Estimation of content and quantum yield of the fluorescent fractions of dissolved organic matter

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Studies of characteristics and properties of dissolved organic matter (DOM), its fluorescent fractions (FDOM) in waters of Yenisei and Angara rivers are presented. The studies have been carried out by measuring spectral absorption coefficients (in the spectral range from 400 to 800 nm), as well as the intensity of DOM fluorescence at three wavelengths (505, 525, and 540 nm) excited by radiation at 390 nm. The following parameters were used: coefficients characterizing the slopes of the spectral distribution of light absorption and DOM fluorescence, specific absorption coefficients of DOM and FDOM at the wavelength of radiation exciting the fluorescence (κ_{sp}, $\kappa_{f.sp}$), energy and quantum yields of DOM and its fluorescent fraction, and specific fluorescence of DOM and FDOM. The fraction of FDOM in the total DOM varies, in waters studied, from 8 to 40% with the mean value being about 19%. The quantum yield varies from 0.18 to 0.40 (the average value is 0.28). Specific absorption coefficients of DOM and FDOM are on the average close to each other ($\kappa_{f.sp}/\kappa_{sp}$ makes 0.93).

Introduction

A lot of organic compounds, mainly in dissolved form, are contained in waters of oceans, rivers, and lakes. The content of dissolved organic matter (DOM) is important biohydrochemical indicator of the state of water medium, which enables one to judge about suitability of this medium for bioproductive development of natural ecosystems. Because of the variety of composition and properties of the organic matter, its insignificant concentration, and difficulty of detection, its distribution in natural waters has been poorly studied.

A certain part of DOM fluoresces under the influence of exciting radiation of ultraviolet and visible ranges.[1–3] It would be interesting to establish whether the content of fluorescent fraction of DOM is related to DOM content. In some cases, correlation is observed between the absorption coefficient at the wavelength of excitation and the intensity of fluorescence. However, one cannot completely identify the absorption and fluorescent fractions of DOM.

Among physical characteristics of DOM fluorescence, its spectral distribution has been studied best of all. The shape of the fluorescence spectrum changes little, if spectral composition of the exciting radiation has been constant. On the contrary, the changes of the latter strongly affect the position of the maximum and the shape of the fluorescence band of DOM.[1] As the wavelength of exciting light decreases, the maximum of fluorescence of DOM is shifted to the short-wave spectral range, and its relative value increases. The maximum of the fluorescence band is shifted relative to the line of excitation by 80–100 nm toward longer wavelengths. This is the difference between the fluorescence of DOM from fluorescence of solutions, the position and the shape of the fluorescence band of which do not depend on the spectral composition of the exciting radiation.

Variations of the intensity of DOM fluorescence in seawaters were carried out by many researchers.[1–6] Such investigations have also been carried out in the inland water reservoirs.[5,7,8]

In this paper the study is presented of the characteristics and properties of the dissolved organic matter, estimation of the fraction of fluorescent DOM (FDOM), and their parameters.

Objects and methods of the study

Investigations were carried out on Angara river (August 1990 and July 1991 from its mouth and upstream the dam of Ust'-Ilimsk hydroelectric power station), Yenisei river, and Krasnoyarsk water reservoir, and the characteristics of model solutions of pure humid acids of three different concentrations were measured.

In measurements the spectral absorption and scattering coefficients (κ and σ, respectively) were determined. Determination of the total concentration of DOM (C) was carried out using measurement data on the chemical consumption of oxygen (by standard method of bichromate oxidation) using the generally accepted recalculation coefficient of 0.75.

Measurement of the fluorescence intensity I_f of dissolved organic matters was carried out with a LflI fluorimeter of our own design at the wavelengths of 505, 525, and 540 nm and using excitation of

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fluorescence by radiation at the wavelength $\lambda = 390$ nm. To take into account the spectral selectivity of the device, the measured I_f values were corrected using the integral transmission coefficients of the filters and the spectral sensitivity of the PMT used. In the case of Krasnoyarsk water reservoir the spectral distribution of the intensity of fluorescence of DOM was measured in the range from 430 to 600 nm.

In many water reservoirs, the significant fraction of organic matter coming to water medium in dissolved form is adsorbed on living and lifeless suspensions. Therefore, the measurements of all parameters were carried out directly in water samples (without filtering) in order to determine not only the dissolved organic matter but also the organic matter adsorbed from the dissolved phase (AOM) on the particles of a suspension, which varied in the waters under study from 10 to 40% depending on the content of suspended matter. In the majority of cases, fluorescence of chlorophyll of phytoplankton (on average, the content of chlorophyll was about 2.6 µg/l) causes insignificant distortions of the spectra of DOM fluorescence.

Results and discussion

Study of variations of the intensity of fluorescence of dissolved organic matter together with determination of hydrooptical characteristics enables one to obtain some parameters, which make it possible to judge about qualitative change of the composition and properties of DOM.

It is known that the spectral dependence of the absorption coefficient κ of the dissolved organic matter in the wavelength range 250 to 600 nm is approximated by an exponential function[9]:

$$\kappa(\lambda) = K\exp(-\mu\lambda), \qquad (1)$$

where K is the coefficient of proportionality. The coefficient μ characterizing the slope (derivative of the logarithm of the absorption index with respect to wavelength $\mu = d\ln(\kappa)/d\lambda$) of the spectral distribution of the absorption coefficient, obviously, represents qualitative changes of the composition of DOM. The coefficient μ was calculated using the wavelengths 400 and 425 nm as follows:

$$\mu = [\ln\kappa(400) - \ln\kappa(425)]/25. \qquad (2)$$

The values μ for the studied waters varied from 0.012 to 0.020.

One also can approximate the spectral dependence of the intensity of fluorescence of DOM I_f in the wavelength range 500 to 600 nm (Fig. 1) by the exponential function $I_f(\lambda) = K_1\exp(-\mu_f\lambda)$.

The coefficient μ_f was calculated using the wavelengths 505 and 525 nm:

$$\mu_f = [\ln I_f(505) - \ln I_f(525)]/20. \qquad (3)$$

It is seen in determination of μ_f and μ, that the changes of qualitative composition of only fluorescent

fraction of DOM affect the value μ_f, while the value μ is affected by absorption of light by both FDOM and other components of DOM.
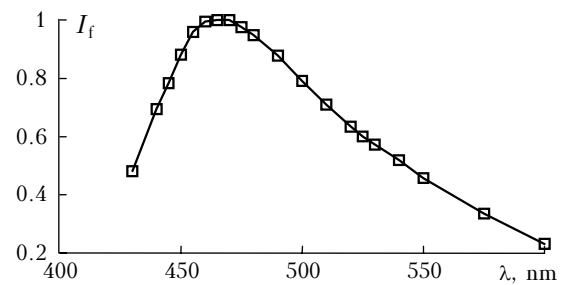

Fig. 1. Spectral distribution of the intensity of fluorescence normalized to the maximum at excitation by radiation at the wavelength of 390 nm.

The specific absorption coefficients of DOM and FDOM also depend on their composition:

$$\kappa_{sp}(\lambda) = \kappa(\lambda)/C = K\exp(-\mu\lambda)/C,$$
$$\kappa_{f.sp}(\lambda) = \kappa_f(\lambda)/C_f = K_1\exp(-\mu_f\lambda)/C_f, \qquad (4)$$

where C_f is the content of FDOM.

Naturally, the values of energy yield of fluorescence of the total DOM (I_y) and its fluorescent fraction FDOM ($I_{f.y}$):

$$I_y = I_f/I_a = I_f/I_0 [1-\exp(-\kappa l)] \approx$$
$$\approx I_f/I_0\kappa l \approx I_f/I_0\kappa_{sp}Cl, \qquad (5)$$
$$I_{f.y} = I_f/I_{f.a} = I_f/I_0 [1-\exp(-\kappa_f l)] \approx$$
$$\approx I_f/I_0\kappa_f l \approx I_f/I_0\kappa_{f.sp}C_f l, \qquad (6)$$

and the values of specific fluorescence of the total DOM (I_y) and its fluorescent fraction FDOM ($I_{f.y}$):

$$I_{sp} = I_f/C = I_f \kappa_{sp} /\kappa, \qquad (7)$$
$$I_{f.sp} = I_f/C_f = I_f \kappa_{f.sp} /\kappa_f, \qquad (8)$$

where I_0 is the flux of exciting radiation, W; I_a, $I_{f.a}$ are the intensities of radiation absorbed by the total DOM and FDOM, respectively, W; l is the layer thickness (in the fluorimeter chamber), m. The approximate relationships $[1 - \exp(-\kappa l)] \approx \kappa l$ and $[1 - \exp(-\kappa_f l)] \approx \kappa_f l$ are used in Eqs. (5) and (6) after expansion of the expression in brackets into a series in exponents.

These parameters are correlated between each other to one or another degree. The ratio of the values of the specific fluorescence and the yield of fluorescence is proportional to the specific absorption coefficient of the total DOM ($I_{sp}/I_y \approx I_0 l\kappa_{sp}$), and analogous ratio for the fluorescent fraction of DOM is proportional to the specific absorption coefficient of the fluorescent components of DOM.

Approximation of the correlations (linear trends) between specific characteristics of the emission and absorption as well as the coefficients μ and μ_f for the studied waters shows that, as μ_f increases, the specific fluorescence of DOM and the specific absorption coefficient at the wavelength of

excitation of fluorescence increase. As μ increases, these parameters decrease. It should be noted that the main characteristics of the DOM composition are different in different periods of investigations. The values μ_f and μ weakly affect the fluorescence yield that is evidence of not very essential changes of the quantum yield of FDOM from sample to sample.

Different directions of the effect of the processes of emission and absorption of light by DOM on the considered parameters are observed. One can conclude that the ratio $q = \mu_f/\mu$ should represent the changes of the characteristics of fluorescence and absorption of light. The dependences of the specific fluorescence on the value q are shown in Fig. 2.
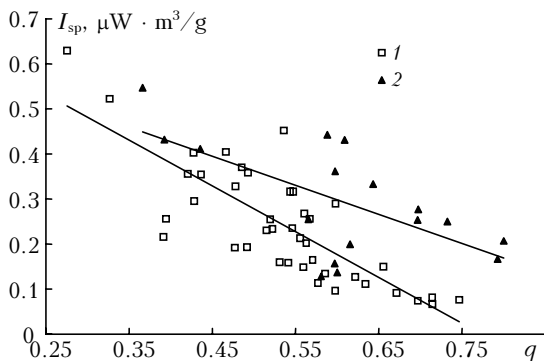

Fig. 2. Dependences of the specific fluorescence on the ratio between coefficients μ_f and μ: (*1*) measurements in 1991, (*2*) in 1990.

The trends are presented by the relationships: $I_{sp} = -1.01q + 0.78$ at $r^2 = 0.65$ (*1*); $I_{sp} = -0.65q + 0.68$ at $r^2 = 0.40$ (curve *2*). The specific absorption coefficient is approximated by the following relationships: in 1990 $\kappa_{sp} = -1.00q + 0.89$ at $r^2 = 0.41$; in 1991 $\kappa_{sp} = -1.59q + 1.32$ at $r^2 = 0.53$.

Weak dependence is observed in the studied water objects between the intensity of radiance I_f and absorption by DOM (at $\lambda = 390$ nm), on the one hand, and the concentration of DOM on the other hand: $I_f = 0.17C$ at $r^2 = -0.06$ (1990) and $I_f = 0.27C$ at $r^2 = 0.46$ (1991); $\kappa = 0.18C$ at $r^2 = 0.10$ (1990) and $\kappa = 0.39C$ at $r^2 = 0.24$ (1991). For humic acids $I_f = 0.32C$ at $r^2 = 0.99$ and $\kappa = 2.64C$ at $r^2 = 0.98$. It is seen that the characteristics of humic acids are different from that of natural waters, especially for the absorption of radiation.

As is noted for seawaters,[1] independence of fluorescence and absorption of light on C means that the part of DOM responsible for both processes is a small fraction of DOM present in water. This conclusion is correct if excitation of fluorescence and measurement of absorption have been realized in the UV wavelength range (less than 250 nm). All organic substances absorb UV radiation, and weak correlation between the absorption coefficient at the wavelength of 390 nm and the content of DOM can be determined by the fact that the contribution of some components of DOM in this range can be not proportional to their content in water. It is especially

correct for the fraction of DOM, the absorption maximum of which is shifted to vacuum UV and, hence, the long-wave part of electron absorption band in the visible wavelength range represents the content of these components to a lower degree.

Approximation of the absorption coefficient by the exponential function (1) makes it possible to estimate its value in experimentally unmeasured UV wavelength range. The correlation coefficients between the absorption coefficient and the content of DOM (Fig. 3) increase with decreasing wavelength of light (up to X-ray radiation). The correlation coefficient between these characteristics in solutions of humic acids remains practically constant at all wavelengths.
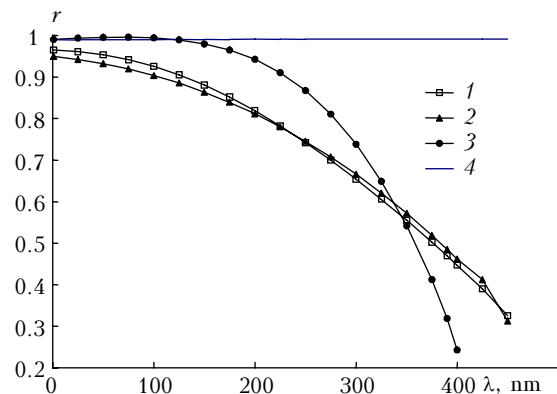

Fig. 3. Correlation coefficients between the absorption coefficient and the content of DOM: (*1*) for the united data array, (*2*) measurements in 1991, (*3*) measurements in 1990, (*4*) solutions of humic acids.

One can estimate the fraction of DOM, which contributes to light absorption at the wavelength of 390 nm, based on the following ideas. Let us normalize the spectral absorption coefficients to the absorption coefficient at a fixed wavelength (λ_0) in the UV range (Fig. 4 for $\lambda_0 = 200$ nm).
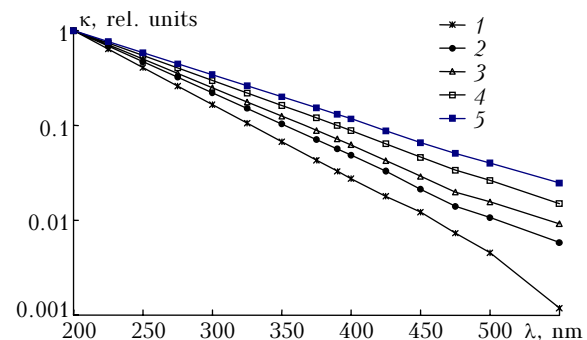

Fig. 4. Normalized spectral characteristics of light absorption: (*1–4*) r. Angara (635, 215, 30, 41 km, respectively), (*5*) solution of humic acids.

One can assume that all components of DOM increase light absorption at the wavelength λ_0 by the values proportional to the content of the component in water, but this relationship becomes essentially different at long wavelengths. Proportionality of the

content of humic acids and absorption is kept at all wavelengths (see Fig. 3). Based on this fact, the fraction of DOM responsible for absorption at the wavelengths longer than λ_0 can be determined as follows:

$$C_d/C = K_2 \exp[-(\mu - \mu_h)(\lambda - \lambda_0)], \qquad (9)$$

where μ_h is the coefficient μ for solution of humic acids, C_d is the content of the fraction of DOM absorbing light at the wavelength λ, K_2 is the coefficient determining the fraction of chromophores of humic acids contributing to light absorption at the wavelength of fluorescence excitation.

Calculation for the wavelength of excitation of fluorescence and $\lambda_0 = 200$ nm gives variability of the value C_d/C for the studied waters from 0.20 to 0.68 with the mean value of 0.36. Regression equations for the relation between κ and C_d for separate periods of measurements approach each other, that makes it possible to consider the united data array. The equation for the relation based on the united array is $\kappa = 1.18 C_d$ ($r^2 = 0.75$). The higher correlation is observed between the intensity of fluorescence and C_d: $I_f = 0.82 C_d$ at $r^2 = 0.58$. The relation for humic acids is represented by the equation $\kappa = 3.20 C_d$ ($r^2 = 0.98$). The choice of $\lambda_0 = 200$ nm is caused by the facts that it is unknown, whether exponential dependence (1) is valid at the shorter wavelengths. There are no such data in literature. Although it is interesting to note that in using shorter wavelengths, correlation between κ and C_d increases. At $\lambda_0 = 75$ nm r^2 is already equal to 0.90. The difference between tangents of the slope angle of the regression equations for natural waters and humic acids decreases: 2.07 and 3.22, respectively.

In order to estimate the content of FDOM, one needs to know the specific fluorescence $I_{f.sp}$. The dependences of the values of the specific fluorescence on the parameter q taking into account the calculated values C_d are the following:

$$I_{sp} = -0.50q + 1.25 \text{ (in 1990)},$$
$$I_{sp} = -2.21q + 1.81 \text{ (in 1991)}.$$

Based on the assumption that the general behavior of the dependences of the specific yield of fluorescence on the value q is the same for the fluorescent components of DOM one can apply, to estimate the content of FDOM, the algorithm for calculation using the formulas:

$$I_{f.sp} = -0.50q + A, \ \ I_{f.sp} = -0.50q + B, \quad (10)$$

where A and B are the coefficients, the values of which are limited by certain relationships.

Varying the coefficients A and B in Eq. (10) and using Eq. (7), one can determine the probable limits of variations of C_f depending on the value q calculated from the experimentally measured absorption coefficients and the intensity of fluorescence. The criterion for the range of variability

of A and B in the most general case is fulfillment of the relationship $0 < C_f/C_d \leq 1$.

Further calculations of the values $I_{f.y}$, $\kappa_{f.sp}$ will enable to better determine the used limits, because one can also impose restrictions on the values of the yield of FDOM fluorescence and the specific absorption coefficient of FDOM. It follows from the estimate of the radiative lifetime[1] ($\tau = 6$ ns) of seawaters DOM fluorescence that the quantum yield of fluorescence of DOM Q cannot be high, because shorter radiative lifetimes (of the order of 1 ns) correspond to the molecules of the substances characterized by high Q values, but usually the time of the fluorescence decay is on the order of 10 ns.[10] The probable quantum yield of fluorescence of FDOM within the maximum of the irradiation band can be no more than 0.6. The maximum of fluorescence of FDOM of inland water reservoirs at excitation at $\lambda = 390$ nm is observed at the wavelength $\lambda = 465$ nm (see Fig. 1). Taking into account this fact, the quantum yield of FDOM Q_f at $\lambda = 505$ nm is not greater than 0.55. It is clear that the value Q_f is always greater than the value Q calculated for the total content of DOM.

Besides, the absence of correlation between the specific light absorption coefficient and the concentration of DOM, as well as between κ_{sp} and the intensity of FDOM radiance is evidence of the fact that the specific absorption coefficients of DOM and FDOM cannot be significantly different, i.e., their ratio, on the average, is close to unity.

Thus, calculation of the possible parameters of FDOM was carried out using the revealed limitations: $0 \leq C_f/C_d \leq 1$; $Q \leq Q_f \leq 0.55$; $\kappa_{f.sp}/\kappa_{sp} \approx 1$. The calculations have shown that the most probable values of the relative content of fluorescent fractions C_f/C in the studied waters vary from 8 to 40% with the mean value of 19%. The relative content of fluorescent fractions C_f/C_d taking into account the components of DOM, which make approximately proportional contribution to light absorption at the wavelength of 390 nm, varies from 22 to 100% with the mean value of 57%. Then it follows that not all light-absorbing DOM fluoresce.

Some decrease of the portion of fluorescent DOM fractions is observed at increase of both total content of DOM and the content of FDOM, that is evidence of different relationship between FDOM and non-fluorescent fractions of allochthonous OM, due to which main variations in the content of DOM occur, and in autochthonous OM formed in the water reservoir due to photosynthesis.

Relation between the specific absorption coefficient and the value q for the united data array using the calculated values of the content C_d is represented by the regression equation $\kappa_{sp} = -2.35q + 2.38$ at $r^2 = 0.52$. The values of the specific absorption coefficient of FDOM were calculated by equation $\kappa_{f.sp} = -2.35q + E$. Varying the free term E of the equation, it was found that the best fulfillment of the established limitations is observed

when the quantum yield of fluorescence Q_f at the wavelength of 505 nm (at excitation of fluorescence at the wavelength of 390 nm) have varied within the limits from 0.18 to 0.40 with the mean value of 0.28. The mean ratio of the specific absorption coefficients $\kappa_{f.sp}/\kappa_{sp}$ is 0.93.

Relation between the intensity of fluorescence in the studied waters and humic acids with the calculated content of FDOM is represented by the general equation $I_f = 1.41 C_f$ ($r^2 = 0.96$).

Let us note for conclusion that the spectra of absorption and fluorescence of FDOM can be determined only by means of direct measurements of the parameters and properties of fluorescent fractions of DOM. However, the necessary methods for fractioning DOM are absent, so researchers have to consider only comparison of the fluorescence of DOM with measurements in the spectral ranges, where absorption of DOM is prevalent.[1] But it is not quite correct, because not all light-absorbing DOM fluoresce.

It is shown in this paper that not all components of DOM make contribution to light absorption in the visible and near UV wavelength ranges, proportional to their content in water medium. The technique is proposed for calculation of the relative content of the DOM fraction, which absorbs light in the aforementioned spectral ranges proportionally to its concentration. Also, the possibility is shown of estimating the parameters of fluorescent fractions of DOM based on the study of the spectral intensity of fluorescence of dissolved organic matter together with determination of the spectral absorption coefficients of DOM.

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