Research Article

Coprecipitation Method of Synthesis, Characterization, and Cytotoxicity of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) Nanoparticles

Maksim S. Pudovkin ¹, Pavel V. Zelenikhin,¹ Victoria Shtyreva,¹ Oleg A. Morozov,¹ Darya A. Koryakovtseva,¹ Vitaly V. Pavlov,¹ Yury N. Osin,¹ Vladimir G. Evtugyn,¹ Albert A. Akhmadeev,^{1,2} Alexey S. Nizamutdinov,¹ and Vadim V. Semashko¹

¹Kazan Federal University, Kazan, Tatarstan 420008, Russia

²Tatarstan Academy of Sciences, Institute of Applied Research, Bauman str., 20, Kazan 420111, Russia

Correspondence should be addressed to Maksim S. Pudovkin; jaz7778@list.ru

Received 29 November 2017; Accepted 26 February 2018; Published 1 April 2018

Academic Editor: Marco Rossi

Copyright © 2018 Maksim S. Pudovkin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) nanoparticles were characterized by means of high-resolution transmission electron microscopy, X-ray diffraction, optical spectroscopy, energy dispersive X-ray spectroscopy, dynamic light scattering, and MTT assay. It was revealed that the average diameter of all the NPs is around 14–18 nm. The hydrodynamic radius of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) nanoparticles strongly depends on the medium. It was revealed that hydrodynamic radii of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) nanoparticles in water, DMEM, and RPMI-1640 biological mediums were 18 ± 5 , 41 ± 6 , and 186 ± 8 nm, respectively. The $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) nanoparticles were nontoxic at micromolar concentrations toward COLO-320 cell line. The lifetime curves were fitted biexponentially, and for the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs, the luminescence lifetimes of Pr^{3+} ions were 480 ± 2 and 53 ± 5 nanosec.

1. Introduction

Bionanomaterials engineering is one of today's most promising fields of materials science [1]. Nanomaterials are utilized in different fields of biomedicine and biology [2, 3]. Among the huge number of nanomaterials, rare-earth doped trifluoride nanoparticles (NPs) have a special place mainly because of their excellent photostability, long luminescent lifetimes, and sharp emission bands which are highly important for industrial and biomedical applications [3]. Particular success has been achieved in the development of luminescent NPs based on rare-earth doped trifluorides which are utilized as nanoscentillators for “hybrid” radiotherapy—photodynamic therapy (PDT) [4]. Also, NPs are used for in vivo imaging of biological objects [5]. In this case, they should operate in the so-called biological window (650–1300 nm). In case of photo-thermal therapy and subtissue thermal sensing [6–8], the luminescent intensity ratio of some luminescent peaks of NPs should be dependent on temperature in the physiological

temperature range (20–50°C). Due to these unique properties of fluoride NPs, the methods of NP synthesis providing excellent optical properties and low toxicity are under the intense investigation [9, 10]. Hence, NPs which are expected to be used in different areas including biomedicine should be characterized very carefully in order to guarantee the excellent physical and chemical properties and low toxicity.

Among the lanthanides, Pr^{3+} as a dopant seems to be very attractive. It has been reported in [11–13] that, because of thermally coupled $^3\text{P}_1$ to $^3\text{P}_0$ electronic states of Pr^{3+} ions, Pr^{3+} doped nanomaterials can be used as nanothermometers operating in broad temperature range including physiological one which paves the way toward thermal sensing of a single cell in vitro and toward thermal imaging of integrated circuits [14]. Also, the biological activity of PrF_3 nanoparticles is under investigation. For these reasons, Pr^{3+} doped fluoride NPs are considered as a very promising material for different applications.

The main purpose of the work is to characterize $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs (molar ratio of Pr^{3+} and La^{3+} ions

are 3:97, 7:93, 3:22, 1:4, and 3:7, resp.) by different methods in order to provide foundation for further studying of temperature sensing properties and biological activity of $\text{Pr}^{3+}:\text{LaF}_3$ NPs.

The NPs were characterized by means of HR TEM, X-ray diffraction, and optical spectroscopy. For chosen $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs, we additionally study their hydrodynamic radii in water, DMEM, and RPMI-1640 biological mediums and cytotoxicity toward human cancer cells (COLO-320 cell line) via the MTT assay.

2. Materials and Methods

2.1. Sample Requirements and Methods of Characterization. The size and shape of NPs can be studied via high-resolution transmission electron microscopy (HR TEM) and the phase composition is commonly studied via X-ray diffraction [9].

NPs which are expected to be used in biomedical application should be nontoxic and stay chemically stable under light irradiation so that toxic components of reaction product are not delivered to the cells [15]. Generally, toxicity of inorganic substances depends on their solubility in water [1]. Also, rare-earth doped fluorides demonstrate the lowest solubility among other rare-earth based materials (oxides and so on). For instance, the CeF_3 and other trifluorides with tysonite crystal structure demonstrate solubility around 10^{-5} – 10^{-6} mol/L and, as a consequence, low toxicity [1]. Also, NPs itself can affect the cell life cycle [16]. Hence, the toxicity of NPs must be studied very carefully. Commonly, the toxicity of NPs can be estimated in vitro via colorimetric MTT assay, described in [17].

In such important works [6–8] where rare-earth doped LaF_3 NPs demonstrate their efficiency in biomedical application, coprecipitation method of synthesis of NPs is used. Indeed, this method is usually user-friendly and inexpensive. It allows synthesizing NPs in 10–100 nm size range. Use of water as the exchange reaction medium instead of other solvents which could be toxic also can be important for the protection of the environment and for biomedical application [18]. But one of the most prominent disadvantages of the coprecipitation method is that the NPs usually contain large amount of adsorbed water [11], which essentially affects spectral properties of the synthesized nanoparticles, including their luminescent features [18, 19]. Moreover, in [17, 20], it is reported that the NPs synthesized via coprecipitation method contain captured water molecules in the lattice because of fast growth of NPs in chemical reaction in an aqueous solution. Anyway, regardless of its disadvantages, this simple method gives the opportunity to study properties of NPs for many research teams without using expensive equipment.

After the synthesis, the residues of precursors which were used during the synthesis should be removed very carefully. The degree of purification of NPs from the precursors is controlled by means of energy-dispersive X-ray spectroscopy (EDX spectroscopy) [21]. But, in [22], the absence of nitrate ion impurities in fluoride NPs is determined by standard qualitative reaction with diphenylamine (diphenylamine test). Hence, the degree of purification of NPs should be controlled very carefully.

One of the unique properties of NPs is protein corona formation phenomenon [23]. After suspending of NPs in biological medium or physiological liquids, they will inevitably come into contact with a huge variety of biomolecules including proteins and sugars. These biomolecules immediately coat the NP surfaces and form the so-called “protein corona.” This fact increases the sizes of NPs in a biological medium and causes its tendency to form agglomeration [24]. The “protein corona” is difficult to resolve via transmission electron microscopy because of low electron density of surface organic ligands [24–26]. On the other hand, the average sizes of NPs and their agglomerations in different mediums can be measured by means of DLS [27, 28]. In this method, the information about size can be extracted from the Stocks–Einstein equation [29] and size is classified as a hydrodynamic radius of NPs. The hydrodynamic radius depends on biological medium in which the NPs are suspended [30]. In [31], it is shown that the values of the hydrodynamic radii of gold NPs demonstrate significant difference for DMED and RPMI-1640 biological mediums.

In order to study optical properties of NPs, conventional techniques for bulk crystal are used.

2.2. Synthesis and Characterization of $\text{Pr}^{3+}:\text{LaF}_3$ Nanoparticles. $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs (molar ratio of Pr^{3+} and La^{3+} ions are 3:97, 7:93, 3:22, 1:4, and 3:7, resp.) were synthesized via coprecipitation method using common chemical reaction for rare-earth elements described in [13]. For example, in order to synthesize $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 30\%$) NPs, 0.6 g of Pr_2O_3 and 1.2 g of La_2O_3 were added to 110 mL of 10% nitric acid in a glass beaker. The mixture was heated to 50°C and stirred for 45 min; then, a transparent light-green solution appeared. The pH of the solution was 1. Then, the mixture was filtered, poured in a polypropylene glass, and placed in an ultrasonic cleaner (model UD100SH-2LQ, ultrasonic power 100 W), and a solution of 0.69 g of NaF in 100 mL of distilled water was added. Then, the pH was adjusted to 4 by adding 25% solution of ammonium hydrate. Then, the mixture was stirred for 10 minutes under the ultrasonic treatment. The precipitate was purified with distilled water by centrifugation (12,000 rpm, centrifugation time was 15 min) 8 times. In order to synthesize $\text{Pr}^{3+}:\text{LaF}_3$ NPs with other Pr:La ratio, the suitable ratio of Pr_2O_3 and La_2O_3 and stoichiometric amount of NaF were taken. Then, nanoparticles were dried in air. The structure of the material was characterized by X-ray diffraction method with Shimadzu XRD-7000S X-ray diffractometer. Analysis of samples was carried out in a transmission electron microscope Hitachi HT7700 Exalens. Samples were prepared as follows: 10 microliters of the suspension was placed on a formvar/carbon lacey 3 mm copper grid and drying was performed at room temperature. After drying, the grid was placed in a transmission electron microscope using a special holder for microanalysis. The analysis was held at an accelerating voltage of 100 kV in the TEM mode; the elemental analysis was carried out in the STEM mode, at the same parameters using Oxford Instruments X-Max™ 80T detector. The control of amount of nitrates in colloidal

solution of the NPs after each stage of centrifugation was performed by identification test using diphenylamine (diphenylamine test).

2.3. Measurement of Hydrodynamic Radii of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=7\%$) NPs and/or Their Agglomerations in Distilled Water, DMEM, and RPMI-1640 Biological Mediums. In order to measure NP sizes and the rate of NP agglomeration in different mediums the size distributions and average hydrodynamic radii in water, DMEM, and RPMI-1640 biological mediums were characterized by means of DLS with Photocor-FC spectrometer. DMEM and RPMI-1640 biological mediums were purchased from Paneco (Moscow, Russia) and were used without further modification. 1 ml of 10 mM water colloid solution of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=7\%$) NPs was added to 4 ml of distilled water, DMEM, and RPMI-1640 biological mediums, and then after 10 minutes of sonication (ultrasonic cleaner model UD100SH-2LQ, ultrasonic power 100 W), all necessary measurements were done.

2.4. Cells Preparation and Cytotoxicity of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=7\%$) NPs. COLO-320 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C and 5% CO_2 in a humidified atmosphere.

The cytotoxicity of the NPs was analyzed via the colorimetric MTT assay. The test protocol for cytotoxicity evaluation was adopted from elsewhere [17]. Nanoparticle suspension in distilled water was added to the cultural medium in a ratio of 1/10 (v/v) for each concentration. Then, the obtained suspension was sonicated for 10 min until the suspension appeared homogeneous to the naked eye. COLO-320 cells were treated with the NPs at 0.5, 1, 2.5, and 5 mM. Exposure time was 24 h at 37°C in humid air (98%) containing 5% CO_2 . Three hours prior to the end of the exposure period, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide, Sigma-Aldrich, #M5655) solution in PBS (5 mg/ml, 20 μl /well) was added to the cells. After the completion of the exposure period, the supernatant was removed and 100 μl /well solution containing 10% SDS (Sigma-Aldrich, #L3771) in PBS was added. Absorbance at 570 nm of each well was measured using a microplate reader (Biorad, xMark). Each experiment was repeated 2 times, with five replications.

2.5. Optical Spectroscopy of $\text{Pr}^{3+}:\text{LaF}_3$ NPs. The luminescence spectra were recorded using CCD spectrometer (StellarNet), which detects the emission in 200–1100 nm spectral range with a spectral resolution of 0.5 nm. The optical parametric oscillator laser system (420–1200 nm) from JV LOTIS TII was used for excitation of the luminescence of the samples. The pulse width and the pulse repetition rate were 10 ns and 10 Hz, respectively. The spectral width of laser radiation was less than 0.15 nm. The luminescent lifetimes of Pr^{3+} ions were detected using BORDO 211A (10 bit, 200 MHz bandwidth) digital oscillograph. The experiments were carried out at room temperature.

3. Results and Discussion

3.1. NPs Characterization. Transmission electron microscopy (TEM) data indicate that obtained $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=3, 30\%$) ($\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=7, 12, 20\%$) samples are not shown here for the sake of brevity) samples consist of nearly monodisperse well-crystallized NPs. An average diameter of all the samples is around 14–18 nm (Figures 1(a)–1(d)), and it does not depend on the concentration of Pr^{3+} ions. The average diameters of the NPs are listed in Table 1. Also, the sample has regular almost spherical shape. Selected area electron diffraction (SAED) patterns correspond to hexagonal crystal structure and do not contain any reflections from impurity phases. The presence of circular rings in the SAED patterns indicates polycrystallinity of $\text{Pr}^{3+}:\text{LaF}_3$ samples. No signs of orientation ordering or oriented attachment of the nanoparticles are observed. According to the X-ray diffraction data (Figure 2), all the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=3, 7, 12, 20, 30\%$) NPs were hexagonal structured crystals. Sharp peaks of the patterns confirm good crystallinity of the NPs. The peaks exhibit a little right shift for the samples with the Pr doping level enhancing because of the crystal lattice distortion. The radius of Pr^{3+} (1.05 Å) is smaller than that of La^{3+} (1.13 Å) due to the lanthanide contraction, so the cell volume of $\text{Pr}^{3+}:\text{LaF}_3$ reduces with more Pr^{3+} replacing La^{3+} , which results in the XRD peaks shifting to higher degree. The lattice parameters are listed in Table 1. The lattice parameters for PrF_3 (JCPDS–46–1167) and for LaF_3 (JCPDS–32–0483) are $a=0.7079$ nm and $c=0.7238$ nm and $a=7.7186$ Å and $c=7.352$ Å, respectively. The average nanoparticle size was calculated using the Debye–Scherrer formula:

$$D = \frac{K\lambda}{\beta_{\text{hkl}} \cos \theta}, \quad (1)$$

where D is the mean size of an NP, K is the shape factor (we used $K=0.9$), λ is the X-ray wavelength (0.15418 nm), β_{hkl} is the line broadening at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle (in degrees). The D values of all the NPs are around 13 nm which is in good accordance with HR TEM data (D values are listed in Table 1). It can be concluded that the peak broadening of the XRD spectra is mainly related to the nanoscale dimensionality of the crystalline particles.

3.2. Control of Purification of the NPs. EDX spectroscopy indicates that the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}}=7\%$) NPs contain Pr, F, O, and trace quantity of Si as it is shown in Figure 3. The presence of Cu and C is related to using the copper grid during the HR TEM measurements. Presence of O can be attributed to the presence of adsorbed water [20], and trace quantity of Si is probably related to using glass beakers during the reaction of oxides with nitric acid. The chemical reactions with NaF were performed in polypropylene glasses. Additionally, the diphenylamine test did not reveal presence of nitrates in the NPs colloidal solution after the third stage of centrifugation (Figure 4(a)). Also, the sensitivity of the diphenylamine test was estimated. For this

FIGURE 1: (a) TEM images of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3\%$) nanoparticles (inset: SAED patterns of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3\%$) nanoparticles). (b) The size distribution of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3\%$) nanoparticles. (c) TEM images of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 30\%$) nanoparticles (inset: SAED patterns of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 30\%$) nanoparticles). (d) The size distribution of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 30\%$) nanoparticles.

TABLE 1

Molar concentration of Pr^{3+} ions in $\text{Pr}^{3+}:\text{LaF}_3$ NPs (%)	a parameter ($\text{\AA}$)	c parameter ($\text{\AA}$)	D (nm) (the Debye-Scherrer formula)	D (nm) (TEM data)
3	7.174 (3)	7.312 (2)	13.2 (2)	16.2 (2)
7	7.123 (2)	7.297 (4)	13.7 (3)	16.4 (3)
12	7.098 (3)	7.281 (2)	12.9 (3)	15.6 (2)
20	7.081 (3)	7.273 (2)	12.2 (4)	14.9 (2)
30	7.072 (2)	7.254 (4)	11.9 (3)	15.9 (2)

purpose, NaNO_3 was taken as a source of nitrates. It was revealed that the threshold concentration of nitrates which can be detected via the diphenylamine test was around 0.01 M in distilled water (Figure 4(b)). According to both methods, 4 stages of centrifugation (12,000 revolutions/min 10 min) in water guarantee thorough absence of nitrates and other precursors.

3.3. Measurement of Hydrodynamic Radii of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs and/or Their Agglomerations in Distilled Water, DMEM, and RPMI-1640 Biological Mediums. According to DLS data, in water, more than 90% of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs have the mean hydrodynamic radius in distilled water around 18 ± 5 nm and it does not change for at least 21 days. The quantity of the NPs agglomeration with

FIGURE 2: X-ray diffraction data of all the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs.

FIGURE 3: EDX spectroscopy of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) nanoparticles.

mean hydrodynamic radius more than 400 nm is less than 10%. Those facts indicate a very low degree of huge particle agglomeration in distilled water colloid solution. The value of the mean hydrodynamic radius is two times more than the mean NPs radius, obtained via TEM. It can be suggested that NPs form small agglomerates consisting of a few NPs as it was shown in [21] via TEM. Also, it should be noted that unlike TEM, hydrodynamic radius does not determine real NP size but can be calculated from the Stocks–Einstein equation in DLS method [29]. The time dependence of the hydrodynamic radii of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs in water, RPMI-1640, and DMEM biological mediums is shown in Figure 5. It was revealed that, in distilled water, the hydrodynamic radius is stable for at least 21 days which can be attributed to suitable value of Z-potential of the NPs [31]. Hydrodynamic sizes of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs in RPMI-1640 and DMEM biological mediums were also measured (Figure 5). In RPMI-1640 biological medium, 75.0% of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs have the hydrodynamic radii about

186 ± 60 nm, and it slightly increases up to 200 nm during 20 hours and then demonstrates stability. In DMEM biological medium 79, 4% of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs have the hydrodynamic radii about 43 ± 3 , and it does not change significantly for 72 hours. It was also detected that the rate of huge agglomerations with diameter more than 800 nm is less than 20% for both DMEM and RPMI 1640. These facts mean that colloidal solutions of the NPs in both biological milieus are stable but the NPs form agglomerations. As it was shown in [28, 31, 32], the stability of colloidal NPs can collapse due to screening of the electrostatic interactions. This screening can result in aggregation, and multivalent electrolytes were found to be more efficient than monovalent ions at suppressing the stabilizing effect of the electric double layer [33]. According to the chemical content of DMEM and RPMI-1640 biological mediums and [28], RPMI-1640 contains approximately 5-fold more phosphate ions (PO_4^{3-}) (5.63 mM) compared to DMEM (0.92 mM), and these multivalent phosphate ions suppress the colloid stability of the NPs more efficiently in RPMI-1640 than in DMEM. As it was already mentioned above, in RPMI-1640, the hydrodynamic radius of the NPs increases during 20 hours unlike DMEM, which probably can be attributed to the dynamic behavior of the protein corona in RPMI-1640 [30, 34], and some proteins with low affinity in RPMI-1640 can be adsorbed and then can be desorbed or/and replaced after a while [30]. For example, unlike DMEM, RPMI-1640 contains human plasma which serves as a main source of proteins with low affinity [30].

It can be concluded that although the hydrodynamic radii of the NPs differ between each other in water, DMEM, and RPMI-1640, the colloidal solutions of the NPs in water and DMEM are stable at least for 72 hours. In case of RPMI-1640, a slight change in the hydrodynamic radius takes place which at least can be attributed to the complicated physicochemical processes at the NP-medium interface [25]. Probably, the DMEM medium demonstrating minor change in the value of hydrodynamic radii of the NPs is more appropriate for toxicity study of LnF_3 -based NPs.

3.4. Toxicity of $\text{Pr}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs. The toxicity of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs was studied in a 0.05–25 mM concentration range toward COLO-320 cell line. The viability ranges of COLO-320 are shown in Figure 6. The NPs are nontoxic at micromolar concentrations. It was mentioned above [1] that the toxicity of materials generally depends on their solubility in the water. As it was mentioned above, the lanthanum fluoride has the lowest solubility among other rare-earth entities (oxides, phosphors, and so on) around 10^{-5} – 10^{-6} mol/L, which correlates substantially with that of fluor spar CaF_2 , which is ranked as safe [1]. This is unlike with quantum dots (CdSe) having higher solubility and releasing toxic Cd and Se ions [25]. It can be suggested that the main mechanism of toxicity is still under the discussion, but at least it is not related to release of La, Pr, and F ions or residues of precursors, or their impact on the cells is neglectable.

3.5. Optical Spectroscopy and Luminescence Lifetime $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs. Room temperature luminescence

FIGURE 4: (a) The diphenylamine test data. The supernatant (1) after the first stage of centrifugation, (2) after the second stage of centrifugation, and (3) after the third stage of centrifugation. (b) The diphenylamine test data for different concentrations of NO_3^- .

FIGURE 5: Hydrodynamic radii of $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) in distilled water, DMEM, and RPMI-1640 biological mediums as functions of time.

FIGURE 6: Survival rate of COLO-320 cells.

spectra of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs excited by laser beam at 444 nm are presented in Figure 7. The emission from $^1\text{D}_2$ state was not found under the excitation condition and at the studied temperature range. Probably, the emission from $^1\text{D}_2$ is not observed because of the lack of

FIGURE 7: Normalized (by maximum at 486 nm) emission spectrum of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs under 444 nm excitation.

FIGURE 8: Luminescence lifetime curves of $^3\text{P}_0$ state of Pr^{3+} ions of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs.

nonradiative relaxation of $^3\text{P}_j$ to $^1\text{D}_2$ due to the low cutoff phonon frequency in LaF_3 ($350\text{--}400\text{ cm}^{-1}$), which is 2 times less than the one for YAG ($700\text{--}865\text{ cm}^{-1}$). [13]. The analogic case is observed in the $\text{Pr}^{3+}:\text{NaYF}_4$ system [35].

The lifetime curves of $^3\text{P}_0$ state of Pr^{3+} ions are shown in Figure 8. Although the emission from $^1\text{D}_2$ is not observed, the curves are fitted biexponentially. A given possible explanation for this is a different probability of nonradiative decay for ions at or near the surface and ions (τ_2) in the core of the particles (τ_1) [36]. It is clearly seen that the luminescence lifetime τ_1 of Pr^{3+} ions decreases while the concentration of Pr^{3+} ions increases (Table 2)

TABLE 2

C_{Pr} (%)	τ_1 (nanosec)	τ_2 (nanosec)
3	560 ± 4	51 ± 4
7	480 ± 2	53 ± 5
12	330 ± 1	54 ± 4
20	240 ± 3	37 ± 6
30	230 ± 1	40 ± 6

This τ_1 lifetime decrease can be related to migration of energy to quenching centers (defects) [37]. Also, in [14], it was shown that in PrF_3 NPs uniform distribution of defects over the particle structure takes place and this factor determines the luminescence lifetime of Pr^{3+} ions in the core of the NP (τ_1). For the Pr^{3+} ions located into the core of the NP, the migration energy process to these defects is probably the main mechanism of luminescence quenching although, in [20] it was shown that the NPs synthesized via coprecipitation method contain captured water molecules in the lattice because of fast growth of NPs in chemical reaction in an aqueous solution. The role of this captured water molecules in luminescence quenching process is still under the discussion and we suggest that in the core of the NPs migration energy process dominants over quenching by captured water molecules. On the other hand, one of the most significant differences between nanosized crystals and bulk crystals is the increased role of surface and, as a consequence, interaction of ions with surface ligands becomes very significant [39]. The adsorbed OH groups can act as main quenching units on the surface of the NPs. It can be suggested that at the surface area the luminescence quenching can be attributed to nonradiative transitions from rare-earth excited state ($^3\text{P}_0$ in case of Pr^{3+}) to vibrational states of OH molecule [19, 39] and this process dominates over the energy migration one. The τ_2 lifetime does not change significantly while the concentration of Pr^{3+} ions increases from 3 to 30%. As we proved via HR TEM, the size of the NPs does not change while the concentration of Pr^{3+} ions increases from 3 to 30%. Hence, the number of adsorbed water molecules probably stays constant and their contribution to the luminescence quenching process is also constant approximately.

Low doped nanoparticles ($C_{\text{Pr}} = 3, 7\%$) have the largest lifetimes around 560 ± 4 and 480 ± 2 nanosec, respectively, and these values are at least 10 times less than lifetimes for bulk $\text{Pr}^{3+}:\text{LaF}_3$ crystals [40]. Probably, in the case of NPs, these small values of lifetimes are related to nonradiative relaxation on the OH group [41] of adsorbed and captured water molecules, and in order to remove these water molecules, the appropriate measurements such as microwave-assisted treatment [14] or treatment with high temperature [6] should be done.

4. Conclusions

The $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 3, 7, 12, 20, 30\%$) NPs were synthesized via coprecipitation method. The NPs were characterized by means of HR TEM, X-ray diffraction, optical spectroscopy, EXD spectroscopy, DLS, and MTT assay. It was revealed that

all the NPs are well-crystallized hexagonal structured nanosized particles and are almost spherical in shape with the average diameter of all the NPs around 15–20 nm and it is regardless of concentration of Pr^{3+} ions. EXD spectroscopy and the diphenylamine test did not reveal any presence of nitrates and other precursors after 4 stages of centrifugation (12,000 revolutions/min 10 min). Also, the sensitivity of the diphenylamine test was estimated. The hydrodynamic radius of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs strongly depends on the medium. It was revealed that hydrodynamic radii of the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs in water, DMEM, and RPMI-1640 are 18 ± 5 , 41 ± 6 , and 186 ± 8 nm, respectively, after 2 hours. The $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs are nontoxic at micromolar concentrations toward COLO-320 cell line. The lifetime curves are fitted biexponentially, and for the $\text{Pr}^{3+}:\text{LaF}_3$ ($C_{\text{Pr}} = 7\%$) NPs, the luminescence lifetimes of Pr^{3+} ions are $0.56 \pm 0.04 \mu\text{sec}$ and 51 ± 4 nanosec.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Optical spectroscopy and the synthesis were supported by the state assignment in the sphere of scientific activities (Project no. 3.1156.2017/4.6 and no. [3.5835.2017/6.7]). Microscopy studies were carried out at the Interdisciplinary Center of Analytical Microscopy of Kazan Federal University. The microscopy studies were funded by the subsidy of the Russian government (Agreement no. 02.A03.21.0002) to support the Program of Competitive Growth of Kazan Federal University among World's Leading Academic Centers. A. Maksim S. Pudovkin was supported by the Foundation for Assistance to Small Innovative Enterprises (FASIE) (Agreement no. 11271GU/2017). The authors thank Ajrat Kiyamov (Kazan Federal University, Kazan, Tatarstan 420008, Russia) for performing the X-ray diffraction experiments.

References

- [1] A. B. Shcherbakov, N. M. Zholobak, A. E. Baranchikov, A. V. Ryabova, and V. K. Ivanov, "Cerium fluoride nanoparticles protect cells against oxidative stress," *Materials Science and Engineering C*, vol. 50, pp. 151–159, 2015.
- [2] S. S. Lucky, K. C. Soo, and Y. Zhang, "Nanoparticles in photodynamic therapy," *Chemical Reviews*, vol. 115, no. 4, pp. 1990–2042, 2015.
- [3] H. Dong, S.-R. Du, X.-Y. Zheng et al., "Lanthanide nanoparticles: from design toward bioimaging and therapy," *Chemical Reviews*, vol. 115, no. 19, pp. 10725–10815, 2015.
- [4] D. Bekah, D. Cooper, K. Kudinov et al., "Synthesis and characterization of biologically stable, doped LaF_3 nanoparticles co-conjugated to PEG and photosensitizers," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 329, pp. 26–34, 2016.
- [5] B. Dong, B. Cao, Y. He, Z. Liu, Z. Li, and Z. Feng, "Temperature sensing and in vivo imaging by molybdenum sensitized visible upconversion luminescence of rare-earth oxides," *Advanced Materials*, vol. 24, pp. 1987–1993, 2012.
- [6] E. C. Ximendes, U. Rocha, K. U. Kumar, C. Jacinto, and D. Jaque, "LaF₃ core/shell nanoparticles for subcutaneous heating and thermal sensing in the second biological window," *Applied Physics Letters*, vol. 108, no. 25, p. 253103, 2016.
- [7] U. Rocha, C. J. Silva, W. F. Silva et al., "Subtissue thermal sensing based on neodymium-doped LaF₃ nanoparticles," *ACS Nano*, vol. 7, no. 2, pp. 1188–1199, 2013.
- [8] E. C. Ximendes, W. Q. Santos, U. Rocha et al., "Unveiling in vivo subcutaneous thermal dynamics by infrared luminescent nanothermometers," *Nano Letters*, vol. 16, pp. 1695–1703, 2016.
- [9] E. M. Alakshin, A. V. Klochkov, E. I. Kondratyeva et al., "Microwave-assisted hydrothermal synthesis and annealing of DyF₃ nanoparticles," *Journal of Nanomaterials*, vol. 2016, Article ID 7148307, 5 pages, 2016.
- [10] E. M. Alakshin, R. R. Gazizulin, A. V. Klochkov et al., "Annealing of PrF₃ nanoparticles by microwave irradiation," *Optics and Spectroscopy*, vol. 116, no. 5, pp. 721–723, 2014.
- [11] V. K. Rai, D. K. Rai, and S. B. Rai, "Pr³⁺ doped lithium tellurite glass as a temperature sensor," *Sensors and Actuators A*, vol. 128, no. 1, pp. 14–17, 2006.
- [12] M. Kaczkan, Z. Boruc, B. Fetlinski, S. Turczynski, and M. Malinowski, "Temperature dependence of 3P₀ Pr³⁺ fluorescence dynamics in Y₄Al₂O₉ crystals," *Applied Physics B*, vol. 113, no. 2, pp. 277–283, 2013.
- [13] I. Kamma, P. Kommidi, and B. Rami Reddy, "High temperature measurement using luminescence of Pr³⁺ doped YAG and Ho³⁺ doped CaF₂," *Physica Status Solidi (C)*, vol. 6, no. S1, pp. S187–S190, 2009.
- [14] G. Tessier, M. Bardoux, C. Boué, C. Filloy, and D. Fournier, "Back side thermal imaging of integrated circuits at high spatial resolution," *Applied Physics Letters*, vol. 90, p. 171112, 2007.
- [15] D. Jaque and F. Vetrone, "Luminescence nanothermometry," *Nanoscale*, vol. 4, no. 15, pp. 4301–4326, 2012.
- [16] M. Mahmoudi, K. Azadmanesh, M. A. Shokrgozar, W. Shane Journeay, and S. Laurent, "Effect of nanoparticles on the cell life cycle," *Chemical Reviews*, vol. 111, no. 5, pp. 3407–3432, 2011.
- [17] H. Tada, O. Shiho, K. Kuroshima, M. Koyama, and K. Tsukamoto, "An improved colorimetric assay for interleukin 2," *Journal of Immunological Methods*, vol. 93, no. 2, pp. 157–165, 1986.
- [18] P. P. Fedorov, A. A. Luginina, S. V. Kuznetsov, and V. V. Osiko, "Nanofluorides," *Journal of Fluorine Chemistry*, vol. 132, no. 12, pp. 1012–1039, 2011.
- [19] J. K. Krebs, S. P. Feofilov, A. A. Kaplyanskii, R. I. Zakharchenya, and U. Happek, "Non-radiative relaxation of Yb³⁺ in highly porous c-Al₂O₃," *Journal of Luminescence*, vol. 83–84, pp. 209–213, 1999.
- [20] E. M. Alakshin, D. S. Blokhin, A. M. Sabitova et al., "Experimental proof of the existence of water clusters in fullerene-like PrF₃ nanoparticles," *JETP Letters*, vol. 96, no. 3, pp. 181–183, 2012.
- [21] L. Ma, W.-X. Chen, Y.-F. Zheng, J. Zhao, and Z. Xu, "Microwave-assisted hydrothermal synthesis and characterizations of PrF₃ hollow nanoparticles," *Materials Letters*, vol. 61, pp. 2765–2768, 2007.
- [22] S. V. Kuznetsov, A. A. Ovsyannikova, E. A. Tupitsyna et al., "Phase formation in LaF₃–NaGdF₄, NaGdF₄–NaLuF₄, and NaLuF₄–NaYF₄ systems: synthesis of powders by co-precipitation from aqueous solutions," *Journal of Fluorine Chemistry*, vol. 161, pp. 95–101, 2014.
- [23] D. Docter, D. Westmeier, M. Markiewicz, S. Stolte, S. K. Knauer, and R. H. Stauber, "The nanoparticle biomolecule corona: lessons learned–challenge accepted?," *Chemical Society Reviews*, vol. 44, no. 17, pp. 6094–6124, 2015.

- [24] L. Shang, K. Nienhaus, and G. U. Nienhaus, "Engineered nanoparticles interacting with cells: size matters," *Journal of Nanobiotechnology*, vol. 12, no. 1, p. 5, 2014.
- [25] A. E. Nel, L. Mädler, D. Velegol et al., "Understanding biophysicochemical interactions at the nano-bio interface," *Nature Materials*, vol. 8, no. 7, pp. 543–557, 2009.
- [26] A. Verma and F. Stellacci, "Effect of surface properties on nanoparticle–cell interactions," *Small*, vol. 6, no. 1, pp. 12–21, 2010.
- [27] A. C. Sabuncu, J. Grubbs, S. Qian, T. M. Abdel-Fattah, M. W. Stacey, and A. Beskok, "Probing nanoparticle interactions in cell culture media," *Colloids and Surfaces B: Biointerfaces*, vol. 95, pp. 96–102, 2012.
- [28] T. L. Moore, L. Rodriguez-Lorenzo, V. Hirsch et al., "Nanoparticle colloidal stability in cell culture media and impact on cellular interactions," *Chemical Society Reviews*, vol. 44, no. 17, pp. 6287–6305, 2015.
- [29] J. K. Lim, S. P. Yeap, H. X. Che, and S. C. Low, "Characterization of magnetic nanoparticle by dynamic light scattering," *Nanoscale Research Letters*, vol. 8, no. 1, p. 381, 2013.
- [30] P. Foroozandeh and A. A. Aziz, "Merging worlds of nanomaterials and biological environment: factors governing protein corona formation on nanoparticles and its biological consequences," *Nanoscale Research Letters*, vol. 10, no. 1, p. 221, 2015.
- [31] G. Maiorano, S. Sabella, B. Sorce et al., "Effects of cell culture media on the dynamic formation of protein_nanoparticle complexes and influence on the cellular response," *American Chemical Society*, vol. 4, no. 12, pp. 7481–7491, 2010.
- [32] L. H. Allen and E. Niatijevec, "Stability of colloidal silica," *Journal of Colloid and Interface Science*, vol. 31, no. 3, pp. 287–296, 1969.
- [33] L. Zha, J. Hu, C. Wang, S. Fu, and M. Luo, "The effect of electrolyte on the colloidal properties of poly (N-isopropylacrylamide-co-dimethylaminoethylmethacrylate) microgel latexes," *Colloid and Polymer Science*, vol. 280, no. 12, pp. 1116–1121, 2002.
- [34] P. Satzer, F. Svec, G. Sekot, and A. Jungbauer, "Protein adsorption onto nanoparticles induces conformational changes: particle size dependency, kinetics, and mechanisms," *Engineering in Life Sciences*, vol. 16, no. 3, pp. 238–246, 2016.
- [35] S. Zhou, G. Jiang, X. Wei, C. Duan, Y. Chen, and M. Yin, "Pr³⁺-doped NaYF₄ for temperature sensing with fluorescence intensity ratio technique," *Journal of Nanoscience and Nanotechnology*, vol. 14, no. 5, pp. 3739–3742, 2014.
- [36] J. W. Stouwdam and F. C. J. M. van Veggel, "Near-infrared emission of redispersible Er³⁺, Nd³⁺, and Ho³⁺ doped LaF₃ nanoparticles," *Nano Letters*, vol. 2, no. 7, pp. 733–737, 2002.
- [37] P. M. Selzer, D. S. Hamilton, R. Flach, and W. M. Yen, "Phonon-assisted energy migration in Pr³⁺: LaF₃," *Journal of Luminescence*, vol. 12–13, pp. 737–741, 1976.
- [38] S. P. Feofilov, "Spectroscopy of dielectric nanocrystals doped by rare-earth and transition-metal ions," *Physics of the Solid State*, vol. 44, no. 8, pp. 1407–1414, 2002.
- [39] K. Ramasesha, L. De Marco, A. Mandal, and A. Tokmakoff, "Water vibrations have strongly mixed intra- and intermolecular character," *Nature Chemistry*, vol. 5, no. 11, pp. 935–940, 2013.
- [40] G. P. Morgan, D. L. Hubed, and W. M. Yen, "Quenching of fluorescence by cross relaxation in LaF₃:Pr³⁺," *Journal de Physique*, vol. 49, no. C7, pp. C7-25–C7-29, 1985.
- [41] M. S. Pudovkin, O. A. Morozov, V. V. Pavlov et al., "Physical background for luminescence thermometry sensors based on Pr³⁺:LaF₃ crystalline particles," *Journal of Nanomaterials*, vol. 2017, Article ID 3108586, 9 pages, 2017.