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Synthesis and optical properties of lanthanides doped ultrasmall $NaYF_4$ markers for bio-medical applications

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Aim. *To synthesise $NaYF_4$ 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, $NaYF_4$.

Introduction. Introducing a concept of optical markers has changed in tremendous way the investigations methods of recent medicine and biology [1]. This was mainly due to strong development in imaging techniques which recently allow us to investigate both static as well dynamic properties of living cells. The visualization of single cell components and investigation of their interactions with internal and external factors (e. g. drug treatments) contribute to understanding the mechanisms in living cells.

The currently used molecular markers including organic dyes, fluorescent proteins and lanthanide chelates have several significant limitations. Most of them emit light only in visible spectral range, their emission is weak and restricted by efficient bleaching and blinking excluding them from long term analyzes. Moreover, multicolor staining is limited by requirement of additional markers injection. The emission decay times are very short in most of the cases (ns) and PL overlap temporally with autofluoroscence giving poor quality images.

One of the alternatives for molecular markers are inorganic quantum dots (QD) (e. g. CdSe, CdS, CdSe/ZnS, GaN) [2–5], which are recently commonly used in many academic studies. However, even if they are much better from physico-chemical point of view, from the application point of view at this moment they are far from the commercial use. This is mainly due to many doubts about their toxicity what suppress all the advantages of using them for *in vivo* imaging, since the toxicity risk is high and side effects are still not well known. These doubts focus recent research in this field on the issue of nanocrystals toxicity. Most of the conclusions are similar to those gives by Derfus et al. [6] who have shown that process parameters, environmental conditions and used model (hepatocyte culture model etc.) could dramatically affect the observed level of toxicity. From their data it appears that surface oxidation

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 $NaYF_4$: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 $NaYF_4$ 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 nanophosphors (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 $NaYF_4$ 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 $NaYF_4$ nanocrystals doped by different lanthanide ions [Eu, Tb, Gd] aiming obtaining their different optical properties. Firstly, rather simple system of Eu doped $NaYF_4$ 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 $NaYF_4$ nanocrystals by Eu and Tb ions simultaneously. In this case, due to efficient energy migration between these two ions, different colors from green ($NaYdF_4$:Tb) to red ($NaYdF_4$:Eu) became possible to achieve depending on relative ions concentrations. In third approach (Fig. 1, *c*, see inset), $NaGdF_4$ 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:Eu^{3+} hexagonal nanocrystals*. The synthesis method of the β-NaYF4:Eu^{3+} NC's followed the single step co-thermolizys method and originally proposed by Shan et al. [13].

The sodium trifluoroacetate Na (CF_3COO) (98 %), yttrium (III) trifluoroacetate hydrate $Y(CF_3COO)_3 \times$ $\times xH_2O$ (99 %), europium (III) trifluoroacetate trihydrate $Eu(CF_3COO)_3 \cdot 3H_2O$ (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 $Na(CF_3COO)$, 0.485 mmol $Y(CF_3COO)_3$, 0.039 mmol $Eu(CF_3COO)_3$ was dissolved in 26 mmol TOPO. 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 ℃ under vacuum for 30 min. To synthesis high quality hexagonal nc the temperature was increased to 350 ℃ 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 NaYF4:Eu^{3+} NC's and a centrifuge was use to isolate them. The β-NaYF4: Eu^{3+} (5 %), Tb^{3+} (5 %) and β-NaGdF4: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 (Photon 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 90o 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 $NaYF_4$:Eu 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.

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А. Подгородецький, М. Банський, Я. Місевич

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 термолізису, а оптичні властивості вивчали методом фотолюмінесценції (ФЛ), фотолюмінесцентної спектроскопії збуд-*

ження і гасіння ФЛ. ***Результати****. Ефективне випромінювання у видимому діапазоні спектра спостерігали для всіх проаналізованих зразків. Пояснено механізм збудження основного емісійного центра.* ***Висновки****. Показано, що головним механізмом збудження іонів європію є передавання енергії від іонів тербію або гадолінію. Крім того, встановлено, що одержані нанокристали характеризуються сильною емісією, що робить їх потенційно ефективнішими оптичними маркерами для біології або медицини.*

Ключові слова: нанокристали, оптичні маркери, лантаноїди, $NaYF_4$.

А. Подгородецкий, М. Банский, Я. Мисевич

Синтез и оптические свойства нанокристаллов $NaYF_4$, легированных с лантаноидами, как биомедицинских маркеров

Резюме

Цель*. Синтезировать нанокристаллы $NaYF_4$, легированные или ко-легированные различными ионами лантаноидов (Eu, Tb, Gd), и исследовать их оптические свойства для получения эффективных оптических маркеров.* ***Методы****. Образцы синтезировали методом совместного термолизиса, а оптические свойства изучали методом фотолюминесценции (ФЛ), фотолюминесцентной спектроскопии возбуждения и гашения ФЛ.* ***Результаты****. Эффективное излучение в видимом диапазоне спектра наблюдалось для всех исследованных образцов. Объяснен механизм возбуждения основного эмиссионного центра.* ***Выводы****. Показано, что главным механизмом возбуждения ионов европия является передача энергии от ионов тербия или гадолиния. Кроме того, установлено, что полученные нами нанокристаллы характеризуются интенсивной эмиссией, что делает их потенциально более эффективными оптическими маркерами для биологии или медицины.*

Ключевые слова: нанокристаллы, оптические маркеры, лантаноиды, $NaYF_4$.

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