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LUMINESCENCE OF ORGANIC COMPOUNDS–COMPONENTS OF NATURAL DISSOLVED ORGANIC SUBSTANCE

K. G. Evsyukhina, S. V. Patsaeva, and V. I. Yuzhakov

The spectral-luminescent properties of compounds–chemical analogues of natural components of dissolved organic substance in fresh-water basins are reported. The compounds studied in aqueous solutions of various concentrations included aromatic amino acids tyrosine, phenylalanine, and tryptophan, hydroquinone, and gallic acid. The pH dependences of the absorption and luminescence spectra were determined.

INTRODUCTION

This work is concerned with the spectral characteristics of the simplest components of natural dissolved organic substance. The fluorescence spectra of natural water excited at wave lengths shorter than 270 nm contain two bands. The UV band has a maximum at 300–350 nm; in the literature, this band is assigned to the fluorescence of protein-like compounds [1–3]. The second band has a luminescence maximum in the blue region and refers to humus compounds dissolved in natural water [4, 5].

The previous studies performed in this laboratory showed that the UV luminescence band of water from natural basins included contributions of not only proteins and aromatic acids but also of phenols and polyphenol compounds such as tannin and lignin.

Collecting data on the fluorescence of phenol compounds and aromatic amino acids of various concentrations will help to estimate the contributions of chemical compounds of various classes to the luminescence band of natural water. A study of acidity effects on the electronic spectra of compounds constituting complex natural associations will promote investigations of biological molecular systems with H-bonds and proton transfer.

METHODS AND OBJECTS OF STUDY

We studied hydroquinone, gallic acid (3,4,5-trihydroxybenzoic acid), and aromatic amino acids tyrosine, tryptophan, and phenylalanine.

The compounds were dissolved in thoroughly purified water; the criterion of water purity was the absence of luminescence. Solution acidity (pH) was varied by adding small amounts of aqueous hydrochloric acid and potassium hydroxide, which do not luminesce noticeably in the spectral range of interest (200–500 nm), and measured with the use of a pH-340 pH-meter.

The absorption spectra were recorded on a Specord M-40 (Germany) spectrophotometer.

The luminescence excitation and emission spectra were obtained with the use of a Jobin Yvon 3CS (France) spectrofluorimeter. The quantum yields were calculated by a relative method from the areas under the luminescence spectra of samples and absorption at the excitation wave length. The quantum yields for all solutions were found relative to a $C = 5$ mg/l tryptophan solution, the quantum yield of which was assumed to be 0.20 [6, 7].

Moscow State University, Faculty of Physics.

Table 1

Spectral-Luminescent Characteristics of Aqueous Solutions of Aromatic Amino Acids.

Amino acid	λ^{abs}_{max}, nm	$\varepsilon \times 10^{-3}$, l/(mol cm)	λ^{fl}_{max}, nm	B_{rel} ($\lambda_{exc} = 220$ nm)
Phenylalanine	210	10	285	0.06
Tyrosine	222	6.8	305	0.22
	275	1.3		
Tryptophan	220	40	355	0.20
	285	8		

Table 2

Spectral-Luminescent Characteristics of Aqueous Solutions of Hydroquinone and Gallic Acid

Compound	pH	λ^{abs}_{max}, nm	$\varepsilon \cdot 10^{-3}$, l/(mol cm)	λ^{fl}_{max}, nm nm	B_{rel} ($\lambda_{exc} = 266$ nm)
Hydroquinone	7	221	5.1	330	0.67
		287	2.9		
	10	221	—	330	0.24
		245	—	290	
	7 (return motion)	214	—	330	0.21
		290	—	—	
	5 (return motion)	—	—	—	0.20
	3	221	5.1	330	0.42
		287	2.9	—	
Gallic acid	7	212	21.4	350	0.11
		260	7.1	390	—
	10	212	15.2	360	—
		285	7.3	400	—
	3	212	18.6	360	—
		272	8.2	390	—

RESULTS AND DISCUSSION

Aromatic amino acids. Proteins contain three amino acid residues that can contribute to UV fluorescence, namely, tyrosine, tryptophan, and phenylalanine. These aromatic amino acids have systems of delocalized π electrons in their aromatic nuclei and absorb light at 240–300 nm.

The experimental maxima of absorption and luminescence spectra (λ^{abs}_{max} and λ^{fl}_{max}, respectively), the extinction values (ε), and the relative quantum yields (B_{rel}) for neutral aqueous solutions of the studied aromatic amino acids are listed in Table 1.

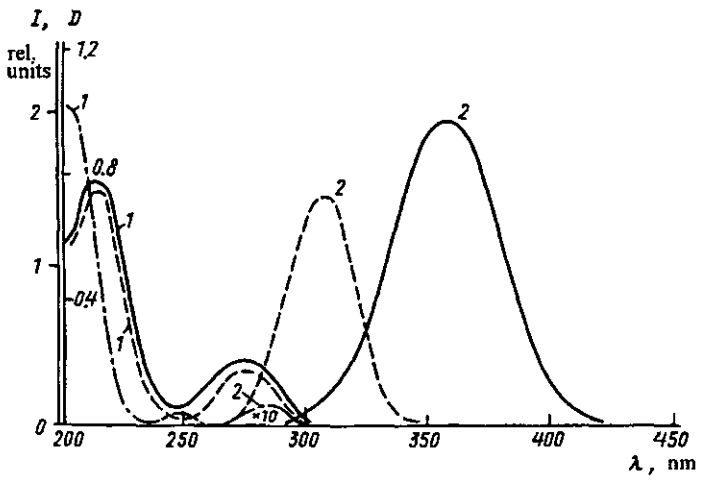

Fig. 1

Absorption (*1*) and fluorescence (*2*) spectra of aqueous solutions of amino acids: tryptophan (C = 5 mg/l, λ_{exc} = 266 nm, solid lines), tyrosine (C = 20 mg/l, λ_{exc} = 266 nm, dashed lines), and phenylalanine (C = 20 mg/l, λ_{exc} = 220 nm, dot-and-dash lines).

The absorption and luminescence spectra of the amino acids are shown in Fig. 1. No substantial changes in spectral curve shapes or band positions were observed when the concentration was varied from 1 to 100 mg/l.

Fluorescence of most of the proteins is largely due to tryptophan residues containing indole rings, which are exceptionally sensitive complex fluorophores. Luminescence of phenylalanine is determined by the benzene ring. According to [6, 7], the quantum yield of phenylalanine is as low as 0.04. Our experimental quantum yield values (Table 1) are in close agreement with the literature data. The emission spectrum of tryptophan is controlled by the indole ring; the quantum yield in neutral aqueous solutions equals 0.20, which is two times smaller than the quantum yield of indole [6, 7]. The emission spectrum of tyrosine is controlled by the phenol ring.

In addition to concentration, we studied pH dependences of tyrosine luminescence spectra. Emission spectrum shapes and wave lengths of emission maxima were virtually independent of pH in the pH range 2–10. The quantum yield for the neutral tyrosine solution was 0.22. In an acid medium (pH 2.8), the quantum yield increased almost twofold. In alkaline media, the quantum yields of phenol and its derivatives such as tyrosine were largely controlled by OH group ionization causing fluorescence quenching. In these media, the quantum yield of tyrosine decreased to 0.16.

Note that tyrosine fluorescence can be quenched by neighboring carboxyl or uncharged amino groups [6, 7]. This quenching involves proton transfer to these proton acceptors during excited state lifetime, as was proved in the study of fluorescence of tyrosine–glutamate and tyrosine–lysine copolymers [8, 9]. The mechanism of quenching may include hydrogen transfer from phenol in the ground or excited state [10].

Hydroquinone and gallic acid were studied as phenol analogues of the simplest components of natural dissolved organic substance.

The spectral-luminescent characteristics of the solutions of hydroquinone and gallic acid measured at C = 5 mg/l are listed in Table 2.

Gallic acid is a structural unit of natural polymers such as tannin and lignin and is formed as a result of the degradation of natural dissolved organic substance of fresh-water basins.

The spectral characteristics of solutions at various pH were studied following two procedures. During the first procedure, pH was initially increased to 9–10 by adding alkali and then decreased to a neutral or pH = 3 value (Scheme I). During the second procedure, pH was first decreased to 3–4 by adding an acid and then increased to a neutral or higher pH value (8–9) (Scheme II). This was done to see whether the processes occurring under pH variations were reversible.

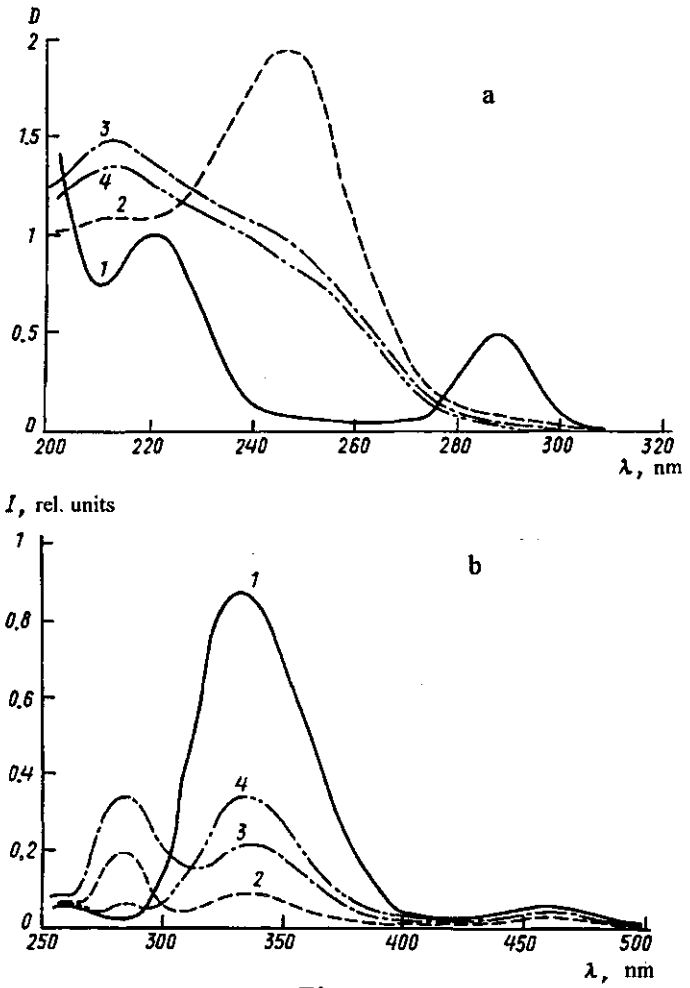

Fig. 2

Absorption (*a*) and fluorescence (*b*) spectra of hydroquinone (C = 20 and 5 mg/l, respectively, λ_{exc} = 230 nm) at various pH values (Scheme I): *1*, neutral solution; *2*, pH 10; *3*, pH 7 (return motion); and *4*, pH 3 (return motion).

The pH dependences of the absorption and the fluorescence spectra of hydroquinone are shown in Figs. 2 and 3. When the solution pH increased to 10, new bands appeared in the luminescence and absorption spectra, and the quantum yield of luminescence decreased (Table 2). A decrease in solution acidity to pH 2.5 did not cause noticeable changes in the absorption and emission spectra. The luminescence quantum yield decreased from 0.67 in a neutral to 0.42 in an acid medium.

The absorption spectra of the aqueous solutions of gallic acid (c = 5 mg/l) recorded under pH variations according to Schemes I and II are shown in Fig. 4. The absorption spectrum of the neutral solution of gallic acid contained two maxima at λ = 212 and 260 nm, the ratio between the intensities of which was 1 : 0.3. In the alkaline medium (pH 10), a new absorption maximum appeared at λ = 285 nm. The absorption spectra taken at various pH values had two isobestic points at λ = 245 and 273 nm. When the neutral solution was acidified, the long-wave absorption maximum shifted toward the red to λ = 272 nm.

The emission spectrum of the neutral solution contained a maximum at λ = 350 nm with a shoulder at λ = 390 nm, the intensity ratio between the maximum and the shoulder being 1 : 0.5. In the excitation spectrum, the corresponding maxima were located at λ = 268 and 246 nm, respectively. This observation is the evidence of the presence of two different gallic acid forms in neutral solutions. When the acidity of the aqueous solutions of gallic acid was varied, the fluorescence spectrum experienced reversible changes

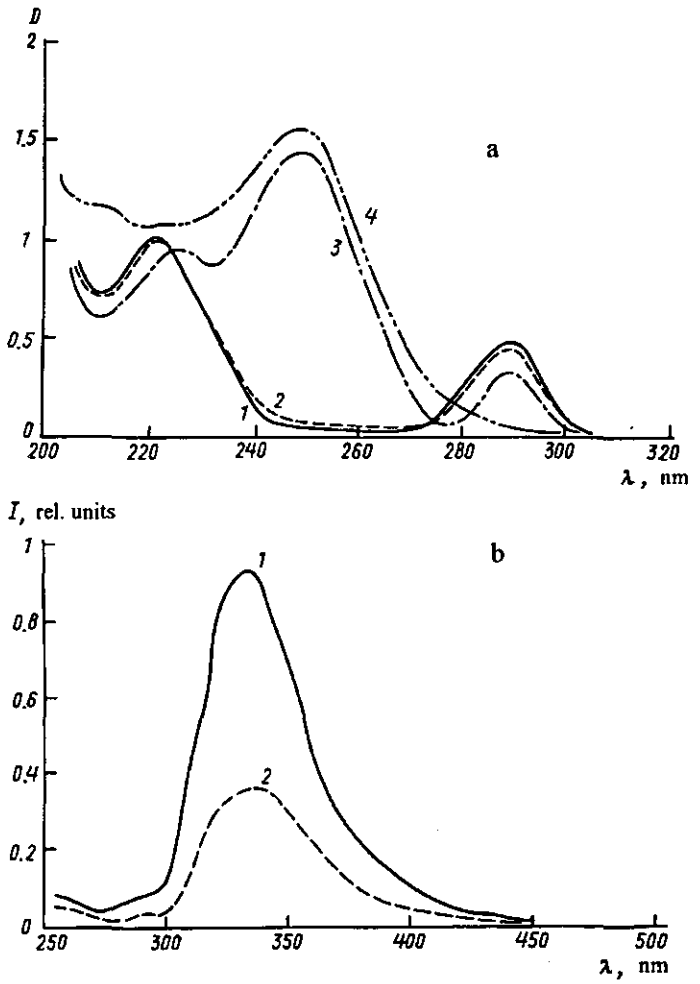

Fig. 3

Absorption (*a*) and fluorescence (*b*) spectra of hydroquinone (C = 20 and 5 mg/l, respectively, λ_{exc} = 230 nm) at various pH values (Scheme II): *1*, neutral solution; *2*, pH 3; *3*, pH 7 (return motion); and *4*, pH 10 (return motion).

irrespective of the direction of acidity variations. In both acid (pH 3) and alkaline (pH 10) media, the principal fluorescence maximum at λ = 390 nm shifted by 10 nm to the longer waves. The intensity ratio between the λ = 350 and 390 nm fluorescence bands was 1 : 2.5 in the acid and 1 : 0.8 in the alkaline medium.

CONCLUSION

The amino acids and phenol compounds studied in this work make contributions to the UV fluorescence band of natural water. The irreversibility of processes that occur in the aqueous solutions of these compounds under pH variations should be taken into account in analyzing the absorption and emission spectra of natural dissolved organic substance.

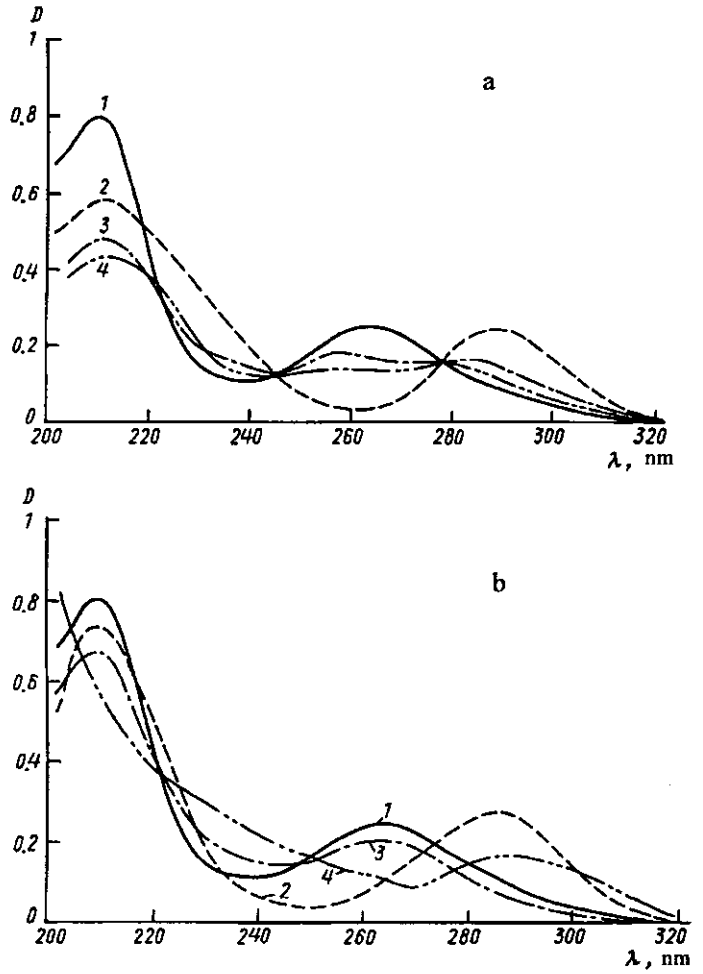

Fig. 4

Absorption spectra of gallic acid ($C = 5$ mg/l) at various pH values; (*a*) Scheme I: *1*, neutral solution; *2*, pH 10; *3*, pH 7 (return motion); and *4*, pH 3 (return motion); (*b*) Scheme II: *1*, neutral solution; *2*, pH 3; *3*, pH 7 (return motion); and *4*, pH 10 (return motion).

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