

ORGANIC FLUORESCENT NANOCRYSTALS FOR LABELLING AND BIO-IMAGING

A. Ibanez¹, C. Philippot¹, F. Dubois¹, M. Maurin², P.L. Baldeck²

¹ Institut Néel, CNRS & Université Joseph Fourier, BP166, F-38042 Grenoble Cedex 9, France

² Equipe MOTIV, Laboratoire de Spectrométrie Physique Université Joseph Fourier & CNRS UMR 5588, BP87, F-38402 Saint Martin d'Hères cedex, FRANCE

Introduction

Cerebral imaging has become an important diagnostic tool in medicine. Many therapies are now tested on small animals by optical imaging methods at the micro scale. The technique uses a fluorescent dye that is injected in the blood vessels. In many cases, it is necessary to visualize capillaries deeply as possible in the cortex, for example, to image the tumour microvascularization (angiography). The relative difficulty is to synthesise non diffusible labels (diameter around 100 nm), highly fluorescent and biocompatible nanoparticles. For this purpose, we have recently developed an original method using a spray-drying process to obtain new luminescent tracers for biological applications constituted by organic nanocrystals grown in silicate spheres [1].

Experimental

These hybrid nanoparticles of silicate were obtained from sol-gel solutions constituted by mixed silicium alkoxides (TMOS, MTMOS, TMSE) and organic dye in a large volume of solvent (THF, alcohols), aged for few days at 60°C and finally atomized (atomizer model 3076 - TSI INC., U.S.A.). The droplets were dried under air laminar flux in a furnace and collected by an electrostatic filter. We have first involved small organic molecules such as CMONS (a-[(4'-methoxyphenyl)methylene]-4-nitro-benzene-acetonitrile molecule) which exhibits a strong fluorescence in the crystal state. During the droplet drying, the formation of a crust takes place first at their surfaces leading then to amorphous silicate shells. Then, at

the end of the solvent evaporation, the confined nucleation of CMONS nanocrystals occurs into the cores of the resulting sol-gel particles. Thus, through a one-step and self-assembly process we obtain new core-shell hybrid nanoparticles whose size and morphology were characterized using Field Emission Scanning Electron Microscopy (FESEM – Zeiss Ultra +).

Results and discussion

Control of all the parameters to obtain an “ultra bright” label

The aim was to prepare non diffusible (size between 50-100 nm), highly fluorescent (through large amount of CMONS molecules), photostable (crystal core) and biocompatible (silicate shell) particles (Figure 1-c). It has been possible, through a careful control of the physical and chemical parameters, to obtain a reproducible and generic preparation of core-shell hybrid organo-mineral nanoparticles. The main experimental parameters are :

- The temperatures and the temperature differences between the tubular drying furnace and that of electrostatic filter (where the nanoparticles are collected) to avoid the organic dye condensation around the particle (Figure 1-a).
- The gas flow must be carefully controlled for a total particle drying
- The dye amount can very high, around 100-120 wt% referred to the silica matrix but at higher concentrations some CMONS needles can be observed outside the particles (Figure 1-b).

- The nature of the alkoxides, to optimize the particle biocompatibility and to embed a large amount of CMONS. The best results were obtained by using sol-gel mixture of TMOS – TMSE shell precursors (Figure 1-b).

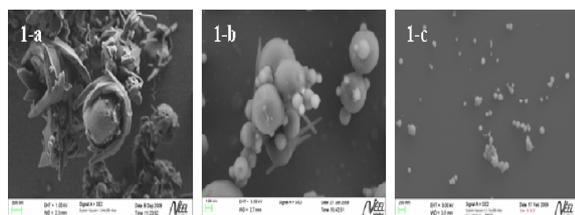


Figure 1: FESEM pictures of hybrids core/shell nanoparticles: a) Non optimal temperatures; b) Dye amount too high and poor embedding ability of the silicate shell ; c) Good control of all the parameters

Highlight of the single crystal character of organic cores

The silica shells of the particles were totally dissolved to observe then by FESEM the organic cores which exhibit faceted morphologies of typical single-crystals. (Figure 2-a) This single crystal character of the organic core will be very interesting for fluorescence properties particularly to have single emitter behaviors.

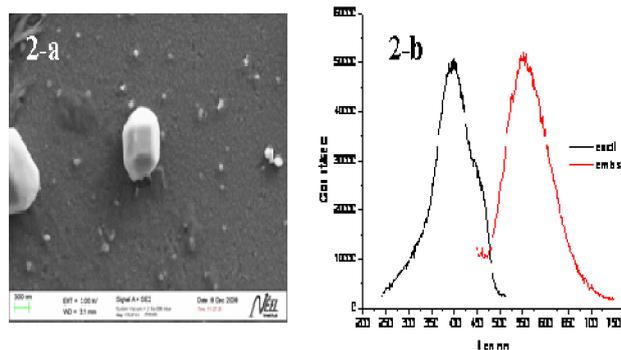


Figure 2: a) FESEM pictures of a faceted organic crystal after total dissolution of the silica shell
b) Excitation at 400 nm for emission spectrum and detection at 575 for excitation spectrum.

First optical characterizations

CMONS fluorophore was selected because it is a good candidate for two-photon fluorescence imaging. It exhibits in the crystal state a strong yellow fluorescence ($\lambda = 596$ nm) under the wavelength excitation of a Ti:Sa laser ($\lambda = 1064$ nm). Fluorescence spectroscopy was first carried out on core-shell hybrid particles well dispersed in biological solutions. These CMONS doped nanoparticles are very promising for two-photon fluorescence imaging because there is no overlapping between absorption and emission spectra (Figure 2-b).

Conclusion

The sol-gel chemistry combined to a spray drying process allowed us to control the formation of original hybrid core-shell nanoparticles constituted by organic single crystal embedded in biocompatible silicate spheres. With a good management of all the physical (gas flows, temperatures) and chemical (dye, solvent and alkoxide natures, relative concentrations, hydrolysis condensation conditions) parameters, we optimized a one-step, low cost and reproducible process allowing to obtain large amounts (several grams) of nanoparticles exhibiting promising optical properties such as ultra bright fluorescent labels for medical imaging. Moreover, the presence of Si-OH functions on the silicate shell surface make possible to functionalize these fluorescent nanoparticles by grafting biomolecules (peptides) for targeting properties. For example, specific targeting of receptors in glioma cells or glioma vascular endothelial cells is in progress.

Reference

1. Patent PCT/FR2009/000294, C. Philippot, N. Marcellin, E. Djurado, A. Ibanez