Synthesis and optical properties of lanthanides doped ultrasmall NaYF₄ markers for bio-medical applications

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Aim. To synthesise NaYF₄ nanocrytstals doped or co-doped with different lanthanide ions (Eu, Tb, Gd) and to investigate them optically to achieve efficient optical markers.

Methods. Samples have been synthesized by using co-thermolizys method and optical properties have been investigated by using photoluminescence (PL), PL excitation and PL decay spectroscopies.

Results. Efficient emission in visible spectra range has been observed for all investigated samples. The excitation mechanism of the main emission centre has been explained.

Conclusions. It has been shown that the main excitation mechanism of Eu ions is through energy transfer from Tb or Gd ions. Moreover, it has been shown that obtained by us nanocrystals characterize by strong emission which make them potential as efficient optical markers in biology or medicine.

Keywords: optical markers, nanocrystals, lanthanides, NaYF₄.
of the CdSe nanocrystals during processing, via air-induced or UV-catalyzed oxidation, resulted in release of Cd surface atoms and consequently high toxicity. These findings indicate that processing strategies, surface coatings, and use in biological applications that prevent surface oxidation should improve biocompatibility of CdSe core QDs, whereas oxidative environments should promote their cytotoxicity. Thus, many efforts have been taken recently to cover CdSe nanocrystals by additional shell. However, it has been demonstrated that even if the QDs are covered by the shell like ZnS their are not cytotoxic only at low concentrations (<0.25 mg/ml) and relatively short periods of UV exposure (<1 h). Thus, surface coatings such as ZnS and BSA were shown to significantly reduce, but not eliminate cytotoxicity. In the framework of above discussion, we can conclude that we are still far from the commercial use of Cadmium based quantum dots and new concepts of optical markers are still desired.

One of the solution combining the advantages of both concepts (molecular markers and quantum dots) and overcoming the issue of high toxicity of quantum dots is to make inorganic Cadmium free nanocrystals doped with lanthanide ions. However, to date, there are very limited published studies that have either directly or indirectly assessed the toxic effects of phosphors fluorescent nanocrystals. Nevertheless, it seems that the toxicity of this kind of nanocrystals is much less then in the case of II–VI and III–V compounds. For example, Chen et al. [7] showed that the NaYF₄:Yb, Er nanocrystals showed mild toxicity to skeletal myoblasts and BMSCs cells. Both cell types showed good tolerance to various concentrations of the nanocrystals, ranging from 1 to 100 mg/ml.

Except lower toxicity, phosphor nanocrystals additionally could be characterized by a narrow emission or excitation bands in UV-infrared spectral range, by long emission decay times (μs or ms) and simultaneously exhibits magnetic properties. All these facts have strong consequences for application potential of these markers. For the reasons discussed above increasing number in papers dedicated to NaYF₄ nanocrystals doped or co-doped by lanthanide ions can be observed recently [8–11]. However, in most of the cases, obtained nanocrystals are characterized by size of tens of nanometers (20–200 nm) and are designed as excited in infrared spectral range as up-converting phosphors. However, the size of the smallest reported nanoporphors (25–30 nm) is not optimal for their use as bioimaging probes. For example, as a labeling material for biomolecules, especially for the sensitive determination of molecules such as DNA, RNA, or proteins, nanosized phosphors with monodispersed size distribution and high luminescence efficiency are required [12].

Thus, synthesizing ultrasmall NaYF₄ nanocrystals (<10 nm) with good size distribution and efficient emission could give a high impact for development of inorganic optical markers technology. In this work, we will present the optical and structural results obtained for NaYF₄ nanocrystals doped by different lanthanide ions [Eu, Tb, Gd] aiming obtaining their different optical properties. Firstly, rather simple system of Eu doped NaYF₄ nanocrystals (Fig. 1, a, see inset) has been investigated. Nevertheless, even in such a simple system by varying ions concentration we became able to control emitted colors due to excitation energy transfer and cross-relaxation between the ions. The second approach (Fig. 1, b, see inset) based on co-doping NaYF₄ nanocrystals by Eu and Tb ions simultaneously. In this case, due to efficient energy migration between these two ions, different colors from green (NaYdF₄:Tb) to red (NaYdF₄:Eu) became possible to achieve depending on relative ions concentrations. In third approach (Fig. 1, c, see inset), NaGdF₄ nanocrystals have been doped with Eu and Tb ions where Gd ions substitute Y ions. The gadolinium ions gave us another important benefit in this case, namely magnetic response of the marker while due to Eu and Tb co-doping the advantages of previous approach are still present. Additionally, presence of Gd ions changing slightly the excitation mechanism of Eu ions what can be seen in Fig. 3 (see inset).

Materials and methods. Synthesis of NaYF₄:Eu³⁺ hexagonal nanocrystals. The synthesis method of the β-NaYF₄:Eu³⁺ NC’s followed the single step co-thermolizys method and originally proposed by Shan et al. [13].

The sodium trifluoroacetate Na (CF₃COO) (98 %), yttrium (III) trifluoroacetate hydrate Y(CF₃COO)ₓ × xH₂O (99 %), europium (III) trifluoroacetate trihydrate Eu(CF₃COO)ₓ·3H₂O (98 %) and trioctylphosphine oxide (TOPO, 90 %) were purchase from «Al-
drich» (USA). All chemicals were used as received without further purification. A mixture of 1.25 mmol Na(CF$_3$COO), 0.485 mmol Y(CF$_3$COO)$_3$, 0.039 mmol Eu(CF$_3$COO)$_3$ was dissolved in 26 mmol TOP. The Schlenk technique was used to remove oxygen and water form the solution. The mixture was rigorously stirred in 100 ml three-neck flask heated at 120 °C under vacuum for 30 min. To synthesis high quality hexagonal nc the temperature was increased to 350 °C within 10 minutes and the solution was kept in a presence of nitrogen for the one hour. Then the reaction was stopped. Excess amount of ethanol was added to cooled solution to precipitate NaYF$_4$:Eu$^{3+}$ NC’s and a centrifuge was used to isolate them. The β-NaYF$_4$: Eu$^{3+}$ (5 %), Tb$^{3+}$ (5 %) and β-NaGdF$_4$:Eu$^{3+}$ (5 %), Tb$^{3+}$ (5 %) NC’s synthesis procedures have been similar to described above. As the excitation source for all optical measurements 1 ms pulse xenon lamp coupled with two Czerny-Turner monochromators ($f = 200$ mm, 1200 line/mm 300 nm blaze) has been used. Optical signal has been recorded by using strobe detector (Photom Technology International) coupled with Czerny-Turner monochromator ($f = 200$ mm, 1200 line/mm 400 nm blaze). The excitation spectra have been divided by the excitation source characteristic and all measurements have been done in 90° geometry for samples placed in high quality quartz cuvette.

Results and discussion. Fig. 2 (see inset) shows TEM image together with XRD pattern recorded for obtained for representative sample (NaYF$_4$:Eu). The sample consist small nanocrystals (diameter 9–10 nm) with well crystallized hexagonal structure.

Fig. 3 (see inset) shows the emission spectra obtained for all three markers discussed by us in introduction (Fig. 1, see inset). The excitation spectra for all samples are very similar. The most intensive emission is obtained when the samples are excited by 395 or 285 nm. Nevertheless, these wavelengths, from the application point of view, are rather useless since they can damage the sample and cause many unwanted effects. However, for samples with both ions (Eu and Tb) additional channels of Eu excitation through the f-f transition are observed at 465, 490 or 530 nm wavelengths. The possibility of exciting our markers by the visible light makes them very promising from the application point of view.

Fig. 3 (see inset) shows also the emission spectra for all samples obtained at 285 nm excitation wave length. Emission obtained for NaYF$_4$:Eur nanocrystals is characterized by intense red light with long emission decay time (~6 ms). For used in our case ions concentrations (5 % Eu and 5 % Tb), very similar color of emission has been also obtained for two other samples (Fig. 4, see inset) which emissions are also characterized by long emission decay times (~5 ms). The shortening in the emission lifetime is due to interaction between Tb and Eu ions through efficient energy transfer. The origin of observed emission lines in all cases is due to intra shell f-f transitions within the Eu and Tb ions and has been discussed in more details elsewhere [2].

Fig. 4 (see inset) shows the real emission of all discussed samples when the 500 nm edge filter has been used to cutoff the excitation beam.

Conclusions. In this work, NaYF$_4$:Tb,Eu optical markers characterized by different optical properties have been synthesized by using co-thermolizys method and have been characterized optically and structurally. It has been shown that obtained by us ultrasmall nanocrystals (<10 nm) characterize by narrow emission bands, long emission decay times and varieties of possible emission wavelengths. Small size and efficient red emission make synthesized nanocrystals very attractive from the application point of view in biology and medicine. Nevertheless, more studies on toxicity of our samples must be done to prove their real application potential.

Acknowledgments. Authors would like to thanks M. Afzaal and P. O’Brien from The University of Manchester for their help with samples preparation. Moreover, the authors (A. P.) would like to acknowledge for financial support to Iuventus Plus program (no. IP2010032570).

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Синтез і оптичні властивості нанокристалів NaYF$_4$, легованих лантаноїдами, як біомедичних маркерів

Резюме

Мета. Синтезувати нанокристали NaYF$_4$, леговані або ко-леговані різними іонами лантаноїдів (Eu, Tb, Gd), та дослідити їхні оптичні властивості для отримання ефективних оптичних маркерів. Методи. Зразки синтезували методом спільного термолизису, а оптичні властивості вивчили методом фотолюминесценції (ФЛ), фотофлуоресцентної спектроскопії збуд-
Синтез і оптичне світло нанокристалів NaYF₄, леговань з лантанідами, як біомедіцинських маркеров

**Резюме**

Синтезувати нанокристали NaYF₄, леговань або не, ефективно і безпечні для біомедичних використань. Метаєм синтезувати комбінацію атомів оксиду та гідроксиду, які притаманні для біологічних властивостей. Нанокристали використовуються у біомедичному виділенні для визначення біологічних процесів, таких як тромбізація, інтенсивність, або кількість. Нанокристали мають потенціал для використання в біомедичних маркерах.

**Ключові слова:** нанокристали, оптичні маркери, лантаноїди, NaYF₄

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