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Revision 1

Dissolution reaction and surface iron speciation of UICC crocidolite
in buffered solution at pH 7.4: a combined ICP-OES, XPS and TEM
investigation

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Abstract

The dissolution reaction and the surface modifications of crocidolite asbestos fibres incubated for 0.5, 1, 24, 48, 168 and 1440 h in a phosphate buffered solution at pH 7.4 with and without hydrogen peroxide were investigated. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to monitor the ion release into solution, X-Ray Photoelectron Spectroscopy (XPS) was performed to unveil the chemistry of the leached surface, and High Resolution Transmission Electron Microscopy (HR-TEM) was carried out to monitor the structural modifications of the fibres. No significant differences were observed between dissolution experiments carried out with and without H$_2$O$_2$ with the exception of results after the first hour, from which it may be inferred that the dissolution proceeds faster in the presence of H$_2$O$_2$ but only in its very early steps. Congruent mobilization of Si and Mg from crocidolite was observed, increasing with time especially in the range between 1 and 48 h, while Ca decreased after 48 h and Fe was not detected at any incubation time. In the under-saturated conditions (0-48 h), dissolution rate of UICC crocidolite fibres has been estimated to be d(Si)/dt = 0.079 µmol h$^{-1}$. The fibre surface modification is continuous with time: XPS results showed a regular depletion of Si and Mg and enrichment of Fe along dissolution. The Fe2p$_{3/2}$ signal on the surface was fitted with four components at 709.0, 710.5, 711.6 and 712.8 eV binding energy values corresponding to: i) Fe(II)-O and ii) Fe(III)-O surrounded by oxygen atoms in the silicate structure, iii) Fe(III)-OOH as a product of the dissolution process, and iv) Fe in a phosphate precipitate (Fe-P), respectively. The evolution of Fe speciation on the crocidolite surface was followed by integrating the four photoemission peaks, and results showed that the oxidative environment promotes the formation of Fe(III)-O (up to 37% Fe$_{tot}$) and of Fe-P species (up to 16% Fe$_{tot}$), which are found on the fibre surface at the end of the dissolution experiment. HR-TEM showed that the crocidolite lattice structure, the fibrous habit and the high aspect ratio are preserved upon leaching, while Fe-bearing nanoparticles, likely amorphous and possibly displaced on top of the fibres, become clearly visible. As a conclusion, coating of the crocidolite fibres was demonstrated to occur due to precipitation of Fe-rich phases (both phosphates and oxide-hydroxides). The occurrence of such iron armouring may modulate asbestos toxicity and possibly be the initial step in the formation of asbestos ferruginous bodies.

Keywords: asbestos, crocidolite, dissolution, surface chemistry, X-ray Photoelectron Spectroscopy (XPS), High-Resolution Transmission Electron Microscopy (HR-TEM)
1. INTRODUCTION

Asbestos materials are made of six hydrate silicate minerals with fibrous morphology: chrysotile, the only fibrous member of the serpentine mineral group, with ideal chemical formula \( \text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4 \); anthophyllite, belonging to the amphibole supergroup, ideally \( \text{Mg}_7\text{Si}_8\text{O}_{22}(\text{OH})_2 \); grunerite amphibole, ideally \( \text{Fe}^{2+}_7\text{Si}_8\text{O}_{22}(\text{OH})_2 \), usually referred to colloquially as “amosite” (from the acronym AMOS, Asbestos Mines of South Africa); tremolite amphibole, ideally \( \text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2 \); ferro-actinolite amphibole, ideally \( \text{Ca}^2_2\text{Fe}^{2+}_5\text{Si}_8\text{O}_{22}(\text{OH})_2 \); riebeckite amphibole, ideally \( \text{Na}_2(\text{Fe}^{2+}_3\text{Fe}^{3+}_2)_{\sum^2}\text{Si}_8\text{O}_{22}(\text{OH})_2 \), usually referred to colloquially as “crocidolite” (from the Greek κροκος, nap of cloth, on account of its nap-like appearance).

Innumerable epidemiological studies have shown that exposure to asbestos is related to several health problems and respiratory diseases. The molecular basis of asbestos-induced lung disease has not been fully elucidated yet (Liu et al., 2013; Pascolo et al., 2013). However, it is widely held (e.g., Stanton et al., 1981; Kamp and Weitzman, 1999) that size— in particular the aspect ratio—, surface reactivity, and biopersistence are the three main factors in determining the pathological response to asbestos. This paradigm holds for many hazardous inhaled particles and fibres (Hochella, 1993; Fubini et al., 1995; Fubini, 1997; Hohr et al., 2002; Fubini et al., 2011). The most robust mechanism-based structure-activity relationship for asbestos includes generation of iron-mediated reactive oxygen species (ROS) (Fubini and Otero-Areàn, 1999; Shukla et al., 2003). Such chemical activity received considerable attention by the biomedical community and was related to the presence and the bioavailability of Fe. Both the presence and the structural coordination of Fe were showed to be important factors of asbestos toxicity (Turci et al., 2011). Tests performed on isolated mitochondria in contact with iron-rich crocidolite showed that crocidolite causes severe damage to cells and enhances the mitochondrial production of ROS (Bergamini et al., 2007).

Furthermore, only the Fe on the fibre surface, and in particular Fe(II), was considered to play a primary role for the ROS production (Hardy and Aust, 1995; Pacella et al., 2010, 2012; Fantauzzi et al., 2010; 2012). The increase in the surface concentration of Fe on asbestos fibres upon treatment with murine tumor cells and culture medium was reported by Seal et al. (1996, 1997). However, no attempts of quantification of Fe(II) and Fe(III) speciation were done by the authors. Studies on crocidolite dissolution in the presence of Fe chelators showed that the presence of chelators dramatically increases Fe(III) release, with Fe-release lifetime estimated to be on the order of 10 years (Werner et al., 1995). It was reported that Fe can be
mobilized from crocidolite in lung cells with a rate similar to that observed in vitro when crocidolite was incubated with citrate, enhancing asbestos ability to catalyse damages to DNA (Lund and Aust, 1992; Chao et al., 1994). Endogenous chelators present in the lung lining layer fluid, such as ascorbic acid, were shown to be responsible for an effective iron mobilization from crocidolite fibres (Martra et al., 2003). It was also demonstrated by in vitro studies that ferruginous bodies, mainly constituted by ferrihydrite, might precipitate on asbestos fibres in contact with human cells (Shen et al., 2000). In addition, precipitation of calcium phosphates was observed upon interaction between chrysotile asbestos and simulated lung fluids, and SEM analyses of calcified pleural plaques detected the presence of hydroxyapatite secondary phases in lung tissues (Taunton et al., 2010).

The aim of the present work is describing the dissolution dynamics and the surface modifications of crocidolite asbestos incubated at 37 °C in a hydrogen peroxide solution buffered at pH = 7.4. The extreme incubation conditions used are based on the approach previously adopted by some of us for measuring the surface reactivity of the UICC crocidolite (Pacella et al., 2012). Even being far from mimic a real cellular environment, such conditions were chosen to promote the dissolution dynamics that may occur in vivo in a reasonable experimental time. A sample of reference crocidolite, supplied by the Union Internationale Contre le Cancer (UICC), hereafter named UICC crocidolite, was suspended in the leaching solution from 0.5 h to 1440 h (two months) and then investigated by a multi-analytical approach. Ion release was monitored by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Modification of surface chemistry, including Fe(II) and Fe(III) speciation, was investigated by X-ray Photoelectron Spectroscopy (XPS); structural state of the fibres before and after dissolution experiments was studied by High Resolution Transmission Electron Microscopy (HR-TEM).

2. EXPERIMENTAL

2.1. Materials

The sample investigated consists of fibres of UICC crocidolite selected under microscope and gently ground for 1 min under acetone in an agate mortar. Chemical formula is 

\[
[(\text{K}_{0.01}\text{Na}_{1.64}\text{Ca}_{0.14}\text{Mg}_{0.16})_{\Sigma=1.95}(\text{Fe}^{2+}_{2.13}\text{Fe}^{3+}_{2.30}\text{Mg}_{0.55}\text{Mn}_{0.01}\text{Ti}_{0.01})_{\Sigma=5.00}(\text{Si}_{7.82}\text{Al}_{0.02})_{\Sigma=7.84}\text{O}_{22}(\text{OH})_{2}]_{2.1},
\]

fairly close to that of the ideal crocidolite \(\text{Na}_2(\text{Fe}^{2+}_3\text{Fe}^{3+}_2)_{\Sigma=5}\text{Si}_8\text{O}_{22}(\text{OH})_2\). The
morphism of the fibres was investigated by Field-Emission Scanning Electron Microscopy (FE-SEM), and surface area was measured by nitrogen physisorption (BET).

2.2. Dissolution experiments

For the preparation of the leaching solution “ultrapure” deionised water (18.2 MΩ cm at 25°C) obtained from a MilliQ Element system (Millipore, France) and the following reagents and materials were used: potassium dihydrogen phosphate (KH₂PO₄ - RPE - Carlo Erba Reagenti, Italy), 30% hydrogen peroxide (H₂O₂ - “suprapure” - Merck, Germany); Polypropylene Falcon Tubes (Blue Max)™, syringes BD Plastipack™ and 0.22 µm GSWP nitrocellulose membrane filters (Millex HA, Millipore).

An amount of 68.0 g of KH₂PO₄ was dissolved in “ultrapure” water in a 1 dm³ volumetric flask; 10 cm³ of 30% H₂O₂ were added (to obtain a concentration of 0.3% in the 1 dm³ final volume of the solution); pH was adjusted to 7.4 with a 1N potassium hydroxide solution (Normex - Carlo Erba Reagenti, Italy) and finally “ultrapure” water was added up to the final 1 dm³ volume. The experimental conditions used here are based on the approach described by Nejjari et al. (1993) and adopted by Pacella et al. (2012) for measuring surface reactivity of the UICC crocidolite.

For the dissolution experiments an amount of 25 mg of fibres of UICC crocidolite, placed in a Falcon™ polypropylene tube, was suspended in 2 cm³ of the potassium phosphate/hydrogen peroxide buffer solution above described. The tube was continuously shaken in a thermostatic oscillating bath at 37°C. Independent experiments were conducted for 0.5, 1, 24, 48, 168 hours (1 week) and 1440 hours (2 months). For each single experiment leaching tests were performed in triplicate and a blank procedure was always carried out. In addition, the UICC crocidolite fibres were also tested in the potassium phosphate buffer solution at pH 7.4 prepared without H₂O₂, in order to evaluate the effect of the oxidizing ambient on the cation dissolution.

For each experiment the solution was sampled with a syringe from the tube, after centrifugation at 3000 revolution per minute (rpm) for 5 min, and filtered in nitrocellulose membrane filter of 0.22 µm. From each filtered solution 1 cm³ diluted 1:20 with a 1% nitric acid solution was analysed by ICP-OES (see paragraph 2.3).
The fibres were recovered from the tubes on filters, rinsed with ultrapure deionised water to eliminate the residues of the solution, dried and then stored under argon prior to the XPS measurements.

2.3. ICP-OES investigation

One cm$^3$ of each filtered solution was diluted (1:20) with a 1% nitric acid solution and analyzed by ICP-OES in order to measure the concentration of leached Si, Mg, Ca and Fe from the fibers. All measurements were performed using a Perkin Elmer Optima 2000 DV ICP-OES spectrometer (Perkin Elmer, USA) equipped with a cross flow nebulizer placed inside a Scott spray chamber. ICP Aristar (BDH) standard solutions in nitric acid for Si (10,000 mg dm$^{-3}$), Mg, Ca, Fe (1000 mg dm$^{-3}$) were used to prepare the calibrating solutions for ICP-OES analyses. The standard solutions used for the calibrations were prepared as the samples using potassium phosphate buffer solution, with 0.3% H$_2$O$_2$ (or, in case, without), diluted 1:20 with a 1% nitric acid solution. To ensure adequate quality assurance, the measures of the standard solutions were regularly repeated after the measurements of each single experiment. Data reported are the mean values of triplicate measurements (corrected considering data from the blank procedure).

2.4. XPS investigation

XPS analyses were performed on a Theta Probe (Thermo Fisher Scientific, Waltham MA, USA). Fibrous samples were deposited on polycarbonate filters (0.4 μm) and mounted on a standard sample holder for XPS measurements with copper clips. Spectra were collected using a monochromatic source (Al $k\alpha_{1,2}$ energy = 1486.6 eV). The spot size was 300 μm and the beam was operated at 4.7 mA and 15 kV (70 W). The residual pressure into the main chamber was lower than 10$^{-7}$ Pa. The instrument is also equipped with a neutralizer for charge compensation. Survey spectra were acquired in fixed analyser transmission mode (FAT) using a pass energy (PE) of 200 eV, while the high-resolution spectra of C1s, O1s, Si2p, Mg1s, Ca2p, Na1s, K2p, Fe2p and P2p were collected with a PE of 100 eV selecting the standard lens mode; the full-width at half-maximum of the peak height, FWHM, of the silver Ag3d$_{5/2}$ signal for the high-resolution spectra was 0.83 eV; step size of 1 eV and 0.05 eV were set respectively. The emission angle is of 53°, and the angle between the source and the analyser axis is 63.78°. To verify the linear response of the instrument, periodic calibrations were
performed according to ISO 2001. When analysing the fibres the neutralizer was used to compensate for sample charging and the binding energy values were further corrected with reference to the adventitious aliphatic carbon at 285.0 eV. Data were acquired under computer control (Avantage v 3.45). Three different areas were analysed on each sample.

To determine the peak areas and the elemental composition, the spectra were processed using CASAXPS software (Fairley, 1999-2003). Before applying the curve-fitting procedure, the background was subtracted according to the Shirley-Sherwood background subtraction method (Shirley, 1972). The product of Gaussian and Lorentzian functions was used for curve fitting. Quantitative analysis of fibre surfaces was performed using the first-principle method (Seah, 2003) on the assumption that the sample was homogeneous. Peak areas were corrected for the sensitivity factors calculated using Scofield’s photoionization cross-sections $\sigma$ (Scofield, 1976); the asymmetry parameters (Reilman et al., 1976), the inelastic mean free paths (IMFP) and the intensity/energy analyser response were determined according to the procedure provided in Avantage Software v. 3.45. IMFP were calculated according to Gries (1996). The accuracy of the calculated atomic concentrations is estimated to be ± 10%.

Binding energy values and atomic percentages are reported in this work as means on at least three independent measurements with their corresponding standard deviations.

### 2.5. HR-TEM investigation

The morphology and crystalline structure of the samples before and after dissolution experiments were investigated by JEOL 3010-UHR HR-TEM equipped with a LaB$_6$ filament operated at 300 kV, beam current 114 µA and equipped with a 2k x 2k pixels Gatan US1000 CCD camera. Elemental analysis was performed by Oxford INCA X-ray energy dispersive spectrometer (X-EDS) with a Pentafet Si(Li) detector. Crocidolite fibres were dispersed in ultrapure water (MilliQ system, Millipore), sonicated for 20 minutes and a droplet was deposited on lacey carbon Cu grids.

### 3. RESULTS

The UICC crocidolite fibres appear straight, rigid and very tiny under binocular microscope. FE-SEM images show that most of the fibres have a polygonal cross section with major diameter in the range of 0.5-1 µm. Notably, high resolution images show that the micrometric fibres have the possibility of parting into fibrils with diameter of ca. 0.1 µm (Fig. 1).
Results of ICP-OES analyses after suspension experiments in the phosphate buffer solution with H$_2$O$_2$ reveal that some dissolution occurred from early steps, as evidenced by the release of Si, Mg and Ca (Table 1). In particular, Si release ranges from 624 mg/kg after 0.5 h to 5120 mg/kg after two months of dissolution time, and Mg ranges from 180 mg/kg to 590 mg/kg in the same period of time. Calcium release equals 877 mg/kg after 0.5 h, reaches a maximum about 1500 mg/kg after 24-48 h and then markedly decreases down to 866 mg/kg. The analysis of the progression of cation dissolution reveals the existence of two regions: 1) between zero and 48 h, representing the under-saturation conditions, favourable to fibre solubility; 2) after 48 h and markedly after 168 h—representing the near-saturation conditions, where element release is very low or below the detection limit (Fig. 2). In particular, Ca release is observed to slow down after 24 h experiment, and to stop after 48 h, suggesting a possible Ca precipitation. Notably, mobilization of Fe was not observed for any dissolution time, even extending the experiments up to two months. For the experimental conditions adopted here the detection limit for Fe was 50 µg dm$^{-3}$, approximately corresponding to a leaching of less than 0.03% of the total Fe content in the crocidolite sample.

The dissolution experiments were repeated from 1 to 168 hours without H$_2$O$_2$: results of ICP-OES analyses did not show marked differences with the exception of results after the first hour of incubation (Table 1), from which it may be inferred that the dissolution proceeds faster in the presence of H$_2$O$_2$ but only in its very early steps. In both conditions Si and Mg are released congruently, that is in a proportion roughly corresponding to crocidolite bulk and surface stoichiometry (Fig. 3), in agreement with results obtained by Gronow (1987). On the contrary, Ca release is definitely in excess, being the maximum value in solution three times that of Mg (whereas in the crystal chemical formula of the UICC crocidolite Ca is one fifth of Mg). This may be due to Ca-bearing accessory phases present in the sample but not detectable in sample characterization.

Kinetics of the UICC crocidolite dissolution are described on the basis of ICP results. During silicate dissolution it is well known that the Si release is the rate-limiting step controlling dissolution rates (Oze and Solt, 2010, and references therein). In our case, Si release reaches a close-to-saturation condition after 168 h, with Si in the solution corresponding to less than 2% of the total Si of the suspended fibres. If steady-state conditions were maintained and the observed trend was extended in time, more than 100 years would be required to release only 4% of Si, leading to fibre dissolution rate extremely low (or close to zero). However, it is known from literature that body fluids are continually replenished and fibre dissolution rates...
are calculated for undersaturated conditions at constant pH (Hume and Rimstidt, 1992; Oze and Solt, 2010). Accordingly, for UICC crocidolite the fibre dissolution rate was quantified in the undersaturated initial conditions (0-48 h). Following previous authors, the rate of asbestos dissolution was modelled by the simplified equation: 
\[ \frac{d(Si)}{dt} = k \cdot [A]^n \]
where \( \frac{d(Si)}{dt} \) is the rate of Si release (µmol h\(^{-1}\)), \( k \) is the rate constant (µmol m\(^{-2}\) n h\(^{-1}\)), \( [A] \) is the total surface area (in m\(^2\)), and \( n \) is the reaction order. In our case, the rate of Si release is estimated using the linear regression described by the equation \( \frac{d(Si)}{dt} = 0.079 \) µmol h\(^{-1}\), \( R^2 = 0.77 \) and the surface area was measured by BET (8.66 m\(^2\) g\(^{-1}\)). At the moment, data available do not allow the calculation of the reaction order because leaching experiments were performed without using different surface areas.

Surface quantitative analysis of UICC crocidolite fibres treated with H\(_2\)O\(_2\) was obtained by XPS, assuming as first approximation a homogeneous composition throughout the sampling depth, and results are reported in Table 2. The identification of the elements present on the UICC crocidolite fibres was possible by the XP-survey spectra (data not shown – Figure A.1 in the Appendix A). The elements O, Si, Fe, Na and Mg were detected on both the untreated and treated samples, whereas the photoemission signals of K, P and Ca only appeared on treated fibres. In particular, the Ca signal appeared after 24 h. Table 2 also shows the results obtained only considering Si, Ca, Mg and Fe, to be compared with the ICP-OES results. Binding energy (BE) values of the curve-fitted spectra are summarized in Table 3. Si 2p peak was resolved using the doublet Si\(_{2p}^{3/2}\) and Si\(_{2p}^{1/2}\) with an energy separation, \( \Delta E \), of 0.805 eV and an area ratio of 2. The BE of the Si\(_{2p}\) peaks (102.4 ± 0.2 eV) was not influenced by the cation mobilization and it is in agreement with the BE value reported in Fantauzzi et al. (2010) for untreated UICC crocidolite (corresponding to \( t = 0 \) h in Table 3). Mg\(_{2p}\) and Na\(_{1s}\) BE values were also independent from the dissolution time and are in agreement with those reported in Fantauzzi et al. (2010). The BE of Ca\(_{2p}^{3/2}\) (347.8 ± 0.2 eV) is in agreement with both Ca\(_{2p}^{3/2}\) in tremolite (Fantauzzi et al. 2010) and Ca\(_{2p}^{3/2}\) in CaHPO\(_4\) and CaHPO\(_4\)*2H\(_2\)O (Landis and Martin, 1984). In addition, the BE in a calcium metaphosphate glass has been measured to be 347.0 eV (Mura, 2010). The observed presence of K (K\(_{2p}^{3/2} = 293.1 \pm 0.2\) eV) on the fibre surfaces after incubation is due to the potassium phosphate used for buffer preparation, that is very likely adsorbed onto the fibres. The same evidence was provided by Gold et al. (1997). Due to adsorbed buffer on the fibre surfaces, phosphorus signals were detected on the sample surfaces at 133.8 ± 0.2 eV (Table 3), which is the BE of phosphorus in phosphates (Crobu et al., 2010 and references cited therein). It is worth noting that the P content increases with the dissolution time (from 4% after 1 h to 7% after 168 h, Table 2).
Significant variations were observed for the Fe2p\textsubscript{3/2} spectrum when comparing data previously obtained on untreated fibres (Fantauzzi et al., 2010), with those obtained in this work for the fibres suspended in the buffered solution. According to Fantauzzi et al. (2010), the Fe2p\textsubscript{3/2} curve fitting was performed using the convolution of Gaussian – Lorentzian function based on a multiplet-splitting approach (Fig. 4). The Fe peak of untreated UICC crocidolite was fitted with three components at 709.0, 710.5 and 711.6 eV assigned to: 1) Fe(II) bonded to the O atoms in the silicates cavities [hereafter indicated as Fe(II)-O]; 2) Fe(III) bonded to the O atoms in the silicates cavities [hereafter indicated as Fe(III)-O]; and 3) Fe(III) oxide-hydroxide [hereafter indicated as Fe(III)-OOH], respectively (Table 3). In the present case, a fourth component appeared at 712.8 eV. This component is attributable to an iron phosphate phase (Rossi et al., 2006; Crobu, 2012) [hereafter indicated as Fe-P] which is insoluble in neutral solutions. The intensity of the Fe(II)-O signal reaches the maximum value of 29% of the total peak area after 1 h of dissolution and then keeps constant at about 25%; the Fe(III)-O signal increases almost regularly with time from 18% to 37%; the Fe(III)-OOH signal decreases from 62% to 20%, following a nearly exponential trend in the first 24 h and then a linear trend up to 168 h; the Fe-P signal is zero at \( t = 0 \) and increases almost regularly from 8% to 16% when increasing the dissolution time (Fig. 5). Notably, when comparing data of surface composition obtained on fibres suspended in the solution with \( \text{H}_2\text{O}_2 \) with those obtained on fibres suspended in the solution without \( \text{H}_2\text{O}_2 \), no significant differences were observed (data not shown – Figures A.2 and A.3 in the Appendix A).

To further investigate the effect of the leaching solution on UICC crocidolite fibres, HR-TEM images have been collected on fibres incubated in hydrogen peroxide buffered solution for one week (168 h), and pristine fibre was examined for comparison. Representative low- and high-resolution images are shown in Figures 6 and 7, respectively. The pristine sample shows bundle of long and thin fibres with size and morphology comparable with previous reports (see for example Gunter et al., 2007). The fibre bundle splits in several thinner fibres up to structure of two/three associated single-crystal fibrils (Fig. 6C). High-resolution image (Fig. 6D) clearly shows diffraction fringes arising from the well-ordered crystalline lattice of the amphibole, as witnessed by the presence of lattice fringes with \( d_{hkl} \) fully compatible with those of the crocidolite lattice (Table 4). In this respect, for instance, the spacing 4.876 Å evidenced in Figure 6D corresponds to the (-1 1 1) lattice planes (JCPDS card no. 19-1061). The dissolution experiments with \( \text{H}_2\text{O}_2 \) did not significantly alter the crocidolite even after 168 h: in fact, fibrous habit and high aspect ratio are indeed preserved after dissolution (Fig. 7A). The crystalline structure of treated crocidolite remains also largely unaltered as indicated
by the lattice fringes evidenced by HR-TEM (Fig. 7D and Table 4). This is consistent with
the well-known high persistence of amphibole structure. However, at higher magnifications,
the HR-TEM analysis shows the alteration the crocidolite underwent, because Fe-bearing
nanoparticles become clearly visible (Fig. 7 B–D). These nanoparticles are likely amorphous
and possibly displaced on top of the fibres, since the continuum of crocidolite fringes is
preserved throughout the new-formed particles.

4. DISCUSSION

The dissolution of UICC crocidolite leads to the release of Si, Mg and Ca from the mineral
bulk (Table 1). The amounts of released Si, Mg and Ca correspond to 2%, 4% and 17% of the
total content, respectively. On the contrary, release of Fe was not observed for any dissolution
time, likely due to the negligible solubility of iron in the adopted experimental conditions.
These results are in good agreement with those obtained via XPS on the fibre surface, where a
depletion of Si and Mg of treated fibres with respect to the untreated ones was observed
(together with a significant increase of Fe content up to ca. 30% relative (Table 2). Possible
explanations for this latter increase are that, in the chosen experimental conditions, Fe is not
released and therefore accumulates on the fibre surface or, in alternative, it is mobilized from
the fibre surface but immediately precipitates from the solution. In the second hypothesis, part
of the Fe(III) present in the oxidised external layer is supposed to have been mobilized and to
have immediately reacted with phosphate ions in the buffer solution, with consequent
precipitation on the fibre surface as iron phosphate, insoluble at neutral pH. This would lead
to an increase of iron phosphate with dissolution time paralleled by a decrease of Fe(III)
oxide-hydroxide, and is well supported by XPS results (Fig. 5).
Notably, the occurrence of iron phosphates together with remaining Fe(III) oxide-hydroxides
on fibre surfaces is well in agreement with the Fe-bearing nanoparticles observed by TEM
and with P traces also observed in TEM-EDS analysis. The occurrence of new phases during
the leaching process, also described for other asbestos minerals (e.g., chrysotile asbestos,
Turci et al., 2007), accounts for the observed presence of iron-rich nanoparticles and is
relevant to modulate asbestos toxicity (Favero-Longo et al., 2009). An increase of surface Fe
concentration was already reported in Seal et al. (1996, 1997) for asbestos in contact with
both cell-bearing and cell-free biological media. Moreover, the precipitation of Fe(III)
phosphate was observed during the interaction between Fe oxyhydroxide and orthophosphate
ions (Lijklema, 1980), and the formation of FeOOH-orthophosphate surface complexes
during silicate dissolution in neutral aqueous solutions was evidenced by Schott et al. (1983).

According to Person et al. (1996), the direct bond between surface metal iron and phosphate involves the formation of monodentate surface complexes. At pH 7.4 the most thermodynamically stable Fe(III) phosphate complexes are -FePO₄H⁻ and -FePO₄²⁻. The binding energy value of Fe-P signal observed in the present case (712.8 eV) is intermediate between BE values of Fe(II)-P and Fe(III)-P (712.3 and 713.8 eV, respectively) synthesised as glasses and characterized by XPS and ToF-SIMS (Crobu, 2012). The presence of a mixture of the two phosphates cannot be ruled out and the shift at higher BE might be also due to the particle sizes that according to the TEM results are in the nanometer range. On the other hand a possible contribution to Fe(II)–phosphate might also be due to the X-ray induced degradation of the iron phosphate (Crobu, 2012).

In minerals as well as mineral nanoparticles, the dissolution process promotes the interaction between bulk and surface, and this interaction may depend on particle size, morphology and surface structure (Echigo et al. 2012). The untreated UICC crocidolite fibres have the bulk enriched in Fe(II) with respect to the surface (Fe²⁺/Fetot ratios are 48% and 20%, respectively, Fantauzzi et al., 2010). On this basis, it can be claimed that the dissolution process promotes bulk Fe(II) sites to occur on the fibre surface. This is very relevant due to the recognised primary role of Fe(II) in the ROS production (Pacella et al., 2012 and references therein). In our experiments the dissolution of the fibres is particularly vigorous in the first hour (Table 1); the consequent promotion of Fe(II) sites on the fibre surface is maximum in the first hour and is likely faster than oxidation rate, as revealed by the abrupt increases of Fe(II) content at the surface (from ca. 20% to ca. 30% of the total Fe content, Figure 5). For longer dissolution time the two processes likely approach equilibrium, as the Fe(II) content on the surface remains almost constant and Fe(III) bonded to the silicate structure increases almost regularly (Figure 5). The subsequent formation of Fe oxide-hydroxide and precipitation of Fe phosphate may account for coating of the crocidolite fibres, which in turn may modulate asbestos toxicity or eventually represents one of the mechanisms of formation of ferruginous bodies, one of the key marker for the histopathological assessment of asbestosis in lung and surrounding human tissues (Pascolo et al. 2013).

In addition to precipitation of Fe phosphate, the precipitation of Ca phosphate on fibre surface may be invoked during dissolution of crocidolite, as suggested by combining the ICP and XPS data. In fact, the rate of Ca release significantly decreases after 24-48 h (Table 1), and a Ca2pₓ/₂ signal is measured starting from 24 h experiment (Table 2). The Ca-phosphate precipitation was proved by Lu and Leng (2005) to occur at circa-neutral pH in simulated
body fluids containing 1.6-2.5 mmol*dm$^{-3}$ (about 1/10 of the presently measured Ca amount).

According to thermodynamical data, at pH 7.4 octacalcium phosphate [OCP: $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_2 \cdot 5\text{H}_2\text{O}$] and hydroxyapatite [HA: $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$] may precipitate from simulated body fluids. The BE of calcium in our sample is in good agreement with BE of Ca in both HA and OCP (Chuesuei et al., 1999). In spite of the highest nucleation rate of OCP with respect to HA, it is interesting to note that the formation of HA as a secondary phase from dissolution of chrysotile and brucite was already observed under simulated lung conditions, without any assistance from cells (Taunton et al., 2010). In addition, ions such as Fe$^{3+}$, Fe$^{2+}$, Mg$^{2+}$, Na$^+$ and K$^+$ may be incorporated in Ca deficient hydroxyapatites (Morrisey et al., 2005), therefore the precipitation of HA in our conditions seems to be very likely.

5. CONCLUSIONS

In this study we have investigated by ICP-OES, XPS and HR-TEM the dissolution process and progressive surface modifications of UICC crocidolite fibres after suspension in a hydrogen peroxide solution buffered at pH 7.4 for 0.5 h, 1 h, 24 h, 48 h, 168 h (one week) and 1440 h (two months). The dissolution experiments were repeated with and without H$_2$O$_2$: after the first hour results did not show marked differences. The dissolution process evidenced two steps: 1) between zero and 48 h, representing the undersaturation conditions, favourable to fibre solubility; 2) after 48 h – and markedly after 168 h – representing the near-saturation conditions, where element release is very low or below the detection limit. Both undersaturated (initial) and close-to-saturation (steady state) dissolution rates were tentatively retrieved. Congruent dissolution of Si and Mg are observed in a proportion roughly corresponding to crocidolite stoichiometry (both bulk and surface), Ca is released in excess (possibly due to a contaminant impurity) and later precipitates, and Fe seems to be not released. Consequently, fibre surface chemistry is progressively enriched in Fe coming from the bulk and oxidized, firstly in the form of Fe(III) silicate, then in the forms of Fe(III) oxide-hydroxide and Fe phosphate. This latter is presumably a mix of Fe(III) and Fe(II) phosphates, even if a contribution to Fe(II) might also be a consequence of Fe(III) reduction under X-rays. A conceivable explanation is that bulk Fe(II) is oxidized to Fe(III) and then mobilized from the fibre surface, but immediately precipitates after reaction with phosphate ions in the buffer solution, with consequent precipitation on the fibre surface of a Fe phosphate, insoluble at neutral pH. In fact, crocidolite structure, fibrous habit and high aspect ratio are indeed preserved after dissolution, but a fibre coating enriched in Fe(III) oxide-hydroxide and Fe(III)
phosphate occurs and Fe-bearing nanoparticles are clearly visible on fibre surface after dissolution. This is highly relevant, because the process of coating of the fibres with Fe-rich phases is candidate to modulate asbestos toxicity and may eventually represent one of the mechanisms of formation of ferruginous bodies.

ACKNOWLEDGMENTS

J. Fournier is gratefully acknowledged for providing UICC crocidolite sample, G. Ferraris for measuring surface area by BET, and A. Cavallo for collecting FE-SEM images. GBA benefited of FARI funds from Sapienza University of Rome.

APPENDIX A. SUPPLEMENTARY DATA

REFERENCES

Avantage Software v. 3.45, Thermo Fisher Scientific Inc. – Micro Focus Ltd.


Captions to Figures

Figure 1: FE-SEM images of untreated UICC crocidolite fibres at increasing magnification: large, polygonal fibres as well as single fibrils (coming from partition of previous ones) are evident.

Figure 2: Dissolution of UICC crocidolite fibres in phosphate buffered solution at pH 7.4 with H$_2$O$_2$ in the range 0-1440 h: a) released Si; b) released Mg; c) released Ca (in this case the interval 0-168 h was plot to better highlight the Ca precipitation after 48 h).

Figure 3: Dissolution of UICC crocidolite fibres in phosphate buffered solution at pH 7.4: released Si and Ca vs. Mg. Data of solution with and without H$_2$O$_2$ are plot and no differences are observed.

Figure 4: XPS high-resolution spectra after background subtraction and curve fitting of Fe2p$_{3/2}$ peak of UICC crocidolite fibres after 0.5, 1, 24, 48 and 168 h of incubation in phosphate buffered solution at pH 7.4 with H$_2$O$_2$. Four components, Fe(II)–O (BE = 709.0 ± 0.2), Fe(III)–O (BE = 710.5 ± 0.2), Fe(III)–OOH (BE = 711.6 ± 0.2) and Fe–P (BE = 712.8 ± 0.2), have been resolved.

Figure 5: Quantitative evolution of the Fe2p$_{3/2}$ components (in % of Fe2p$_{3/2}$ total peak area) detected on the surface of the UICC crocidolite fibres after 0.5, 1, 24, 48 and 168 h of incubation in phosphate buffered solution at pH 7.4 with H$_2$O$_2$.

Figure 6: Representative TEM images of untreated UICC crocidolite fibre taken at increasing magnification. Fibre bundles and single asbestos fibrils are clearly visible in the low-to-medium magnification images (A, B and C). HR-TEM image (D) displays the highly ordered crystal lattice of the pristine fibre.

Figure 7: Representative TEM images of a UICC crocidolite fibre leached in phosphate buffered solution at pH 7.4 with H$_2$O$_2$ for 168 h. Fibre bundles and single asbestos fibrils are clearly visible in the low-magnification image (A). At higher magnification (B, C and D) the occurrence of a neo-formed nanoparticle is observed (white arrows). Though crocidolite
crystal lattice is preserved after dissolution (D), some possibly amorphous nanoparticle are visible on top of the asbestos fibre.

Figure A.1: XP-survey spectra.

Figure A.2: Surface composition obtained on fibres suspended in the solution with and without H₂O₂.

Figure A.3: Fe₂p₃/₂ components (in % of Fe₂p₃/₂ total peak area) obtained on fibres suspended in the solution with and without H₂O₂.
Table 1. Results of ICP-OES analyses of UICC Crocidolite fibres after incubation in phosphate buffered solution at pH 7.4 with and without H$_2$O$_2$ for 0.5, 1, 24, 48, 168 and 1440 h. Standard deviations (in brackets) were calculated over three measurements.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Mg (mg/kg)</th>
<th>Ca (mg/kg)</th>
<th>Si (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>Ca (mg/kg)</th>
<th>Si (mg/kg)</th>
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<tr>
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<td>877(67)</td>
<td>624(79)</td>
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<tr>
<td>1</td>
<td>224(17)</td>
<td>1082(46)</td>
<td>1110(107)</td>
<td>184</td>
<td>775</td>
<td>467</td>
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<tr>
<td>24</td>
<td>370(19)</td>
<td>1508(122)</td>
<td>3011(176)</td>
<td>331</td>
<td>1384</td>
<td>2977</td>
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<tr>
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<td>1531(121)</td>
<td>3772(37)</td>
<td>403</td>
<td>1470</td>
<td>3417</td>
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<tr>
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<td>1239(141)</td>
<td>4087(327)</td>
<td>447</td>
<td>1398</td>
<td>4066</td>
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<tr>
<td>1440</td>
<td>590(7)</td>
<td>866(25)</td>
<td>5120(72)</td>
<td>-</td>
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</table>
Table 2: Surface analysis of UICC crocidolite fibres by XPS before (t = 0 h) and after dissolution in phosphate buffered solution at pH 7.4 with H$_2$O$_2$ for 0.5, 1, 24, 48 and 168 h: atomic percentages of elements (at. %) for the various incubation times. In the lowest part of the Table the surface composition is recalculated considering only Si, Mg, Ca and Fe. Standard deviations (in brackets) were calculated over three measurements.

<table>
<thead>
<tr>
<th>Dissolution time (h)</th>
<th>Fe</th>
<th>O</th>
<th>Si</th>
<th>Na</th>
<th>Mg</th>
<th>Ca</th>
<th>P</th>
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Composition: Fe – Si – Ca – Mg (at. %)

<table>
<thead>
<tr>
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<th>Si</th>
<th>Mg</th>
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<tr>
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<td>5 (1)</td>
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</tbody>
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Data for t = 0 h and for bulk composition of UICC crocidolite (Na 4.1; Si 20.7; O 61.5; Fe 10.7; Mg 2.2; Al 0.1; Ca 0.7 at. %) are from Fantauzzi et al. (2010).
Table 3: Mean binding energy values of the most intense XPS signals of UICC crocidolite before (t = 0 h) and after dissolution in phosphate buffered solution at pH 7.4 with H₂O₂ for 0.5, 1, 24, 48 and 168 h. Standard deviations (in brackets) were calculated over three measurements.

<table>
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<th>Mg2p</th>
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<th>Na1s</th>
<th>P2p₃/₂</th>
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<th>Fe₂p₃/₂ Fe(III)-O</th>
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<td>--</td>
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<td>711.6 (0.2)</td>
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Data for t = 0 h are from Fantauzzi et al. (2010)
Table 4. HR-TEM data of UICC crocidolite before and after dissolution in phosphate buffered solution at pH 7.4 with H\textsubscript{2}O\textsubscript{2} for 168 h: calculated diffraction fringes distance and crystallographic planes assigned from Joint Committee on Powder Diffraction Standards (JCPDS) reference no. 19-1061

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Figure 1
Figure 2
Figure 3
Figure 4

![Graph showing binding energy over time with different markers for experimental and theoretical data points.](image)
Figure 5
Figure 6
Figure 7
Figure A.2
Click here to download Electronic Annex: Figure A.2.JPG