PROBLEMS OF LASER FLUOROMETRY OF ORGANIC ADMIXTURES IN NATURAL WATER

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Received January 17, 1994

In this paper we analyze some new possibilities of applying laser fluorescence spectroscopy to diagnostics of organic matter in natural waters like, for example, oil products, dissolved organic matter, and proteins which mainly determine the ecological conditions in natural water. In particular we analyze a direct (without sampling) laser fluorescence technique for identification of such admixtures at excitation with radiation at 266 nm *wavelength. The results obtained in this study are the next step in solving the problem on remote laser monitoring of natural water.*

1. INTRODUCTION

In this paper we analyze some possibilities of laser diagnostics of two classes of organic compounds which are of paramount importance for functioning the water ecological systems. They are dissolved organic matter (DOM) and oil hydrocarbons (OH). The problem of determining the parameters of oil film on a water surface will be considered in the other publications.

The proposed methods of diagnostics are based on fluorometry with continuous calibration of a fluorescence signal with respect to an internal reference mark, i.e., a signal of Raman scattering of water or an organic solvent used for extracting oil hydrocarbons from water. Fluorometry enables one to carry out both a quick analysis of water samples and remote sounding of a water layer.

The major problem, which is solved in this paper, is a separate determination of a dissolved organic matter and oil hydrocarbons. When solving this problem, we faced the need for determining one more class of organic compounds, namely, proteins.

The state of a water ecological system is largely characterized by the content of a dissolved organic matter in water (a composite organic complex of natural origin). This unique matter occurring in all types of soils, bottom sediments, and waters contains the basic reserve of organic carbon on the Earth and is the heart of the global cycle of organic carbon.[1] A fresh interest to study this object in water media was brought on in 1974 by the report[2] on capability of the dissolved organic matter to form organochlorine pesticides during a chlorination of drinking water, which are particularly detrimental to public health. Since then DOM inherent in water has become not only an important ecological and geochemical characteristic but also the subject of investigation in such fields of science as medicine and particularly toxicology. The DOM originates from chemical and biological decomposition of vegetable and animal remains and cannot be attributed to one of the following classes of substances: proteins, polysacharides, and polynucleotides. The DOM is the final product of decomposition of any organic material, however in spite of its omnipresence and availability it is one of the least studied and intelligible natural objects.

The nature of the DOM fluorescence band is still obscure. In recent years some serious efforts were made to study this problem. The results were reported at the conference ISPRS'92 (Ref. 3) in USA and in the near future they will be published in an individual paper.

It is important here to list the following properties of the DOM fluorescence band: invariability of shape and position (λ_{max}^{fl} = (421 ± 5) nm, $\Delta\lambda_{fl}$ = (98 ± 3) nm) at any excitation wavelength which is shorter than λ = 350 nm, and displacement of the band to a long–wave region with simultaneous decrease of intensity and the band half–width with the increase of λ_{exc} .

Of a rich variety of pollutants flowing into the World Ocean the most potentially harmful to human health are those chemical compounds, which have global distribution, a permanent character of supply, and a pronounced negative effect on living organisms. These are toxic metals, long–lived artificial radionuclides, pesticides. Crude oils and oil products are at the head of this list. Tens of thousands of scientific and popular science papers as well as some monographs concerned with the study of petroleum pollution of the World Ocean and reconstruction of sea ecological systems polluted with oil spills are yearly published in the world. This fact testifies that the problem under study is currently very serious and urgent.

The extensive program of observations for the content of oil hydrocarbons in different natural and industrial objects requires that accessible, highly sensitive, selective and quick methods of analysis be developed. All available analytical methods for determining oil hydrocarbons can be divided into two basic classes:

– methods of quantitative determination of the total content of oil hydrocarbons;

– methods of identification and quantitative determination of individual hydrocarbons.

The comparative analysis of the methods was made in some papers.[4,5] Our paper does not concern with this analysis but is dedicated to studying new potentialities of fluorescence, including laser, spectroscopy for oil hydrocarbon diagnostics in natural waters. Therefore, we shall give only a brief description of advantages and disadvantages of this method.

0235-6880/94/04 231-07 $02.00

The fluorescence spectroscopy exhibits two very important advantages, i.e., its high sensitivity and capacity for rapid detection, which set it off from the other methods of determining oil hydrocarbons in water. The possibility of remote measurements is one more advantage of this method, which originated from a development of laser technique. Moreover, during excitation by ultra–short laser pulses it becomes possible to record the fluorescence kinetics. In this case not only spectra but also a curve of fluorescence decay are recorded and the obtained lifetime values t_3 are used as supplement spectral information for identification of oils and oil products.[6]

The other variant of fluorescence spectroscopy is the method of TLS–diagrams (total luminescence spectra) based on record of fluorescence spectra during excitation at different wavelengths and construction of patterns with equal radiation intensity.[7] The additional third coordinate in the form of fluorescence intensity makes it possible to construct visual three–dimensional plots which enable one to determine concentration and to identify the oil pollution.

The possibilities, which are provided with TLS–diagrams and fluorescence lifetime measurements for fluorescence spectroscopy, are reduced because of the loss of the basic advantages of this method, i.e., quickness, since it takes much more time to extract information, and simplicity, since special instrumentation, which is not always widely available, is required for the investigations to be performed.

Therefore, when detecting oil hydrocarbons in water the conventional fluorescence spectroscopy is more preferential, in particular, for field measurements, and new efforts are carried out to increase its potential. Some of such efforts are described in this paper.

One more important organic component determining an ecological state of natural water media is amino acids and their derivatives, protein compounds. Amino acids have been found in sediments, suspension, and dissolve state in all water reservoirs (seas and oceans, rivers and lakes).

The total content of dissolved amino acids in various natural waters is in direct proportion to the total concentration of
organic matter in water. Thus, in Ref. 8, the carbon of dissolved amino acides was found to vary from 5 to 7% of the total content of the organic carbon and the typical mean concentration of dissolved amino acids for the majority of natural waters ranges from a few tens to hundreds of μg/l.

The fluorescent properties of proteins are considered in Section 6, when the problem of separate determination of fluorescence contributions of the dissolved organic matter and oil hydrocarbons to the general fluorescence band of organic mixtures in water has been outlined.

2. SOME PROBLEMS OF DETERMINING THE OIL HYDROCARBON CONCENTRATION IN WATER BY THE FLUOROMETRY METHOD

As noted above, the UV fluorescence spectroscopy is widely used for diagnostics of oil pollution. The UV fluorometry yields to such methods as gas chromatography, highly efficient liquid chromatography, mass–spectroscopy or combination of these methods in selectivity. But it benefits from the ease and rate of information extraction. The standard method of oil hydrocarbon identification accepted by the American Society on verification and testing of materials is based on comparing fluorescence spectra of different oil and oil–product solutions in an organic solvent (cyclohexane).[9] Moreover, the fluorescence method in its extract variant was accepted by UNESCO (Ref. 10) as a standard method for determining the total content of oil hydrocarbons in natural waters and was repeatedly tested under field conditions. These tests have shown that the standard technique is highly sensitive, provides for good reproducibility of the results, high rate of extracting information "on hot blazing scent" and is insufficient the first and necessary step in monitoring natural waters, especially for rapid diagnostics of vast marine areas. If the information obtained using this method is inadequate, then the same samples can be thereafter investigated using the above listed methods to derive more comprehensive information about a qualitative content of the sample.

The standard fluorescence method accepted by UNESCO has many advantages. Nevertheless, its disadvantages should always be kept in mind. In this paper we attempt to search for ways of tackling these problems. The UNESCO method exhibits the following limitations:

1. The fluorescence spectroscopy is a highly sensitive method for detecting oil hydrocarbons since aromatic oil hydrocarbons are constituents of these substances but it does not provide for the information about other hydrocarbons being incorporated into the oil content. Crude oils from different deposits, like their oil fractions obtained during sublimation, contain different quantity of methane, naphthene, and aromatic hydrocarbons. Therefore, this brings up the problems of choosing a standard sample of oil to calibrate a fluorescence signal against the oil hydrocarbon concentration.

2. Concentration of oil hydrocarbons can not be determined directly from the spectra of their fluorescence in water. First the oil hydrocarbons dissolved and emulsified in water are converted into hexane by extraction. The solution can be concentrated by evaporation on a rotor evaporator as the need arose. Thereafter the concentration of oil hydrocarbons in water is determined based on the fluorescence spectra of the obtained hexane extracts. Such multistage preliminary operations and indirect measurements reduce the efficiency of the method and can introduce some errors, in particular, due to different states of oil in water and hexane (in water it is, for the most part, in an emulsified state and in hexane it is, for the most part, in a dissolved state[11]).

Moreover, it should be noted that the problem of converting the other compounds not related to oil hydrocarbons to a hexane extract remains unsolved. The natural dissolved organic matter does not convert to hexane during extraction. However, the analysis performed in Ref. 11 showed that in addition to oil hydrocarbons the hexane extracts contain some fluorescent polar compounds of unknown nature. Thus this problem calls for further investigation.

3. The most critical moment in the UNESCO fluorescence technique is, what we believe, nonoptimal choice of wavelengths for excitation and recording of fluorescence (λ_{exc} = 310 nm and λ_{em} = 360 nm). Following this technique the oil hydrocarbon concentration is determined from the relation $C = \alpha I$, where C is the oil hydrocarbon concentration, I is the fluorescence intensity, and α is the coupling coefficient found from the calibration dependence $C(I)$ against the standard in the capacity of which chrisen or any crude oil is used.

The calibration dependences for different oils, oil products, and temperature fractions of oils were investigated in Refs. 12 and 13. The calculated values of α under excitation at wavelength λ_{exc} = 337 nm for the samples under study differ by the order of 3 or 4. The same result was obtained at λ_{exc} = 310 nm. Such dependence of α on the type of oil hydrocarbon implies that the same oil hydrocarbon which occurs in water and is the source of pollution must be used as a standard for calibration. But, as a rule, this condition is difficult to be realized since we are, more often than not, unaware where the pollution source is. Thus, the UNESCO technique is not an all–purpose one. But we believe that the situation is not disparate since the spread of the values of the coefficient α can be attributed to both different chemical compositions of original oils and oil hydrocarbons containing different quantity of aromatic oil hydrocarbons and poor choice of wavelengths of the fluorescence excitation and recording. The oil hydrocarbons of light fractions are excited with the least efficiency by radiation at λ_{exc} = 337 nm and 310 nm (Refs. 12 and 13). Hence, small concentrations of these oil hydrocarbons in water (according to our estimates C = 10 μg/l and lower) cannot be detected using the fluorescence technique.

In the same papers the authors made the proposal that the decrease of wavelength λ_{exc} would extend the spectrum of excited oil hydrocarbons thus decreasing the spread of α values and making the fluorescence technique more versatile. In our studies this hypothesis was supported quantitatively.

As was previously noted, the use of short–wave radiation (λ_{exc} = 250, 254, and 290 nm) is favourable for exciting oil hydrocarbons. The results of these investigations formed the basis for the fluorescence method of oil hydrocarbon identification.[9] However, they were not used for quantitative determination of oil hydrocarbon content in water. Our investigations have roughly supported the possibilities of a quantitative analysis at λ < 270 nm and established new details, which can be used to identify oil hydrocarbons.

In this paper a modification of the UNESCO technique is proposed, which is founded on the use of new wavelengths of excitation and recording of fluorescence. Quantitative estimates described here prove the correctness of such choice, and the possibilities appeared enable one to use the UNESCO technique both for determining concentration and identifying oil hydrocarbons. It turned out that the use of these wavelengths made it possible to progress in direct diagnostics of oil hydrocarbons in water.

3. EXPERIMENTAL INSTRUMENTATION AND PREPARATION OF SAMPLES

The corrected spectra of fluorescence and fluorescence excitation of water samples as well as hexane extracts and hexane solutions of different oils and oil products were recorded on a lamp fluorescence spectrophotometer HITACHI 850 with automated correction of spectra.

Simultaneously with these investigations the corrected fluorescence spectra of all samples were registered by a small laser spectrometer. The radiation of the fourth harmonic of the YAG:Nd^{3+} laser (λ_{exc} = 266 nm, power P = 50 kW per pulse, duration τ = 10 ns, and pulse repetition rate ν = 10 Hz) was directed to the quartz 4 cm^3 cell with a sample under study in it. The radiation scattered at angle of 90° entered the entrance slit of the monochromator, and the spectrum was recorded in the regime of successive detection of PM and ADC of an integral type "discharge–time" with subsequent microcomputer processing. The fluorescence intensity of the samples under study was expressed in units of the dimensionless parameter $\Phi = I_{fl}/I_{rs}$, where I_{fl} and I_{rs} are intensities in maxima of fluorescence and RS (Raman scattering) bands of water or hexane. The weakest reliably detected signal of fluorescence was Φ = 0.05.

We have studied spectral–luminescence characteristics of hexane extracts and solutions of ten different oils and oil products. Samples of bi–distilled water with oil hydrocarbons in a dissolved–emulsified state in it were extracted. To prepare such samples we have used dividing 0.5 liter funnels filled with bi–distilled water. After filling up several milligrams of an oil sample into the funnel it was shaken (about 50 times) and then the content of the funnel had settled during three days. During the first of 5 hrs a cover of the funnel was opened and all the rest of the time it was kept closed at room temperature under natural illumination. Based on the known facts we believed that the time was enough for all essential processes of evaporation, solution, emulsification, and, possibly, oxidation came about and have used then our samples as model samples of natural water for the analysis. The remaining content of the funnels was kept closed during three months and then was analysed too. The shape of excitation and fluorescence spectra of fresh and sustained samples were qualitatively similar what testified to a similar chemical composition of the samples and their subsequent minor destruction. Thus, the samples prepared under equal conditions allowed us to do away with the problem of oil "aging".

The basic part of our work was concerned with solution to the problem of separating spectral contributions of different organic compounds in natural water and particularly separation of contributions made by oil hydrocarbons and dissolved organic matter as the most important ingredients.

To obtain different concentrations of oil hydrocarbons in water the mid–portion of a sample was decanted and dissolved with different volumes of bi–distilled water. The final concentration of the sample was selected so that the fluorescence signal of oil hydrocarbon in water in a dissolved–emulsified form under excitation at λ = 266 nm could be compared to a water Raman–scattering signal (Φ = 0.3 ... 1). Such situation is typical for water areas subjected to anthropogenic action with the level of oil pollution varying from 0.1 to 10 maximum permissible concentration (MPC for oil hydrocarbon is 50 μg/l, Ref. 14) and hence is of greatest practical interest.

The prepared water samples with final concentration of dissolved–emulsified oil hydrocarbons were divided into two portions each being then analyzed independently. One portion of the sample was extracted by hexane and the other one was taken as a pattern of an oil water sample and its spectral–luminescence characteristics were then studied. The samples were extracted following the traditional UNESCO technique, with the only difference that high sensitivity of the used laser spectrometer enabled us to record the oil–hydrocarbon fluorescence signal in an hexane extract with a small value of the concentration coefficient $K = V_w/V_h = 10$ (V_w and V_h are the water and hexane volumes during extraction).

Further concentrating of the sample by evaporation with a rotor evaporator was not needed that significantly reduced the time of preparing samples for analysis (t = 15 min). In this case the minimum detected concentration of oil hydrocarbons in water varied from 0.5 to 3 μg/l for different hydrocarbons. In order to reduce this limit the values of K can be increased since, following the standard technique, it can attain the value K = 800.

Solutions of oil hydrocarbons in hexane with concentration C = 10 ... 500 μg/l were prepared by dissolving in hexane the volumes of oil products calibrated using a micro–injector that provided for required accuracy and reproducibility of the experiments performed.

4. EXTRACTION TECHNIQUE AT SHORT–WAVE LASER EXCITATION OF OIL HYDROCARBON FLUORESCENCE

Depicted in Figs. 1 *a* and *b* are the corrected spectra of fluorescence of some hexane extracts under excitation at λ_{exc} = 266 and 310 nm. The comparison of these figures testifies to obvious advantages of shorter–wave excitation: first, the fluorescence spectra under such excitation contain more information, second, fluorescence of oil hydrocarbons is excited more advantageously at λ_{exc} = 266 nm. In most cases (for 7 oil and oil–product samples studied) the band of fluorescence of hexane extracts and solutions at λ_{exc} = 266 nm has two pronounced maxima at wavelengths λ_{1max} = 308 nm and λ_{2max} = 360 nm, and a "shoulder" in the range of 385 nm. In the spectrum of diesel fuel there is only one short–wave band (λ_{max} = 308 nm) and in spectra of gasolines there is a band with maximum at 290 nm and a "shoulder" within 300 ... 310 nm. Such qualitative similarity of spectra is due, from our standpoint, to identical fluorophors (aromatic oil hydrocarbons) occurring in all oils and oil products. The responsibility for short–wave fluorescence at λ_{1max} = 290 and 308 nm is apparently on oil hydrocarbons with one or two aromatic rings and the one for long–wave fluorescence is on those with several (3, 4) rings.

It should be noted that such dependence of spectro–luminescence characteristics on structure of oil–hydrocarbon molecules has long been observed. Regularities in behavior of aromatic–molecule spectra were described at length[15] based on studying electron spectra of absorption, emission, and excitation of fluorescence of a large number of polycyclic aromatic hydrocarbons. It is known that for condensed aromatic hydrocarbons, as a number of benzene rings and conjugated bonds increases in the molecular chain there is a tendency to displacement of absorption and luminescence bands to long–wave region.

Similar regularities in variations of positions of fluorescence spectrum maxima and degree of spectra structurizability depending on complexity of molecule structure have been studied for the majority of other groups of aromatic oil–hydrocarbons. In the wavelength range between 250 and 280 nm there is maximum of the first absorption band for one– and two–ring structures and maximum of the second absorption band for oil hydrocarbon molecules with 3 or 4 aromatic rings. The radiation at 310 nm hardly excites light oil hydrocarbons (1 and 2 rings) and scarcely best excites more complicated structures. The fluorescence analysis made in Ref. 15 corroborates the presence of maximum in excitation spectra in the range between 250 and 280 nm for the majority of aromatic oil hydrocarbons (more than 70 of 90 studied oil hydrocarbons consisting of 1, 2, 3, 4, and 5 aromatic rings of different configuration). Thus, in the excitation spectrum of chrysin–standard suggested by the UNESCO technique for determining oil concentration in water, the intense maximum is observed at 269 nm (during recording at λ_{em} = 361 nm) which, for an uncorrected spectrum, is a factor of two more intense than that at 310 nm. These results are indicative of explicit advantages of shorter–wave (than it is accepted in the standard technique) radiation in the region from 260 to 280 nm. It excites fluorescence of a large number of oil hydrocarbons more efficiently, and the structure of the obtained spectra enables one to make rough identification of oil hydrocarbons for basic classes. Figure 1 illustrates these advantages.

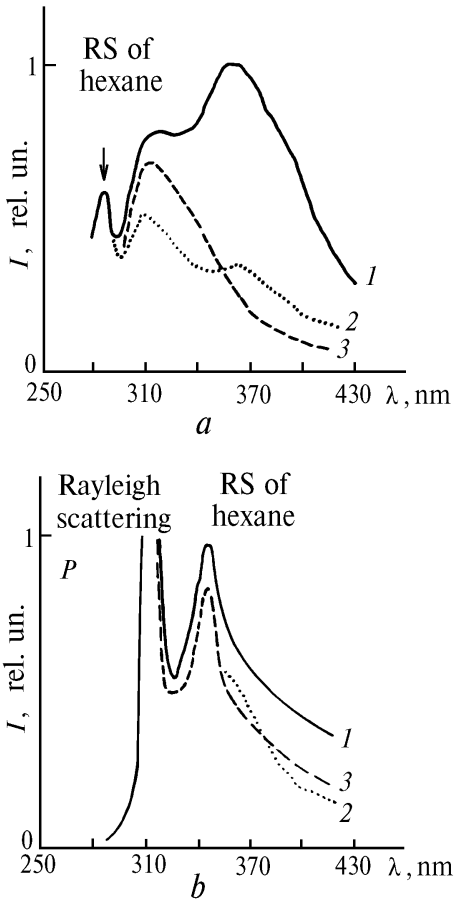

FIG. 1. Corrected fluorescence spectra of hexane extracts: fuel oil (1), Saratov oil (2), and diesel fuel (3). Fluorescence excitation wavelengths are 266 *nm (a) and* 310 *nm (b).*

At the next step of our investigations we analyze concentration dependences of hexane solutions of different oil hydrocarbons and determine the coefficients $\alpha_1 = \alpha(\lambda_{1max})$ and $\alpha_2 = \alpha(\lambda_{2max})$ in the relation $C = \alpha\Phi$ for two fluorescence bands at λ_{1max} = 308 nm and λ_{2max} = 360 nm, i.e., investigate possibilities of calibration of fluorescence signals of the two bands Φ_1 and Φ_2 based on the oil–hydrocarbon concentrations. The obtained coupling coefficients are listed in Table I (for a lamp spectrofluorimeter α_1 and α_2 have been corrected to the

final width of an excitation line). From our standpoint, it is more reasonable to search for a connection between the oil–hydrocarbon concentration in hexane and the value of the fluorescence parameter Φ_1 for the band at λ_{1max} = 308 nm. Such choice is caused by the fact that short–wave fluorescence, in contrast to the band at λ_{2max} = 360 nm, is observed in spectra of all oil products. As a consequence, in values α_{1max} there occurs a smaller spread: it reduced from the value variation from 10^2 to 10^3 (typical for a standard version of the technique) to those from 3 to 5. Thus, the ratio $C = \alpha_1\Phi_1$ must be used when determining the concentration of oil hydrocarbons.

This technique was tested in August 1991 in the mission along the coast of the Black Sea and fully justified all expectations about sensitivity and quickness of execution. The oil–hydrocarbon concentration in water (minimum 0.5 μg/l) was determined not longer than in 15 min after sampling. Typical spectra of hexane extracts of coastal water are represented in Fig. 2. The results of ecological analysis of the sea water showed that the mean oil–hydrocarbon concentration in the 0.5–m surface water layer varies from 1 to 14 μg/l. The maximum content of oil hydrocarbons was detected in the region of Adler. To determine concentrations we used the mean value of the coefficient α_1 = 280 μg/l.

TABLE I. Coefficients α_1 (λ_{1max} = 308 nm) *and* α_2 (λ_{2max} = 360 nm) *for hexane solutions of different oil products.*

Type of oil product	α_1, μg/l	α_2, μg/l
Crude oil, West Siberia	163	116
––––––"–––––– , Saratov	373	183
––––––"–––––– , Shaimskaya	126	31
––––––"–––––– , Libyan	337	183
Heavy Fuel Oil	128	98
MARED	100	100
Oil for jet engines	352	438
Diesel Fuel	166	10^3
Gasoline A–70	568	$> 10^4$
Gasoline A–93	555	$> 10^4$

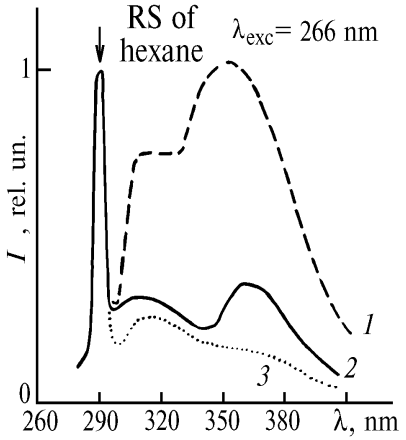

FIG. 2. Typical corrected fluorescence spectra of hexane extracts of water samples in different regions of the coast of the Black Sea. Concentration of oil products: 13.5 *(1),* 5.5 *(2), and* 4.0 μg/l *(3).*

Thus, our modification of the UNESCO standard fluorescence technique increases, to a great extent

– sensitivity of the technique due to more efficient excitation of fluorescence;

– quickness of execution due to simplified procedure of preparing the samples for the analysis;

– information content due to possible identification of oil hydrocarbons for basic classes;

– versatility due to increase of the range of excited oil hydrocarbons.

5. DIRECT FLUORESCENCE DIAGNOSTICS OF OIL HYDROCARBONS IN A DISSOLVED–EMULSIFIED STATE IN WATER

The UNESCO technique, the modification of which is described in the previous section, is very convenient and sensitive method of determining the oil hydrocarbon concentration in natural water. However, it is a contact technique, which requires preliminary preparation of samples to be analyzed. That is why the idea of direct determination of oil hydrocarbons in water from fluorescence spectra of water samples remains actual and attractive. This problem is shown by the earlier investigations to be too complicated.[4] The problems of direct determination of oil hydrocarbons in water by the method of fluorescence spectroscopy are caused by

a) existence of oil in water in different states (dissolved, emulsified, and in the form of a film);

b) "aging" of oil, i.e., change of its chemical composition, and with it the spectral characteristics under the action of some outside factors;

c) superposition of fluorescence spectral bands of oil hydrocarbons and other dissolved organic substances in water.

There are only few papers, which concern with spectro–luminescence characteristics of oil hydrocarbons in their different state in water. This is accounted for by, first, difficult preparation of real solutions of oil hydrocarbons in water due to their very low solubility.[16] Second, it is not so promising to carry out this laborious work since under natural conditions the situation with only dissolved or only emulsified oil hydrocarbons seems to be unlikely. In actual practice we always deal with dissolved–emulsified oil hydrocarbons in a water volume, and it is precisely the characteristics of these samples, which are needed for solving the problems of diagnostics.

We did not consider the oil "aging" at different stages of the process at length in this paper. To solve this difficult problem one should simulate the natural conditions on special stands, which we did not have at that time. It is conceivable that we will return back to this problem in the future. In this paper we analyzed the samples prepared under the same conditions as it was described and substantiated in Section 3.

The possibility of direct diagnostics of oil hydrocarbons in water was studied. The search for an optimal wavelength range of excitation of oil hydrocarbons fluorescence in water must be the first and necessary stage of the solution to this problem.

To this end we studied spectro–luminescence characteristics (corrected spectra of fluorescence (SF) and fluorescence excitation (SFE)) for ten different oil products in water in a dissolved–emulsified state. The spectra of fluorescence of water samples obtained at different wavelengths of excitation (λ_{exc} = 250, 266, 280, 308, and 337 nm) differ dramatically in shape and position of maxima. The structure of spectra is simplified with the increase of λ_{exc} .

Such behavior of SF testifies to a complicated and multicomponent composition of water samples of oil containing different quantity of various aromatic oil hydrocarbons. These fluorophors, each absorbing UV light in its manner, fluorescent and form the total spectrum of oil hydrocarbons in water. By analogy with SF of oils in hexane it is possible to assume that in the short–wave range of spectrum (270 ... 310 nm) the light from mono– and bicyclic oil hydrocarbons (benzene, toluene, naphthalene, etc.), which enrich a water–soluble fraction of oil due to their good solubility in water, give the most contribution to fluorescence. In a longer–wave region (310 ... 380 nm) the contribution to the fluorescence band can be made by 3–4–ring aromatic structures.

It is difficult now to identify the oil hydrocarbons more accurately since a comprehensive chemical analysis of the hydrocarbons occurring in the samples was not made by us. That required special experiments and was not the goal of our studies. We have analyzed spectral characteristics of water samples of oils and can agree with the opinion of authors of Refs. 4, 9, 12, and 13 that the form and structure of SFs (irrespective of excitation wavelength) correlate with their fraction composition. Thus, the oil hydrocarbons can be identified with respect to the main classes using the spectral information obtained.

Let us consider the spectra structure in more detail. It should be noted that there is always a band with maximum at λ_{max} = 320 nm in SF of water samples with different values of λ_{exc} (λ_{exc} = 220, 250, 266, and 280 nm). This constant situation with maximum position provoked our interest to SFE studying with recording at λ_{em} = 320 nm.

As seen in Fig. 3, all of SFE in the region from 260 to 280 nm contain a local maximum (the band at λ_{max} = 296 nm corresponds to RS of water). The SFs derived at different excitation (λ_{exc} = 220, 250, 266, 280, and 308 nm) corroborate the existence of maximum in SFE: a fluorescence signal is maximum at λ_{exc} = 266 nm (Table II). Hence the range from 260 to 280 nm is an optimal range of fluorescence excitation of oil hydrocarbons in water. The fourth–harmonic excitation wavelength of the YAG:Nd^{3+} laser lies in this region that accounts for high efficiency of this laser for diagnostics of oil hydrocarbons.

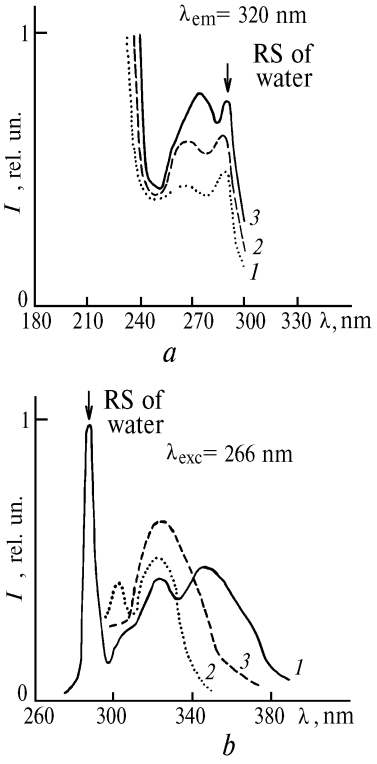

FIG. 3. Corrected spectra of excitation (a) and fluorescence (b) dissolved–emulsified oil products in water: 1 – fuel oil (15 μg/l), 2 – fuel for jet engines (50 μg/l), and 3 – Shaimskaya oil (25 μg/l).

The SFs of different samples recorded at such optimal excitation with laser fluorometer are given in Fig. 3*b*. The spectra have an inhomogeneous structure and, as was mentioned above, maximum at 320–nm wavelength. In the spectrum of heavy fuel oil there is an intense long–wave band (λ_{max} = 360 nm), while in the spectra of light oil products the most intense are short–wave bands at λ_{max} = 308 and 320 nm. In SF of crude oil all three bands are usually pronounced (Fig. 3*b*).

TABLE II. Comparison of a fluorescence parameter Φ for different oil products in water.

Type of oil product	$\frac{\Phi(\lambda_{exc} = 266\ nm)}{\Phi(\lambda_{exc} = 250\ nm)}$	$\frac{\Phi(\lambda_{exc} = 266\ nm)}{\Phi(\lambda_{exc} = 310\ nm)}$	$\frac{\Phi(\lambda_{exc} = 266\ nm)}{\Phi(\lambda_{exc} = 337\ nm)}$
Fuel Oil	1.5	1.6	2.2
Fuel for jet engines	2.1	7.8	8.0
Saratov oil	–	1.6	1.7
MARED	2.0	5.1	8.3

Thus, one can observe good agreement between the structure of spectra obtained at λ_{exc} = 266 nm and chemical (fractional) composition of oil hydrocarbons, i.e., it is possible to identify roughly the pollution with respect to the basic classes. A minimum detectable fluorescence signal related to concentrations varying from 5 to 10 μg/l for different samples of oil products. These limiting values we have found using the UNESCO hexane technique with our modification.

The use of UV radiation varying from 260 to 280 nm wavelength for exciting oil hydrocarbons in water has one more advantage: the problem of separating the spectral band of oil hydrocarbons and dissolved organic water in water is solved at once. As noted above, variation of the excitation wavelength in the range from 250 to 310 nm does not lead to displacement of maximum in the DOM fluorescence band and noticeable variation of the contour shape. In the spectrum of a model sample (Fig. 4) one can see that the fluorescence bands of oil hydrocarbons and dissolved organic matter at

λ_{exc} = 266 nm are resolvable, and the maximum of fluorescence is at 320 nm wavelength for oil hydrocarbons and at 420 nm for DOM.

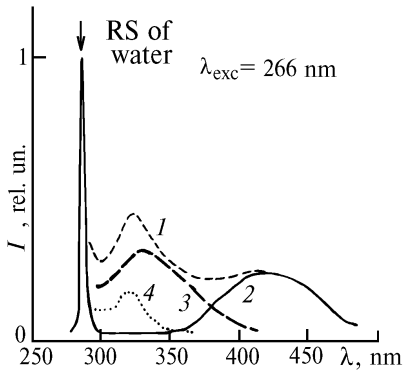

FIG. 4. Fluorescence spectra of a model water sample (1) with different organic admixtures. Fluorescence bands of DOM (2), dissolved protein (3), and dissolved–emulsified oil (4).

The determination of optimal wavelength of fluorescence excitation and possibility of spectral separation of the oil–hydrocarbon and DOM bands in water at such excitation enable us to look forward to a successful solution to the problem of diagnostics of oil hydrocarbon based on fluorescence spectra of water samples. However, field investigations have shown that the problems originated here are much more numerous than it might be expected. In addition to oil hydrocarbons and DOM the short–wave UV radiation excites protein compounds, which necessarily exist in natural water.

Thus, the fluorescence spectra of natural water excited at 266 nm contain information about at least three important components, which are responsible for an ecological state of water medium. So the problem is to extract this information from the spectra recorded.

6. FLUORESCENCE OF PROTEIN COMPOUNDS IN WATER

All protein compounds existing in natural water consist of amino acids, the proteins containing the same set of about 22 amino acids. Depending on their chemical structure amino acids are divided into seven groups. One of these groups responsible for protein fluorescence is a group of aromatic and heterocyclic amino acids: phenylalanine, tyrosine, and tryptophan.

The fluorescence properties of these aromatic amino acids have been known for a long time.[17] When amino acids are present in protein, their spectral characteristics change that leads to significant difference between SF of free amino acids and those of proteins. It should be noted that contribution of the three aforementioned amino acids to the total fluorescence of proteins is different. The main conclusions about the character of fluorescence of proteins and free amino acids drawn from this study[18] are as follows:

– due to low quantum yield (~ 4%) fluorescence of phenylalanine is not in fact observed in proteins. Moreover, in most experiments fluorescence of proteins is excited in the maximum of absorption bands of tyrosine and tryptophan (280 nm) that further decreases the phenylalanine contribution;

– the SFs of proteins containing tyrosine and phenylalanine and clear of tryptophan are close to the spectrum of free tyrosine at λ_{max} = 303 nm;

– the SFs of proteins containing all three amino acids have a fluorescence band at λ_{max} = 330 nm, which is typical for tryptophan but with small displacement of maximum position to the region of shorter wavelength (~ 20 nm) that results from screening tryptophan remainders from water with a protein matrix;

– the position of fluorescence of different proteins varies strongly since indole rings of tryptophan remainders are very sensitive to polarity, *pH*, and temperature of the solvent, and reaction of association and denaturation.

The results of our experiments on studying spectral characteristics of aromatic amino acids and some proteins are in good agreement with the foregoing results. The direct measurements with a small laser spectrometer at λ_{exc} = 266 nm have shown the minimum detected concentrations to be 15 μg/l for tryptophan and to vary from 100 to 200 μg/l for the proteins under study. These values correspond to mean concentrations of amino acids in natural water of the World Ocean.

All existing fluorescence methods for determining amino acids in natural water are based on addition of special derivators and sensitizers and give the total content of amino acids. However, to perform the analyses one should make special many–hour preparation of samples during which their content changes and some side reactions occur.

The direct laser method for determining protein compounds might be free from these disadvantages. However, due to a great deal of different protein compounds in natural water the problem of choosing the protein or one free amino acid as a standard requires special studies.

7. CONCLUSION

As seen from the results described in this paper the problem of separate determination of the main classes of organic compounds existing in water is very complicated and remains to be studied. The authors are hopeful they have made a step forward in that direction. There is good reason to believe that it will be possible to separate the fluorescence bands of proteins and oil hydrocarbons and this stage of the work will be brought to completion (without taking into account the effect of the other fluorescent compounds, e.g., phenols).

Furthermore, one must solve the other complicated problem of transition from fluorescence characteristics, in particular the parameter $\Phi = I_{fl}/I_{rs}$, to qualitative and quantitative parameters of the controlled organic complexes and compounds. As discussed earlier, crude identification of dissolved organic matter, oil hydrocarbons, and proteins can be made from fluorescence spectra during excitation at λ = 266 nm. After publication of Ref. 19 we were pinning our hopes on nonlinear fluorometry (saturation fluorometry), since the saturation parameters turned out to be different for different oil hydrocarbons. We continued these studies using more perfect instrumentation and technique, which made it possible to exclude the effect of photochemical processes using high–power laser excitation.[3] Thus, the search for fine identification of oil hydrocarbons and other organic compounds in water is now in progress.

The quantitative diagnostics of DOM also runs into some problems. When determining the DOM

concentration the main indicator is now concentration of organic carbon C_{org}, the instrumentation and technique for determining it being an individual complicated problem.[20] There is not always high correlation between fluorescence intensities of DOM and C_{org}. The reasons for such situation are not yet studied. This motivates our studies of the nature of DOM fluorescence band. During the mission on board the motor–ship "Il'ya Repin" in July, 1993 the comparison of the parameters Φ and C_{org} was made for severely polluted water of rivers.[21]

Transition from the parameter Φ for oil hydrocarbons in water to their concentrations faces the problem of choosing a calibrating standard solution, which could best simulate an oil hydrocarbons really existing in water (at least their fluorescent fraction). Coincidence of fluorescence spectra of oil hydrocarbons dissolved in hexane and extracted by hexane from water (see Section 4) testifies to competent usage of the same coupling coefficient α_1 for these both objects. However, one should also exercise caution and take into account the possible variety of natural situations where oil hydrocarbons dissolved–emulsified in water are formed.

These are some of the problems, which must be solved for developing the scientifically–justified methods of laser diagnostics of organic compounds in water.

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