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	Experiment title: Antioxidative activity of Cerium Oxide nanoparticle in biological environments studied by XANES	**Experiment number**: CH-4478
Beamline: BM08	**Date of experiment**: from: 01/10/2015 to: 06/10/2015	**Date of report**: 20/11/2015
Shifts: 12	**Local contact(s)**: Francesco d'Acapito, Alessandro Puri	*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.

Aim To address some of these questions and further elucidate the possible mechanism involved, we planned to investigate the Ce(III)/Ce(IV) ratio, on the surface of cell internalized CNP using XANES spectroscopy at the Ce-L_{III} edge.

Experimental description We used human epithelial cultured cells (HeLa) incubated with CNP with a diameter of 5-10 nm, which allowed a reasonable ratio of surface sites/bulk sites and therefore a better contrast between (surface) Ce(III) and (bulk) Ce(IV). Prior to cell incubation, the CNP were exposed to both highly oxidative and highly reducing environment in order to modify significantly the Ce(III)/Ce(IV) ratio on their surface. After 1, 6 and 24 h of incubation with the CNPs, cells were recovered and the Ce oxidation state of the internalized CNPs analyzed. All the biological samples, were immediately quenched to liquid nitrogen temperature to prevent modification in the oxidation state of CNP during storage and transport to the ESRF. The XANES samples analyses were performed at 80 K. Due to the low amount of Ce present into the cells, the spectra have been acquired in fluorescence mode.

Results The XANES spectra of the CNP exposed to oxidizing (sample Z6) and reducing (sample Z5) media showed that the reducing environment effectively produces CNP (Figure 1) with a significant amount of Ce(III), *in addition to Ce(IV)*. The main evidence is a shift towards lower energy of the main edge position (as evidenced by the derivative spectra shown in Fig. 1). By fitting the edge structure of sample Z5 with a linear combination of the spectra of CeO_2 and $Ce(NO_3)_3$ an amount of Ce(III) equal to ca. 4 % (atomic percentage) was estimated. Once extablished the Ce oxidation state of the CNP just before entering the cells, we then investigated whether the process of internalization modified the initial Ce(III)/Ce(IV) ratio. By analyzing the internalized reduced CNP incubated with the cells for different times (sample 22, incubated for 1 h; sample 30, incubated for 6 hours; sample 13, incubated for 24 hours), the amount of Ce(III) is observed to decrease first after 1h of incubation (sample 22), and then to increase further at 6 and 24h of incubation (samples 30 and 13). In this last case, the same fitting procedure described above gives an amount of Ce(III) equal to 8% (atomic fraction). This result suggested that cells can "process" CNP, with a complex chemistry that initially favours the complete oxidation of Ce, while by increasing time the formation of Ce(III) is recovered (see Figure 2). Further information can be obtained by comparing the spectra of oxidised CNP incubated for 24 h in the presence of t-BHP (tert-Butyl hydroperoxide) for ROS induction (sample 8), that of reduced Sm-doped CNP incubated for 24 h (sample 5C) and that of reduced CNP incubated for 24h (sample 13). We observe a decrease in the amount of Ce(III) by Sm doping, while the addition of t-BHP/ROS induction seems to favour the formation of Ce(III) (Figure. 3).

Conclusion By "feeding" cells with NP characterized by a known Ce(III)/Ce(IV) ratio, we could detect an intracellular transformation showing a modification in the level of Ce(III), that first decreases and then increases. This provided the first direct evidence the Ce oxidative states can be altered intra-cellularly by an active cell-mediated process. Further investigation for better determining the pertinent kinetics and mechanisms of the process is required.

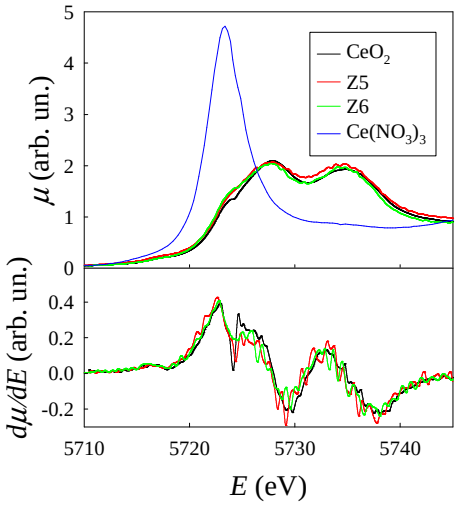

Fig.1

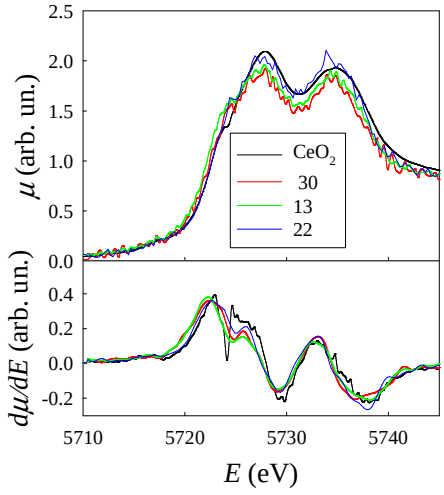

Fig. 2

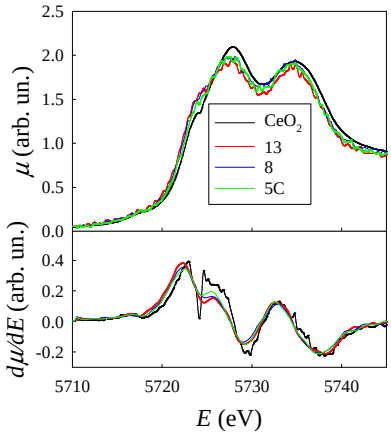

Fig. 3