Investigation of UV fluorescence of DOM in centrifuged seawater samples

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Fluorescence of centrifuged seawater samples taken from a depth of 20 to 200 m in the Black Sea was investigated. Intense ultraviolet fluorescence of dissolved organic seawater matter with two peaks near 345 and 358 nm was found. The fluorescence is excited by a band with two peaks at 260 and 290 nm. With depth, the intensity of the fluorescence decreases, and the shape of the fluorescence spectrum changes. In the sea photic zone, the character of ultraviolet fluorescence of the dissolved organic matter changes in different seasons.

It is well-known that fluorescence of dissolved organic matter (DOM) in seawater looks like a broad structureless band with a half-width of 100–140 nm. The main spectral range of DOM fluorescence falls on the region of 360–520 nm (blue fluorescence) with a peak at 420–430 nm in the case of short-wavelength excitation. This peak shifts depending on the exciting radiation wavelength. The Stokes shift of the fluorescence is 80–120 nm. The excitation band of this fluorescence has a half-width of 80–90 nm, and its peak also shifts for different wavelengths of fluorescence recording. The shifts of this kind are indicative of the DOM complex composition, whose nature remains poorly studied yet.

In Ref. 1, fluorescent characteristics of samples were studied, obtained from suspension of seawater plankton organisms concentrated by 40 times through filtering. A distinctive feature of those spectra was the presence of two peaks both in excitation (~ 282 and ~ 365 nm) and in fluorescence (~ 350 and ~ 450 nm) spectra.

In Ref. 2, as filtered samples were excited by radiation at $\lambda \sim 285$ nm, an unclear fluorescence arm was observed nearby 350 nm in some cases. A distinctive feature was that the arm was excited only within the narrow spectral range 270–300 nm. Comparing the results obtained with the results of Ref. 1, Brown (Ref. 2) supposed that peaks at 280 nm in the excitation spectrum and at 350 nm in the fluorescence spectrum are connected with the presence of proteins, which could be released upon destruction of cells.

The results obtained by Traganza and Brown are of undoubted interest. They suggest that the study of UV fluorescence of the seawater DOM will yield new data on its composition and origin.

The aim of this work is to continue the study into DOM nature and the mechanism of its appearance in seawater with the aid of the spectrofluorimetric technique.

Instrumentation of investigations

When developing the measurement technique, it was assumed that organic matter dissolved in seawater is neither a product of vital phytoplankton excretion[3] nor a product of a Meyer's-type reaction proceeding directly in seawater, but is formed inside the organic suspension. It releases in the marine environment as a result of diffusion and washing-out from organic matter, decaying due to ageing and bacterial action in the process of sedimentation.

In this work, seawater was sampled in the eastern part of the Black Sea 8–10 miles offshore from depths of 20–200 m with a viniplast bathometer. Before sampling, the depth range of the living phytoplankton occurrence was determined with the Variosens immersible fluorimeter.

The process of the organic suspension destruction and washing-out of dissolved organic matter, which proceeds under natural conditions while the organic suspension sedimentation in seawater, was modeled in the accelerated form upon centrifugation of seawater samples.

Seawater samples were centrifuged on the Opn-8 medical centrifuge in 20-ml plastic test tubes at a rate of 8000 rpm for 30 min. After centrifugation, the top part (part *a*) of a sample was collected by a pipette and the bottom part (part *b*) was discharged. It was believed conditionally that the top part of the sample contained mostly the light fraction of organic matter, while the bottom part contained the heavier fraction and residues of organic suspension.

Changes in transmission spectra of centrifuged seawater samples and their excitation and fluorescence spectra were studied.

Differential transmission spectra between centrifuged (parts *a* and *b*) and initial seawater samples were recorded on a KSVU-23 system with a scanning step of 2.5 nm. In measurements, the specially manufactured 1-cm quartz cell was used.

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Fluorescence and fluorescence excitation spectra in parts *a* and *b* of centrifuged seawater samples were recorded on an SDL-2 setup in the photon-counting mode with a scanning step of 1 nm. Fluorescence excitation spectra were recorded in the spectral range 240–320 nm, while fluorescence spectra were recorded in the range 330–400 nm at room temperature.

Experimental results and discussion

Differential transmission spectra have shown that after centrifugation of seawater samples a broad absorption band of a complex shape appeared in the spectral range 230–340 nm. A distinctive feature of this band was the presence of absorption peaks at ~ 242, ~ 265, and ~ 284 nm. The complex shape of this absorption band and its spectral range are indicative of appearance of chromophoric groups of amino acid molecules, proteins, and their derivatives in the seawater solution.

Figure 1 shows the differential absorption spectrum between the initial sample collected in August from a depth of 20 m and different parts of the centrifuged sample (CS).

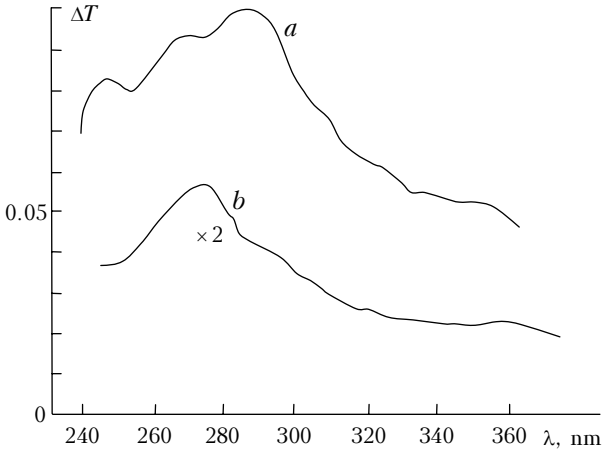

Fig. 1. Differential transmission spectra of seawater CS sampled from a depth of 20 m.

Similar results were obtained in Ref. 4, in which light absorption by an individual chloroplast was studied with a microspectrophotometer. As is well-known, chloroplasts contained in phytoplankton cells are organoids responsible for synthesis or accumulation of organic substances inside a cell. The measurement have revealed the presence of absorption peaks in the UV spectral region at 265 and 290 nm, which were attributed to the presence of proteins in chloroplasts.

The study of fluorescence of centrifuged seawater samples has shown the following. Intense UV fluorescence with a pronounced structure was found at $\lambda_{exc} = 298$ nm in centrifuged seawater samples from a depth of 20 m. This fluorescence band was characterized by a half-width of ~ 30 nm and two peaks at 345 and 358 nm. The intensity of the UV fluorescence tens times exceeded that at a peak of the usually recorded seawater DOM fluorescence (at 420–430 nm).

For the both peaks observed in the fluorescence spectrum, the fluorescence excitation spectra were identical and looking like an intense narrow almost symmetric band with a half-width of ~ 20 nm, a main peak nearby ~ 290 nm, and a small peak nearby ~ 260 nm. Radiation at 260 and 290 nm identically excited the discovered fluorescence. The fluorescence excitation spectra were not calibrated, which did not allow us to estimate the true intensity ratio of these peaks. Figure 2 shows the obtained excitation and UV fluorescence spectra of centrifuged seawater samples.

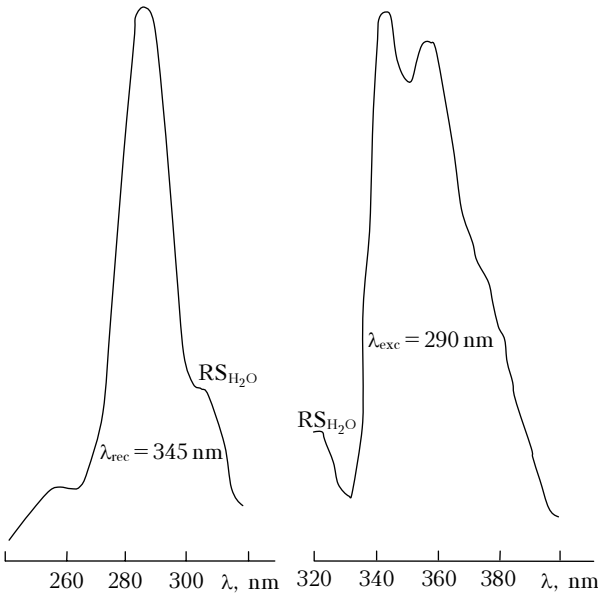

Fig. 2. Spectra of excitation and UV fluorescence in part *a* of the centrifuged seawater sample collected from a depth of 20 m (RS_{H_2O} is the Raman scattering of water).

The spectral range of the excitation band and the discovered fluorescence, as well as the obtained differential transmission spectra, are indicative of the presence of proteins and their derivatives in the studied seawater samples.

From the investigation of the molecular composition of the photosynthetic reactive center of bacteria and algae, it is known that the protein of the reactive center consists of polypeptide subunits of different masses, the heaviest of which can be separated from the reactive center under conditions of soft denaturation.[5] This fact apparently takes place at filtration and, especially, at centrifugation of seawater samples.

Changes in UV fluorescence of seawater CS with depth were studied in seawater samples collected in August from depths of 20, 35, 50, 80, 100, 150, and 200 m. The measurements have shown that the UV fluorescence spectra do not change, as the depth increased down to the hydrogen sulfide zone (~ 100 m), which was determined by the characteristic smell in collected samples. Differences appeared in part *a* of

the seawater sample from a depth of 150 m. Here, a single low-intensity peak nearby ~358 nm was observed against the background of the long-wavelength wing of blue fluorescence, whose intensity increased with the depth. In samples from a depth of 200 m, the fluorescence of parts *a* and *b* was similar to that of part *a* of the sample from 150 m, and for part *b* an arm was additionally observed at 345 nm. The intensity of the UV fluorescence in the spectral range 330–370 nm at a depth of 200 m roughly halved compared to the intensity at a depth of 20 m.

Changes in the UV fluorescence of seawater in different seasons were studied in February–July. The fluorescence spectra of seawater CS for February and June included two peaks of roughly identical intensity at 345 and 358 nm. The fluorescence excitation spectra were also characterized by two intense peaks centered at 258 and 290 nm. An arm was observed in the range 230–250 nm and at 284 nm.

A single peak centered at 345 nm mostly predominated in the fluorescence spectra of seawater CS collected in May, and in parts *a* of the seawater samples from 20 and 50 m only this peak was present. In parts *b* of the seawater samples from 50 and 80 m, as well as in the both parts of the sample from 150 m, the peaks centered at 345 and 358 nm had the same intensity. In part *a* of the sample from 200 m, the peak centered at 358 nm had a higher intensity than that at 345 nm.

In the fluorescence excitation spectrum for λ_{rec} = 345 nm, the highest-intensity peak at 290 nm was accompanied by an arm nearby 284 nm. The intensity of the peak at 260 nm in the excitation spectra was at the level of an unclear arm, whereas the intensity of the peak in the range 230–250 nm increased markedly, and unclear peaks at 235 and 248 nm were observed here.

Analyzing the results obtained, we can conclude that in the photic zone of the sea the character of UV fluorescence of seawater changes in different seasons. Thus, in the period of phytoplankton bloom, organic substances having mostly a single peak (nearby 345 nm) are present in upper sea levels. The excitation spectrum of this fluorescence mostly includes a single peak at 290 nm. The intensity of the peak at 260 nm exciting this fluorescence decreases considerably, but in the range 230–245 nm the intensity increases. Before and after phytoplankton bloom, the fluorescence spectrum includes two peaks centered at 345 and 358 nm, which are excited by radiation with peaks at 260 and 290 nm.

It is obvious that substances fluorescing at 345 nm are short-lived, since in addition to seasonal changes the intensity of this peak in the fluorescence spectrum decreases with depth till complete vanishing, whereas the peak at 358 nm is still present down to the maximally studied depth (200 m). Changes in UV fluorescence spectra of photic-zone seawater during a season are caused most probably by biochemical transformations in phytoplankton occurring in the period of its intense bloom.

For further investigation of the discovered fluorescence, part *a* of the centrifuged seawater sample collected during the phytoplankton bloom in the last part of May from depths of 20 and 100 m was two to three times concentrated by evaporation. The evaporation proceeded at the seawater boiling temperature and normal atmospheric pressure. In parallel to evaporation, products of the volatile fraction were collected through a chemical cooler. The UV fluorescence was studied in the samples obtained.

The investigations have shown that the products of the volatile fraction of part *a* of the seawater samples under study had similar intense fluorescence, in whose band a bathochromic shift of about 10 nm occurred. Here, two peaks were observed at 355 and 370 nm along with an arm nearby ~345 nm. For the sample from a depth of 20 m, the peak at 355 nm far exceeded that at 370 nm. For the sample from a depth of 100 m, the peaks had similar intensities.

The excitation spectra of fluorescence and their character did not change compared to the initial ones. For λ_{rec} = 370 nm, the excitation spectrum was characterized by a more intense peak at ~260 nm and a less intense peak at ~290 nm with an unclear arm at ~284 nm. In the excitation spectrum for λ_{rec} = 355 nm, to the contrary, a more intense peak was observed at 290 nm and a less intense peak took place at 260 nm. A broad arm appeared in the range 225–245 nm, and an unclear arm was observed near 280 nm.

We failed to record the fluorescence spectra of concentrated seawater samples because of their turbidity. Only the excitation spectra for λ_{rec} = 345 and 358 nm were recorded. The fluorescence excitation spectra and their character were generally similar to the initial ones, but for the peak at 290 nm the bathochromic shift of 10 nm occurred. For λ_{rec} = 358 nm, the excitation spectrum of the sample from a depth of 20 m included two intense peaks at 300 and 260 nm with an arm in the region 230–250 nm. For λ_{rec} = 345 nm, the peak at 260 nm was absent, while a very intense peak at 248 nm and an arm at 255 nm appeared along with the peak at 300 nm. An unclear arm was observed near 235 nm. In the excitation spectra of the concentrated sample from a depth of 100 m for λ_{rec} = 358 nm, the most intense peak was observed at 296 nm and two pronounced peaks were seen at 260 and 245 nm. For λ_{rec} = 345 nm, one peak was observed at 296 nm and another intense peak took place at 246 nm.

Thus, the investigations have shown that the dissolved organic matter having the UV fluorescence characterized by the peaks at 345 and 358 nm is released into the seawater solution during centrifugation. These peaks are excited in the regions of 248, 260, and 290 nm. In the region of 284 nm, an additional peak is present in the excitation spectrum, but it is seen as an arm due to the closeness to the peak at 290 nm. The substance released upon centrifugation has a volatile fraction, which also has the UV fluorescence.

This investigation allows us to conclude that centers responsible for the fluorescence at 345 and 358 nm are most probably different, as indicated both by seasonal variations in the fluorescence spectra and by the excitation spectra of concentrated seawater samples. The investigation has shown that the peak at 345 nm in the fluorescence spectrum is excited predominantly near 290 nm, while the fluorescence peak at 358 nm has one more rather intense peak centered at 260 nm, which may be absent in the excitation spectrum of the peak at 345 nm.

The comparison of the obtained fluorescence excitation spectra with differential transmission spectra has shown that the peaks in the excitation spectra nearly coincide with the transmission peaks (~ 242, ~ 265, ~ 284 nm), that is, their chromophore and fluorophore centers are close. The coincidence of peaks in the UV fluorescence excitation spectra (260, 290 nm) with those of chloroplast absorption (265, 290 nm) described in Ref. 4 suggests that the organic substance released during centrifugation is contained in phytoplankton cells.

The studied excitation and fluorescence spectra of centrifuged seawater samples confirm and complement the results obtained in Refs. 1 and 2 for other seas. The similarity of the spectra obtained allows us to suppose that the nature of fluorescence in waters of different seas and oceans is identical.

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