FLUORESCENCE QUENCHING OF CERIUM NITRATE WITH GRAPHENE OXIDE

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The fluorescence quenching of Ce^{3+} ions in an aqueous solution of cerium nitrate with colloidal graphene oxide (GO) was studied. By measuring the relative fluorescence intensities and the lifetimes of the excited states of these ions from the concentration of GO plates and using the Stern-Volmer equations, it was shown that electron excitation of Ce^{3+} is accompanied by static fluorescence quenching. The measured Stern–Volmer association constant of aquacomplexes of cerium with GO amounts to 24.7 l/g, which is significantly lower than the constants of cationic dyes of rhodamines and metal cations. The result is explained by the presence of negative charges on the surfaces of the interacting particles.

Keywords: cerium nitrate; graphene oxide; absorption spectra; fluorescence; quenching of fluorescence; Stern–Volmer equation.

Introduction

Graphene oxide (GO) is one of the most promising multi-functional nanomaterials. To the surface of GO nanoparticles and to the edges are bound oxygen-containing groups and many aromatic rings with condensed nuclei and a π-conjugated electron system. The presence of oxygen-containing groups gives the plates a negative charge in the solution and ensures the dispersion of GO in water and organic solvents. Currently, research is being conducted on the interaction of carbon nanoparticles with organic and inorganic compounds. The interaction of graphene oxide dispersed in water with positively charged particles (metal ions, cations of organic compounds) is most often studied. Aqueous solutions of trivalent cerium salts with anions that do not have absorption bands in the near ultraviolet region are of considerable interest for practical applications. Their fluorescence with a quantum yield reaching unity is used for the calibration of spectrofluorimeters and the fluorescence of fluorophores. Cerium usually resides in two stable oxidation states (+3 and +4) with the configurations [Xe] $4f^1$ and [Xe] $4f^0$, respectively. The high reduction potential of Ce (IV) makes it a very effective oxidizing agent compared to other cations [1], and for this reason, cerium salts, especially ammonium cerium (IV) nitrate, are widely used as a single-electron oxidizers. Recently, studies have been conducted on the use of cerium in biology and medicine [2]. CeO_2 was studied as a means for intracellular drug delivery and as a framework for the cultivation f stem cells in vitro. Studies are being carried out with cerium salts in order to thermally separate H_2O and CO_2 and to generate fuel. To increase the efficiency of interaction with negatively charged particles, the plate surface is sometimes passivated by groups with a positive charges. In this work, we studied the regularities of the effect of graphene oxide plates dispersed in water on the spectral-luminescent properties of trivalent cerium nitrate in order to clarify the nature of their interaction.

Experimental

Graphene oxide was synthesized from graphite using a modified Hammers method. A concentrated colloidal solution of GO plates in water was used to obtain the required concentrations by diluting it. Different amounts of GO were mixed with a solution of cerium nitrate crystalline hydrate ($Ce(NO_3)_3{\cdot}6H_2O$) with a concentration of 7.8×10^{-5} M/l. The absorption spectra were recorded on a Varian Cary 500 spectrophotometer, and the fluorescence and fluorescence excitation spectra were recorded on a Solar SM 2203 spectrometer. The fluorescence kinetics were measured on a Horiba Jobin Yvon spectrofluorometer. The method of dynamic light scattering (DLS) is used to measure the size of the synthesized graphene oxide particles, which uses the relationship of the DLS and the speed of the translational Brownian motion of particles with a diffusion coefficient *D*. The hydrodynamic size *d* of monodisperse particles is calculated using the Stokes-Einstein equation:

$$d = kT/3\pi\eta D.$$

Here *k* is the Boltzmann constant, *η* is the viscosity of the solution, *T* is the temperature. In the study of non-spherical particles by the DLS method, d is the diameter of a spherical particle, which has the same translational diffusion rate as the GO particle. Graphene oxide production method produces a mixture of microplates of various sizes. To obtain the size distribution of graphene oxide nanoparticles in a suspension of the product in an aqueous solution, an appropriate processing of the autocorrelation function of the light intensity scattered at an angle of 90° was carried out. The maximum of the curve with a half-width of 32 to 54 nm corresponded to a size of about 42 nm (Fig. 1).

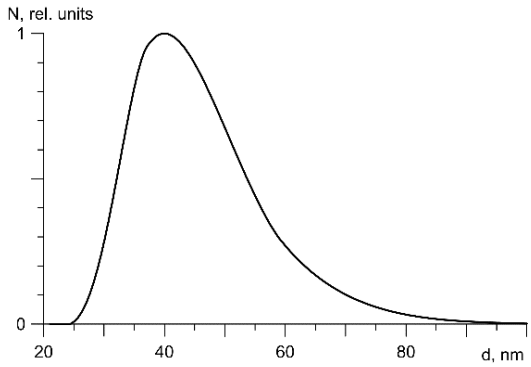

Fig.1. Graphene oxide particle size distribution

Results and discussion

In fig. 2 shown the spectrum of the optical density of GO dispersed in an aqueous solution obtained in the ultraviolet and visible regions.

As can be seen from the figure, there is a band maximum around 200 nm, which corresponds to the π-π* transitions of the C – C bond of aromatic rings, as well as a small shoulder in the spectrum at λ = 300 nm, corresponding to the n – π transitions of carbonyl C = O bonds that is consistent with known data indicating the formation of an GO. The absorption of light by the graphene oxide in the visible range of the spectrum is due to electronic transitions in sp^2–C clusters, the energy of which decreases with increasing their size. Insert in fig. 2 shows the dependence of the change in the optical density of the GO solution on the concentration of particles. The linearity of the dependence indicates the absence of aggregation of GO in this concentration range.

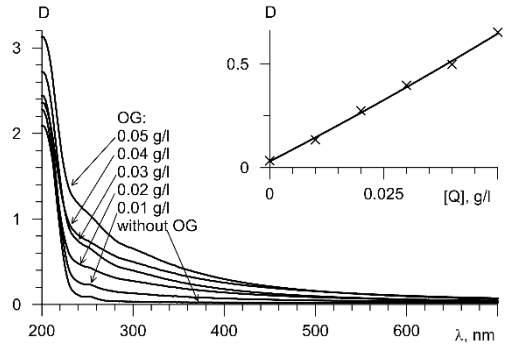

Fig. 2. Spectra of optical density of graphene oxide in a colloidal aqueous solution, depending on the concentration of GO, in the inset is the dependence of optical density solution at λ = 300 nm from the concentration of GO

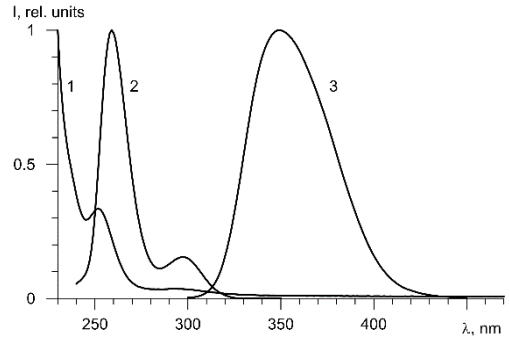

Fig. 3. The absorption spectra of an aqueous solution of cerium nitrate (1), excitation of fluorescence (2, λ_{reg} = 370 nm) and fluorescence (3, λ_{exc} = 260 nm) in this solution of Ce^{3+} ions

In fig. 3 shown the absorption spectra, fluorescence excitation and fluorescence of a solution of cerium nitrate in water. In the absorption spectrum, two bands are observed, the maxima of which correspond to the regions of 300 and 252 nm. The extinction value at the maximum of the last band is about 585 l/mol×cm. The fluorescence excitation spectrum does not coincide with the absorption spectrum, which indicates the dependence of the fluorescence quantum yield on the frequency of the exciting radiation and may be due to photoionization of Ce^{3+}. The maximum at the 252 nm of absorption spectrum of $Ce(NO_3)_3$ in water is shifted to the low-frequency region by ~ 2780 cm^{-1} in a buffer phosphate solution with pH = 7.0, while the extinction value is significantly reduced. The fluorescence spectra of cerium nitrate do not depend on the frequency of the excitation radiation (within 32200 - 40300 cm^{-1}) both in water and in the buffer solution; they coincide in shape and are a continuous band extending from 310 to 430 nm (fig. 4). Similar fluorescence spectra are also observed for other cerium salts ($Ce_2(SO_4)_3$, $CeCl_3$, Ce $(ClO_4)_3$) in aqueous solutions. This behavior is due to the manifestation of a predominantly ionic bond (with some covalent character) of lanthanoid complexes, which can easily undergo ligand exchange reactions. The results show that for different salts the same form of aquacomplex (aquaion) $[Ce(H_2O)_n]^{3+}$ participates in luminescence, where n is most likely equal to 9. The measured anisotropy value for the fluorescence band of cerium nitrate in glycerin with excitation wavelength 260 nm is zero, which indicates a significant orientational depolarization, which is apparently due to the high symmetry of the emitter.

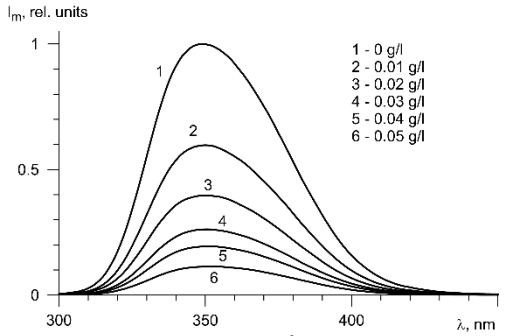

Fig. 4. Fluorescence spectra of Ce^{3+} ions in an aqueous solution of cerium nitrate with various GO additives corrected for the effect of the internal filter. d_{ex} = 5 mm and d_{em} = 10 mm

In fig. 4 shown the fluorescence spectra of aqueous solutions of cerium nitrate with various addition of GO. Due to the large overlap with absorption spectra, these spectra were corrected for the effect of the internal filter using the formula [3]

$$I = I_m \cdot 10^{\left(D_{ex}\frac{d_{ex}}{2} + D_{em}\frac{d_{em}}{2}\right)}, \quad (1)$$

where I and I_m are the calculated and measured fluorescence intensities, D_{ex} and D_{em} are the optical density at the excitation and fluorescence wavelengths, d_{ex} and d_{em} are the path length in the cell of excitation radiation and fluorescence. As can be seen, the maximum of the fluorescence spectrum of the cerium salt without the quencher additive is localized near λ = 349 nm, and with increasing GO concentration a slight shift of the band maximum to 351 nm is observed.

After taking into account the effect of the internal filter, the mechanisms for quenching fluorescence of cerium nitrate with graphene oxide can be dynamic, static, or mixed quenching. In the case of static quenching in the ground state, non-fluorescent supramolecules should be formed, including GO and $[Ce(H_2O)_n]^{3+}$. In this case, the lifetime of excited aquaions that do not participate in complexation does not change, and for purely static quenching, the ratio $\tau_0/\tau = 1$ is performed, where τ_0 and τ are the lifetimes of the excited states $[Ce(H_2O)_n]^{3+}$, respectively the absence and availability of an quencher. At the same time, as the GO concentration increases, the fluorescence intensity decreases. The observed decrease in the fluorescence

intensity of the fluorophore with an increase in the concentration of GO due to static quenching is described by the classic Stern-Volmer equation:

$$I_0/I = 1 + K_S[Q] \quad (2)$$

where I_0 and I are the fluorescence intensities in the absence and presence of the concentration quencher $[Q]$, K_S is the Stern-Folmer association constant. In the case of dynamic quenching, the molecules of the excited fluorophore and the quencher are contacted due to diffusion. As a result of contact, the fluorophore molecule returns nonradiatively to the ground state. Therefore, the average lifetime of the excited state of the fluorophore τ in this case decreases with increasing GO concentration, and the observed decrease in the fluorescence intensity is described by the Stern-Volmer equation of the form:

$$I_0/I = 1 + K_d[Q] \quad (3)$$

where K_d is the dynamic quenching constant. The main feature of the purely dynamic quenching is the proportionality of the decrease in the intensity and the fluorescence decay time, i.e. $I_0/I = \tau_0/\tau$. To distinguish extinguishing mechanisms, the dependence of the lifetime of the excited state of the fluorophore on the quencher concentration is most often used.

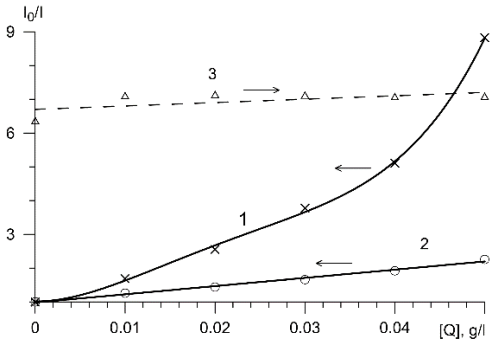

Fig. 5. Dependence of the intensity ratio (1 - without the effect of reabsorption, 2 - taking into account this effect) and the lifetimes of the excited states (3) $[Ce(H_2O)_n]^{3+}$ on the concentration of GO [Q] in water

In fig. 5 shown the dependences of the relative intensities I_0/I (uncorrected (1) and corrected taking into account reabsorption (2)), as well as τ_0/τ on the concentration of GO (3). As can be seen from the figure, due to the effect of the internal filter significantly increases the fluorescence intensity with significant GO weight additives. Due to the small magnitude of the shift of the fluorescence spectra with an increase in the GO concentration as intensity I in formulas (2) and (3), we used the corrected intensities at the maxima of the fluorescence bands, and not the quantum yields. The lifetime of the excited state of the aquaion $[Ce(H_2O)_n]^{3+}$ without a quencher is 33 ns, and with the addition of graphene oxide, the value of τ is constant and is ~ 37 ns. The fluorescence decay curves of the cerium salt within the quencher concentration of 0–0.05 g/l are well modeled by a two-exponential dependence with a short lived component of ~ 2.1 ns and the above long term with statistical weights of 5.5% and 94.5%, respectively, which may be due to the presence of another type of fluorescent centers.

The method of determining the frequency of the electron transition of a molecule from the diffuse absorption, fluorescence and fluorescence excitation spectra serves as a test of the homogeneity of the emitting centers [4]. To determine the position of the frequency of a purely electronic transition from the absorption spectrum and fluorescence excitation, it is more convenient to find the extremum of a function using the expression:

$$ln\frac{\sigma_a(\nu)}{\nu} - \frac{h\nu}{2kT} = \varphi_a(\nu), \quad (4)$$

and on the emission spectrum, i.e. for transition from excited electronic states to the ground one, using the formula:

$$ln\frac{I(\nu)}{\nu^4} - \frac{h\nu}{2kT} = \varphi_e(\nu). \quad (5)$$

Here $\sigma_a(\nu)$ is the absorption cross section and $I(\nu)$ is the fluorescence intensity from vibrationally thermalized states, ν is the light frequency, T is the solution temperature, k is the Boltzmann constant.

The extremes of the functions $\varphi_a(\nu)$ and $\varphi_e(\nu)$ are localized at the electron transition frequencies, i.e. at $\nu = \nu_0$. From the analysis of [4], it follows that in the presence of isomers, conformers and other chromophores with differing localization of extremes in the solution, the observed integral extremum is diffuse or even absent. In our case, such extremums are absent in the excitation spectra. In the fluorescence spectra of an aqueous solution of cerium-free nitrate, it is observed in the region of 32000 cm^{-1} (Fig. 6, a) and is represented only by a shoulder in the same area with such a quencher (Fig. 6, b). The result obtained correlates with the data on the inhomogeneity of the fluorescent centers obtained from the analysis of τ. It also indicates a large heterogeneity of absorbing chromophores.

From the analysis of fig. 6 it follows that in the range of GO concentrations of 0 – 0.05 g/l, the fluorescence quenching process is static due to the constant value τ_0/τ and the linear dependence I_0/I in the studied concentration range. From the slope of the approximated straight fig. 5 (dependence 2) the Stern – Volmer association constant K_s = 24.7 l/g was determined. It is much lower than the constants for cationic rhodamine dyes and metal cations. The result obtained can be explained by weak adsorption of the negatively charged GO surface of $[Ce(H_2O)_n]^{3+}$ aquaions.

One of the highly sensitive methods for detecting and controlling biochemical reactions has recently become spectroscopy with fluorescence anisotropy amplification by immobilizing the studied biological objects on the surface of nanoparticles [5]. The most effective amplifier turned out to be graphene oxide, which, having a unique flat 2D structure with sizes up to hundreds of nanometers, has a lower rotational mobility than spherical 0D-dimensional nanoparticles with the same surface areas. However, GO is a very strong quencher of the fluorescence of dye-labeled probe DNA on GO die to resonance energy transfer and electron transfer. A novel graphene oxide amplified fluorescence anisotropy assay by placing the specifically dye-labeled DNA on the surface of graphene oxide without substantial fluorescence quenching has recently been proposed to control biochemical reactions involving metal ions, single-strand DNA, adenosine and thrombin [6-8]. Model of restricted diffusion is suggested to describe reorientation of molecules partially immobilized on the surface

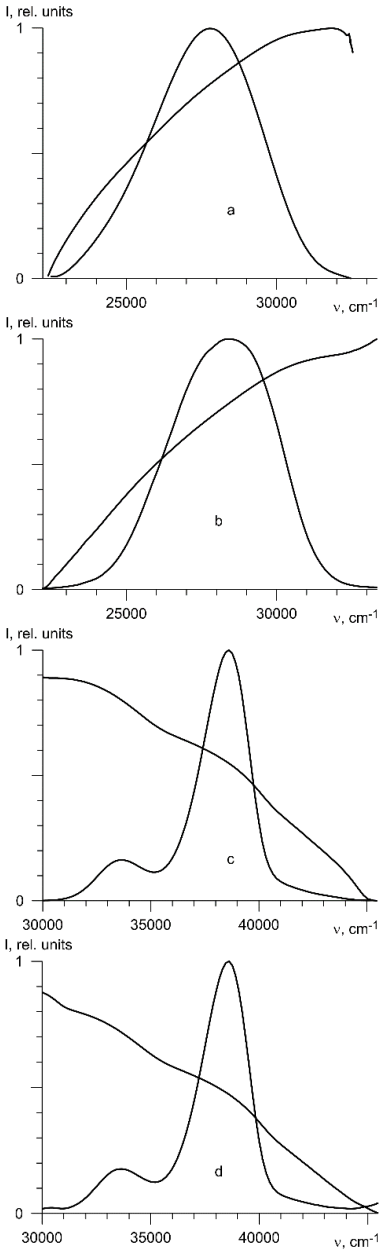

Fig. 6. Fluorescence spectra (a, b) and excitation (c, d) of Ce^{3+} ions in an aqueous solution of cerium nitrate without the addition of GO (a, c), with the addition of GO (b, d) and their corresponding $\varphi_s(\nu)$ functions

of graphene oxide. The orientational order parameters are calculated for molecules with internal rotations adsorbed on the graphene surface. The dependences of the fluorescence anisotropy on the rates of internal rotational diffusion and the orientation angles of the labeled molecules relative to the graphene plane are calculated. From a comparison of calculated kinetic dependencies for the free and associated with nanoparticles biomolecules, it was shown that a significant increase in anisotropy by immobilizing biomolecules to an graphene oxide can be expected if the order parameter is close to 1, i.e., if the diffusion of the attached part of the molecule relative to the graphene plane occurs in a narrow cone around the normal. It is also important that the orientation angles of the transition dipole moments would be close to the direction of the internal axis of rotation. Such conditions will be optimal for amplification of fluorescence anisotropy by immobilizing nanoparticles on the surface of graphene oxide and controlling biochemical reactions.

Conclusions

By measuring the relative fluorescence intensities and the lifetimes of the excited states of an aqueous solution of cerium nitrate versus the concentration of graphene oxide plates and using the Stern-Volmer equations, it has been shown that electronic excitation of salt causes static fluorescence quenching. The association constant is 24.7 l/g, which is significantly lower than the constants of cationic dyes of rhodamines and metal cations. To increase the efficiency of the interaction of carbon nanoparticles with organic and inorganic compounds the surface of the plates should be passivated by a charge opposite to the surface charge of the compound. A theoretical analysis of the method of fluorescence anisotropy amplification by immobilization of labeled biomolecules on the surface of graphene oxide nanoparticles, which is used to control biochemical reactions, has been carried out. Optimal conditions are found under which the differences in the anisotropy of fluorescence of free and nanoparticle-bound biomolecules are most pronounced.

Acknowledgments

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References

1. Sridharan V., Menendez J.C. Cerium (IV) Ammonium Nitrate as a Catalyst in Organic Synthesis. *Chemical Reviews* 2010; 110(6): 3805-3849.
2. Xu C., Lin Y., Wang J., Wu L., Wei W., Qu X. Nanoceria-Triggered Synergetic Drug Release Based on CeO_2 - Capped Mesoporous Silica Host–Guest Interactions and Switchable Enzymatic Activity and Cellular Effects of CeO_2. *Advanced Healthcare Materials*. 2013; 2(12): 1591-1599.
3. Li S., Aphale A.N., Macwan I.G., Patra P.K., Gonzalez W.G., Leblanc J.R.M.. Graphene Oxide as a Quencher for Fluorescent Assay of Aminoacids, Peptides, and Proteins. *ACS Applied Materials Interfaces* 2012; 4(12): 7069-7075.
4. Tolkachev V.A. Determination of 0-0-transition frequencies from diffuse vibronic spectra. *Journal of Applied Spectroscopy.* 2017**;** 84(4): 668-673.
5. Perrier S., Guieu V, Cheovelon B, Ravelet C, Peyrin E. A panoply of fluorescence polarization/anisotropy signaling mechanisms for functional nucleic acid-based sensing platforms. *Analytical Chemistry* 2018; 90(7): 4236-4238.
6. Yu Y., Liu Y., Zhen S.J., Huang C.Z. A graphene oxide enhanced fluorescence anisotropy strategy for DNAzyme assay of metal ions. *Chemical Communication* 2013; 49(19): 1942-1944.
7. Xiao X., Li Y.F., Huang C. Z., Zhen S.J. A novel graphene oxide amplified fluorescence anisotropy assay with improved accuracy and sensitivity. *Chemical Communication* 2015; 51(89): 16080-16083.
8. Zhen S.J., Yu Y., Huang C.M. Graphene oxide amplified fluorescence anisotropy for label-free detection of potassium ion. *Analyst* 2015; (140): 353-357.