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Effect of pH of the medium on fluorescence spectra of carbon dots based on Nile red dye

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The influence of alkaline medium and ultraviolet irradiation on the fluorescence of solutions of carbon dots based on the laser dye Nile red has been studied. It was found that the increase in pH of the medium leads to the quenching of the red emission band dominant in the fluorescence spectrum of carbon dots and to the enhancement of the green band, and the same fluorescence behavior is characteristic of Nile red solutions. It is shown that the quenching of red fluorescence of carbon dots and Nile red is accompanied by a significant weakening of the corresponding bands in the absorption spectra of the fluorophores studied. A similar effect was observed in the fluorescence of solutions of carbon dots and Nile red with neutral $\mathrm{pH} \approx 7$ as a result of their irradiation with ultraviolet light in the spectral range of 248−400 nm. The influence of alkaline environment and ultraviolet irradiation on the optical characteristics of fluorophores is attributed to the chemical and photochemical reactions initiated by these external factors in the systems studied. The possible nature of the emissive centers involved in the reactions is discussed.

Keywords: Carbon dots, absorption spectra, luminescence spectra, pH effect, UV treatment.

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Introduction

The concept of „carbon dots“ was first introduced into the scientific literature in 2006 to specify luminescent carbon nanoparticles synthesized by laser ablation of a carbon target [1]. Carbon dots (CD) are nanoparticles with size of less than 10 nm from crystalline graphite or turbostrat carbon with atoms in the state of sp^2-hybridization [2]. CDs are of interest as a new type of luminophores, which successfully combine such qualities as bright photoluminescence, low toxicity, low cost and relative simplicity of synthesis. This combination of properties sets CDs apart from semiconductor quantum dots. In process of CD synthesis the surface of the graphite core turns out to be enriched with various functional groups (carbonyl, carboxyl etc.), which substantially determine the optical properties, chemical activity, degree of hydrophilic behavior and other characteristics of CDs [3–7]. The ability to functionalize the surface of a nanodot with various functional groups opens the way to the targeted modification of its properties [8,9]. Unique luminescent and physical-chemical properties of CDs, including the ability to modify the radiation wavelength when the exciting light frequency changes, absence of fluttering specific for semiconductor quantum dots [10], provide them with huge potential for wide use. Apart from using in optoelectronics, information storage and transformation devices, catalysis, chemical probing, CDs are of special interest from the point of view of various biomedical applications [11–15]. Successful use of CDs in various applications suggests comprehensive research of impact from various external factors, including environmental factors, at their properties. In a wide range of papers (see review [10]) high sensitivity of fluorescence (FL) of CD is noted to power of hydrogen (pH) of the medium, which, in particular, opens the prospects of CD use as pH-sensors. It was established that the impact of pH of the medium may be expressed in change of intensity [16,17] and spectral shift of CD luminescence bands [18–21]. A relatively complex structure of CD with a graphite core and surface rich in carboxyl, hydroxyl, carbonyl and other functional groups, which may interact with ions $\mathrm{H}^+/\mathrm{OH}^-$ (determining the pH of the solution), complicates the analysis of pH dependence mechanism for CD's FL. Even though by this time several mechanisms were proposed to explain the pH-sensitivity of CD, there is no single opinion about the feature of their structure that determines this sensitivity yet [10]. Therefore it is important to observe the previously unknown features of impact of pH of solution at CD's FL, and to do the comparative study of the pH impact at FL of CDs and of corresponding precursors.

It is known that the CD luminescent characteristics depend on the conditions of their synthesis, including the used precursors, molecules (or fragments) of which in some cases may be included in the composition of synthesized CDs [22]. To that effect the properties of CDs synthesized on the basis of laser dyes are obviously interesting. This paper presents CD's FL spectra obtained by pyrolysis of laser dye Nile red (NR), and the features of influence from high pH of solutions of synthesized CDs on their FL spectrum. It is shown that the increase in pH level of CD solution in various solvents causes quenching of a red band of CD FL and strengthens the green fluorescence. A close analogy is found for the influence of high pH of solutions of CDs and NR on their optical spectra of luminescence and absorption. It was demonstrated that a similar influence on the FL spectra of NR and CD solutions is provided by ultraviolet light. The possible causes of the observed effects are discussed.

1. Experimental procedure

CDs were synthesized in nanopores of spherical particles of the mesoporous silica SiO_2. The precursor was laser dye NR ($C_{20}H_{18}N_2O_2$, Sigma-Aldrich), containing atoms of nitrogen and oxygen, which, as we know, participate in the formation of emitting centers in CDs [23,24]. Particles of mesoporous silica were prepared using the method described in [25]. The particles have an internal system of cylindrical channels of the same diameter (if necessary, it may be varied in the range from 2 to 5 nm). The particle diameter was 500 ± 20 nm, the diameter of nanopores - 3.10 ± 0.15 nm. Silica particles were impregnated with 33 % (vol) NR solution in methanol (99.8 %, Acros), dried at 50°C and annealed on air at 330°C for 2 h. Thermal decomposition of NR led to the formation of highly monodisperse CDs in silica nanopores [26]. To separate the CDs from the silica matrix, the latter was dissolved in fluoric acid (ACS, reagent grade). After dissolution of the silica particles, the CDs sediment was cleaned from SiO_2 dissolution products. CD content in the concentrated water suspension was detected gravimetrically and amounted to 0.06 mass% (~ 0.03 vol.%). The CD concentration in the suspension was $\sim 10^{16}\,\mathrm{cm}^{-3}$ (with volume of single CD $\sim 20\,\mathrm{nm}^3$). The concentrated CD suspension was used to prepare colloidal CD solutions in ethanol. With the CD suspension volume of $100\,\mu l$ per 1.1 ml of ethanol, the CD concentration in the solution was $\sim 10^{15}\,\mathrm{cm}^{-3}$ at $\mathrm{pH} \approx 7$. The solution pH was changed by adding 25 % aqueous solution of ammonia to the produced solution. The maximum pH value was 12, and the CD concentration in the solutions with different pH was maintained invariable.

To detect the functional groups withing the CDs, we studied their IR transmission spectra [27]. Electronic transmission spectra of CD solutions in the area of 200−1000 nm were recorded using spectrophotometer SF2000. CD’s FL was excited by radiation of laser with wavelength of 405 nm. The density of the exciting radiation power did not exceed $0.5\,\mathrm{W/cm}^2$. The CD solution was placed into a standard quartz cuvette 10×10 mm. The exciting radiation was normally incident on the surface of the cuvette, CD’s FL was recorded at the right angle to the direction of the exciting light propagation. The radiation spectra were recorded using a diffraction spectrometer. The analysis of the temperature dependence of CD’s FL anisotropy on the basis of the Perrin equation [28] makes it possible to estimate the dot size as 3.5 ± 0.3 nm, which agrees well with the results of the CD size measurement by electron microscopy.

2. Experimental results and discussion

IR spectrum of CDs is characterized by a series of peaks that may be related to the specific absorption bands of functional groups OH, C−H, C=O, C=C, C−C, C−O, C−O−C [27]. The presence of C=C peak indicates the graphite structure of the CD, whereas the peaks of OH, C=O and COOH indicate there are hydroxyl, carbonyl and carboxyl functional groups on the CD surface [29]. IR transmission spectrum of the CDs in ethanol is hard to identify due to strong absorption of the solvent.

Electronic absorption spectra of CD and NR solutions in ethanol are shown in fig. 1. You can see that the absorption spectra of CD and NR are similar to each other. In the CD spectrum you may identify the absorption bands with maxima at 206, 245 and 480 nm. Short-wave bands in the area of $\lambda < 300$ nm are usually assigned to $\pi\pi^*$-transition with participation of carbon atoms in the state of sp^2-hybridization (excitation of aromatic C=C-bond), and the structure in the area of 300−400 nm — to electron $n\pi^*$-transition with participation of C=O-group in CD carbon core [30–32]. Longer-wave absorption is related to the defects of the CD surface caused by the presence of C=O- and C=N-groups on the surface [24]. In the NR spectrum you can clearly see the absorption bands with maxima at 205, 265, 305 and 552 nm. The longest-wave band 552 nm (fig. 1), matching the maximum of the band in the NR FL excitation spectrum, complies with the photo-induced transfer of electron density from the nitrogen atom in diethyl amino group and the backbone of NR molecule to heteroatoms, mainly, to cyclic nitrogen and carbonyl oxygen (electron transition HOMO → LUMO) [33,34].

FL spectra of CD and NR are presented in fig. 2. As in the cases of absorption spectra, certain similarity is seen

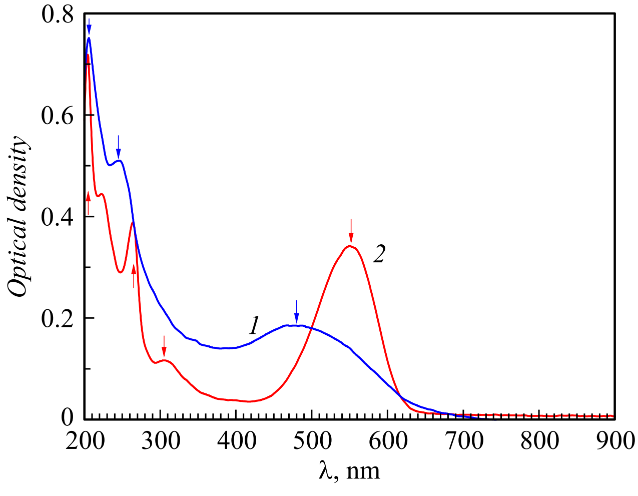

Figure 1. Absorption spectra of CD (*1*) and NR (*2*) solutions in ethanol.

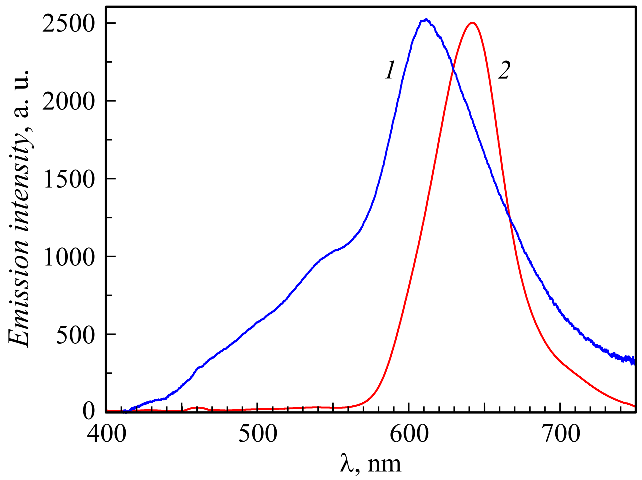

Figure 2. Emission spectra of CD (*1*) and NR (*2*) solutions in ethanol. $T = 300\,\mathrm{K}$.

in both spectra. Each is dominated by the intense band of red FL with wavelength 611 nm (in case of CD) or 642 nm (in case of NR). (642 nm band is due to LUMO → HOMO transition in NR molecule.) In case of CD the FL band is wider and has asymmetrical shape with a noticeable short-wave wing, which is compliant with a more complicated structure of emitting states in the CD compared to the NR molecule.

Increasing the pH of CD solution from pH ≈ 7 up to ≈ 12 was accompanied by change in the color of solution photoluminescence from pink to light green, which was due to the drop in the intensity of the main (red) band of FL and formation of intensive green luminescence in the area of the short-wave wing (fig. 3).

You can see in fig. 3, the alkaline medium provides substantial impact on the condition of the emitting centers in CD, which causes, in particular, the reduction in the number of red FL centers and formation of green FL centers. The intensity of green FL is quite high with account of the fact that the absorption of the solution at the excitation wavelength (405 nm) in the alkali presence drops significantly (fig. 4). The assumption on the destruction of the red luminescence centers matches the changes observed with increasing pH in the CD absorption spectrum (fig. 4). As it was noted above, the CD excitation with the light having wavelength from the absorption band 480 nm (fig. 1) is quite effective from the point of view of the red FL excitation (in this respect this band is similar to band 552 nm in the NR absorption). As you can see in fig. 4, in the medium with pH ≈ 12 the absorption band 480 nm is absent in the spectrum (as band 611 nm in FL spectrum).

As it follows from fig. 3, the impact of alkali at CD’s FL even at pH ≈ 12 shows itself rather slowly, and a noticeable time delay is observed between the moment of solution pH increase and the moment of the actual reaction in the FL spectrum. Therefore, one may assume that the molecules of proton solvent (ethanol) due to formation of hydrogen bonds with the atoms of nitrogen and oxygen in CD form around it a „tight“ solvate shell, which complicates the contact of the alkali molecules and the carbon dot. Note that when ethanol is replaced with aprotic dimethylsulfoxide, similar changes in the CD FL spectrum happen much faster. Also note that FL spectra and spectra of CD and NR absorption in pure ethanol (pH ≈ 7) practically did not change in the same time interval.

In connection with the certain similarity of NR spectra and that of CDs synthesized on its basis noted above it is interesting to find out, how far this similarity goes, and what is the NR behavior in the high pH media. Fig. 5 shows FL spectra of NR in ethanol (pH ≈ 7) and in ethanol with addition of NH_4OH (pH ≈ 12). As you can see in fig. 5, pH-effect in the NR spectrum is qualitatively similar to the

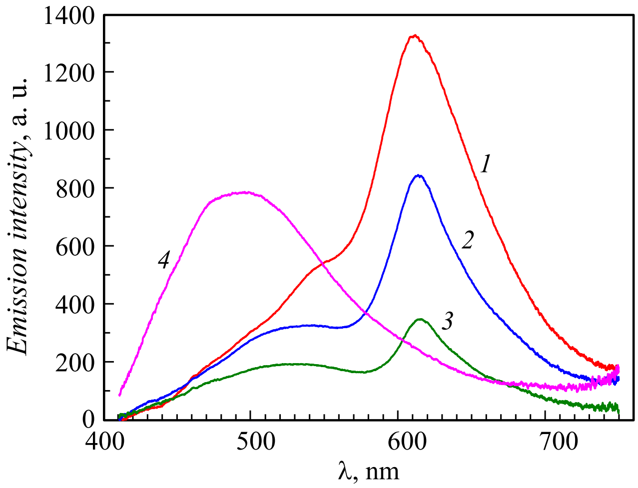

Figure 3. Emission spectra of CD solutions with pH ≈ 7 (*1*) and pH ≈ 12 (*2–4*). Spectra *2–4* were measured in 12 h (*2*), five days (*3*) and two months (*4*) after preparing the solutions.

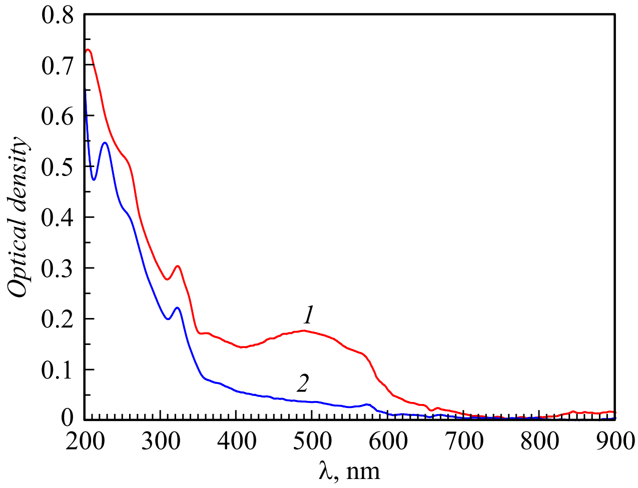

Figure 4. Absorption spectra of CD solutions with pH ≈ 7 (*1*) and pH ≈ 12 (*2*). Spectra are measured in two months after preparation of the solutions.

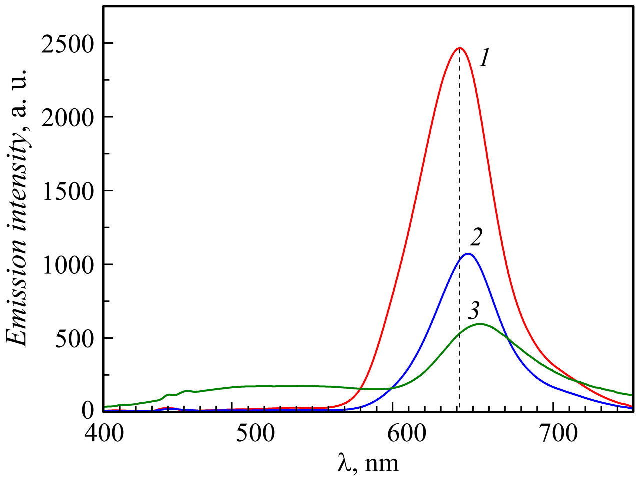

Figure 5. Emission spectra of NR solutions with pH ≈ 7 (*1*) and pH ≈ 12 (*2,3*). Spectra *2* and *3* are measured in 12 h and two months after preparation of the solutions, accordingly.

effect observed in the CDs spectra. Increase of the solution pH causes quenching of red FL and appearance of weak NR’s FL in the green area of the spectrum. It should be noted that energy distances between the maxima of red and green FL bands in NR and CD spectra coincide.

NR absorption behaves in a similar way. In the alkaline medium (pH ≈ 12) a drastic weakening is observed for the primary absorption band 552 nm, but absorption increases in a shorter-wave area of the spectrum. Therefore, influence of the alkaline medium on the FL spectra and long-wavelength structure of the CD and NR absorption spectra is quite similar, which makes it possible to assume there is similarity in the corresponding mechanisms. This similarity may be due to the closeness of the structures responsible for FL, and be related, in particular, to inclusion of NR molecules (or their fragments) into the composition of the CD graphite core layers (for example, its external „polar“ layers).

Therefore, let us also note that the CD and NR solutions demonstrate similar behavior when exposed to UV light. Fig. 6 illustrates the impact of the preliminary UV radiation of CD and NR solutions on their FL spectra. Irradiation was done with the radiation of superhigh pressure mercury lamp DRSh-250 in the spectral interval of 248−400 nm. The degree of UV irradiation impact on FL depends on its duration. As you can see in fig. 6, UV irradiation of CD and NR causes quenching of the red FL in the solutions and strengthens (in case of CD) or causes appearance (in case of NR) of green FL.

UV irradiation of CD and NR solutions reduces optical density of solutions at the excitation wavelength (405 nm) and accordingly reduces the light power absorbed by solutions. Therefore, the FL spectra in fig. 6 are normalized to the power absorbed by the solutions. As in case of the alkaline medium, UV irradiation of CD and NR solutions is accompanied with a drastic weakening of long-wavelength absorption bands coupled with red FL of CD and NR.

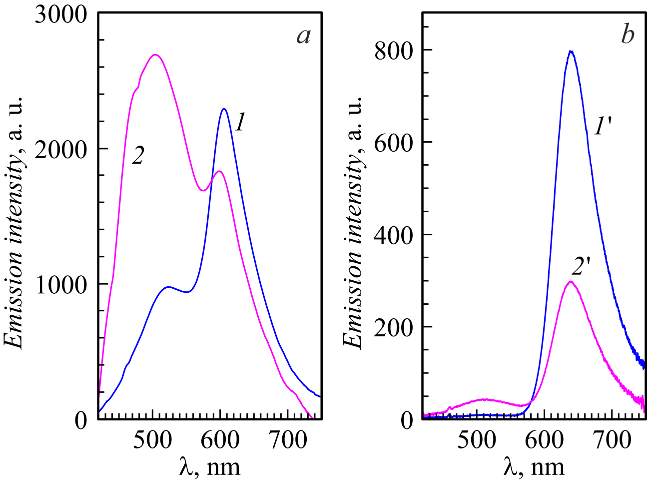

Figure 6. Emission spectra of CD (*a*) and NR (*b*) solutions in ethanol after preliminary UV irradiation for 19 (*1*), 160 (*2*), 25 (*1′*) and 80 min (*2′*). The excitation wavelength is $\lambda_{\mathrm{exc}} = 405$ nm. Solution spectra are normalized to the absorbed excitation power.

One may assume that the impact of the alkaline medium or UV irradiation on the CD and NR is due to the chemical or photochemical reactions happening in the system, which cause a change in the composition and/or structure of both fluorophores. In particular, at high pH values the alkaline medium may cause deprotonation of hydrogen-containing functional groups on the CD surface and formation of new surface states [35,36]. It is also known that the exposure to alkaline medium and UV radiation cause deoxygenation of CD [37–39], including the reduction of the carbonyl and hydroxyl functional groups. As it was noted above, NR FL is due to electronic transitions between the states with the participation of carbonyl oxygen. In this case the transformation of carbonyl groups under the impact of the alkaline medium or UV irradiation should suppress NR FL. On the other hand, transitions with the participation of the modified carbonyl group may cause origination of new FL bands (for example, green FL). If luminescent properties of the studied CDs are due to the inclusions of NR molecules, one may assume that the CD response to the high pH of the medium or UV irradiation will be similar to the NR response.

Conclusion

Therefore, pH of the environment to a substantial degree determines the FL of the CD solutions based on NR. If in the neutral medium with pH ≈ 7 in the FL spectrum of CD solution in ethanol the red radiation prevails, then, as pH rises, the FL red band is quenched with simultaneous formation of a green FL band on the background of its short-wave wing, and this band will prevail in the spectrum at high pH values. It was found that the described effect was qualitatively similar to effect on CD’s FL from CD exposure to UV light. The similarity in the effects of high pH and UV treatment to CD and NR FL makes it possible to assume that FL of CD is due to the inclusion of NR molecules (or their fragments) into the graphene layers of CD. The possible cause for the observed effects is the change in the composition of oxygen-containing functional groups in CD under the effect of alkali and UV radiation, however, the mechanism of the corresponding chemical and photochemical reactions remains unclear and requires further research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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