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Y_2O_3:Eu nanocrystals as biomarkers prepared by a microwave hydrothermal method

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ABSTRACT

Microwave hydrothermal growth of Y_2O_3 crystallites results in needle-shaped aggregates of μm length. Thermal treatment has little influence on the material microstructure, but significant impact on the nanometric level. Nanoparticles doped with europium show an intense red luminescence, related to the $^5D_0 \rightarrow {}^7F_2$ transition of Eu^{3+} ions. The luminescence intensity increases with the calcination temperature and is accompanied by increasing size of Y_2O_3:Eu crystallites. EPR studies show the absence of Eu^{2+} related signals in the material. Y_2O_3:Eu nanoparticles crystallized via a microwave hydrothermal method were employed as luminescent biomarkers in mice. The initial tests confirmed their applicability as biological markers. Persorption of the Y_2O_3:Eu nanoparticles after IG in the adult mouse duodenum, brain and liver is reported.

1. Introduction

Quantum dots (QDs) based biomarkers have attracted significant attention allowing a non invasive way of drug delivery for medical imaging [1]. The most widely investigated markers are based on QDs of CdSe or CdTe [2]. The main disadvantage of their application in living organisms is the release of Cd ions of high cytotoxicity [3]. A partial solution of this problem was the application of CdSe/ZnS core–shell structures. However, also these QDs are biodegradable and cell damage cannot be avoided [4]. Another class of QDs employed in biological applications consists of non-toxic materials, such as Si [5] or carbon [6]. Especially high luminescence activity exhibit also fluoride based nanoparticles, such as fluorite [7] or lanthanide fluorides [8]. However, long-term toxicity in these materials may become a problem [9].

To avoid the toxicity a new generation of biomarkers was developed [10]. Nanoparticles of lanthanide doped ZrO_2 [11], ZnO [12], $ZnAl_2O_4$ [13] were tested for this application. Long-term biocompatibility was tested for most of them [14]. Y_2O_3 usage as new biomarker is proposed in the present work, considering its chemical resistivity, insolubility in water and bioinertness.

Lanthanides, with their precisely defined and characteristic energy structure resulting in 4f–4f emission in visible and infrared regions, are often used as a luminescence activators [15]. Sharp emission lines and low thermal sensitivity of the emission wavelength and intensity are a consequence of f-electrons shielding from the environment [16]. Unfortunately, the atomic-like character of 4f–4f transitions means also that transitions within the 4f shell are weak. These transitions are parity forbidden. This explains the search for more effective channels of excitation, e.g., via parity allowed 4f–5d processes [17].

Yttrium oxide is known as a perfect matrix for Eu^{3+} ions [18]. Moreover, yttrium ions in the oxide form are bioinert, hence, yttrium stabilized ZrO_2 has been used in dentistry for a long time [19]. Trivalent europium ions exhibit bright red emission with sharp emission lines. Its rich energy structure allows optical excitation and emission at a variety of energies. The dominant emission band is due to $^5D_0 \rightarrow {}^7F_J$ intra-shell transitions.

All these properties of both matrix and activator motivate the present study. For the growth of nanoparticles we used the

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microwave hydrothermal method, which allows crystallization of oxides in a hydrous environment. Material properties can be easily controlled, since the process conditions directly influence the resulting product. The application of microwaves in the hydrothermal process promotes rapid crystallization even without a calcination step [20]. The homogenous heating of the whole reaction mixture volume leads to a narrow grain size distribution of the resulting product. This is why we selected this growth method to obtain Y_2O_3:Eu nanoparticles, which are then evaluated for biomarkers applications.

2. Experimental – samples preparation and analyses

2.1. Y_2O_3:Eu nanoparticles

The Y_2O_3:Eu nanoparticles (nanopowders) are prepared by a microwave hydrothermal method. Concentration of europium in the samples was set to 0.5 mol%. The nanopowder was prepared as follows. The nitrate(V) solution of yttrium and europium ions was prepared by dissolving compounds (99.8% and 99.9% respectively provided by Sigma–Aldrich) in distilled water. Then an ammonia solution (25%, Chempur) was added to reach pH = 10 and a white precipitate was created. After washing in distilled water, the obtained residue was processed in the microwave hydrothermal reactor (Ertec) at the pressure of 6 MPa. The wet product was dried and then calcined at temperatures of 400, 800, and 1200 °C in a ceramic boat to promote recrystallization.

The X-ray diffraction measurements were performed with use of a Phillips X'Pert powder diffractometer working in the Bragg–Brentano geometry. The measurements were conducted in the 2Θ range from 20° to 40° with a step of 0.05° and at counting time of 3 s. The Cu Kα radiation (1.54 Å) was used in all the experiments. The calibration was done with an Si sample. Samples were prepared by pressing the powder into steel rings. Processing of raw data was conducted with X'Pert Highscore software.

The scanning electron microscopy (SEM) measurements were conducted with high resolution (1 nm) Hitachi SU-70 microscope, equipped with characteristic radiation detector (EDX) and cathodoluminescence system GATAN Mono CL3. Transmission electron microscopy (TEM) measurements were conducted with FEI Tecnai F20 electron microscope.

The photoluminescence (PL) emission and excitation spectra were taken using Horiba/Jobin–Yvon Fluorolog-3 spectrofluorimeter, equipped with a xenon lamp as excitation source and Hamamatsu R928P photomultiplier. Luminescence decays were obtained with 308 nm excitation pulses (time width 20 ns, repetition rate 10 Hz), provided by a Lambda Physik LPX100 excimer laser. Detection path, in right angle in respect to excitation, consisted of a McPherson 0.67 m monochromator, EMI9659 photomultiplier operating in photon counting mode and a Stanford Research SR430 Multi-Channel Scaler.

The EPR spectra were taken with Bruker spectrometer operating at X-band.

2.2. Biological studies

Graded concentrations ethanol series, paraformaldehyde and xylene were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). Unless stated otherwise, all other media and reagents were purchased from Sigma–Aldrich Sp. z o. o. (Poznań, Poland).

All experiments were approved by the local ethical committee (LEC agreement No. 44/2012). Adult, male, 3–6 month old Balb-c mice were kept in the standard living conditions (12 h day–night cycle), fed *ad libitum* with unobstructed access to water. Following 1 week adaptation period, freshly-prepared reverse osmosis (RO) water suspension of nanoparticles (50 μg/ml) was applied via intragastric gavage (IG, 0.3 ml/mouse). No behavioural changes or discomfort were observed in the animals after the procedure. Following IG, mice were kept for the experiment-imposed period and then sacrificed in the CO_2/O_2 chamber following EU-approved protocol. Internal organs were collected and fixed for 24 h in 4% buffered paraformaldehyde then transferred to 70% ethanol for storage until the embedment procedure. Fixed tissues were dehydrated in a series of alcohol concentrations and embedded in paraffin in the TP1020 Leica tissue processor following the standard histological procedure. Embedded tissues were then cut to 5 μm-thin sections using the Leica RM2235 rotary microtome and fixed to the silane-coated microscope slides. Samples were then deparaffined in xylene, rehydrated in a series of alcohol solutions using the standard histological procedure and rinsed in PBS for 5 min. Afterwards, cell nuclei were counterstained with HOECHST 33342 (0.1 mg/ml, 2 min). Mounting medium for immune-fluorescence was added prior to the application of coverslips. Due to the possible overlap of the fluorescence channels the sequence scan mode was employed. Specimens were visualised under the Leica SP8 confocal microscope with white light laser. One slide from each organ was evaluated for the pathological changes. No signs of the inflammation or abnormal accumulation of the white blood cells was observed.

3. Results and discussion

3.1. Structure and thermal behaviour

3.1.1. XRD

Diffraction patterns of obtained nanopowders are shown in Fig. 1. The structural evolution of the material annealed at different temperatures is presented. The as-grown sample (Fig. 1A) does not contain the crystalline yttrium oxide phase. It seems that processing at hydrothermal conditions leads to hydroxide crystallization. Phase analysis reveals that the phase present after microwave hydrothermal treatment is monoclinic $Y_4O(OH)_9(NO_3)$ (PDF reference code 79-1352). Since the initial residue was precipitated from the nitrate(V) solution, the nitrate group is present in the initial crystallites. In the temperature range of 200–400 °C the decomposition process takes place and cubic Y_2O_3 (PDF reference code 88-2162) starts to form. Heating in the range of 600–1200 °C results in the presence of only one phase of cubic yttrium oxide. Microwave hydrothermal processed hydroxide precursors were reported to crystallize as oxides in many cases, see e.g. [20,21]. However, in this case the process conditions did not induce precursor dehydration. Additionally, the presence of nitrate(V) groups led to crystallization of intermediate yttrium hydroxide nitrate(V) phase.

The mean crystallite size (MCS) values, calculated using Scherrer's equation, depending on heat treatment are shown in the inset of Fig. 1. They vary from ca. 8 nm for material calcined at 200 to ca. 120 nm for nanopowder calcined at 1200 °C.

3.1.2. SEM

SEM images (Fig. 2) show the structure of nanopowders calcined at different temperatures. The grains are longitudinal in all the cases and have few μm of length and 100–400 nm of width. The grains exhibit discrete structure, due to accumulation of needle-shaped structures with similar lengths as the grains, but with widths of several dozen nm. The fine structures do not appear in samples calcined at 1200 °C, as the sintering took place. The observed sizes of the grains are much larger than calculated from the X-ray diffraction line broadening, suggesting that crystallites