

Synthesis, functionalization and toxicity of multicolour bifunctional nanophosphors

Huayna Terraschke^a, Maximilian Felix Toni Meier^a, Yvonne Voss^b, Holger Schönherr^b and Claudia Wickleder^{a,*}

^aInorganic Chemistry, Science and Technology Faculty, University of Siegen, 57068 Siegen, Germany

^bPhysical Chemistry I, Science and Technology Faculty, University of Siegen, 57068 Siegen, Germany

The development of an easily upscalable method for the preparation of nanosized phosphors is reported, which comprises a simplified precipitation method for the synthesis of lanthanide (Ln)-doped Y₂O₃ nanoparticles (NPs). Co-doping the Y₂O₃ NPs with Tb³⁺ and Eu³⁺ led to a gradient of the emission color, this adjustment is very important for applications. The influence of different synthesis parameters on the mean particle size and the size distribution was unravelled and the toxicity of aminosilane functionalized and neat NPs was investigated.

Key words: Y₂O₃ : Eu³⁺, Tb³⁺, Nanoparticles, Luminescence, Toxicity, Chemical precipitation

Introduction

Luminescent nanoparticles (NPs) play a decisive role in technological and biomedical applications due to their remarkable physical and chemical properties, such as high stability, high quantum yield and emission intensity, low light scattering effect and pronounced suspendability in liquid media [1]. However, the currently applied synthesis routes for the production of luminescent NPs, including sol-gel, combustion, polyol, laser ablation as well as ultrasound and microwave-assisted methods [2a-i], are rather energy or time consuming as well as hardly upscalable and reproducible on an industrial scale.

Yttrium oxide doped with trivalent lanthanides (Y₂O₃ : Ln³⁺) comprises a widely studied phosphor system [3] thanks to its high brightness and high efficiency light emission coupled with very good mechanical and thermal stability. To date, few reports on the synthesis of Y₂O₃ : Ln³⁺ NPs by means of co-precipitation methods have appeared in the literature. [4a-d] By means of hydrolysis assisted co-precipitation, for instance, small particles of Y₂O₃ : Eu³⁺ with diameters of 7-14 nm are reached, requiring however a long preparation time of 72 h [4a]. Additional interesting approaches are explored by solving the starting materials in an acidic solution and adding an alkaline solution to it, until a critical pH value is reached and an abrupt nucleation starts [4b-d]. These approaches are especially attractive for applying low-cost materials as hydrochloric acid, sodium hydroxide or urea. Nevertheless, the overall preparation time for these methods also reach ranges of

24-36 h and the crystal growth is difficult to control due to the fast nucleation, often resulting in particle sizes in the range of ~ 100 nm [4c-d].

In contrast to the previously reported methods, in our present work, the HCl acidic solution of the starting materials was added dropwise into an ammonium aqueous solution. The advantage of this co-precipitation method in comparison to the hitherto reported ones [4a-d] is that, when the reactant solution is dropped within the ammonia aqueous solution, the freshly formed Y₂O₃ : Ln³⁺ precursor particles are immediately surrounded and stabilized by ammonium cations. The large ammonium cations form a positively charged layer around the particles, improving the surface stabilization due to electrostatic forces [5], which can be combined with steric effects by the supplementary addition of surfactants to the solution [6]. This method have been applied for the production of magnetic Fe₃O₄ nanoparticles before [7] and used for the production of multicolour Y₂O₃ : Ln³⁺ NPs in this work, to the best of our knowledge, for the first time. Therefore, this work reports a simple precipitation process for the synthesis of multicolour bifunctional aminosilane-coated Y₂O₃ : Eu³⁺, Tb³⁺ NPs, which is able to produce particles down to ~ 8 nm and takes only few hours. In addition to the successful synthesis and detailed NP characterization, we also report on the cytotoxicity of Y₂O₃ : Eu³⁺ NPs.

Experimental Part

All reagents were commercially purchased and used without further purification. In a typical experiment, bulk Y₂O₃ powder (Merck Chemicals, Darmstadt, Germany, 99.9 %) was dissolved in aqueous 3.7% HCl solution together with 1 mol-% LnCl₃ (Ln = Eu, Tb, Alfa Aesar, Karlsruhe, Germany, 99.9 %). This solution was heated and added dropwise to 25-75 mL of a

*Corresponding author:

Tel : +49-271-740-4217

Fax: +49-271-740-2555

E-mail: wickleder@chemie.uni-siegen.de

stirred 25% NH_3 solution. After stirring for 30 minutes, a powder precipitated, which was centrifuged and then washed with water and ethanol. Finally, the powder was sintered at 600-1000 °C for 0.5-5 h in air. Hence, the entire production time took only 2-9 hours. To control and manipulate the mean NP size and the NP size distribution, a non-ionic (Triton X-100, polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, Acros Organics, Geel, Belgium, 98 %) and an ionic (cetyl trimethylammonium bromide, CTAB, Acros Organics, 99+%) tenside were added to the NH_3 solution applied in the NP precipitation. Furthermore, the synthesized $\text{Y}_2\text{O}_3:\text{Eu}^{3+}$ NPs were functionalized with peripheral amino groups by coating the NPs with a layer of 3-aminopropyltriethoxysilane [$\text{NH}_2(\text{CH}_2)_3\text{Si}(\text{OC}_2\text{H}_5)_3$] (APTES, Merck Chemicals, 98+%) in order to improve the interaction with biomolecules [8]. Therefore, 0.1 g of the $\text{Y}_2\text{O}_3:\text{Eu}^{3+}$ NPs were suspended in 200 mL of ethanol and sonicated (3 W, Misonix XL2000, Qsonica LLC, USA) for two minutes. Subsequently, 70 μL of APTES were added to the suspension, which was then vigorously stirred overnight, washed several times with ethanol and dried under vacuum [8].

For the toxicity tests, Patu 8988T [9] cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 5% FBS, 5% Horse Serum, 100 U/ml Penicillin- 100 $\mu\text{g}/\text{ml}$ Streptomycin and 2 mM L-Glutamine. Patu 8988T cells were cultured in a humidified incubator at 37 °C, in 95% air and 5% CO_2 . Cell proliferation was evaluated using the Vybrant® MTT Cell Proliferation Assay Kit, following the manufacturer's instructions. In brief, 32000 cells were seeded in 24-well plates in culture media for 24 h. The cells were then treated with solutions of NPs of various concentrations between 3.125 and 1200 $\mu\text{g}/\text{well}$ (the well area was 1.9 cm^2) for additional 24-48 h, respectively. MTT was added and allowed to react for 4 h, followed by an 18 h SDS treatment. The absorbance (OD) was measured at 570 nm on a Varian Cary 50 Bio Spectrometer. Photoluminescence measurements were

performed on a Fluorolog3 spectrofluorometer F13-22 (Horiba Jobin Yvon) equipped with double Czerny-Turner monochromators, a 450 W xenon lamp and a R928P photomultiplier (Hamamatsu) with a photon-counting system. The emission spectra were corrected for the photomultiplier sensitivity and the excitation spectra were corrected for the lamp intensity. The X-ray diffraction data were measured on a D5000 X-ray diffractometer (Siemens) operating at 40 kV, 30 mA with $\text{Cu-K}\alpha$ radiation ($\lambda = 0.154178$ nm). The microscopy analysis was carried out on a CS44 Scanning Electron Microscope (SEM, CamScan). Dynamic light scattering (DLS) measurements were performed with an ANALYSETTE 12 Dynasizer from Fritsch GmbH.

Results

The NPs samples were analysed by powder X-ray diffraction analysis to determine the crystal structure of the synthesized samples **1-12** and their phase purity. The good match with the theoretical pattern of Y_2O_3 [10] shown in Figure 1a demonstrates the successful synthesis for all experiments. Compared to the standard diffraction pattern, the NP samples exhibited mostly broad reflexes, low intensity and high signal background, which are typical for nano-sized crystals. This interpretation agrees with SEM measurements, which also revealed the spherical shape of the synthesized NPs (Fig. 1b).

From the full width at half maximum (FWHM) of the X-ray diffraction reflexes at $2\theta = 29.1^\circ$, 33.6° and 48.4° shown in Figure 1a, the particle size of **1-12** were estimated using the Scherrer equation [11]. Figure 2a depicts the mean particle size with respect to different synthesis parameters. The lowest mean particle size (8 nm) was hence found to increase for increasing sintering temperatures and times as well as decreasing NH_3 content. At high sintering temperatures and for longer sintering time, an undesired coalescence of the particles occurred, resulting in an increase of the apparent particle diameter up to 35 nm. On the other

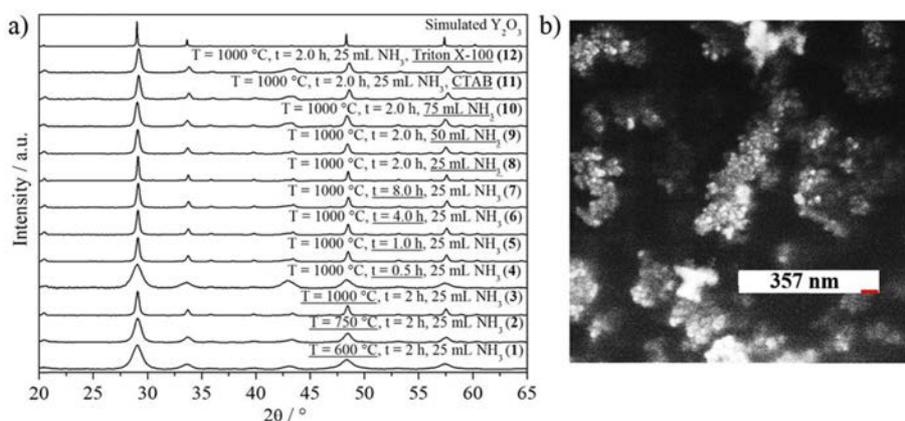


Fig. 1. a) XRD diffractograms of $\text{Y}_2\text{O}_3 : 1\% \text{Eu}^{3+}$ compounds **1-12** in comparison to the simulated Y_2O_3 pattern [10], b) SEM image of **8**, scale bar of 357 nm.

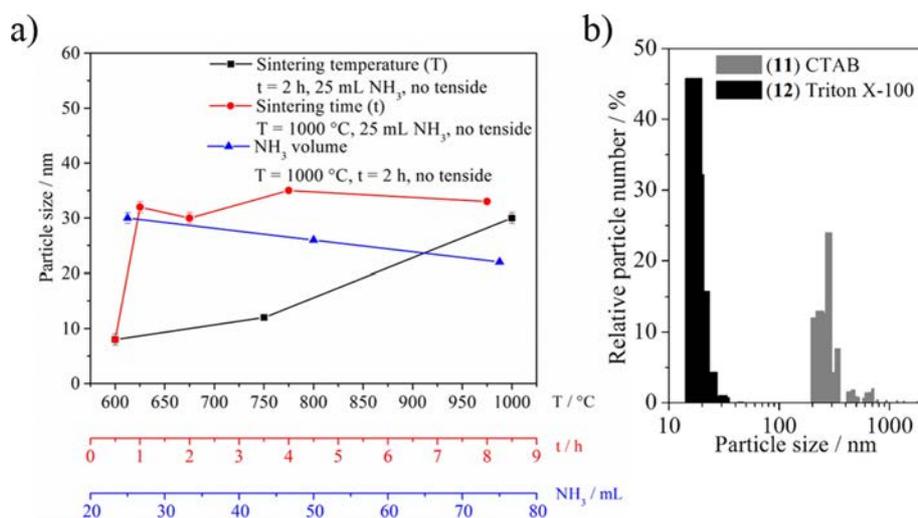


Fig. 2. a) Influence of sintering temperature (T), sintering time (t) and NH₃ content on the particle size, estimated with the Scherrer equation. b) Particle size distribution of compounds **11** (in grey) and **12** (in black), measured with dynamic light scattering.

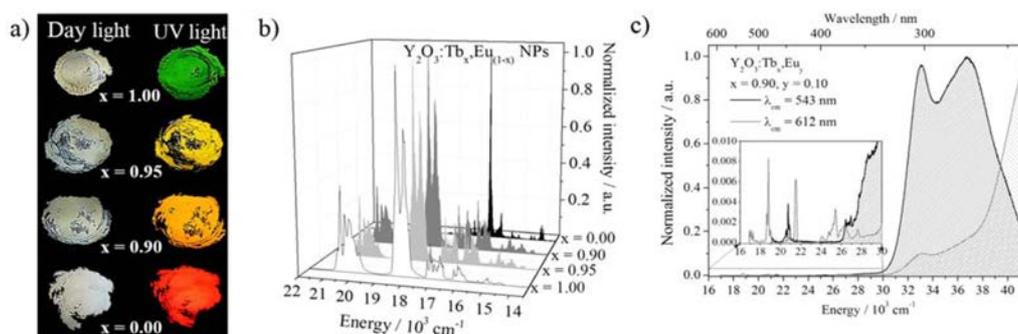


Fig. 3. a) Y₂O₃:Tb_xEu_(1-x) irradiated with day light and UV light for x = 1.00, 0.95, 0.90 and 0.00. b) Emission spectra of Y₂O₃:Tb_xEu_(1-x) NPs (ν = 40000 cm⁻¹). c) Excitation spectra of Y₂O₃:Tb_{0.90}Eu_{0.10} NPs.

hand, when the NH₃ content was increased, the pH of the stock solution as well as the surface charge of the precipitated NPs increased. Consequently, the stabilizing electrostatic repulsion between the NPs was enhanced and helped to avoid the formation of larger agglomerates.

The application of tensides does not demonstrate a significant decrease on particle size in comparison to comparable samples without tensides. However, the size distribution measured by dynamic light scattering (Fig. 2b) reveals that samples stabilized with CTAB (**11**) present more agglomerates and broader size distribution in comparison with samples stabilized with Triton X-100 (**12**). A probable explanation for this effect is the longer organic chain and consequent enhanced steric effect of Triton X-100 in comparison to CTAB.

Figure 3a shows photographs of Y₂O₃:Eu³⁺ samples loaded with different concentrations of Eu³⁺ and Tb³⁺ under daylight and UV light illumination. Under day light, all samples remain colourless, while under UV light the samples exhibited a reddish, green or orange and yellow emission, respectively, which is characteristic of Eu³⁺, Tb³⁺ transitions or the mixture of both ions. This

possibility to scan the emission colour is extremely advantageous for the use of the materials for applications. In the emission spectra (Fig. 3b) of the NPs loaded only with terbium, exclusively the Tb³⁺ ⁵D₄ → ⁷F_J (J = 3-6) [12] transitions were observed, among which the ⁵D₄ → ⁷F₅ at 18416 cm⁻¹ (543 nm) was the most intense one. Substituting 5%-10% of the Tb³⁺ with Eu³⁺ ions, additional peaks corresponding to the transitions of trivalent europium appeared in the emission spectrum. The Tb³⁺ peak at 18416 cm⁻¹ remained the most intense one, in full agreement with results of similar experiments previously reported in the literature [9]. For samples doped only with Eu³⁺, exclusively the sharp ⁵D₀ → ⁷F_J (J = 1-4) transitions were observed [12]. The excitation spectra of Y₂O₃:Tb_{0.90}Eu_{0.10} NPs (Fig. 3c) contained a broad band above 30000 cm⁻¹ together with a few sharp peaks of lower intensities, assigned to the f-f transitions of the respective Ln³⁺ ion. This broad band rises due to a combination of charge transfer processes between oxygen ions in the host lattice and the europium ions and f-d transitions assigned to the terbium ions [3].

Finally, the toxicity of uncoated and APTES-coated Y₂O₃:Eu³⁺ NPs (Fig. 4a) was investigated using the MTT Cell Proliferation Assay. After 24 hours, a

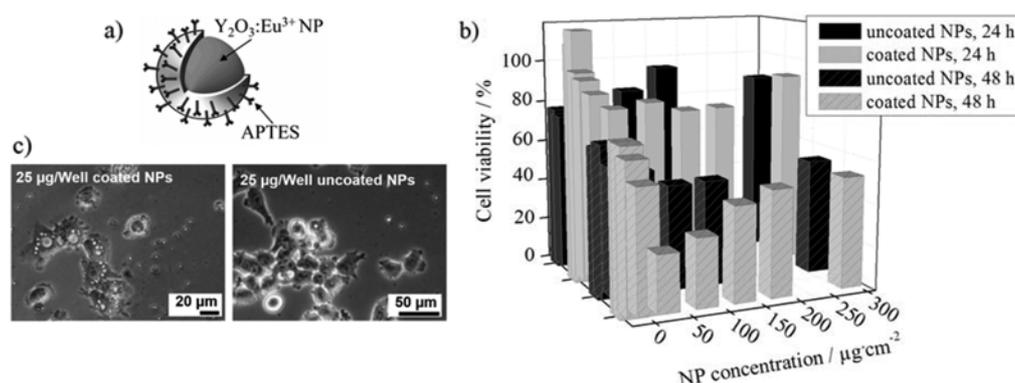


Fig. 4. a) Schematic representation of $Y_2O_3:Eu^{3+}@APTES$ NPs. b) Viability of (un) coated NPs after 24 h and 48 h interaction with Patu 8988T cells. c) Optical microscopy images of Patu 8988T cells in contact with $Y_2O_3:Eu^{3+}$ and $Y_2O_3:Eu^{3+}@APTES$ NPs.

decrease of up to 25% of the cell viability for cells interacting with uncoated NPs was detected. However, this decrease was not found to be related to the NP concentration (Fig. 4b). The nearly same behaviour was observed for APTES-coated NPs, with a decrease of up to 21% in cell viability after 24 h. Similarly, there was a further decrease on the cell surviving rate for coated and uncoated NPs after 48 h, which was independent of the NP concentrations as well. Nevertheless, many small inclusions inside the cells were observed in microscope images of cells cultivated in the APTES-coated NPs in comparison to the non-coated ones (Fig. 4c) (the cells with uncoated NPs also have these inclusions, albeit in much lower numbers), indicating a possible deterioration of the cells under these circumstances. Because the decrease of the cell viability demonstrated to be independent to the presence or concentration of coated and non-coated NPs, the cytotoxicity of these luminescent nanoparticles is considered substantially low. In addition, a brief comparison of these results with MTT tests reported in the literature for other luminescent nanoparticles e.g. bare quantum dot [14] reveals a generally lower cytotoxicity of the nanophosphors produced herein. However, a direct comparison with the literature data is difficult due to the diverging experimental conditions utilized in different works.

Conclusions

In this work, an alternative method for the synthesis of multicolour bifunctional luminescent nanoparticles was developed. NPs were prepared within few hours and at low temperatures in an easily upscalable process. The particle size could be flexibly changed by applying different synthesis conditions. In addition, the spectroscopic properties especially the emission color could be fine-tuned according to the chemical composition of the NPs. According to the MTT Cell Proliferation Assay, the toxicity was lower than that reported e.g. for bare quantum dots [14]. Finally, the particle surface was functionalized with amino groups in a preliminary attempt

to improve the interaction with biomolecules and cells.

Acknowledgments

The authors thank Dr. J. Schnekenburger for providing the cell samples and acknowledge the financial support by the European Research Council (ERC grant to HS, ERC grant agreement No. 279202) and the University of Siegen.

References

1. D. Vollath, in "Nanomaterials: An Introduction to Synthesis, Properties and Applications" (Wiley-VCH press, 2008) p. 145-207.
2. (a) B.-C. Hong, K. Kawano, *J. Alloys Compd.* 451 [1-2] (2008) 276-279. (b) Z. Qiu, Y. Zhou, M. Lu, A. Zhang, Q. Ma, *Acta Mater.* 55 [8] (2007) 2615-2620. (c) C.-H. Lu, S.-Y. Chen, C.-H. Hsu, *Mater. Sci. Eng. B* 140 [3] (2007) 218-221. (d) W. Chen, J.-O. Malm, V. Zwiller, R. Wallenberg, J.-O. Bovin, *J. Appl. Phys.* 89 [5] (2001) 2671-2675. (e) H. C. Streit, J. Kramer, M. Suta, C. Wickleder, *Materials* 6 (2013) 3079-3093.
3. W. M. Yen, S. Shionoya, in "Phosphor Handbook" (CRC Press Inc, 2006) p. 342.
4. (a) R. Srinivasan, N. R. Yogamalar, J. Elanchezhian, R. J. Joseyphus, A. C. Bose, *J. Alloys Compd.* 496 [1-2] (2010) 472-477. (b) G. S. Gowd, M. K. Patra, S. Songara, A. Shukla, M. Mathew, S. R. Vadera, N. Kumar, *J. Lumin.* 132 [8] (2012) 2023-2029. (c) G. Wakefield, E. Holland, J. Dobson, J. L. Hutchison, *Adv. Mater.* 13 [20] (2001) 1557-1560. (d) X. Hou, S. Zhou, Y. Li, W. Li, *J. Alloys Compd.* 494 [1-2] (2010) 382-385.
5. R. Massart, *IEEE Trans. Magn.* 17 [2] (1981) 1247-1248.
6. S. P. Gubin, in "Magnetic Nanoparticles" (Wiley-VCH press, 2009) p. 51.
7. S. H. Othman, S. A. Rashid, T. I. M. Ghazi, N. Abdullah, *J. Nanomater.* 2012 (2012) 1-10.
8. M. Ma, Y. Zhang, W. Yu, H. Shen, H. Zhang, N. Gu, *Colloids Surf, A* 212 [2-3] (2003) 219-226.
9. H. P. Elsässer, U. Lehr, B. Agricola, H. F. Kern, *Virchows Archiv B Cell Pathol* 61 [5] (1992) 295-306.
10. G. Brauer, H. Gradinger, *Z. Anorg. Allg. Chem.* 276 (1954) 209-226.
11. P. Scherrer, *Nach. Ges. Wiss. Göttingen* 2 (1918) 98-100.

12. G. H. Dieke, in "Spectra and Energy Levels of Rare Earth Ions in Crystals" (Interscience Publishers, 1968) p. 242-261.
13. S. Mukherjee, V. Sudarsan, R. K. Vatsa, S. V. Godbole, R. M. Kadam, U. M. Bhatta, A. K. Tyagi, *Nanotechnology* 19 [32] (2008) 325704-325711.
14. S. Deka, A. Quarta, M. G. Lupo, A. Falqui, S. Boninelli, C. Giannini, G. Morello, M. De Giorgi, G. Lanzani, C. Spinella, R. Cingolani, T. Pellegrino, L. Manna, *J. Am. Chem. Soc.* 131 [8] (2009) 2948-2958.