

## Colloidal synthesis and properties of lanthanide orthophosphate nanophosphors

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Nanocrystals water solutions of activated lanthanides orthophosphates  $\text{CePO}_4:\text{Tb}^{3+}$ ,  $\text{LaPO}_4:\text{Eu}^{3+}$  have been obtained by colloidal synthesis. The solution solid phase possesses characteristic luminescent properties. Nanoparticles of about 2 nm size are stable in water and have a negative surface charge. The solution toxicity has been estimated.

Методом коллоидного синтеза получены водные растворы нанокристаллов ортофосфатов редкоземельных элементов  $\text{CePO}_4:\text{Tb}^{3+}$ ,  $\text{LaPO}_4:\text{Eu}^{3+}$ . Твердая фаза растворов обладает характерными люминесцентными свойствами. Наночастицы с размерами около 2 нм стабильны в воде и имеют отрицательный заряд поверхности. Проведена оценка токсичности растворов.

Luminescent nanomaterials acquire ever growing importance in engineering, electronics, biology and medicine [1, 2]. For many physical, chemical and biological objects, the transition from macroscale objects to particles of 1 to 10 nm size has been shown to result in the qualitative modifications of physicochemical properties of individual compounds and systems obtained basing thereon. The properties of nanosized crystals depend essentially on the number of surface atoms, localized electron state and can take new qualitative properties as compared to the volume phase [3–5]. The use of inorganic nanocrystals as biological probes and labels is actual in the modern biology and medicine [2].

The conventional classes of fluorescent probes are organic dyes, fluorescent proteins, quantum dots, and lanthanide chelates. These probes find an application due to such attractive properties as the possibility to use low-intensity exciting sources and availability of standard usage protocol thereof. However, there are some limitations for their usage caused by the prop-

erties of conventional fluorophors. For instance, organic fluorophors show a small Stokes shift, low photostability, wide luminescence bands, and a toxicity. The use of quantum dots is complicated by the fact that biologically incompatible organic solvents are required to synthesize thereof. The semiconductor nanocrystals are toxic, so for biological usage, their passivation is necessary, i.e., the synthesis of so-called core-shell structures [2, 6].

Hence, the use of inorganic dielectric crystals of the activated compounds of rare-earth elements as luminescent labels seems to be of good prospects. First of all, this is connected with the high photostability of dielectric nanocrystals under considerable changes of medium parameters and also at different excitation techniques. Those compounds show an intense luminescence and narrow spectral bands, they are stable under optical irradiation, do not exhibit any pronounced toxicity, and can be obtained in aqueous solutions [7–9] that is of a considerable importance for their biological applications.

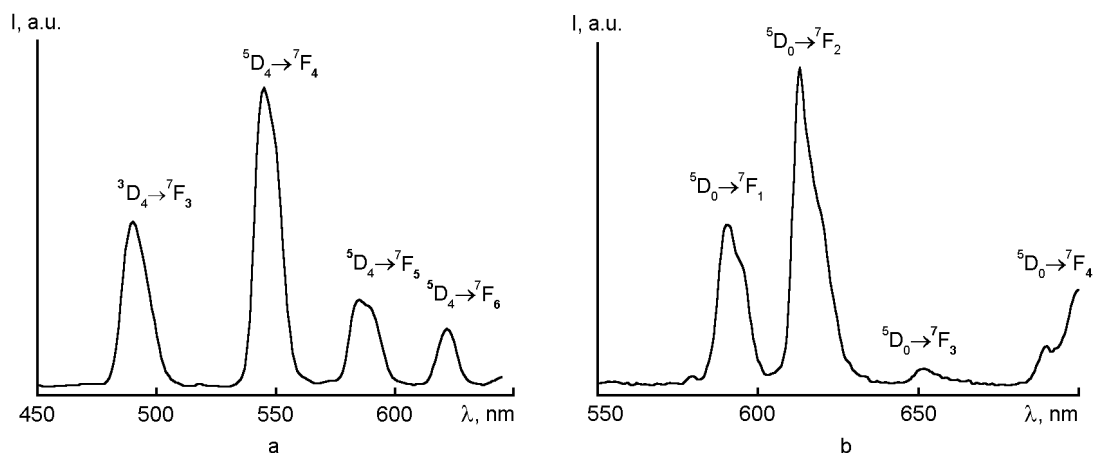


Fig. 1. Luminescence spectra of aqueous colloidal solutions of (a)  $\text{CePO}_4:\text{Tb}^{3+}$  (Helium-cadmium laser,  $\lambda_{exc} = 325$  nm), (b)  $\text{LaPO}_4:\text{Eu}^{3+}$  (Xenon lamp,  $\lambda_{exc} = 395$  nm).

In the present work, orthophosphates of rare-earth elements were selected for studies, being the compounds of best compatibility with biological objects and able to solubilization in organic low-molecular and polymeric molecules. Also it is possible to modify the surface of orthophosphate nanocrystals in order to provide biologically active labels of selective action.

For the synthesis of  $\text{CePO}_4:\text{Tb}^{3+}$  and  $\text{LaPO}_4:\text{Eu}^{3+}$ , colloidal aqueous solutions of rare-earth chlorides from "Acros organics" (99.9 %) and sodium tripolyphosphate M211 from "Macrochem" were used. Absorption spectra were measured using a SPECORD 200 spectrophotometer. To obtain the luminescence spectra, a MDR-23 monochromator with the manipulation and data gathering in KAMAK standard was used. As the excitation sources, a helium-cadmium laser with 325 nm emission wavelength and a 150 W xenon lamp were used. The particle surface charge sign was determined by electrophoresis in U-shaped tube at 100 V DC voltage on the electrodes. As the buffer solution, 0.01 M NaCl was used. The movement direction of nanoparticles was determined visually by the travel of luminescent solution boundary in the buffer under ultraviolet illumination. The transmission electron microscopy was performed using a PEM-125K.

For toxicity evaluation of obtained solutions, biological tests on cells using standard protocols were performed. The influence of nanoparticles on the viability were studied using the rat marrow cells and hepatocytes in conditions of long-time incubation (20 hours). For cell viability evaluation, two methods were applied, — with

propidium iodide and with exclusion of trypan blue [10]. After incubation with the particles, 0.9 mL of 0.4 % trypan blue solution in the phosphate buffer without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  under pH 7.3 have been added to 0.1 mL of cell suspension. The cell calculation was done in Goryaev chamber under optical microscope observation. The cells with the visualized (dyed) nuclei have been evaluated as inviable. The number of inviable cells expressed in per cent to the total cell number in the sample was compared with the control values. The cell suspension incubated in the same conditions but in the absence of nanoparticles was taken as a control. Propidium iodide was introduced in the cell suspension at 1  $\mu\text{M}$  concentration. The number of luminescent nuclei was calculated under the cell observation in an Olympus IX71 luminescent microscope using a 75 W xenon lamp as the excitation source with 450–490 nm excitation filter and emission at 510 nm. The cell material for testing was assigned by the Institute of cryobiology and cryomedicine NASU.

We have used a modified technique permitting to obtain stable aqueous solutions of activated nanocrystals with pre-specified size and phase structure in conditions of controlled colloidal chemical synthesis [8]. The technique specific feature is the dual role of sodium tripolyphosphate precursor (TPP). On the one hand, it is the source of orthophosphate ions ( $\text{PO}_4^{3-}$ ) formed during sodium tripolyphosphate hydrolysis, on the other hand, this compound plays a part of colloidal particle stabilizing agent. The technique essence is as follows. At the first stage, lanthanide (Ln) chlorides are mixed with TPP in P/Ln = 3 ratio resulting in for-

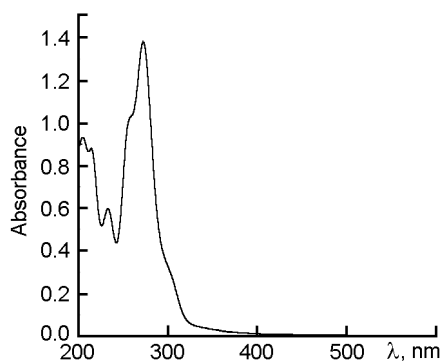


Fig. 2. Absorption spectrum of  $\text{CePO}_4:\text{Tb}^{3+}$  colloidal solution.

mation of  $\text{Ln}^{3+}$ -TPP complex. At the heating up to  $90^\circ\text{C}$ , a fraction of TPP hydrolyzes forming pyrophosphate and orthophosphate ions, some amount of unhydrolyzed sodium tripolyphosphate being remained in the solution, too. Orthophosphate ion reacts with  $\text{Ln}^{3+}$  ion forming insoluble  $\text{LnPO}_4$ . The further clusterization of  $\text{LnPO}_4$  molecules results in formation of nanoparticles coated with polyphosphate molecules playing the part of stabilizing layer for those particles. Then, the crystal phase is formed inside the polyphosphate shell, the presence thereof can be judged from appearance of characteristic luminescence under ultraviolet irradiation of the solutions (Fig. 1). The most intense luminescence was observed at the activator concentration of 20 mol.% for  $\text{CePO}_4:\text{Tb}^{3+}$ , while for  $\text{LaPO}_4:\text{Eu}^{3+}$ ,  $\text{Eu}^{3+}$  concentration in the 10–25 mol.% range has not visually influenced the red luminescence intensity under wideband ultraviolet excitation.

During the reaction, the solution pH value decreases, that influences negatively on the crystal phase formation rate. It has been determined in experiment that when the reaction mixture pH is controlled and maintained at the 7.6–8.0 level, a considerable acceleration of crystal fields formation takes place. Also the reaction time decrease from 3 hours [8] to 30 minutes in our case. After the synthesis in aqueous colloidal solutions, the impurities of sodium, chlorine, pyrophosphate and unreacted tripolyphosphate ions remain in the medium along with formed nanoparticles. The impurities influence considerably the system stability and its optical properties. Therefore, after the synthesis, the solutions were purified from impurities by dialysis on the dialysis membranes (cut-off 12 kD). The purification process was monitored by measuring the

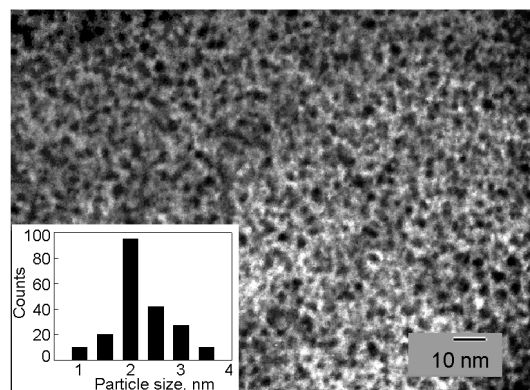


Fig. 3. TEM image data for solid phase of  $\text{CePO}_4:\text{Tb}^{3+}$  colloidal solution.

electroconductivity of the dialyzate flowing through the cell with platinum electrodes. Aqueous colloidal solutions of  $\text{CePO}_4:\text{Tb}^{3+}$ ,  $\text{LaPO}_4:\text{Eu}^{3+}$  nanocrystals have been obtained that are stable for more than 3 months. The solutions are transparent in the transmitted light (Fig. 2) and show a weak opalescence in the side light (Tyndall cone). When passing the exciting light beam through the solutions, an intense luminescence is observed according to the activator ion nature — green for  $\text{Tb}^{3+}$  ( $\lambda_{\text{max}}$  of the most intense band 543 nm) and red for  $\text{Eu}^{3+}$  ( $\lambda_{\text{max}}$  of the most intense band 613 nm). The solid phase concentration in the solutions amounts to 4.0 g/l. Using the vacuum evaporation, the concentration can be increased by one decimal order, but the solution stability decreases at the same time.

Using TEM, dimensions of particles in aqueous solutions have been determined (Fig. 3). As it is seen from the Figure, the particles are polydisperse and have average size of  $2.1 \pm 0.4$  nm. Dimensions of particles remain unchanged for more than 3 months due to polyphosphate shell stabilizing the system. The diffraction pattern for the particles corresponds to the crystal-like solid phase structure. To study the crystal structure of precipitates obtained after colloidal solution evaporation, the X-ray scattering was used. In whole, the results conform with data [8, 11, 12] for rhabdophane type structure. However, the further comparative investigations of the solid phase luminescent properties in the solutions and after their drying do not allow to assert with sufficient reliability that the precipitates dried even in the low-temperature conditions ( $60^\circ\text{C}$ ) have the same crystal structure as the solid phase in colloidal solution.

The pH value of obtained dialyzed solutions changes in the range of 7.0–7.4, that corresponds to the normal value of pH for biological systems. When monitoring the electrophoresis, it was observed that the movement of boundary between sol and buffer solution towards the anode, thus evidencing that the particles in the solution are charged negatively. The study of toxic action of obtained solutions shows that solutions with solid phase concentration up to 50 mg/L do not possess any toxic action on the hepatocyte cells. The marrow cell were stable to the concentrations up to 100 mg/L.

Thus, the considered preparation technique of activated nanocrystals permit enables the preparation of aqueous nanophosphor solution without making use of high-temperature conditions and toxic precursors. The absence of toxicity in the range of solution working concentrations up to 50 mg/L will enable in the future to carry out experiments with the minimal action of luminescent probes on biological objects.

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## Колоїдний синтез та властивості нанофосфорів на основі ортофосфатів лантанідів

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Методом колоїдного синтезу отримано водні розчини нанокристалів на основі ортофосфатів рідкісноземельних елементів  $\text{CePO}_4:\text{Tb}^{3+}$ ,  $\text{LaPO}_4:\text{Eu}^{3+}$ . Тверда фаза розчинів має характерні люмінесцентні властивості. Наночастинки з розмірами близько 2 нм стабільні у воді та мають негативний заряд поверхні. Проведено оцінку токсичності розчинів.