EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Antioxidative activity of Cerium Oxide nanoparticle in biological environments studied by XANES: kinetics of Ce^{3+} formation	**Experiment number**: 08-01-1012
Beamline: BM08 LISA	**Date of experiment**: from: 22-06-2016 to:27-06-206	**Date of report**: 05/10/2016
Shifts: 12	**Local contact(s)**: LEPORE Giovanni Orazio	*Received at ESRF:*

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Report:

Background CeO_2 nanoparticles are at the moment actively investigated as promising agents in the therapy of different pathogenesis involving free radicals or oxidative stress, like Alzheimer and Parkinson. Although the antioxidant property of Ceria nanoparticles (CNP) has been largely demonstrated, the exact mechanism that makes CNP such a powerful tool is not completely elucidated yet. It is not clear, for example, which is the time evolution of the oxidative state of the CNP during the process of cell internalization; how it is influenced by the initial Ce(III)/Ce(IV) ratio; how the presence of a protein corona and surface functionalization can modify the response of CNP as free radical scavenger. In recent experiments carried out at the ESRF (exp. CH-4478 and CH-4716, paper submitted) we were able to demonstrate that CNPs, after internalization by human cells, show a significant degree of reduction, with the formation of Ce(III). However, the kinetics and mechanisms of the chemical reactions leading to this reduction are at the moment not clear at all. Aim of the present experiment was to shed some light on these aspects by carrying out XANES measurements on CNPs after internalization in human cells for different time intervals. In addition, we programmed to perform pulse-chase experiments as well.

Experimental description

Sample preparation We used HeLa cultured cells incubated with CNP with a diameter of 5-10 nm. The small radius of these NPs gives a significant ratio of surface sites/bulk sites, allowing a reasonable contrast between (surface) Ce(III) and (bulk) Ce(IV). After 24, 48, 72, 96, and 120h of incubation with the CNPs, cells were recovered on filter paper and the Ce oxidation state of the internalized CNPs was analyzed.

Results We encountered a lot of problems with the cryostat aligment. As a matter of fact, the first three day were spent in finding a correct configuration. As a consequence, just few samples could be measured by the end of the beamtime, and this resulted in the fact that we could not perform reasonable measurements on the samples programmed for the pulse-chase experiments.

However, some preliminay results could be obtained on samples of CNPs internalised for long periods. Fig. 1 shows the Ce-L_{III} XANES spectra of bulk CeO_2, compared to those of CNPs internalised for 1, 3, 4 and 5 days. The corresponding derivatives are also shown for the sake of better comparison. It is quite apparent that, when the CNPs are internalised for 1 day, they retain a formal oxidation state of Ce(IV). Indeed, the edge energy position, as measured by the first maximum in the derivative spectrum, is identical to that of bulk CeO_2. For longer internalisation periods, the oxidation state of Ce changes. In particular, after 3 days a well defined shoulder appears on the rising edge at an energy corresponding to that of the maximum absorption for Ce(III), and the edge energy position shifts towards lower energies. For what concerns the samples after 4 and 5 days of internalisation, the amount of Ce(III) seems to further increase.

Data treatment is still in progress for the quantification of the amount of Ce(III) that is present in all the samples.

Figure 1. Panel A shows the Ce-L_{III} spectra of CNPs intarlised for 1 day (black line), 3 days (red line), 4 days (green line) and 5 days (blue line). The spectrum of bulk CeO_2 is also shown for reference (pink line). The vertical line marks the energy position of the maximum in the absorbtion for Ce(III). Panel B shows the corresponding derivative spectra. The maximum in the derivative for bulk CeO_2 is here marked by a vertical line for better reference.