EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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:** Biodistribution of SiO2-Fe4O3 core-shell nanoparticles, focus on liver and lung	**Experiment number**: LS-2269
Beamline:	**Date of experiment**: from: 06 February 2014 to: 10 February 2014	**Date of report**: 29/08/14
Shifts:	**Local contact(s)**: Camille Rivard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Stijn Smulders, Peter Hoet

Report:

In this experiment, we focused on the body distribution of SiO2 nanoparticles (NPs) in mice, with special attention to the biodistribution in liver and lung. Mice were exposed to SiO2 core shell NPs, containing a magnetic core of Fe4O3, by two different exposure routes (lung aspiration and intravenous (iv) injection) and organs were analysed by µXRF and XANES. The biodistribution in the lung and liver was assessed. At the same time, the composition of the SiO2 NPs was assessed by looking at the fitting of the Si and Fe signal. If the particles keep their original core-shell structure, both signals fit well.

Millimetric parts of lung and liver were immersed in a water soluble embedding compound resin (FSC 22 from Leica Biosystems, Germany) and were frozen using methyl butane cooled by liquid nitrogen. Samples were sectioned at 15 µm thickness using a cryomicrotome. The sections were transferred to a freeze-drier using pre-cooled Al holders and freeze-dried for 12 hours. The sections were then mounted in a sample holder between two thin layers of ultralene foil.

The beam was focused to 0.3 x 1 (h x v) µm2 by means of Kirkpatrick-Baez mirror arrangement. Fluorescence signal is collected using a large-area (80 mm2) Bruker silicon drift placed at 60° scattering angle. Maps were recorded at 7.12 keV to avoid detector saturation by Fe signal and be able to record at the same time lower element signal like Si. Pixel size was 2 x 2 µm2 for the large maps and 0.5 x 0.5 µm2 for the small ones and integration time 100 and 150 ms per pixels, respectively. Fe K-edge XANES spectra were recorded to determine Fe speciation on Fe-rich pixels identified by XRF. Spectra were collected between 7.05 and 7.35 eV with a 0.6 eV step in continuous scan mode. At least ten spectra were recorded on each selected point in order to optimize the signal-to-noise ratio. XANES spectra were also recorded on the pure SiO2-Fe3O4 ENPs.

Results

Intravenous injection exposure protocol

The elemental distribution of Fe and Si was studied by synchrotron XRF on thin liver sections. No spots of Fe and Si were observed in non-exposed control animals (data not shown). Liver slices of mice 7 and 84 days after intravenous exposure to SiO_2-Fe_3O_4 ENPs show clear spots of Fe and Si, with an overlap of both signals (Fig. 1 and 2A). Maps collected with a 0.5 µm step on the Fe and Si-rich spots show spot sizes of around 5 µm. The XANES spectra of the two Fe-rich spots, collected after 84 days, show no differences in terms of position and shape of the pre-edge, white line (the most intensive peak) and oscillations, in comparison to the reference material (pure SiO_2-Fe_3O_4 ENPs) (Fig. 2B). These results show that the SiO2 NPs are present in the liver, even 84 days after exposure. Furthermore, these results give strong evidence that the ENPs retain their original core-shell structure during the whole observation period

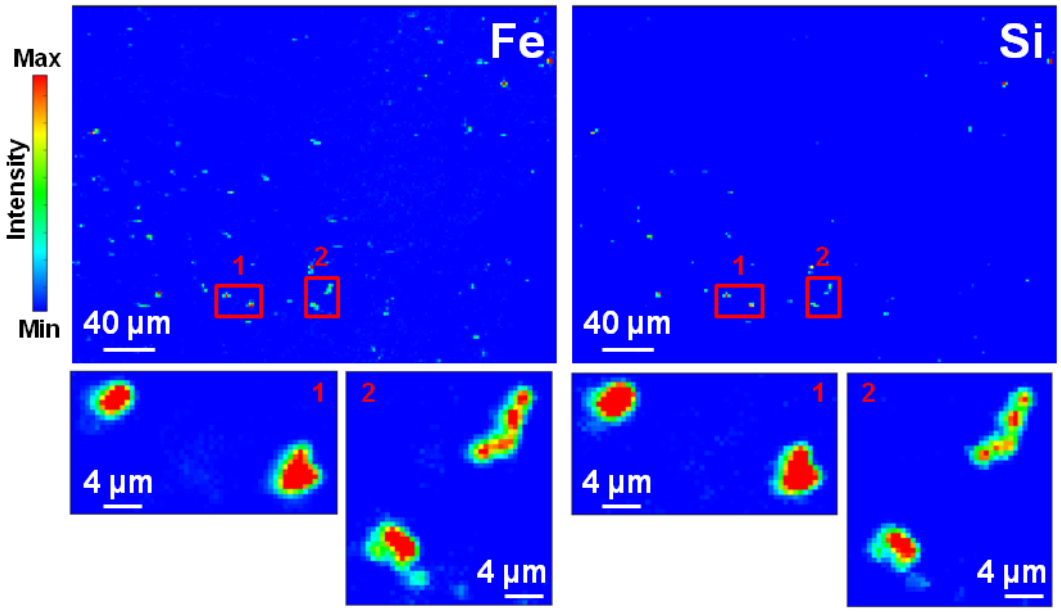

Fig. 1: Distribution of Fe and Si in liver 7 days after exposure.
Fe and Si distribution X-ray fluorescence maps (left and right side, respectively) within liver tissue 7 days after intravenous exposure to SiO_2-Fe_3O_4 core-shell ENPs. Small images represent magnifications of two spots indicated on the larger map.

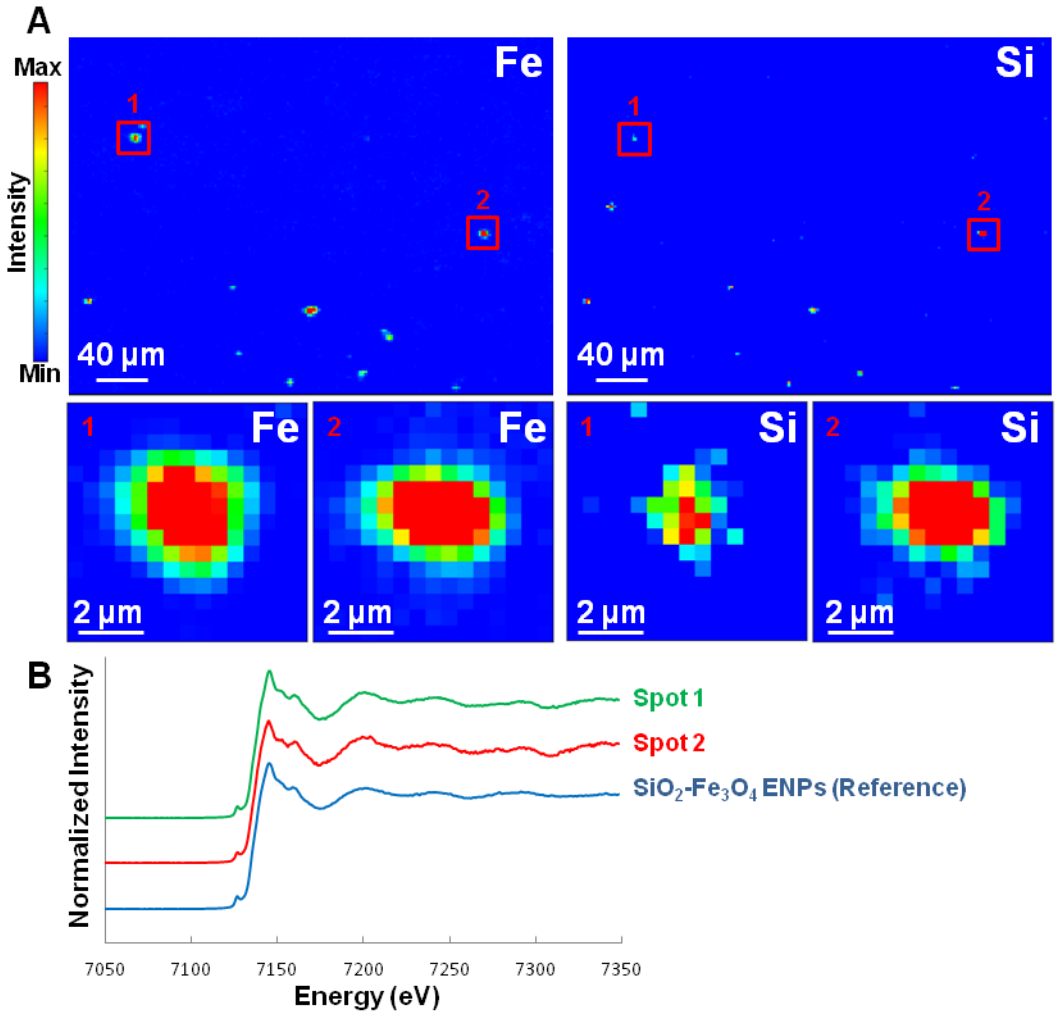

Fig. 2: Distribution of Fe and Si in the liver 84 days after exposure.
A: Fe and Si distribution X-ray fluorescence maps (left and right side, respectively) within liver tissue 84 days after intravenous exposure to SiO_2-Fe_3O_4 core-shell ENPs. Small images represent magnifications of two spots indicated on the larger map. **B:** Fe K-edge X-ray Absorption Near Edge Structure (XANES) of Fe-rich spots, compared to the pure ENPs.

Intratracheal instillation exposure protocol

The elemental distribution of P, Fe and Si was studied by synchrotron XRF on thin lung sections. No spots of Fe and Si were observed in non-exposed control animals (data not shown). Spots of Fe and Si were observed 7 and 84 days after exposure to the ENPs, with an overlap of both signals (Fig.3 and 4). In both times, the P signal, showing cellular structures, correlated quite well with Fe- and Si-rich regions. The number of spots appears lower after 84 days than after 7 days. The XANES spectra of the Fe-rich spots, collected after 84 days, show no difference in terms of position and shape of the pre-edge, white line and oscillations, in comparison to the reference material (pure SiO2-Fe3O4 ENPs) (Fig. 5B). The variations of intensity observed on the spectra of spot 1 can be assigned to self-absorption effect resulting from too high concentration of Fe in the investigated point.
These results show that Fe and Si spots are omnipresent in lung tissue at 7 days after exposure, where only a few spots could be found after 84 days. This indicates that some SiO2 ENPs are still present in the lung. Furthermore, Fe and Si spots correlate quite well with the P signal, suggesting that the ENPs are taken up by cells. We hypothesize that the largest part of the instilled particles are taken up by alveolar macrophages and will be removed from the lung by respiratory mucociliary clearance.

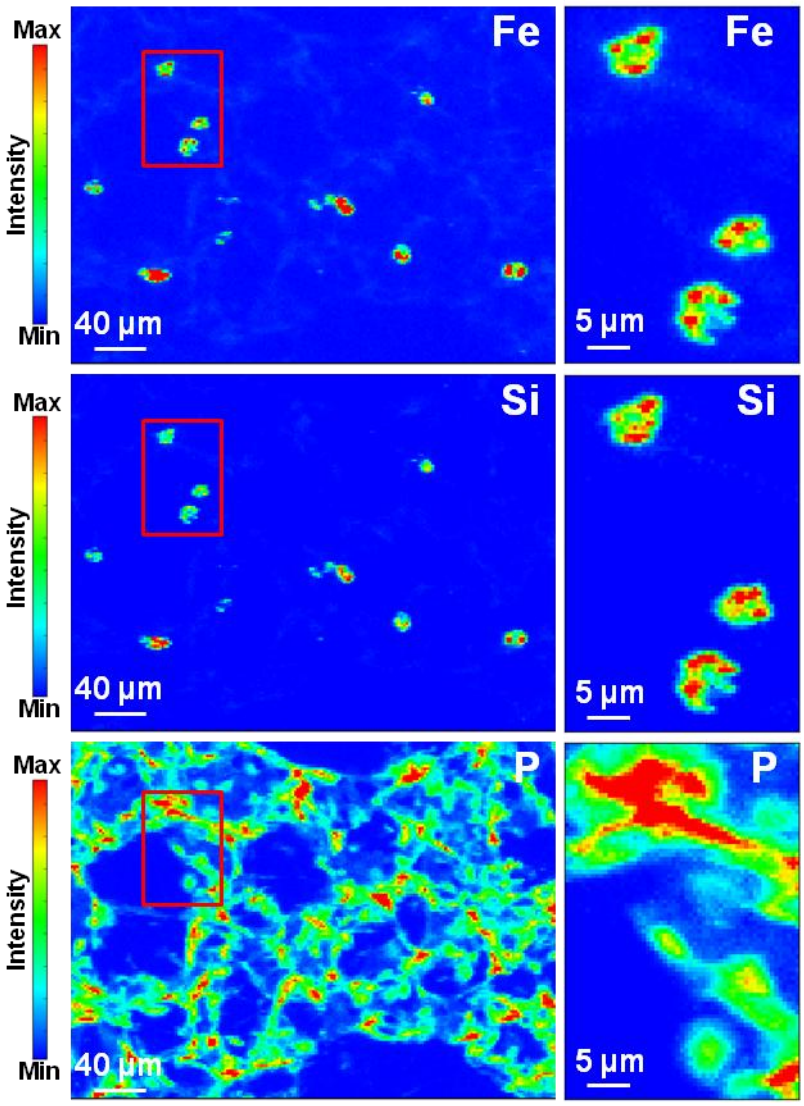

Fig. 3: Distribution of P, Fe and Si in lung 7 days after exposure.
Fe, Si and P distribution X-ray fluorescence maps (top, middle and bottom, respectively) within lung tissue 7 days after intratracheal exposure to SiO_2-Fe_3O_4 core-shell ENPs. Small images represent magnifications of one spot indicated on the larger map.

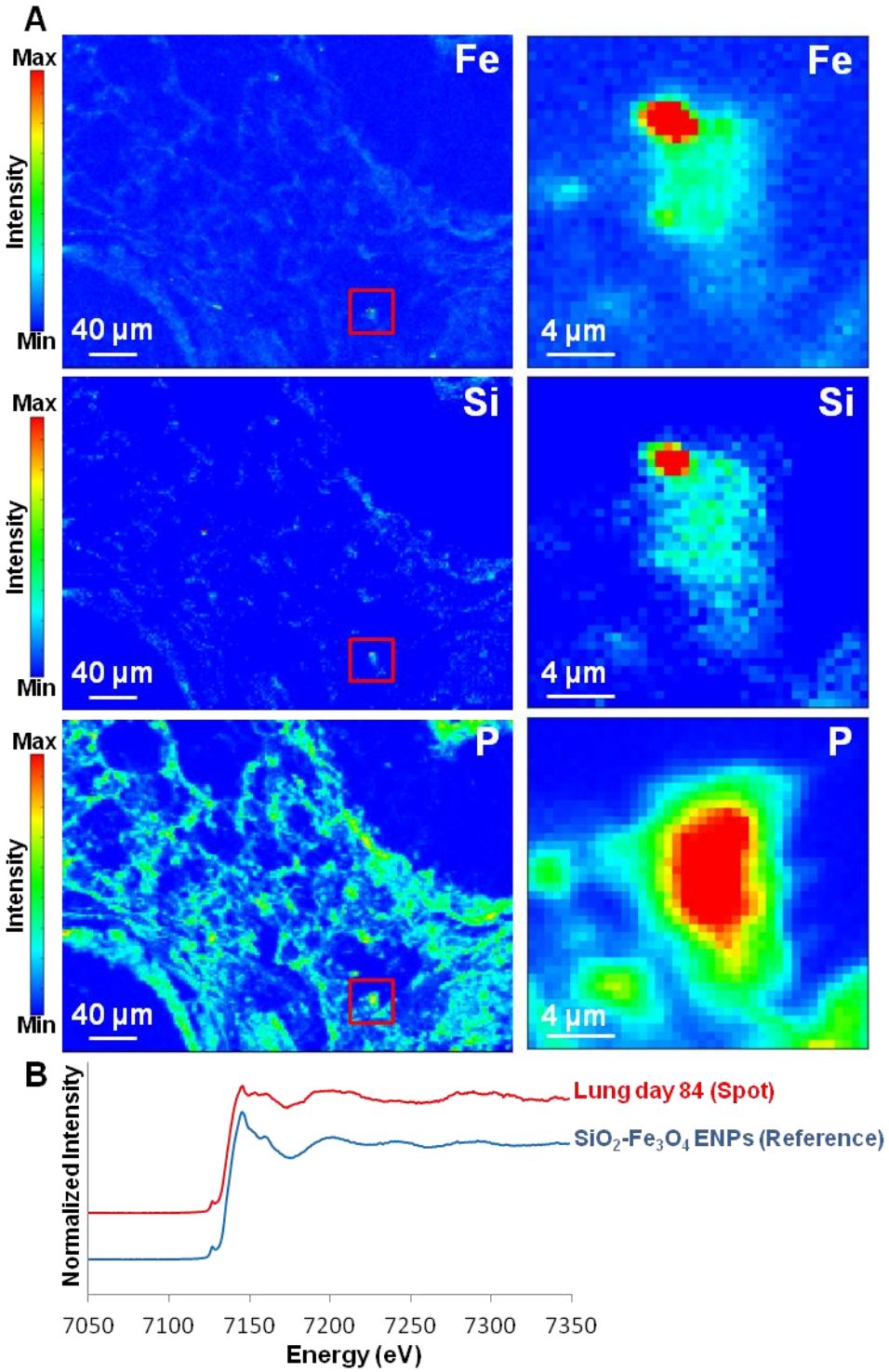

Fig. 4: Distribution of P, Fe and Si in the lung 84 days after exposure.
A: Fe, Si and P distribution X-ray fluorescence maps (top, middle and bottom, respectively) within liver tissue 84 days after intravenous exposure to SiO_2-Fe_3O_4 core-shell ENPs. Right images represent magnification of one spot indicated on the left images. **B:** Fe K-edge X-ray Absorption Near Edge Structure (XANES) of one Fe-rich spot compared to the pure ENPs.

Scientific production

One paper is in review in the journal Particle and Fibre Toxicology:

Abstract:

Nano-silicon dioxide (SiO_2) is used nowadays in several biomedical applications such as drug delivery and cancer therapy, and is produced on an industrial scale as additive to paints and coatings, cosmetics and food. Data regarding the long-term biokinetics of SiO_2 engineered nanoparticles (ENPs) is lacking. In this study, the whole-body biodistribution of SiO_2 core-shell ENPs containing a paramagnetic core of Fe_3O_4 was investigated after a single exposure via intravenous injection or intratracheal instillation in mice. The distribution and accumulation in different organs was evaluated for a period of 84 days using several techniques including magnetic resonance imaging (MRI), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence (XRF) and X-ray absorption near edge structure spectroscopy (XANES). We demonstrated that intravenously administered SiO_2 ENPs mainly accumulate in the liver, and are retained in this tissue for over 84 days. After intratracheal instillation, an almost complete particle clearance from the lung was seen after 84 days with distribution to spleen and kidney. Furthermore, we have strong evidence that the ENPs retain their original core-shell structure during the whole observation period. This work gives insight into the whole-body biodistribution of SiO_2 ENPs and will provide guidance for further toxicity studies.