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Colloidal Nanocrystalline Luminophors Doped by Rare-earth Ions for Biological Testing

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$nReVO_4:Eu^{3+}$ (Re = Gd, Y, Sm, La) luminescent nanocrystals of different shape and size from 2 to 300 nm have been synthesized. Luminescence of $nReVO_4:Eu^{3+}$ nanocrystals are effectively excited under UV and visible irradiation. By means of luminescence microscopy and luminescence microspectroscopy it has been revealed that spherical nanocrystals with an average diameter from 3 to 20 nm tend to accumulate mainly in the isolated rat hepatocyte nuclei. Spherical $nGdYVO_4:Eu^{3+}$ nanocrystals are efficient inorganic markers and can be used in systems of selective delivery of substances into the cell nucleus.

Keywords: Nanoparticles, Luminescence, Cell, Hepatocyte nuclei, Intranuclear accumulation.

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1. INTRODUCTION

Development of nanotechnologies has led to creation of inorganic fluorescent labels and probes, which were more stable to the action of the exciting radiation and allow the continuous monitoring of biological processes. Along with the search for biomarkers for selection of cells, one of the most promising directions is the creation of nanoscale transporting systems targeted to delivery of different compounds to specific biological structures.

Recently, the semiconductor nanocrystals (quantum dots) have found a wide range of commercial applications as nanoprobes [1, 2]. It should be noted that the effects of toxicity, blinking and relatively broad luminescence band sufficiently limit their practical use.

More attention is paid to luminescent nanocrystals based on dielectrics and wide band gap semiconductors doped with rare-earth elements [3, 4]. These materials possess high photostability, large Stokes shift, the absence of blinking and the narrow luminescence bands. Availability of synthesis and modification methods for nanoparticles (NPs), as well as, the absence of significant toxicity make them promising for biological and medical use, but low luminescence intensity of these materials limits their application. Currently, inorganic nanocrystals doped by rare earth elements, are not used extensively in biological studies.

Recently, we have reported the method for the wet-chemical synthesis of brightly luminescent $ReVO_4:Eu^{3+}$ nanoluminophores [5]. One of the attractive features of orthovanadate nanoparticles (VNPs) is a possibility to control their size and shape during the synthesis procedure keeping almost unchangeable such parameters as surrounding, composition, charge, mass concentration, etc. It allows tracing the influence of NPs size and shape on efficiency and pattern of their interaction with biological objects.

In our work $nReVO_4:Eu^{3+}$ (Re = Gd, Y, Sm, La) luminescent nanocrystals have been synthesized in the form of water colloidal solutions. The possibility of NPs detection in hepatocytes was investigated by means of luminescence microscopy and luminescence microspectroscopy.

2. MATERIALS AND METHODS

The synthesis of water colloidal solutions containing spherical NPs has been carried out according to the method reported earlier, using sodium citrate as a stabilizing agent [5]. For obtaining of colloids with particles of spindle-like and rod-like shape the method [6] was used with disodium salt of ethylenediaminetetraacetic acid as the stabilizing agent. All solutions were purged of impurities by dialysis using a 3.5 KDa dialysis membrane (with pore size less than 1.5 nm).

Synthesis duration, temperature and stoichiometric composition were chosen empirically until reproducible geometric parameters of solid phase have been achieved. The composition of spherical particles is $Gd_{(0.6\text{-}0.8)}Y_{(0.1\text{-}0.3)}Eu_{(0.1)}VO_4$, spindle-like – $Gd_{(0.9)}Eu_{(0.1)}VO_4$, rod-like – $La_{(0.9)}Eu_{(0.1)}VO_4$, $Sm_{(0.9)}Eu_{(0.1)}VO_4$

The obtained colloidal systems are transparent solutions scattering the light under the side illumination, aggregative stabile more than 2 months and suitable for biological testing.

Size and morphology of particles were investigated by means of transmission electron microscopy. The samples for microscopy were prepared by evaporating dilute solution droplets onto carbon coated copper discs.

UV-vis absorption spectra of colloidal solutions were measured with a SPECORD 200 spectrometer ("Analytik Jena"). Photoluminescence spectra were recorded by a spectrofluorimeter on the base of a grating monochromator at room temperature under excitation by the xenon lamp at $\lambda = 325$ nm.

Cell visualization was carried out by Olympus IX 71 luminescent microscope with magnification of 1000 in conditions of oil immersion. Luminescence was excited by 75 W xenon lamp using a band-pass 460 – 490 nm excitation filter. Luminescence was collected using an emission long-pass 510 nm filter.

Nanoparticles of $Gd_{0.7}Y_{0.2}Eu_{0.1}VO_4$ have been investigated as biological markers.

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3. RESULTS AND DISCUSSION

In the present work we have investigated colloidal solutions of nanocrystals of different sizes (from 2 to 300 nm) and shapes with the form factor (L/D ratio equal to 1; 3.3 – 5.0 ± 0.2 and 8.0 – 12.5 ± 0.5 (Fig. 1).

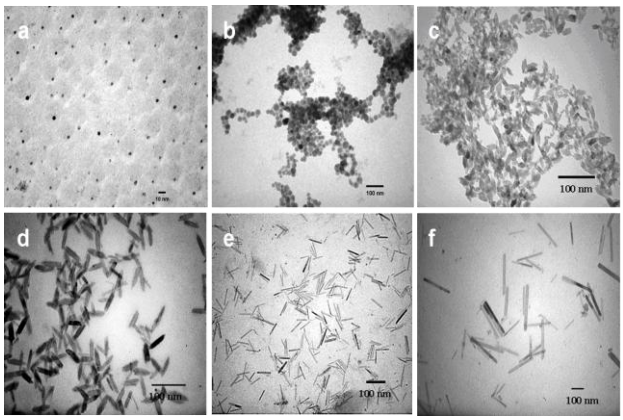

Fig. 1 – TEM images of $ReVO_4:Eu^{3+}$ nanocrystals in colloidal solutions. Spherical particles with an average size of 3 nm – **a**, 20 nm – **b**; asymmetric spindle-like: 3 × 10 nm – **c**, 10 × 50 nm – **d** and rod-like: – 5 × 40 nm – **e**, 28 × 300 nm – **f**

Absorption spectrum consists of the broad band with maximum in the range of 271 – 287 nm, which characterizes the energy transfer from oxygen ligands to the central vanadium ion in the VO_4^{3-} complex [4, 7]. Increase the size of nanoparticles leads to bathochromic shift of the absorption maximum (Fig. 2).

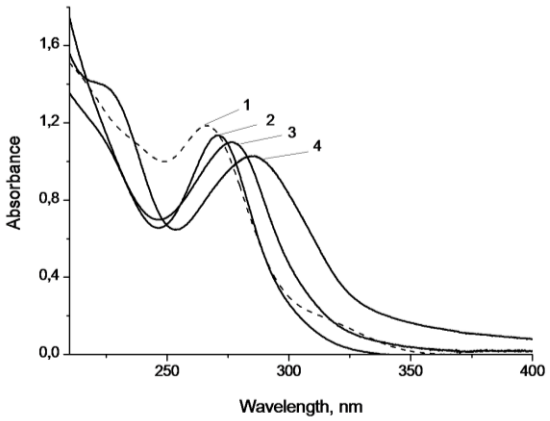

Fig. 2 – UV–vis absorption spectra of Na_3VO_4 (dotted line, 1), spherical $GdYVO_4:Eu^{3+}$ nanoparticles (2) spindle-like $GdVO_4:Eu^{3+}$ (3) and rod-like $LaVO_4:Eu^{3+}$ (4)

One of the most important advantages of RE-doped inorganic nanoparticles is the characteristic narrow luminescence bands of RE ion, which appear to be a reliable instrument for spectroscopic identification of nanoparticles in the investigated medium. Fig. 3 shows the excitation and emission spectra of $nReVO_4:Eu^{3+}$ water solutions. At the excitation in $^7F_{0.1} \rightarrow {}^5L_6$ and $^7F_{0.1} \rightarrow {}^5D_2$ transitions, red luminescence associated with $^5D_0 \rightarrow {}^7F_J$ transitions is observed. The intensity and ratio of the bands in the spectra of nanocrystals almost does not depend on the type of regular ion (Gd, Y, La), size and shape of the particle.

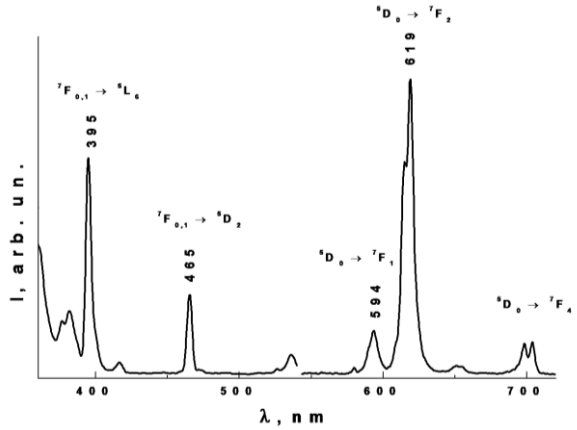

Fig. 3 – Excitation (left, $\lambda_{vis} = 619$ nm) and emission (right, $\lambda_{ex} = 465$ nm) spectra of $ReVO_4:Eu^{3+}$ luminophor in water solution

It should be noted that a significant Stokes's shift for $nReVO_4:Eu^{3+}$ nanocrystals allow to separate the autofluorescence of biological material from the fluorescence of the probe. The Stokes shift for fluorescence nanocrystals $nGdYVO_4:Eu^{3+}$ is more than 200 nm, while the Stokes shift for organic fluorescent probes is usually less than 150 nm.

One of the objectives of this study was to investigate the possibility of rare-earth orthovanadate based nanocrystalline systems application as the fluorescent probes for long-term monitoring of the movement and localization of cells in biological experiments.

For incubation of NPs with cells, salt-free buffer 5 % glucose solution was used. It has been revealed that 1.5 h incubation was enough to observe NPs accumulation in both isolated cells and isolated nuclei of rat hepatocytes. In the control sample (cell without NPs), bright green autofluorescence of cell cytoplasm is observed, while cell nucleus does not possess any luminescence (Fig. 4a).

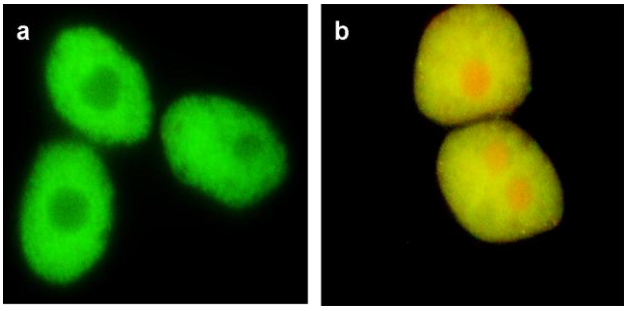

Fig. 4 – Luminescence microscopy images of hepatocytes and isolated cells. Autofluorescence of hepatocytes – **a**, fluorescence of hepatocytes under incubation with NPs – **b**

Luminescence images of cells incubated with NPs reveals red luminescence in hepatocyte nuclei region, which is characteristic for trivalent europium in crystal field of orthovanadate matrix (Fig. 4b). The intensive luminescence of the nanoparticles in the nuclei of hepatocytes is observed.

The ability of cells to accumulate particles was confirmed by the results of spectroscopic studies. Luminescence spectra recorded from different regions within the same cell reveal a broad declining band correspond-

ing to cell autofluorescence and a narrow one centered at 619 nm corresponding to the most intensive transition $^5D_0 \rightarrow {}^7F_2$ in $nReVO_4:Eu^{3+}$ nanoluminophor (Fig. 5). The intensity of this narrow band depends on the registrable region and is maximal in the cell nucleus region.

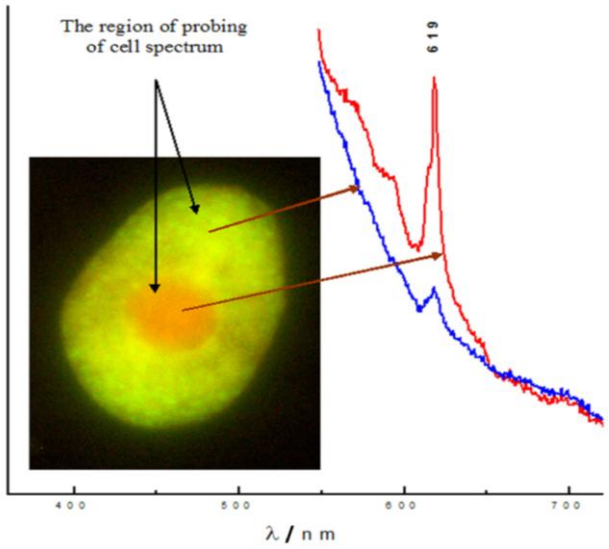

Fig. 5 – Fluorescence spectra of cell local regions

The maximum intensity of this band is in the nucleus of hepatocytes. Hereby, both visual observation and luminescence spectra recorded from the different region within the hepatocyte cells provide strong evidence that spherical particles with average diameters of 3 and 20 nm are concentrated in the cell nucleus region.

It should be noted that the effect of nanoparticles penetration is observed only for spherical ones (see Fig. 1a, b). Nanoparticles with spindle-like and rod-like shapes were not found in cells and in isolated nuclei.

4. CONCLUSIONS

Water colloidal solutions of $nReVO_4:Eu^{3+}$ (Re = Gd, Y, La) luminescent nanocrystals with different shape and size from 2 to 300 nm have been obtained.

By means of luminescence microscopy and luminescence microspectroscopy the transport of NPs based on orthovanadates of rare-earth elements into rat hepatocytes and their accumulation in and around the cell nucleus has been revealed for the first time.

Asymmetric spindle-like and rod-like orthovanadate NPs are not capable to penetrate into cells, only transport of spherical particles, with an average diameter of 3 and 20 nm, occur in biological experiments on rat hepatocytes.

The ability of the obtained $nGdYVO_4:Eu^{3+}$ particles to penetrate into the nucleus, can be used as a tool to study the mechanisms of intracellular transport of molecular forms and provides a basis for the creation of nanocontainers for the selective delivery of drug compounds.

REFERENCES

1. M.Jr Bruchez, M. Moronne, P. Gin et al., *Science* **281**, 2013 (1998).
2. A.P. Alivisatos, W. Gu, C. Larabell, *Annu. Rev. Biomed. Eng.* **7**, 1 (2005).
3. C.R. Patra, R. Bhattacharya, S. Patra et al., *Clinical Chemistry* **53** No11, 2029 (2007).
4. A. Huignard, T. Gacoin, J.-P. Boilot, *Chem. Mater.* **12**, 1090 (2000).
5. V.K. Klochkov, *Russ. Nanostrukturnoe materialovedenie* **2**, 3 (2009).
6. V.K. Klochkov, A.I. Malyshenko, O.O. Sedyh, Yu.V. Malyukin, *Functional materials* **1**, 111 (2011).
7. W. Fan, Y. Bu, X. Song, S. Sun, *Cryst. Growth Des.* **7** No **11**, 2361 (2007).