The continuous hydrothermal synthesis and characterisation of citric acid coated (Y_{0.96}: Eu_{0.04}) OOH phosphor nanoparticles for cell imaging applications


*Department of Chemistry, University College London, 20 Gordon street, London, WC1H 0AJ
**Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK

ABSTRACT

Herein, we report a green and continuous hydrothermal process for the direct synthesis of phosphor nanoparticles ((Y_{0.96}: Eu_{0.04}) OOH) surface functionalised with citric acid. The particles are obtained directly from the process as a stable aqueous suspension with a crystallite size of 25.57 ± 7.08 nm. Photoluminescence spectroscopy was used to assess the excitation and emission spectra of the materials which showed broad UV excitation and red emission due to \(^{3}D_0 \rightarrow ^{3}F_1\) and \(^{3}D_0 \rightarrow ^{3}F_2\) transitions in europium. To demonstrate the feasibility of these materials to serve as optical probes in biological applications, an in-vitro fluorescence microscopy assay was performed using an immortal cell line (COS7) at an excitation wavelength of 470 nm and emission at 620 nm (allowing particle visualisation without significant cellular autofluorescence). The particles were successfully visualised within cell bodies and freely diffusing in solution.

Keywords: hydrothermal, nanoparticles, phosphor, imaging

1 INTRODUCTION

Lanthanide-doped phosphors have been applied to a variety of technological applications including security inks and materials for biomedical application [1, 2]. A common dopant for the synthesis of nanoparticle phosphors is trivalent europium (Eu\(^{3+}\)) due to its broad UV excitation and strong red emission spectrum originating from \(^{3}D_j (J = 0,1,2) \rightarrow ^{3}F_j (J = 0,1,2,3)\) transitions. Europium (Eu\(^{3+}\)) doped rare-earth oxides, oxysulfides, hydroxides, phosphates and fluorides have been investigated as possible alternatives to conventional fluorescent probes [3, 4]. Briefly, the literature highlights lanthanide-doped phosphor materials as having several advantages over both conventional organic molecular probes and functionalised Quantum Dots; including, high photochemical stability (non-blinking), narrow emission, low cytotoxicity and tuneable emission [3, 5].

Phosphor nanoparticles (NPs) have been synthesized by a number of synthetic methods based on both bottom-up and top-down strategies; solid state, sol-gel, co-precipitation and combustion syntheses are all represented in the literature [4, 5]. Typically, bottom-up strategies require lengthy heat treatment and grinding/milling steps to produce a nanosized material. Resultantly, complex formulation and material refinement steps are often required to produce a biocompatible material. Recent efforts to produce phosphor nanoparticles for biological applications have focused on colloidal synthesis methods capable of producing non-polar solvent/aqueous dispersions of nanoparticles which can be converted to biocompatible materials through a suitable formulation strategy (coupling, conjugation, ligand exchange etc) [5]. Hence there is interest in methods which can rapidly produce stable aqueous dispersions of phosphor nanoparticles which require fewer post-synthesis processing steps.

The process of continuous hydrothermal flow synthesis (CHFS) has been used to make crystalline nanoparticles of many different materials e.g. components of solid oxide fuel cells, photo-catalysts and materials with biomedical applications [6, 7, 8]. CHFS can be described as a green process that uses water as a solvent and reagent. The process uses water soluble metal salts (e.g. metal acetates, nitrates etc) as precursors for the synthesis of many nanomaterials. Nanoparticles form under controlled conditions when a superheated water feed (typically, 350-450 °C, 24.1 MPa) meets a flow of a metal salt solution at ambient temperature (R in figure 1). The unique reaction conditions in the CHFS process produce nanoparticles through rapid particle nucleation (with minimal growth) via the hydrolysis and dehydration of precursor salts.

In this work we present our recent investigations into the synthesis of citric acid coated (Y_{0.96}: Eu_{0.04}) OOH phosphors using a modified CHFS reactor. Citric acid (CA) was chosen as a capping agent for this work due to its widespread application in many materials intended for biological application/bioconjugation. As-synthesized citric acid coated (Y_{0.96}: Eu_{0.04}) OOH phosphor nanoparticles were characterised using XRD, TEM, DLS, Zeta-potential measurements, FTIR, TGA, photoluminescence measurements and fluorescence microscopy.

2 MATERIALS AND METHODS

Figure 1 is a flow diagram of the modified CHFS process which allows for the introduction of a capping agent shortly after the formation of nanoparticles. The general CHFS process has been described in detail in our previous publications [6, 7, 8]. The reaction point R in figure 1 was a...
concentrations (5 mM NaCl) using NaOH and HCL as titrants. Fluorescence was captured using an Olympus microscope (BX51WI) with a 60x Olympus objective coupled to an EM-CCD camera (Ixon, Andor).

3 RESULTS AND DISCUSSION

Figure 1 shows a schematic of the CHFS process used for the synthesis of nanoparticles. A reaction point temperature of ca. 380 °C (above the supercritical point of pure water at 24.1 MPa) was found to be crucial for the direct synthesis of visibly bright phosphor materials (figure 2a inset). In this temperature regime monoclinic (Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH is obtained as the reaction product (synthesis optimisation data not shown). Reaction point temperatures of < 330 °C typically yielded mixed phase products of Y\textsubscript{0.96}Eu\textsubscript{0.04} (OH)\textsubscript{3}/(Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH as determined by XRD. The synthesis of citric acid coated phosphor materials was first evaluated by materials synthesis to define an operational space in which stable dispersions of particles could be produced. A material which showed good dispersion properties and narrow hydrodynamic diameter distribution was chosen and characterised prior to florescence microscopy. The characterisation details are presented within the text.

Figure 2 shows the XRD pattern obtained for the stable phosphor dispersion reported herein. The diffraction pattern is in good agreement with ICDS pattern number 28442 (monoclinic YOOH P\textsuperscript{21}/M\textsubscript{1}). The unit cell volume of the material was calculated using the le-bail method as 87.65 Å\textsuperscript{3}, the reported unit cell volume of YOOH is 86.84 Å\textsuperscript{3} providing evidence for the incorporation of europium into the host lattice (Thereafter, referred to as (Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH). The crystallite size was calculated using the scheerer equation as 29.20 nm . Figure 2 b shows a TEM image representative of (Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH nanoparticle synthesised using CHFS. The average crystallite size was determined as 25.57 ± 7.08 nm (ca. 350 particles measured), which is consistent with the crystallite size determined from XRD.

Figure 3 shows photoluminescence measurements taken of the as-synthesised (Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH nanoparticles. The excitation spectrum showed relatively broad lines in the 200-500 nm region. Absorbance in the 200-300 nm region originates from transitions to charge transfer states and absorbance in the region 350-500 can be attributed to transitions from ground state \^3F\textsubscript{0} electrons to excited states [9, 10]. Figure 3 (inset) shows the emission spectra of (Y\textsubscript{0.96}: Eu\textsubscript{0.04}) OOH measured at 360 nm. The spectra show strong red emission in the 600-630 nm region corresponding to transitions of \^3D\textsubscript{0}→\^3F\textsubscript{2} (electronic dipole transitions). A weak emission region (580-600 nm) was also observed and can be assigned to the transitions of \^3D\textsubscript{0}→\^3F\textsubscript{1} (magnetic dipole transitions). The origin of each emission peak is denoted on figure 3 [9, 10].
Figure 2: (A) Diffraction pattern obtained for CHFS synthesised \((Y_{0.96} \text{: Eu}_{0.04})\) OOH compared to ICDS pattern 28442 (YOOH). Inset shows a photograph of \((Y_{0.96} \text{: Eu}_{0.04})\) OOH phosphor particle dispersion after dialysis (Solids 0.05 mg/mL) under UV irradiation (\( \lambda = 254 \) nm). (B) TEM image of CHFS synthesised \((Y_{0.96} \text{: Eu}_{0.04})\) OOH nanoparticles (scale bar = 100 nm).

ATR-FTIR was used to confirm the presence of citric acid on the surface of \((Y_{0.96} \text{: Eu}_{0.04})\) OOH nanoparticles. Spectra showed modes originating from (\(v\) C=O (\(~1730 \text{ cm}^{-1}\)), (\(v_s\)) COO\(^{-}\) (\(~1400 \text{ cm}^{-1}\)), (\(v_{as}\)) COO\(^{-}\) (\(~1569 \text{ cm}^{-1}\)) and (\(v\)) OC\(-\text{OH}\) (\(~1200 \text{ cm}^{-1}\)) confirming the presence of citric acid within the sample (data not shown). The grafting density of citric acid was calculated using the theoretic density determined from diffraction data (4.710 g/cm\(^3\)), the crystallite size determined by TEM (25.62 nm) and the weight loss measured by TGA in the region 200-300°C as 3.65% (attributed to the thermal decomposition of citric acid). A percentage surface coverage of 53.26 % was determined (1.14 x 10\(^6\) molecules of CA per particle). The weight loss attributed to material dehydration (350-700 °C) was 6.38% which is in good agreement with the theoretical weight loss associated with the dehydration of YOOH\(\rightarrow\)\(Y_2O_3\) (5.02 %) and in good agreement with diffraction data (data not shown).

The sample’s hydrodynamic diameter was measured using DLS before and after filtration through a 0.22 μm filter as 128.2 (0.149) and 65.3 (0.201) nm, respectively (PDI). The as-synthesised material showed a multi-modal size distribution and relatively large polydispersity index, indicative of a polydisperse suspension (figure 4). The particle yield after passing the raw material through a 0.22 μm filter was ca. 40% by mass. The electrophoretic mobility of the particles was measured against pH to determine the material’s surface charge, the zeta-potential calculated for the as-synthesised and filtered samples are shown in figure 4 (inset). The dispersion was characterized as stable above pH 7 with zeta-potential > - 30 mV. The surface charge reduced around pH 5 which is consistent with the suppression of dissociation of the weakest acid group reported for citric acid (pKa 6.40). This is in agreement with much literature data on the behavior of citric acid coated nanoparticles in aqueous dispersions (commonly referred to as electrostatically stabilised dispersions) which are typically stable in basic to weakly acidic conditions [11].

Figure 3: Photoluminescence excitation and emission spectra recorded for \((Y_{0.96} \text{: Eu}_{0.04})\) OOH nanoparticles synthesised using CHFS. To demonstrate the potential application of citric acid coated \((Y_{0.96} \text{: Eu}_{0.04})\) OOH NPs as fluorescent probes we assessed the visualisation of the material under conditions analogous to a typical in-vitro fluorescence imaging experiment. In the assay phosphor NPs (filtered) were dispersed in PBS to a concentration of 166 μg/mL and COS7 cells were incubated in the solution for 4 hours (we predicted that constitutive uptake of the particles would occur). Figure 5a shows a bright-field image of a COS7 cell following incubation with NP, showing some sub-micron particle agglomerate formation surrounding the cell membrane and the field of view within the image. Figure 5b shows a COS7 cell imaged using 470 nm excitation / 620 nm emission showing resolvable fluorescence from the particles. A degree of cellular autofluorescence was observed using 470 nm excitation / 620 nm (likely to arise from flavoproteins). Figure 5a (inset) shows the extent of cellular autofluorescence observed using instead a 470 nm excitation / 540 nm emission optical configuration. However, no fluorescence from the phosphor nanoparticles was observed at this measurement wavelength, consistent
with our fluorescence measurements. Using 470 nm excitation/620 nm emission free particles were also visualised diffusing in solution (data not shown). In the assay the formation of agglomerates was anticipated as electrostatically stabilised nanoparticles suffer suppression of the electrostatic double layer at electrolyte concentrations approaching those of physiological buffers [11]. DLS was used to assess the evolution of hydrodynamic diameter as a function of time in a 150 mM NaCl solution. The hydrodynamic diameter was shown to increase to 200-400 nm within 5 minutes whereas a control remained stable (data not presented), explaining some of our observations in the bright-field image (figure 5 a).

**Figure 4:** Comparison of the hydrodynamic size determined for citric acid coated (Y_{0.96}: Eu_{0.04}) OOH phosphors, as-synthesised (circles) and after filtration through a 0.22 µm syringe filter (squares), inset shows the zeta-potential titration for the sample as-synthesised (circles) and after filtration (squares).

**4 CONCLUSIONS**

In summary, we have used a rapid and continuous process for the production of aqueous dispersions of surface functionalised (Y_{0.96}: Eu_{0.04}) OOH nanoparticles. This novel adaptation of the CHFS process allows for the bottom-up synthesis of nanoparticle phosphors which form stable aqueous dispersions without the requirement for post-synthesis modification (a large benefit over many other synthetic strategies). The excitation and emission spectra recorded for (Y_{0.96}: Eu_{0.04}) OOH nanoparticles showed broad UV/visible excitation and red emission originating from the ^5D_0 → ^7F_1 and ^5D_0 → ^7F_2 transitions in europium. The dispersions are electrostatically stabilized and show pH-dependent surface charge consistent with the presence of free acid groups on the surface of the particles. The surface functionalisation was confirmed by FTIR, TGA and zeta-potential measurements. Particles were resolved in a simple fluorescence assay using 470 nm excitation and emission detected at 620 nm in both cell bodies and as discrete entities freely diffusing in solution. Emission from nanoparticles could be resolved separately to cellular autofluorescence by using combinations of filters. In addition, free carboxylic acid terminal groups of the coated NPs can provide a useful synthetic handle for further bioconjugation [11].

**Figure 5:** Live-cell imaging of COS7 cells using citric acid coated (Y_{0.96}: Eu_{0.04}) OOH nanoparticles. (A) Bright-field image of a cell incubated with NPs, inset shows cellular autofluorescence measured at 470 nm excitation / 540 nm emission (B) Upconverted luminescence following 470 nm excitation / 620 nm emission shows uptake of NPs into COS7 cell (scale bar, 30 µm). Images are shown on the same intensity scale.

EPSRC are thanked for funding project EP/E040551/1.

**5 REFERENCES**

7. Chaudhry AA et al. 2006. Chemical Communications2286-8