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Experimental report

Monitoring of biotransformations and biosynthesis of iron oxide-based nanoparticles in cells using XAS and XES

The biotransformations of iron oxide-based nanoheterostructures for applications in biomedicine were monitored over time, at the cellular environment using **X-ray absorption spectroscopy (XAS)**. XAS spectra at the near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) regimes were measured at Fe (7112 eV) and Co (7709 eV) K-edges to study the evolution of different iron oxide-based nanomaterials within cells that were previously confined at different stages of maturation.

Results:

XANES and EXAFS experiments were performed at the **Fe** and **Co K-edges** to quantitatively investigate the valence states and structural properties of the metal ions and the evolution of the ferrite phases with time **as a probe of the biotranformations that can occur in the biological environment**. XANES is a sensitive probe of the coordination and oxidation state of absorbing ions and EXAFS gives information about their local environment, including interatomic distances and coordination numbers of surrounding shells. These two complimentary techniques can thus reveal the short-range geometry and identify the phases.[1-2] They are considered non-damaging techniques and were recently applied by this group to study the biodegradation of nanomaterials as advanced characterization techniques.[3-4]

Fe K-edge XAS spectra of **bio-synthesized nanosystem** during tissue maturation in cells (at day 36) were determined in fluorescence mode and compared with initial iron-based precursors in solution and with reference samples (Fe_3O_4, γ-Fe_2O_3, $Fe_2O_3 \cdot 0.5H_2O$). Each XAS spectrum of cells was an average accumulation of 5-10 acquisitions merged to improve the signal-to-noise ratio. Biotransformations *in situ* of initial precursors led to formation of iron oxide-compounds inside cells over time (**Figure 1**). In overall, it corresponded to a change of iron oxide structure to Fe^{3+} compounds in cells.

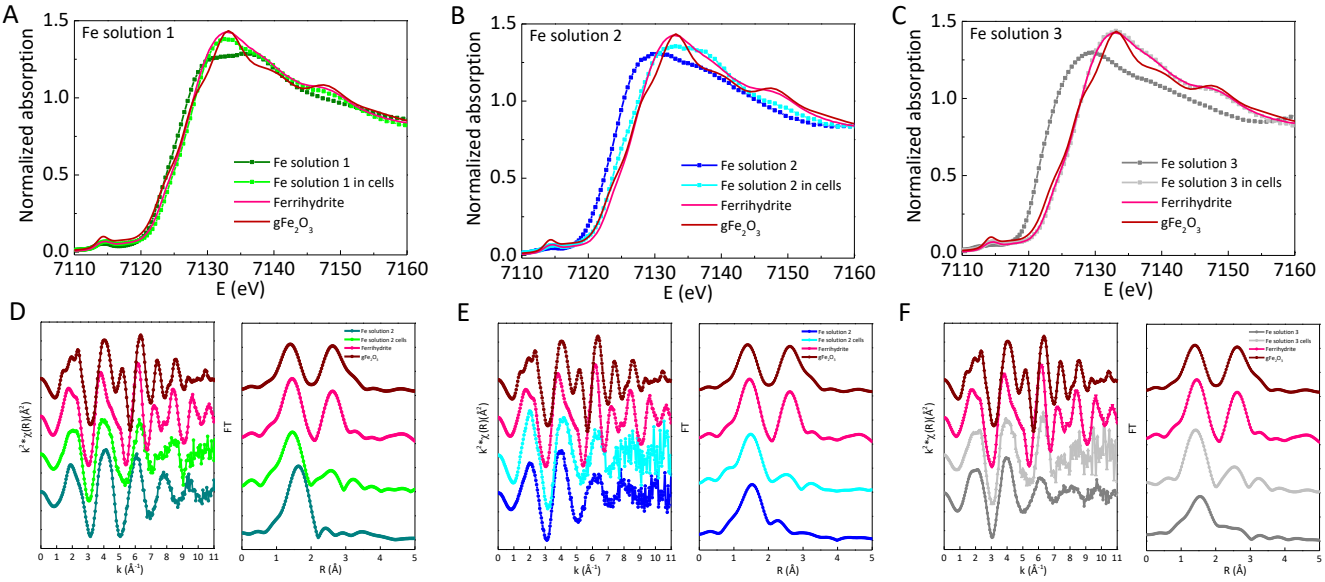

Figure 1: (A-C) XANES absorption spectra at the Fe K-edge of solutions 1, 2 and 3. They display a shift of energy edge of iron in cells revealing an oxidation state of 3+ (on average) that matches with the existence of different proportions of maghemite and/or ferrihydrite (and other phases based on N, C, etc). (D-F) EXAFS signal and modulus of the FT of previous compounds.

Fe and **Co K-edge XAS** measurements were performed in **Fe-Co-O** prepared nanoparticles in solution and internalized in cells (at 4 different stages of maturation/degradation (at day 0, 3, 9 and 21)). The XANES

spectrum of the solution at **Fe K-edge** corresponded to a Fe-Co-O mixed phase with Fe in 2+/3+ oxidation state (mainly Fe_3O_4 or/and partial doping of Fe_3O_4 with Co, Co:Fe_3O_4) (**Figure 2A**).[5] An energy shift to higher oxidation state (Fe^{3+}-based oxides) is detected in all stages of maturation of cells (**Figure 2B**). EXAFS analysis provided direct structural evidence of the two bond distances (Fe-O and Fe-Fe), characteristic of the magnetic spinel references.[3-4]

XANES and EXAFS at the **Co K-edge** probed the local environment of Co atoms in Fe-Co-O prepared nanoparticles in solution and internalized in cells (at day 0, 3, 9 and 21). **Figure 3A** shows the XANES spectrum of Fe-Co-O solution. It is comparable to that of Co^{2+} (similar to $CoFe_2O_4$).[5] The Co oxidation state of Fe-Co-O nanoparticles in cells was maintained after internalization and no biotranformations of spectra over time were observed (D0 = D21) (**Figure 3B**).

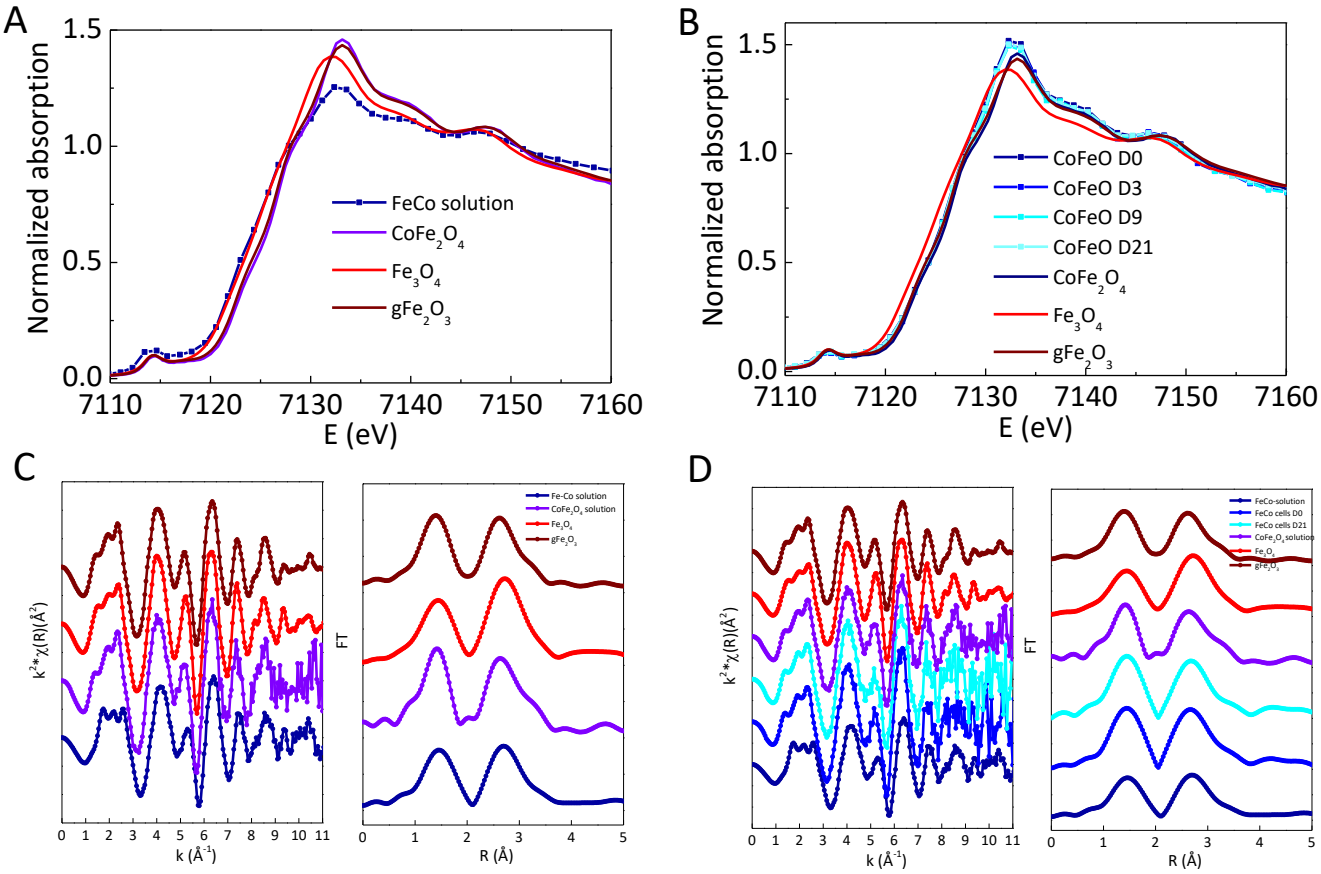

Figure 2: (A, B) XANES absorption spectra at the Fe K-edge of initial solution Fe-Co-O nanoparticles and internalized in cells at days 0, 3, 9 and 21. The spectra were compared to the magnetite, cobalt ferrite ($CoFe_2O_4$) and maghemite reference compounds. Fe oxidation state of solution fitted with 2+/3+ valence and shifted to 3+ after internalization in cells. No degradation was detected over time. (C-D) EXAFS signal and modulus of the FT of previous compounds.

We can conclude that these Fe-Co-O nanoparticles present robustness upon transformations inflicted by the cellular environment and low degradability regarding their biological kinetics. These results shed light in the pursue of new materials that have a long lasting therapeutic effect in the future clinical applications of multifunctional nanomedicines.

Notes: X-ray emission spectroscopy (XES) ($K_{\beta 1,3}$ (main line) and $K_{\beta'}$ (satellite) emission peaks) of samples were not finally measured since the XAS experiments covered all the beamtime. XANES at the Zn K-edge was not considered in view of the low stability of the Zn-based samples. These measurements were discarded and communicated in advanced to the beamline BM23 staff.

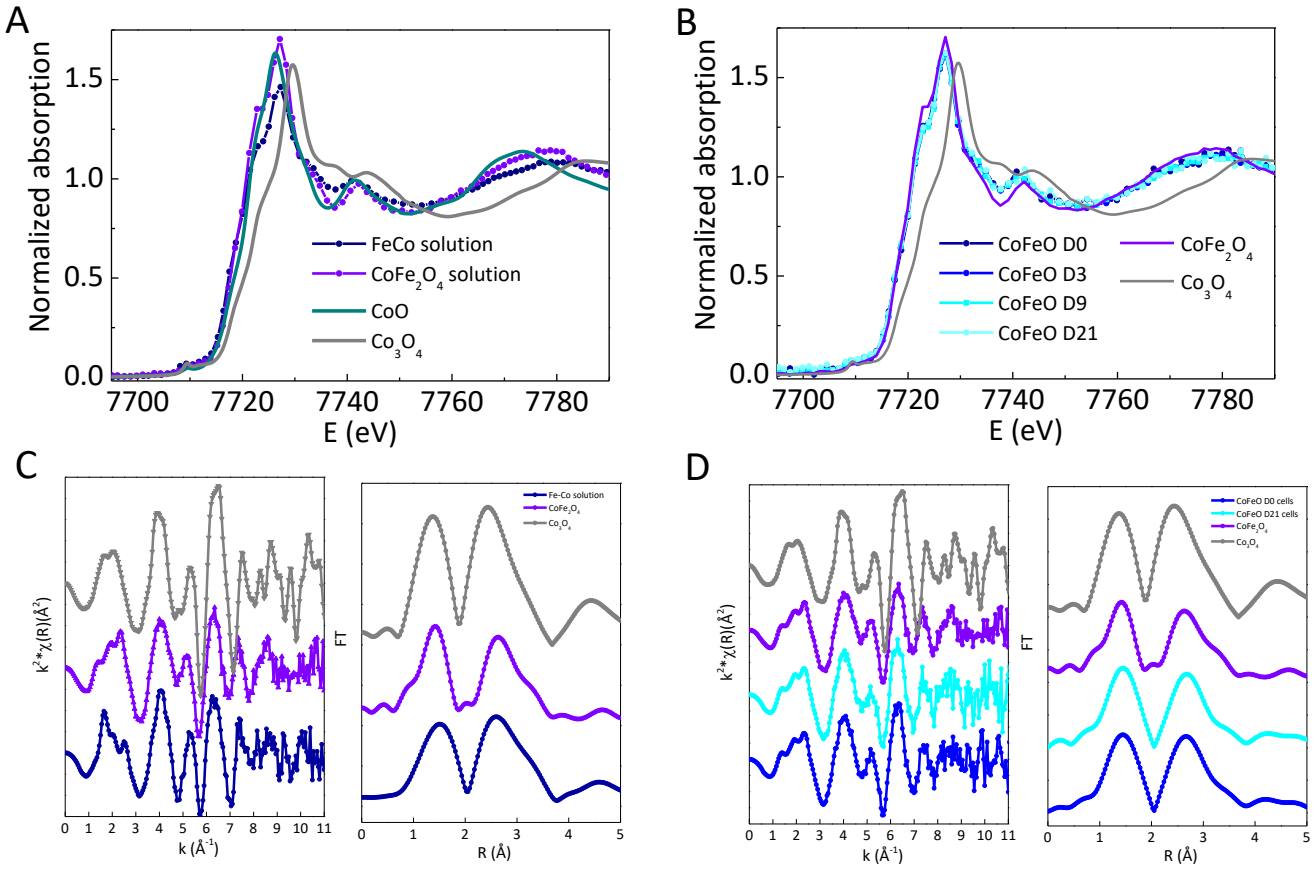

Figure 3: (A, B) XANES absorption spectra at the Co K-edge of initial solution Fe-Co-O nanoparticles and internalized in cells at days 0, 3, 9 and 21. The spectra were compared to that of CoO, cobalt ferrite ($CoFe_2O_4$) and Co_3O_4 reference compounds. Co oxidation state of solution and in cells fitted with 2+ valence. No degradation was detected over time. (C-D) EXAFS signal and modulus of the FT of previous compounds.

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