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REAL-TIME SURFACE/LIQUID INTERACTIONS OF FIBROUS ZEOLITES IN CONTACT WITH SIMULATED LUNG FLUIDS (SLFs) BY ATOMIC FORCE MICROSCOPE (AFM)

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In recent years, a growing concern has developed regarding the potential risks associated with environmental and occupational exposures to erionite in different countries, such as Turkey, U.S.A., Mexico, Iran and Italy (Carbone *et al*., 2011; Saini-Eidukat & Triplett, 2014; Ortega-Guerrero *et al*., 2015; Ilgren *et al*., 2015; Giordani *et al*., 2017). The exposure of erionite fibers to humans has been unambiguously linked to malignant mesothelioma, and *in vivo* studies have demonstrated that, at present, erionite is the most carcinogenic mineral in the world (IARC, 1987). Moreover, there is another zeolite, named offretite, which is closely related both structurally and chemically to erionite. Despite commonly occurring as prisms, offretite has also been found under asbestiform habit (Mattioli *et al*., 2018), suggesting that some mineralogical aspects have still to be discovered. Due to these similarities and to their possible intergrowth, the distinction between erionite and offretite can be hampered. Despite the great number of researches, the relationships among mineralogical features and biological activity of erionite have not been fully understood, while there are no studies regarding a potentially hazard of offretite fibers and it is unclear whether the mineralogical distinction between erionite and offretite has any health implications.

The epidemiological, mineralogical and toxicological studies on erionite have multiplied (*e.g.*, Ballirano *et al*., 2015; Ortega-Guerrero *et al*., 2015; Ballirano & Pacella, 2016; Bloise *et al*., 2016; Giordani *et al*., 2016; Gualtieri *et al*., 2016; Pacella *et al*., 2016; Giordani *et al*., 2017; Gualtieri *et al*., 2018). Studies regarding erionite fibers have been conducted on their interacting capability and on the interactions occurring with micelles and model membranes (Mattioli *et al*., 2016; Cangiotti *et al*., 2017). Significant results were obtained on human cell cultures (Pollastri *et al*., 2016; Cangiotti *et al*., 2018) and from *in-vivo* studies (Gualtieri *et al*., 2017), which demonstrated as erionite is extremely biopersistent and could spread its cytotoxic action over decades. Moreover, the biological activity of erionite samples from different localities has been demonstrated to be similar (Carbone *et al*., 2011). The structural analysis of erionite fibers leached with artificial simulated lung fluids (SLFs) (Ballirano & Cametti, 2015; Matassa *et al*., 2015; Gualtieri *et al*., 2018) highlighted a partial amorphisation and a slight reduction of the fibers diameter, probably because of partial dissolution occurring during the leaching processes.

Although these studies were extremely useful in determining the reaction of erionite fibers in contact with organic matter or with lung fluids environment, they do not provide information on modifications occurring at nanometric scale at the fiber surface. Considering that the chemical reactions implied in pathogenesis mainly take place at the solid-liquid interface (Guthrie & Mossman, 1993; Fubini *et al*., 1995), this kind of information is of paramount importance. Moreover, studies focused on the characterization of zeolites surface demonstrated that external surfaces do not always match with the bulk-terminated structure (Komiyama *et al*., 2001; Sugiyama Ono *et al*., 2001). Thus, results obtained from the investigation of bulk processes can be significantly different from those extrapolated when analyzing the surface (*i.e.*, first atomic layers) of the same sample.

The aim of the project entitled "*Real-time surface/liquid interactions of fibrous zeolites in contact with simulated lung fluids (SLFs) by Atomic Force Microscope (AFM)*" was to investigate the surface/fluid interactions of fibrous zeolites in contact with SLFs. In particular, the research was focused on the determination of the zeolite-surface microtopography and morphology under the effect of simulated pulmonary solutions. To monitor how the external surface of a mineral is affected by the interaction with fluids, the Atomic Force

Microscope (AFM) is up to now one of the most powerful tools. The innovative aspect of this project is the possibility to track *in situ* the processes occurring at mineral surfaces.

The investigated samples were: *i*) erionite: prismatic crystals with a tendency to split in a great number of fibrils of smaller dimension, from Bog Hill Quarry, Northern Ireland; *ii*) offretite: prismatic to acicular crystals, some of which with compact aspect, while others show a high fragmentation, from Val Grossa, Valdagno (VI), Italy. Two solutions were used: *i*) artificial lysosomal fluid (ALF pH of 4.5; Moss, 1979), and *ii*) Gamble's solution (pH of 7.4; Marques *et al*., 2011). ALF is analogous to the fluid with which inhaled particles would come into contact after phagocytosis by alveolar and interstitial macrophages. Gamble's solution simulates the interstitial fluid deep within the lung. The selection of the two fluids has been performed following the suggestion of National Institute for Occupational Safety and Health (NIOSH) for solubility assays (Nelson *et al*., 2009). ALF/Gamble's solutions were prepared according to standard formulation (Marques *et al*., 2011).

The solution/surface interactions of both samples in contact with SLFs were analyzed by *in situ* Atomic Force Microscope (AFM). Moreover, the samples were analyzed also by *ex situ* AFM at different interaction times of ~20 and ~90 days with fluids. The sample preparations, as well as the AFM measurements, were performed at the Institute of Geological Sciences, Bern University, in collaboration with Prof. S. Churakov. The laboratory is equipped with a Cypher ES© instrument. Preliminary chemical and structural characterizations were performed on both samples by Scanning Electron Microscope with Energy Dispersive Spectroscopy (SEM-EDS) and single-crystal X-ray diffraction (SC-XRD) respectively, at the Institute of Geological Sciences, Bern University. The measurements at AFM were initially performed in an environment-controlled fluid cell in a tapping mode at different imaging conditions (drive amplitude, attractive/repulsive mode) to probe different parameters on the surface structure. To investigate the role of the fluid surface interaction, additional measurements were performed in water and air using both tapping and contact mode.

RESULTS

The natural surface of offretite is characterized by a widespread presence of small particles attached on the surface, in variable amount, and clean surfaces are rarely observed. The particle sizes range from less than 40 nm in diameter and ~1 nm in height, to ~2 µm in diameter and ~250 nm in height for the bigger ones. In contrast, the surface of erionite is usually cleaner than that of the offretite. In some areas it is possible to observe particles (sizes range from less than 30 nm in diameter and ~2 nm in height, to ~400 nm in diameter and ~30 nm in height for the bigger ones) attached at the surface, but generally they are very scarce (Fig. 1). It is worth noting that due to their small size most of these particles are not detectable by SEM.

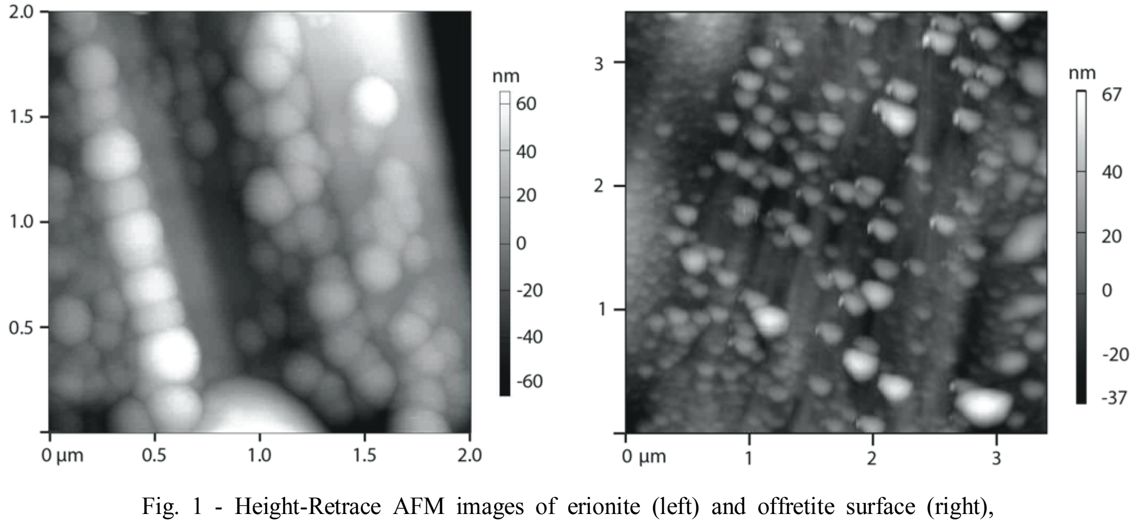

Fig. 1 - Height-Retrace AFM images of erionite (left) and offretite surface (right), acquired in H_2O, where small particles diffused on the crystals surface are present.

Under ALF solution, both the zeolites erionite and offretite showed general dissolution, but with a strong dependence on local topography. The most striking effect was the detachment of the particles from the surface that became relevant starting from approximately 30 minutes of interaction-time, which continues until almost complete removal of particles after several hours (Fig. 2).

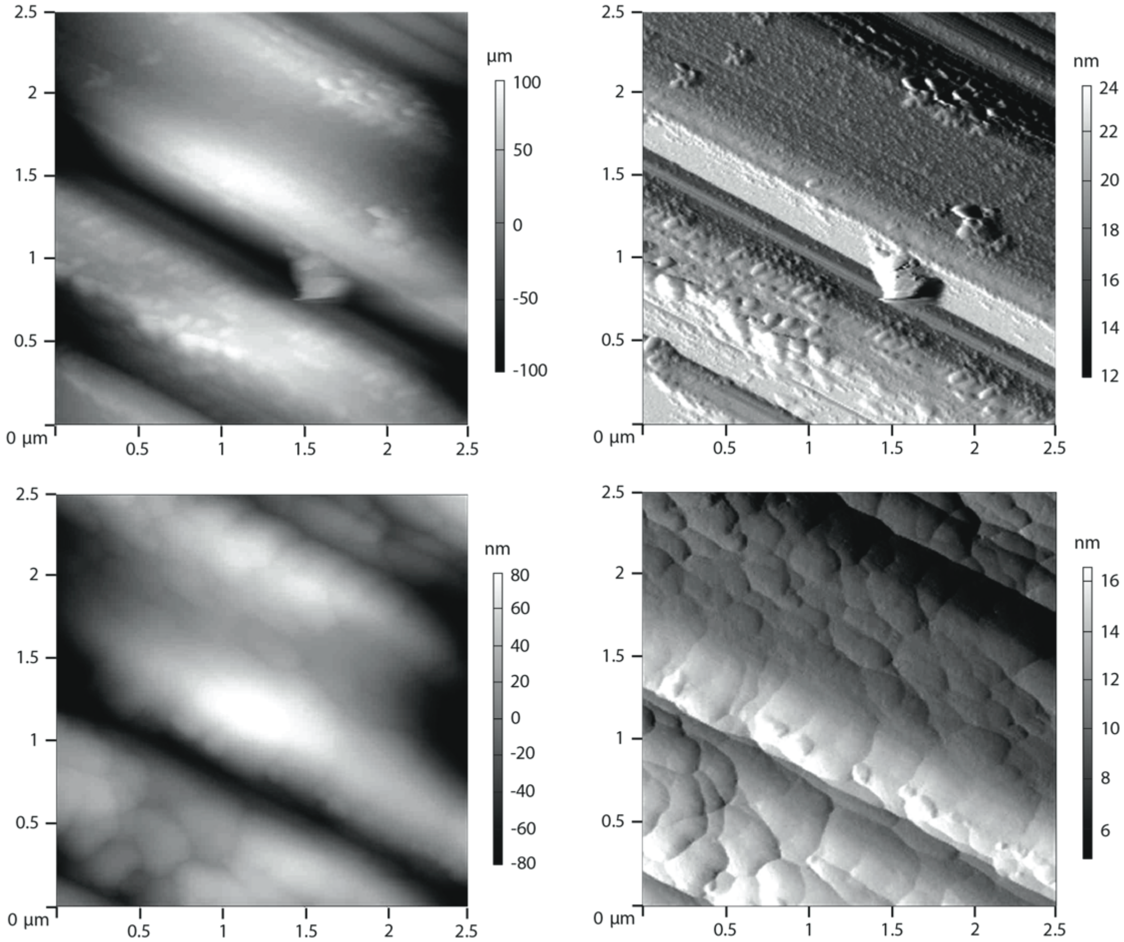

Fig. 2 - Height-Retrace (left) and Amplitude-Retrace (right) AFM images of offretite surface at 37°C in ALF solution (a) and same area ~7 hours later (b).

Comparing the process at different interaction temperatures, 25°C and 37°C (to simulate the body temperature), no significant differences emerged.

In Gamble's solution, the behavior was different. For both zeolites, in some areas the dissolution was notable but less pronounced than that observed in ALF whereas in other zones the surfaces were completely covered by a thin layer (few nanometers thick) of another substance/phase which made the original crystal-surface unrecognizable (Fig. 3).

The results at the different 25°C and 37°C temperatures showed surficial variations only related to faster layer coverage on the zeolites surface at the higher temperature.

A plausible dissolution-model to interpret the obtained results is currently being prepared and it will be submitted to *Minerals* journal.

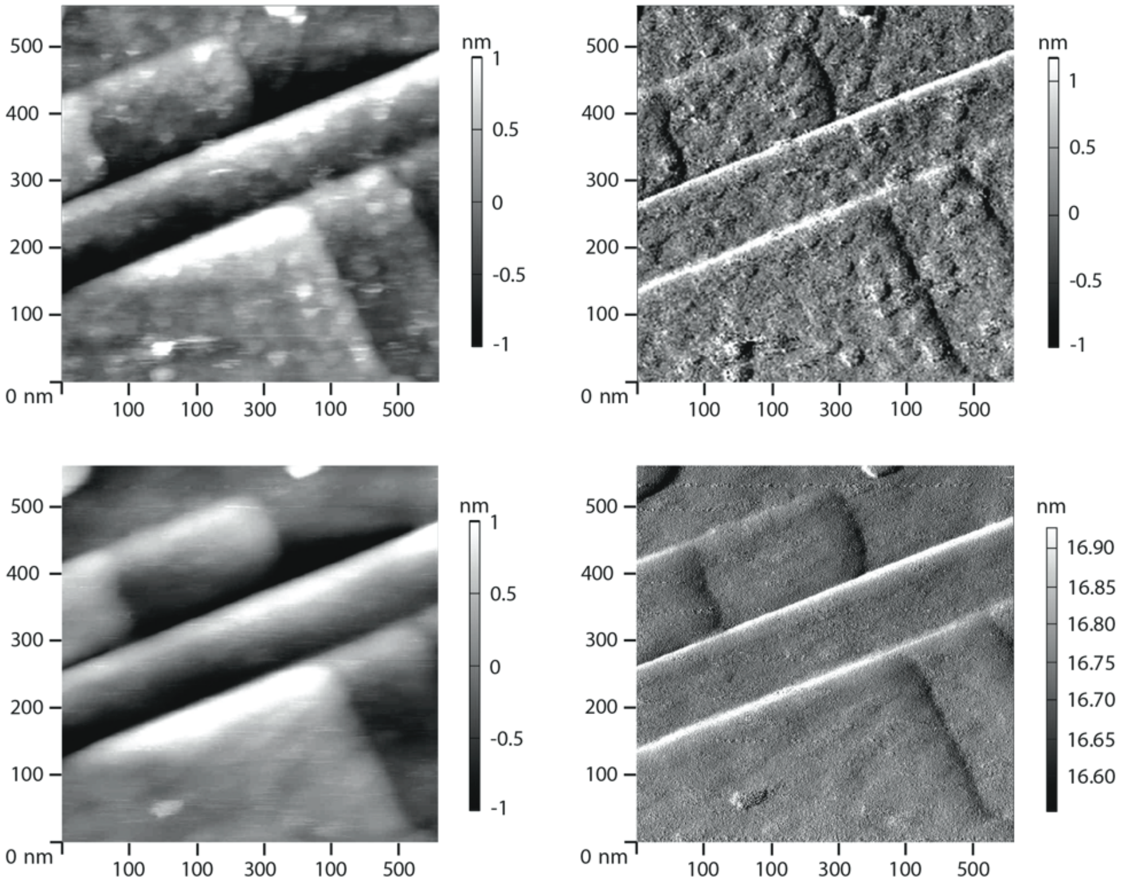

Fig. 3 - Height-Retrace (left) and Amplitude-Retrace (right) AFM images of erionite surface at 37°C in Gamble's solution (a) and same area ~4 hours later (b).

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