow shows the Raman line of water.

secondary radiation allowed us to separate the PL signal from the Raman signal (synchronous with the exciting pulse). Analysis of the form of the low-frequency Raman spectra of water allowed us to evaluate the degree of structuring of water related to its gustatory quality and digestability by the animal bodies. It is shown that the technique developed allows one to detect microorganisms in water with a threshold sensitivity of 10 ml^{-1}. The form of the PL spectra of microorganisms in the ultraviolet region provides information on their type and concentration in aqueous suspension as well as the degree of destruction under the influence of various disturbing factors (hard ultraviolet radiation, heating, and so on).

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (Grant No. 02-02-16221).

References

1. G. E. Walfaren, *J. Chem. Phys.*, **40**, 3249 (1964).
2. V. S. Gorelik, *J. Russ. Laser Res.*, **20**, 152 (1999).
3. P. K. Narayanasvamy, *Proc. Indian Acad. Sci.*, **27A**, 3 (1948).
4. G. Bolla, *Nuovo Cimento*, **9**, 290 (1932).
5. M. Maget, *Annal. Phys. (Paris)*, **6**, 109 (1936).
6. G. I. Zatsepina, *Physical Properties and Structure of Water* [in Russian], Moscow State University Press, Moscow (1987).