Synthesis of Upconverting Nanophosphors micelles. A new class of nanoparticles with a potential application for optical biomedical imaging.

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Cell and molecular imaging are nowadays of paramount importance in order to understand the origin and evolution of pathological mechanisms and, even more important, for an early diagnostic of the diseases. Among the different existing techniques, fluorescence imaging presents the distinctive advantage of a high sensitivity without ionizing radiation. Recently, fluorescence imaging has been equally described as a potential tool for providing anatomical and physiological information[1].

The most common luminescent probes currently in use are fluorescent proteins, organic dyes, metal complexes and recently semiconductor quantum dots. The majority of them bring into play down-conversion fluorescence. This can explain intrinsic limitations of fluorescence imaging: tissue damaged because of long exposition time and high energy radiation, significant auto-fluorescence of the biological tissues, low penetration depth or inner toxicity of the probe[1]. Very recently the rare-earth upconverting nanophosphors (UCNPs), a new generation of luminescent probes has been studied as interesting alternative. These materials present the unique feature of converting low energy NIR light into higher visible light and/or NIR emission throw 2 or 3 sequential photon absorption, together with energy transfers. This singular property confers very attractive advantages for a UCNP-based fluorescence imaging: excitation less harmful, no auto-fluorescence, high penetration depth, no toxicity, low cost techniques[2].

One of the objectives of the Laboratory of the Molecular Probes and Nanotechnology at CNIC is the synthesis of new multifunctional nanobiomaterials for lung and cardiovascular imaging. Lung imaging with conventional techniques in magnetic resonance imaging (MRI), such as the widely used T_2-weigthed sequences, is difficult because of its low proton density. Thus, fluorescence imaging with UCNPs appears like an option of paramount importance.

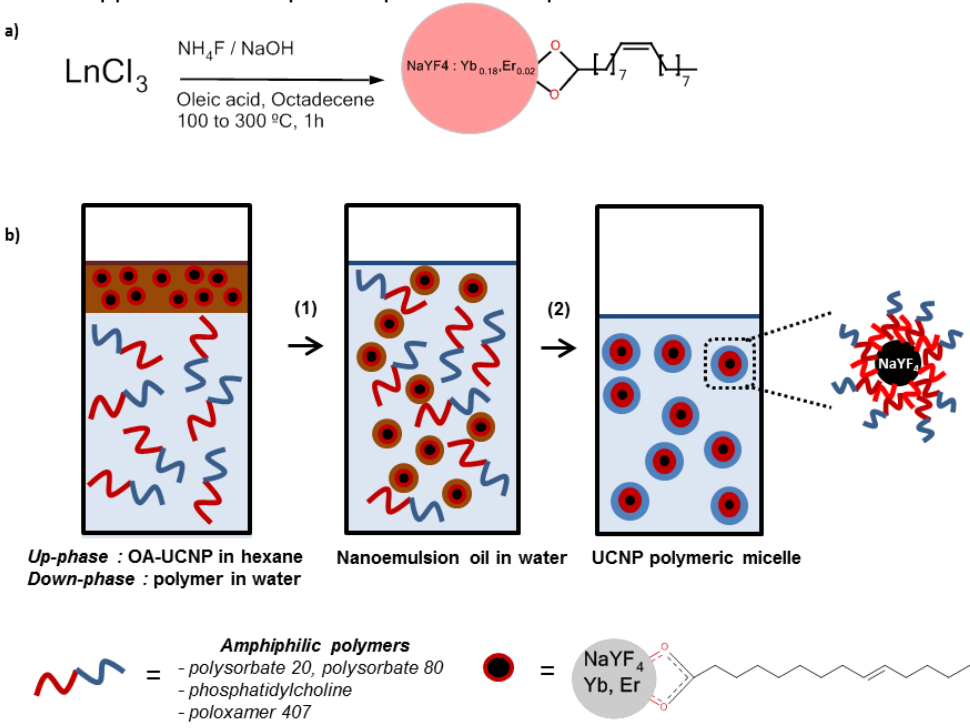

Figure 1. Synthetic methodology for the UCNP micelle preparation. (a) Step 1 preparation of the oleic acid coated $NaYF_4$:Yb,Er (OA-UCNP). (b) Step 2 coating of the OA-UCNP with amphiphilic polymers 1) sonication, stirring 2) hexane evaporation, centrifugation, dialysis.

For *in vivo* small animal imaging, current literature describes the $NaYF_4$ in hexagonal phase as the best host doped material for upconverting fluorescence[3]. Then, the dopants most used are Yb, Er, and Tm. Therefore we focused first on the golden standard β-$NaY_{0.8}F_4$: $Yb_{0.18}$, $E_{r0.02}$ as proof-of-principle for all our future chemistry. The adopted strategy was an initial synthesis in organic solvent, as it provides the nanocrystals of the highest quality[4], followed by a step to render the nanoparticles hydrophilic and suitable for *in vivo* applications (figure 1).

Following an already published method we prepared oleic acid capped $NaYF_4$: Yb, E_r (OA-UCNP) by decomposition at high temperature of lanthanides chloro-precursors in a high-boiling solvent mixture.[5] Then we opted for a non-covalent coating of the OA-UCNP in a hydrophilic matrix. Mimicking a nanoemulsion method we previously used with iron oxide nanoparticles, we coated the particles by the formation of micelles with four amphiphilic polymers known for their antibiofouling properties: polysorbate 20, polysorbate 80, phosphatidylcholine, and poloxamer 407 (figure 1)[6]. The micelles were fully characterized; they displayed a high *in vitro* fluorescence under 980 nm excitation and excellent aqueous stability which make them promising candidates for *in vivo* fluorescent probes. We believe that the same methodology can be applied to others upconverting host doped material. (figure 2).

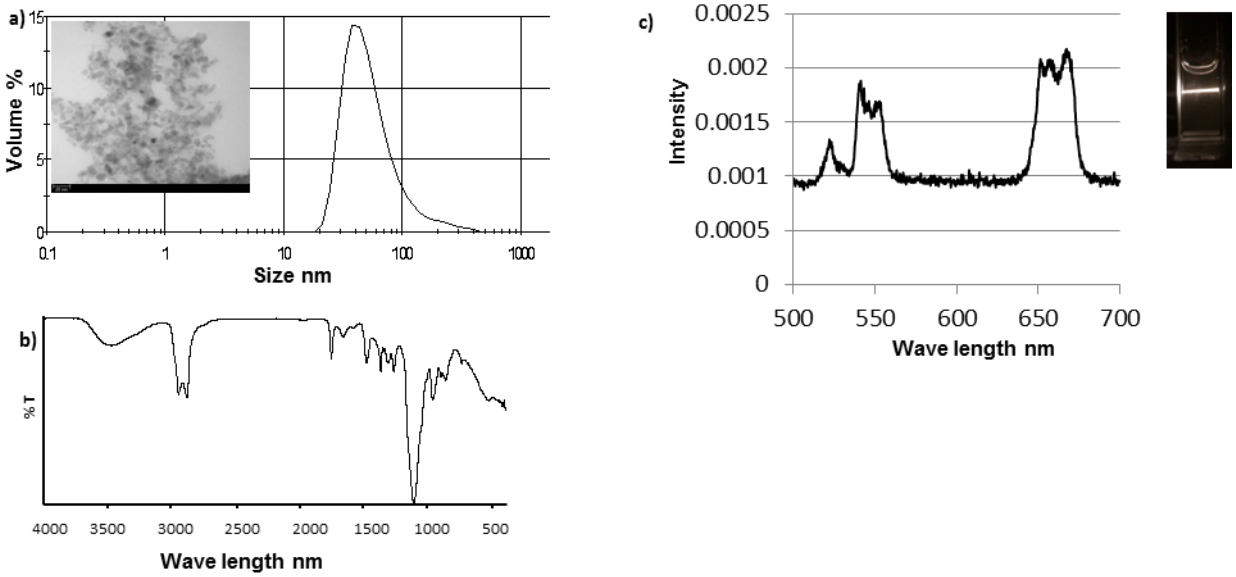

Figure 2. Characterization of a polysorbate 20 coated $NaYF_4$:$Yb_{0.18}$,$Er_{0.02}$ micelle (a) UCNP z-average size in volume; inset TEM image of the OA-$NaYF_4$ precursor. (b) UCNP FTIR spectra. (c) UCNP fluorescence emission under a 980 nm excitation; inset photograph of the visible emission of the OA-$NaYF_4$ precursor under a 980 nm excitation.

References

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