

Luminescent Terbium/Europium Chelates-Based Silica Nanoparticles : Stability and Incorporation Efficiency measured by Radioactive Probe

N. Wartenberg*, O. Raccurt*, D. Imbert**, M. Mazzanti**, E. Bourgeat-Lami***

*Laboratoire de Chimie et de Sécurité des NanoMatériaux, DTNM/LITEN/CEA

**Laboratoire de Chimie de Coordination et de Reconnaissance Ionique, LCIB/INAC/CEA
CEA, 17 Rue des Martyrs – 38054 Grenoble Cedex 9, France. nicolas.wartenberg@cea.fr

***Laboratoire de Chimie, Catalyse, Polymères Procédés, UMR 5265 CNRS/UCBL/CPE Lyon -
Villeurbanne, France

ABSTRACT

Two chelates were embedded into silica nanoparticle using a reverse microemulsion process. The incorporation was achieved without covalent link between the organolanthanide and the silica matrix. To investigate the efficiency and stability of the incorporation, a method based on radioactive europium probe could be efficiently used as it will be developed in the poster presentation.

Keywords: Silica nanoparticles, Organolanthanide, Radioactive probe, Luminescence

1 INTRODUCTION

The design of multichromophoric nanostructures is of high current interest for the development of new functional material with potential application in material science (diode lasers and optical fibers for telecommunication, tracers in counterfeiting, sensors) and multimodal biomedical-imaging [1-3].

Internally dye-doped silica nanoparticles (Nps) present several advantages over other nanostructures that should lead to a variety of biological/technological applications [3, 4-6]. Their most remarkable properties include: i) signal amplification, because they allow to concentrate a large amount of chromophores in a single target binding site; ii) biocompatibility and versatility in the immobilization of biomolecules because they are easily functionalized; iii) photostability, as a result of the matrix shielding effect with respect to oxygen and water, iv) hydrophilicity; v) efficient dye-immobilization preventing self-quenching. Lanthanide-containing nanosized architectures are particularly attractive because of the unique spectroscopic and magnetic properties of these ions (large Stokes' shift, very narrow emission bands spanning from the visible to the near-infrared, long life-time allowing time gated detection) [7]. Moreover the emission of lanthanide ions can be tuned and optimized by their inclusion in organic ligands carefully tailored according to the properties of the chosen ion [8-9]. Multiple doping of silica nanoparticles with different lanthanide chelates is of particular current interest for the

development of materials with tunable emission and of efficient tags for multiplexed and highly sensitive bioassays. It is generally believed, partly as a result of the limited performance of the classic characterization methods, that, to avoid leakage, the chelates should be covalently linked to the silica matrix [10]. This requires lengthy and difficult ligand functionalizations and can result in a decreased stability. The incorporation without covalent link is then more flexible but needs to be accurately characterize.

2 DISCUSSION

The synthesized nanoparticles are highly luminescent and display different emissions which can be excited by a single or multiple source. The tripodal ligands tpatcn [12, 13, 14] and thqtcnSO₃ [11] which present a very good metal-ligand complementarity and a rigid coordination environment prevent the formation of hydrates and subsequent luminescence quenchings particularly in the NIR range and minimizes the vibrational de-activation processes (Fig. 1).

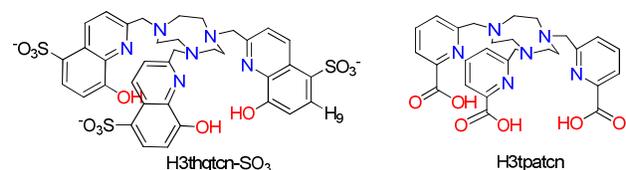


Figure 1 : Structure of the chelate used

The lanthanide chelates Ln-tpatcn (Ln = Eu, Tb, Nd) and Yb-thqtcnSO₃ presented among the best efficiencies described in the literature for the sensitization of visible- and NIR-emitting lanthanide ions [9, 15]. They have been successfully included in spherical Nps of uniform size (50 nm) using a reversed micro-emulsion process. The lanthanide-chelate doped nanoparticles are synthesized by a hydrolysis-condensation reaction [6] of the silica precursor (TEOS) in the presence of the lanthanide chelate, catalyzed by aqueous ammonia (pH = 9) in a water/oil micro-emulsion. Preliminary radioactive measurements based on europium (¹⁵²) allowed us to investigate and

compare the incorporation and the stability of the two different chelates.

This process provides a very efficient method for the encapsulation of the water stable lanthanide complexes presented which does not involve the lengthy and difficult ligand functionalization necessary for covalent bonding of the complexes to the silicate matrix. But this process requires to accurately investigate both the stability and the incorporation efficiency. These characterizations could be achieved by using a radioactive probe. The presence and the position of the lanthanide chelates inside the Nps has been confirmed by Transmission Electron Microscopy (TEM) which has revealed porous doped Nps with homogeneous sizes.

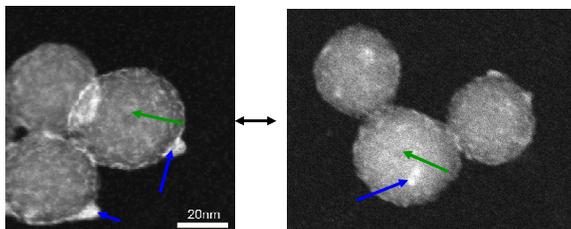


Figure 2. NpEuTb. HAADF STEM images, blue and green arrows correspond to electron densities from complexes and silica matrix, respectively.

HAADF STEM images (Fig. 2) show clearly that the complexes are internally localized and distributed on the surface of the Nps with two types of contrasted areas. Nps were also prepared from the Yb-thqtcnSO₃ complex alone (NpYb) or from a 1:1 mixture of Yb-thqtcnSO₃ and Nd-tpatcn (NpNdYb). The tomographic reconstruction (NpNdYb, Figure 3) shows unambiguously that the Yb complexes (high contrast) are localized in the center of the Nps. Their higher solubility results in a higher percentage of encapsulated complexes and in a deeper localization of the complexes in the silica matrix.

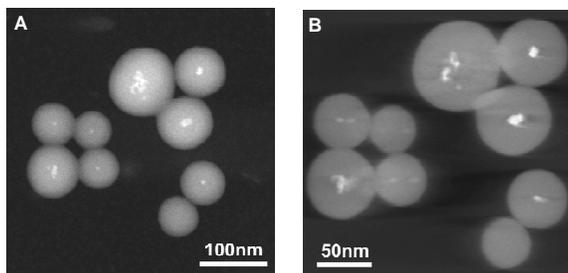


Figure 3. NpNdYb. (A) HAADF STEM images, white and grey regions correspond to electron densities from complexes and silica matrix, respectively. (b) x-y slice from the reconstructed volume.

Gamma emitting europium (¹⁵²) was used with the two ligands presented. It was implicitly assumed that the replacement of lanthanides used by Eu(III) would have no influence on the incorporation efficiency. Europium (¹⁵²) was used as radioactive probe to quantify the final content

of organolanthanide embedded and to estimate the influence of washing. This method could also provide information about the stability of resulting Nps over leakage.

3 EXPERIMENTAL SECTION

3.1 General Details

Solvents and starting materials were obtained from Aldrich, Fluka, Acros and Alfa and used without further purification unless otherwise stated. Solvents were dried over the appropriate drying agents when required. Water and H₂O refer to high purity water with resistivity value of 18 MΩ.cm, obtained from the “Millipore/MilliQ” purification system.

3.2 Luminescence measurements

Low-resolution luminescence measurements (spectra and lifetimes) were recorded on a Fluorolog FL 3-22 spectrometer from Spex-Jobin-Yvon-Horiba with double grating emission and excitation monochromators, and a R928P photomultiplier. For measurements in the NIR spectral range, the spectrometer was fitted with a second measuring channel equipped with a FL-1004 single grating monochromator and light intensity was measured by two Jobin-Yvon solid state InGaAs detectors (i) DSS-IGA020L, cooled to 77 K (range 800-1600 nm). and (ii) DSS-IGA020A (range 800-1700 nm) Lifetimes were measured in time-resolved mode and are averages of three independent measurements, which were made by monitoring the decay at the maxima of the emission spectra. The mono-exponential decays were analyzed with Origin 7.0[®]. Quantum yields of the complexes in solution at pH 7.4 and in solid state were determined using a home-modified integrating sphere from Oriel and the previously described procedure.¹ Spectra were corrected for the instrumental function with an absolute method with an integrations sphere.

3.3 Synthesis of the nanoparticles

A microemulsion is created by the consecutive addition of the oil Cyclohexane (Sigma-Aldrich), the co-surfactant 1-Hexanol (anhydrous, >99%, Sigma-Aldrich), the non ionic surfactant Triton X-100 (Sigma-Aldrich) and a certain amount of an aqueous solution of the complexes. The silica precursor TEOS (Tetraethyl Orthosilicate, >99%, Sigma-Aldrich) is then added when the microemulsion has been stabilized. The hydrolysis-condensation reactions are catalyzed by an aqueous ammonium hydroxide solution (Puriss, 30-33% in H₂O, Sigma-Aldrich).

The following preparation of NpYb describes the general procedure for the synthesis of the nanoparticles:

To a mixture of Triton X-100 (1,65 mL, 2.82 mmol), hexanol (1,6 mL), cyclohexane (7,5 mL), 80 μ L of a 0.01 M aqueous solution of the lanthanide complex Yb-thqtcnSO₃ (pH 7.4) is added followed by ammonium hydroxide (100 μ L) and water (400 μ L) up to pH 9. The mixture is stirred 30 min at room temperature and then 100 μ L of tetraethyl orthosilicate (TEOS, 0.678 mmol) are added. After 24 h under magnetic stirring, a large excess of ethanol is added. The nanoparticles are then washed under centrifugation three times by ethanol and one more time by water. This process is followed by dialysis (Roth, MWCO: 10,000 Dalton) one week in water in a 10L volume buffer (changed every day) under magnetic stirring. Then, the resulting solution is washed under centrifugation by water to give the NpYb nanoparticles. The water was tested by different methods (see below) to verify the absence of lanthanide ion free or complexed.

The Nptpatcn are prepared by an analogous synthetic procedure by using 80 μ L of a 0.01 M solution of the ligand tpatcn. The NpNdYb nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Yb-thqtcnSO₃ and 40 μ L of a 0.01 M solution of Nd-tpatcn. The NpEuTb nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Eu-tpatcn and 40 μ L of a 0.01 M solution of Tb-tpatcn. The NpNdYbtpatcn nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Ndtpatcn and 40 μ L of a 0.01 M solution of Yb-tpatcn.

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