

Jerome Rose - CEREGE
Clement Levard - CEREGE
Marie Tella - CEREGE
Mélanie Auffan - CEREGE

Experiment report:
**Cerium speciation in aquatic mesocosms using High Energy Resolution
Fluorescence Detected X-ray Absorption Spectroscopy**

Introduction

The quantification of Ce(III) in Ce(IV)O₂/Ce(III) mixtures remains a difficult task with conventional laboratory tools. XPS, EELS coupled to TEM or even Raman spectroscopy are helpful. However the literature teaches us that EELS and XPS tend to over estimate the Ce(III) content and Raman is not sensitive enough for low concentrations. Ce L_{III} XANES appears as one of the best techniques. However, "standard" transmission or fluorescence spectra do not have the required energy resolution to unambiguously assign the shoulder at 5724 eV on nano-sized CeO₂ XANES to either (i) crystal field splitting into e_g and t_{2g} and/or to (ii) Ce(III) contribution¹⁻³. The development of High Energy Resolution Fluorescence spectrometer (using analyzing crystals) to obtain XAS spectra (*High Energy Resolution Fluorescence Detected X-ray Absorption Spectroscopy*= HERFD-XAS) is a key to lift this uncertainty.

The objectives of this experiment were (i) to clarify the particle size effect on the XANES spectra for pure Ce(IV) compounds, and (ii) to determine the biotransformation of CeO₂ nanoparticles within aquatic mesocosms using the exceptional resolution of the HERFD-XAS equipment of the FAME beamline.

Experimental details

The general frame of the experiment was as follow. Due to the nature of the samples, the experiments were conducted with liquid-He cryostat to avoid any photo-redox phenomena. We used a He bag to decrease the absorption of the photon by the air between the sample, the crystals, and the detector. Four Ge(331) curved crystals were mounted on the high energy resolution spectrometer (radius of curvature: 1m). The intrinsic energy resolution of the spectrometer was close to 1.5 eV. The spectrometer was set at the energy of the L α_1 Ce fluorescence line (4840 eV).

The samples analyzed consisted of surficial sediments and digestive gland of benthic invertebrates exposed in mesocosms to CeO₂ nanoparticles (bare CeO₂-NPs (Rhodia[®]) and citrate coated CeO₂-NPs used as paint additives (Nanobyk[®])). These samples were previously analyzed in March 2013 on the FAME beamline using "standard" fluorescence XAS (experiment 30-02 1052). Since, analyzing diluted samples in HERFD-XAS configuration is time consuming, we selected 10 samples for which the LCF analyses had the highest level of uncertainty in terms of Ce(III) proportion. The concentration of Ce in these samples were ranged between 150 and 500 mg.kg⁻¹ for the

surficial sediments and between 200 and 500 mg.kg⁻¹ of dry matter for the digestive gland of the organisms^{4,5}.

We also used this synchrotron beamtime to analyzed CeO₂ particles with different sizes: ~3 nm, ~5 nm, ~30 nm, ~50 nm, ~80 nm, and ~5 000 nm. Three to five scans were necessary to obtain good signal/noise ratios. Analysis of these samples will allow quantifying the size effect on the HERFD-XANES features.

The entire sample preparation was performed at our home institution (grinding, pellets pressing and freezing for biological samples). All the samples were brought back to our home institution at the end of this experiment.

Main results

Using reference compounds (Ce(III) acetate, Ce(III) cysteine, and Ce(IV) carbonate) we first observed that all the edge and pre-edge features are much more defined using HERFD-XAS than “standard” transmission or fluorescence detection. One of the most interesting feature on the HERFD-XANES is the presence of a pre-edge for both Ce(III) and Ce(IV) compounds with ~2 eV of difference: pre-edge centered on 5719 eV for Ce(III) compounds, and 5721 eV for Ce(IV) compounds (Figure 1).

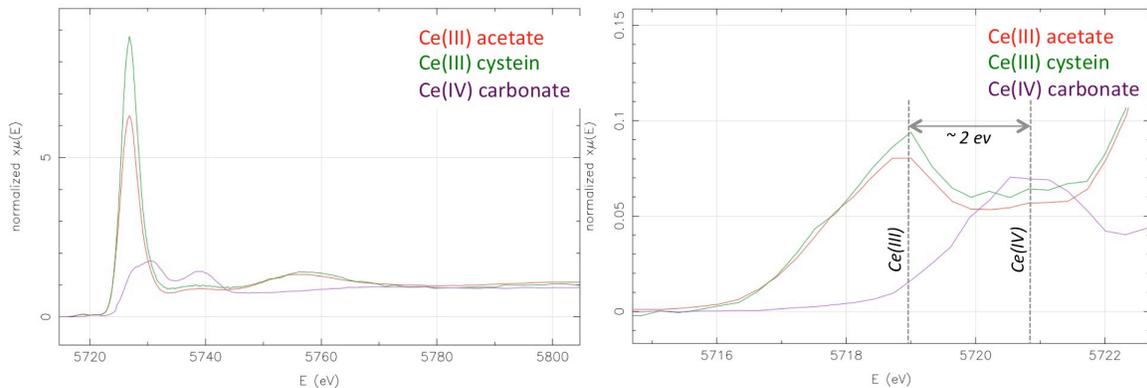


Figure 1. HERFD measurements on Ce reference compounds. (left): XANES. (right): detail of the pre-edge.

Consequently, we used this pre-edge region to better understand whether or not the shoulder at 5724 eV on the nano-sized CeO₂ XANES could be assigned to crystal field splitting or to a Ce(III) contribution. For all the particles tested (between 3 to 5 000 nm), all the pre-edge were centered on 5721 eV and not on 5719 eV (Figure 2). This let us conclude that the peak at 5724 eV that was sometimes attributed to the presence of Ce(III) in the CeO₂ nanoparticles, came from changes in the electronic structure of Ce(IV) and not to the presence of reduced Ce. The intensity of this peak at 5724 eV seems to be size dependent but more work is needed using FDMNES code⁶, to precisely identify the different features and their evolutions with the particle size.

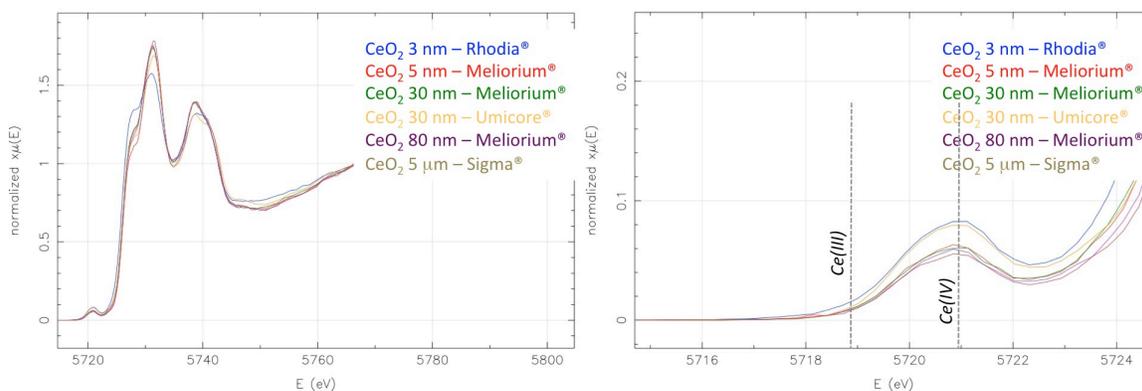


Figure 2. HERFD measurements on CeO_2 particles of different sizes. (left): XANES. (right): detail of the pre-edge.

XAS is a well-suited technique in nano-(eco)toxicology to study changes in speciation of nanoparticles, as nano- CeO_2 in complex matrices with concentrations as close as possible to the predictable concentrations in the ecosystem (in the $\mu\text{g/L}$ - mg/L range). We used HERFD to determine the redox transformation of bare and citrate coated CeO_2 nanoparticles by ecologically relevant organism (snail, *Planorbarius corneus*) exposed to 1 mg/L^{-1} of NPs during 4 weeks in a complex experimental system mimicking a pond ecosystem. Over time, both CeO_2 nanoparticles tend to homo- and hetero-aggregate and to accumulate on the surficial sediments containing bacteria and algae. Figure 3 shows that the Ce present at the surface of the sediments, was only in the Ce(IV) oxidation state. This let us conclude that no reduction of Ce(IV) into Ce(III) occurred after 3 and 4 weeks on the surficial sediments. However, once ingested by the benthic snails, a strong reduction occurs (Figure 3). In the digestive gland (DG) of the organisms, we estimated using linear combination of the pre-edge region (-10 to 0 eV before the edge) that 61 ± 10 to $64 \pm 10\%$ of the Ce(IV) was reduced into Ce(III), depending on the surface coating of the nanoparticles.

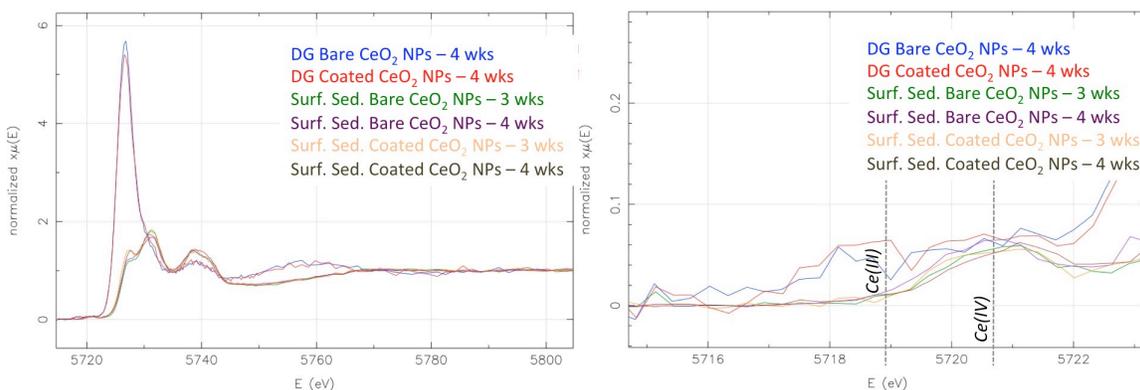


Figure 3. HERFD measurements of (i) nano- CeO_2 settled down at the surface of the sediments, 3 weeks and 4 weeks after injection in indoor aquatic mesocosms mimicking a pond ecosystem and (ii) nano- CeO_2 ingested by benthic organisms exposed 4 weeks in mesocosms. (left): XANES. (right): detail of the pre-edge.

Consequently using the high-energy resolution of the crystal analyzer spectrometer, we were able to thoroughly study the biotransformation and to attribute the reduction to the digestive activity of the snails.

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