Synthesis of upconversion zirconia nanoparticles for bioimaging

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Abstract. In this work, the upconversion Er\(^{3+}/Yb^{3+}\) co-doped ZrO\(_2\) nanoparticles were synthesized and characterized. The nanoparticles were obtained via sol-gel method. To achieve upconversion activity ZrO\(_2\) nanoparticles were doped with lanthanide ion pair of the Yb\(^{3+}\) and Er\(^{3+}\) and further calcined at 800°C. According to X-ray powder and high-resolution transmission electron microscopy the well-crystallized monoclinic nanoparticles were formed, which size ranges from 20 to 30 nm for undoped ZrO\(_2\) and from 30 to 40 nm for doped ZrO\(_2\). The calcined Er\(^{3+}/Yb^{3+}\) co-doped ZrO\(_2\) nanoparticles demonstrated an upconversion luminescence visible by the bare eye. Namely, when excited with NIR-laser, the nanoparticles emit visible green light. Moreover, the Er\(^{3+}/Yb^{3+}\) co-doped ZrO\(_2\) nanoparticles showed red (650-673 nm) and green (520–570 nm) upconversion luminescence due to the \(4\text{F}_{9/2}\) \(\rightarrow\) \(4\text{I}_{15/2}\) of and \(2\text{H}_{11/2}\) and \(2\text{S}_{3/2}\) \(\rightarrow\) \(4\text{I}_{15/2}\) transitions of Er\(^{3+}\) ion, respectively. The obtained nanoparticles exhibited intense luminescence alongside with low toxicity according to colorimetric assay for cellular growth and survival. It was concluded that Er\(^{3+}/Yb^{3+}\) co-doped ZrO\(_2\) nanoparticles are perfect prospective bioimaging agents for using as extremely sensitive detectors of biomarkers or excessive contrast deep tissue indicators.

1. Introduction

The Biomedicine has significantly influenced the successful treatment of many human illnesses. Nevertheless, the clinical development of nanotechnology is only starting to emerge and there are numerous new methods of nanotechnology-based diagnostics and therapeutics being still in the process of clinical development [1]. Up to date, massive preclinical studies have provided vital information on essential design principles for the development of nanomedicine [2]. Although the future of nanomedicine looks bright and widespread, the clinical formulation of nanomedicine results faces the main challenge - it appears to be very complicated to engineer innovative nanoformulations of the drugs providing the ability to track the drug and its behaviour in the organism while not harming its clinical effectiveness [3]. Besides, new drugs are more and more often formulated with nanoparticles (NPs) to address drug delivery challenge such as solubility and therefore agents with any enhanced properties should be formulated in the form of nanoobjects [4].

While nanomedicine develops extremely rapidly its challenges and opportunities are only to be fully realized. Primarily, it is important to highlight that most of our present understanding of NPs behaviour in vivo is grounded on animal data, as a consequence its transformation to NP behaviour in human organism remains mostly unknown. For these purpose we offer optically active NPs intended for modification of biomedicine objects opening approaches for sensing and imaging of biomaterials.
Primarily, NPs with enhanced optical properties are required to identify the effect of nanotherapy, since NPs can influence NP–protein interaction or blood circulation. Inorganic NPs as optical agents suggest the benefit of being exceptionally robust, and hence highly resistant to degradation in the body liquids, which makes them really suitable for imaging and diagnostics [5].

One of the most essential and, simultaneously, the most advanced applications of luminescence is in the medical diagnostics and therapeutics. However, there are some unavoidable difficulties in the employment of photoluminescence based on the UV activation in biomedicine [6]. Human blood is known to absorb intensely both UV radiation and the emission of the optical agent [7]. The solution of this problem can be found by means of employing the upconversion luminescence where the absorption of two or more low-energy photons is followed by the emission of a higher energy photon. Additionally, one of the most reasonable advantages of using near infra-red (NIR) light in biomedicine is that it penetrates deep into tissues causing their minimal autoluminescence [8]. Moreover, there is practically no absorption by the whole-blood in the NIR region. Compared to conventional organic fluorophores upconversion NPs possess such advantages as: low toxicity, high photostability, nonphotobleaching, etc [9,10]. In fact, the upconversion nanoobjects have found a variety of applications in biomedicine as extremely sensitive detectors of biomarkers, excessive contrast deep tissue or single molecule bioimaging agents and many more [11].

Besides, it is worth noting that upconversion nanoobjects can be simply engineered for a multifunctional imaging purposes by integrating some appropriate lanthanide elements into the synthesising system [12]. Therefore, we utilized a well-known lanthanide ion pair of the Yb$^{3+}$ (sensitizer) and Er$^{3+}$ (activator) to produce upconversion bioimaging ability since this pair of ions demonstrates good UCL properties [13].

The zirconium oxide matrix seems to be an ideal medium for producing highly luminescent nanoobjects since it is photochemically and chemically very stable, possess low phonon energy and high refractive index [14]. The use of lanthanide dopants was rationalized to low concentrations for bio-applications because their toxicity is insignificant but the optical response is very high. In this work, we synthesized Er$^{3+}$/Yb$^{3+}$ co-doped zirconium oxide aiming at obtaining a perfect bioimaging agent and studied its optical properties.

2. Synthesis and methods

NPs were prepared using sol-gel method. Sol-gel of zirconium oxide was produced by hydrolysis-polycondensation of zirconium (IV) propoxide solution (70%, Aldrich). To accomplish a controlled hydrolysis-condensation, an esterification reaction of acetic acid (98%, Chemmed) was a source of water and propanol-2 (Chemmed) served as a medium [15]. To achieve upconversion, the rare-earth acetate hydrates were added into the solution of precursors. The ratio of reagents for upconversion NPs was as follows: zirconium propoxide solution 1 ml, isopropanol 13 ml, acetic acid 0.6 ml. First, the desired amounts of erbium (III) acetate hydrate (99.9%, Aldrich) (1 mol%) and ytterbium (III) acetate hydrate (99.95%, Aldrich) (1 mol%) were dissolved in propanol-2 with stirring at 65°C. For undoped NPs, the above described step of adding rare-earth salts was skipped. After that, zirconium (IV) propoxide solution was added to the precursor’s solution in inert atmosphere while stirring for 1 hour at 70°C. Then, acids were added to the solution with stirring for 6 hours at 65°C. Afterwards, the subsequent solution was left for gelation for 48 hours. To reach an upconversion activity zirconium oxide NPs doped with a pair of Er$^{3+}$/Yb$^{3+}$ ions of the NPs were calcined so that dopants embedded in the crystal lattice of the oxide matrix. For an accurate comparison, undoped zirconium oxide NPs were calcined in a similar way. So, the resulting gel was calcined in muffle furnace LOIP-LF at 800°C at heating speed 5° per minute and cooled down to the room temperature at cooling speed 5° per minute.

3. Instrumentation for characterization

Hydrodynamic radius was measured via dynamic light scattering technique using a Photocor Compact-Z analyser. Zeta potential of the hydrosols was distinguished using a Photocor Compact-Z analyser. High-resolution Transmission Electron Microscopy (HRTEM) analysis was performed using
JEOL-2010. The X-ray diffraction (XRD) powder analysis of the calcined NPs was carried out using a D8 Advance Bruker powder X-ray diffractometer with Cu Kα radiation (λ = 1.5406Å) from 5° to 90° at a step of 0.01° per second and speed of 5° per minute. Photoluminescence and transmittance measurements of the NPs were conducted using Ocean Optics QE Pro spectrometer (the excitation was accomplished by HAL 100 and HBO 100). The NIR (980 nm) excitation was provided using a diode laser coupled to a 100 µm (core) optical fiber LASER MASTER PRO LSI980-2000 as a source of light. MTT assay was used to evaluate cytotoxic effects of pure and doped ZrO₂ NPs. The amount of surviving HeLa and human embryonic lung fibroblasts (HELF) (Biolot) cells was determined indirectly by measuring the MTT dye reduction by NAD(P)H dependent cellular oxidoreductase enzyme [16]. The absorption was measured at 570 nm using a plate reading spectrophotometer. HELF and HeLa cells were seeded at density 6×10³ or 10×10³ cells/well, respectively, in 96-well plates.

4. Results and discussion

4.1. Characterization of the NPs in hydrosols

A hydrosol of calcined NPs showed the average zeta potential of 12.3 ± 1.5 mV for pure ZrO₂ and 11.9 ± 1.2 mV for doped zirconia NPs. The average hydrodynamic radius of the calcined NPs in hydrosols (figure 1) observed via dynamic laser scattering analysis lies in the range of 35-40 nm for both pure and doped zirconia NPs. The collected data are in quite a good agreement with the high-resolution transmission electron microscopy (HRTEM) findings, which was performed to study the crystalline structures of the synthesized and calcined NPs.

![Figure 1. Hydrodynamic NPs size in hydrosols: calcined undoped ZrO₂ (orange line) and calcined Er³⁺/Yb³⁺ co-doped ZrO₂ NPs (blue line).](image)

4.2. The crystalline structures of the NPs via HRTEM

Consistent with HRTEM study the NPs size ranges 20-30 nm for undoped ZrO₂ (figure 2 A) and 30-40 nm for Er³⁺/Yb³⁺ co-doped ZrO₂ (figure 2 B). The HRTEM data disclose well-defined crystalline structures of the obtained calcined NPs. Figure 2 (A) reveals distinct lattice fringes of pure ZrO₂ NPs with a P21/a space group and values of 0.3119 nm accurately matching the (-111) planes and 0.3658 nm matching the (110) planes of the monoclinic ZrO₂. The calcined Er³⁺/Yb³⁺ co-doped ZrO₂ NPs demonstrate the similar tendency shown in figure 2 (B). A P21/a space group values of 0.5150 nm matching the (001) planes and 0.3577 nm matching the (011) planes of the monoclinic structure of ZrO₂.
Figure 2. High-resolution TEM images of calcined undoped ZrO$_2$ NPs (A) and calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs (B).

4.3. The phase composition of the NPs via XRD
As seen from the XRD spectra of the both calcined pure and Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ in figure 3, well-crystallized monoclinic NPs were formed, matching correctly with the pattern of standard joint committee of powder diffraction standards (JCPDS) card No. 37–1484, as shown in figure 3 with black bars.

Figure 3. XRD pattern of calcined undoped ZrO$_2$ NPs (orange line) and calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs (blue line), black bars indicate JCPDS-37-1484 card data.

4.4. Study of the optical properties of the NPs
The room-temperature photoluminescence and transmittance spectra in the range of 400-1000 nm of the pure and Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs were measured and presented in figure 4 (A) and figure 5 (A). In the case of calcined undoped ZrO$_2$ NPs, in photoluminescence spectrum, which is shown in figure 4 (A), we observe one broad peak in the range of 420–700 nm, which indicates that no energy transitions take place and, hence, upconversion luminescence of these undoped NPs is impossible to achieve. Moreover, the transmittance spectrum in inset of figure 4 (A) is clear without any peaks. This fact suggests that undoped NPs are not capable of absorbing the NIR light. Additionally, pure ZrO$_2$
NPs demonstrate blue luminescence under UV-excitation as seen in figure 4 (B), which correlates well with photoluminescence and transmittance measurements.

**Figure 4.** Photoluminescence spectrum of calcined undoped ZrO$_2$ NPs (A), the inset shows the transmission spectrum of calcined undoped ZrO$_2$ NPs; an optical micrograph of calcined undoped ZrO$_2$ NPs in photoluminescence microscope (B) under UV excitation.

In the case of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs, we observe the photoluminescence peaks, which are exposed in figure 5 (A), indicating the transitions from the $^4I_{15/2}$ ground state to the excited states of Er$^{3+}$ ion. This fact implies that Er$^{3+}$ ions may absorb the pump light. The transmittance spectrum in inset of figure 5 (A) reveals an evident peak located at 975 nm, resembling the $^2F_{7/2} ightarrow ^2F_{5/2}$ transition of Yb$^{3+}$ ion, which shows that Yb$^{3+}$ ion can absorb the pump light. In addition, the peaks related to the transitions from the $^4I_{15/2}$ ground state to the excited states of the Er$^{3+}$ ion are correspondingly appointed in inset of figure 5 (A) demonstrating the process of energy transfer from Yb$^{3+}$ to Er$^{3+}$ ion. Furthermore, crystals of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs produce visible green light under UV-excitation as seen in figure 5 (B). The obtained data are in a good agreement with peaks in transmittance and photoluminescence spectra.

**Figure 5.** Photoluminescence spectrum of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs (A), the inset shows the transmission spectrum of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs; an optical micrograph of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs in photoluminescence microscope (B) under UV excitation, the inset shows the optical micrograph of calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs in photoluminescence microscope under UV excitation at a higher magnification.
4.4.1. Upconversion luminescence of the NPs. The room-temperature upconversion luminescence spectrum of calcined Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs under NIR (980 nm) excitation was obtained and presented in figure 6 (A). The obtained data reveal two obvious bands in the range of 500-700 nm where two green emissions about 523 nm and 546 nm can be observed. These emissions are correspondingly related to the radiative transitions of \(\text{^2H}_{11/2} \rightarrow \text{^4I}_{15/2}\) and \(\text{^4S}_{3/2} \rightarrow \text{^4I}_{15/2}\) of Er\(^{3+}\) ion. The red emission band lying in the range of 650-673 nm is assigned to the radiative transition of \(\text{^4F}_{9/2} \rightarrow \text{^4I}_{15/2}\) of Er\(^{3+}\) ion. The obtained data correlate precisely and well with the well-known model of upconversion luminescence and the energy transfer process in Er\(^{3+}/\)Yb\(^{3+}\) co-doped samples [17]. The calcined Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs demonstrate an upconversion luminescence visible by the bare eye. Namely, when excited with NIR-laser, the NPs emit visible green light as seen in figure 6 (B).

![Figure 6](image_url)

**Figure 6.** Upconversion emission spectrum of calcined Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs under excitation with NIR laser (980 nm) (A); Digital photo of upconversion luminescence emission of calcined Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs under the excitation with NIR laser (980 nm) (B).

4.5. Cytotoxicity of the NPs

The results of MTT assays concentration-dependent viability of HELF and HeLa cell lines with pure ZrO\(_2\) and Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs are presented in figure 7. The HELF cells were less sensitive to the NPs exposure than HeLa cells. It worth noting, that the death of the studied cell lines does not exceed 20\%, from which it can be concluded that the obtained NPs are low cytotoxic. Moreover, Er\(^{3+}/\)Yb\(^{3+}\) co-doped ZrO\(_2\) NPs show almost identical toxicity as pure ZrO\(_2\) NPs, which means that release of the lanthanide ions doesn’t occur. The obtained data identify that in investigated concentrations the obtained upconversion ZrO\(_2\) NPs are inert to human cell lines and simultaneously they reveal upconversion characteristics making them a promising tool for in vivo diagnostics and therapeutics.
Figure 7. The results of MTT assays of pure ZrO$_2$ and calcined Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs after 72 hours of exposure on HELF cells (A) and HeLa cells (B).

5. Conclusions
It can be concluded that the offered method is applicable for the obtaining of nanoparticles with upconversion activity. The exciting near-infrared light is located in the biological transparency window it can deeply penetrate into living tissues without causing autoluminescence, which is essential for biological imaging, for example, during tissue regeneration processes. The obtained upconversion materials possess well-crystallinity at nanoscale. Furthermore, the developed method allows to obtain stable Er$^{3+}$/Yb$^{3+}$ co-doped ZrO$_2$ NPs hydrosols. This form is very convenient for the inject process for medical applications requiring biovisualization.

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