REPORT OF E.S.R.F. EXPERIMENT HS-4422 AT BEAMLINE BM01A 19-22 OCTOBER 2011

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The aim of the experiment HS-4422 would be to characterize under the crystallographic point of view different mineral fibrous phases incorporated in animal tissues, in particular in mice which developed mesothelioma about 24 months after injection. To contribute to the better understanding the reasons of the toxicity of the fibrous minerals one way is to study the interaction between these minerals and biological matrixes. In fact, all these mineral phases are related to onset peaks of asbestos-related diseases (i.e. Malignant Mesothelioma) but the mechanisms by which these mineral fibers induces cyto- and geno-toxic damage remain unclear. Determination of the changes in the crystal structure after a long period in the biological medium was the goal of the presented experiment. The selected fibrous phases were:

- two different types of erionite (one standard phase from Oregon – USA and one natural sample from Cappadocia – Turkey);
- samples of crocidolite certified UICC.

The latter mineral phase is defined "asbestos" by the law, while erionite is not regulated, but its carcinogenetic potential results higher than that of the "asbestos" phases.

The samples studied were histological thin sections, 5 μm in thickness on glass slides, of different mice tissues (pancreas, liver or spleen) in which the fibres were injected; other sections on polimeric material were also prepared. The slide sections and the localization of the fibers in the slides were determined with care for each slide prior to the experiment at ESRF. The position of the fibers in the tissues was defined with the help of an x-y grid to allow for their quick location during the experiment.

Since the beam dimension at beam-line BM01A was too large (100x100 μm) to allow recording data from single fibre, we carried out measurements on several fibres of the starting material glued on glass slides and on kapton tape. Even if some good measurements where obtained on crocidolite, and very few for the other two phases, the collected powder patterns showed large broad peaks from the components used for the sample preparation (kapton tape and sample holder), which severely overlap with the crocidolite peaks (Fig. 1). Nevertheless, for the starting crocidolite (raw crocidolite), Rietveld refinements were performed obtaining reliable structural parameters.

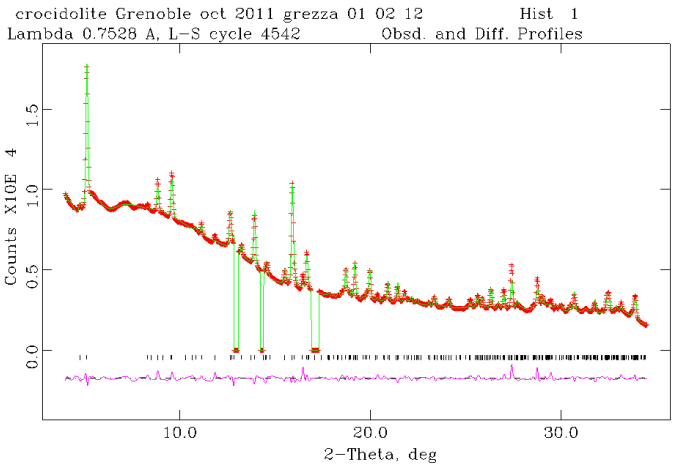

Fig.1 *The Rietveld graphical output of raw crocidolite with the observed pattern (red points), calculated pattern (green line), and difference curve (magenta line).*

To sum up, the experimental setup of BM01A resulted inadequate for the aim of the experiment. The large dimension of the beam spot (100x100 µm) compared to the dimension of the fibres (few µm), and the lack of an optical microscope, prevented the acquisition of an x-ray pattern from a single fibre incorporated in the tissue, which was, in turns, the main aim of the experiment. We tried to record data in regions where many fibres were present, but this was possible for crocidolite and not for erionite. Patterns from some assemblage of crocidolite incorporated in mice were recorded. The structural parameters of the raw crocidolite were imposed for these samples of crocidolite in pancreas and in spleen, obtaining comparison of the calculated cation contents in the different samples. Some differences between the raw crocidolite fibers and those inoculated in organic media were found, although these results are preliminary and not sufficient to clearly show a general picture of the structure dissolution of these fibers in the organic media. On the contrary, in spite of our efforts, no reliable results were obtained for erionite.

To conclude, in order to successfully carry out our project and to obtain more information of the changes on the fiber structure in organic medium, it would be fundamental to have a different experimental setup. a) it is necessary an optical microscope annexed to the instrument to focalize the X-Ray radiation onto an observed single fibre in the tissue; For this, a micro-beam line for single fibre x-ray pattern acquisition (and not from a bunch of fibres as obtained during HS442 experiment) is strongly recommended. Also a sample holder able to minimize background contribution is desirable.

We believe that beamline ID13, which has a micro-focused beam and the possibility to perform simultaneous Raman measurements (in order to gain even more information on the structure of the fibres), would be a suitable choice. A proposal will be prepared and submitted for the next run.