IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Free-radical chemistry as a means to evaluate lunar dust health hazard in view of future missions to the moon

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1560698 since 2021-02-04T15:47:51Z

Published version:

DOI:10.1089/ast.2014.1216

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

12 October 2024

UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on**:*

Questa è la versione dell'autore dell'opera:

Turci et al., Astrobiology, Volume 15, Issue 5, 1 May 2015, Pages 371-380*1155*

The definitive version is available at:

La versione definitiva è disponibile alla URL:

http://online.liebertpub.com/doi/abs/10.1089/ast.2014.1216

Free-radical chemistry as a means to evaluate lunar dust health hazard in view of future missions to the Moon

Francesco Turci[1,2,3,#], Ingrid Corazzari[1,2,#], Gabriele Alberto[1,3], Gianmario Martra[1,2,3], Bice Fubini[1,2,3, *]

[1] Dip. Chimica, University of Torino, via P. Giuria 7, 10125, Torino, Italy.

[2] "G. Scansetti" Interdepartmental Center, University of Torino, via P. Giuria 9, 10125, Torino, Italy.

[3] NIS Excellence Center, University of Torino, via Quarello 11, 10135, Torino, Italy.

[#] These authors equally contributed to the manuscript.

[*] Corresponding author at the Department of Chemistry, University of Torino, via P. Giuria 7, 10125, Torino, phone number: +390116707566, e-mail address: bice.fubini@unito.it

Authors' e-mail address: francesco.turci@unito.it; ingrid.corazzari@unito.it; gabriele.alberto@unito.it; gianmario.martra@unito.it; bice.fubini@unito.it

Running title

Free radicals and lunar dust health hazard

Authors' contribution

FT participated in the coordination of the study and contributed to the experimental design, the interpretation of the results, and the writing of the manuscript. IC contributed to the experimental design, carried out part of the experiments, analyzed the experimental results and contributed in writing the manuscript. GA carried out the fluorimetric assessment of terephthalate. GM participated in the experimental design, helped in analyzing the results and in writing the

manuscript. BF conceived and coordinated the study, helped in the interpretation of the data and in writing the manuscript. All the authors read and approved the final manuscript.

Abstract

Lunar dust toxicity has to be evaluated in view of future manned missions to the Moon. Previous studies on lunar specimens and simulated dusts have revealed an oxidant activity assigned to HO• release. However, the mechanisms behind the reactivity of lunar dust are still quite unclear at the molecular level. In the present study, a complementary set of tests - including TA hydroxylation, free radical release as measured by means of the spin trapping/EPR technique, and cell-free lipoperoxidation - are proposed to investigate the reactions induced by the fine fraction of a lunar dust analog (JSC-1A-vf) in biologically relevant experimental environments. Our study proved that JSC-1A-vf is able to hydroxylate TA also in anaerobic conditions, which indicates that molecular oxygen is not involved in such a reaction. Spin trapping/EPR measures showed that the HO• radical is not the reactive intermediate involved in the oxidative potential of JSC-1A-vf. A surface reactivity implying a redox cycle of phosphate-complexed iron via a Fe (IV) state is proposed. The role of this iron species was investigated by assessing the reactivity of JSC-1A-vf towards hydrogen peroxide (Fenton-like activity), formate ions (homolytic rupture of C-H bond), and linoleic acid (cell-free lipoperoxidation). JSC-1A-vf was active in all tests confirming that redox centers of transition metal ions on the surface of the dust may be responsible for dust reactivity and that the TA assay may be a useful field probe to monitor the surface oxidative potential of lunar dust.

Keywords: lunar dust, JSC-1A-vf lunar dust simulant, free radicals, terephthalate assay, Fenton activity, lipid peroxidation.

1 Introduction

The absence of a protective atmosphere exposes the Moon surface to the effects of cosmic and UV rays, and to frequent collisions with micrometeorites. As a result of such peculiar environmental conditions, the lunar surface is covered by a thin layer of fine dust. During the Apollo missions, several problems caused by lunar dust have been documented, mainly arising from abrasive and irritant behavior of the dust. Besides the detrimental effects on equipment and instruments, the astronauts reported transient eye, throat, and skin irritation after brief exposure to lunar dust (Khan-Mayberry, 2008). Because of the brevity of exposure, long-term health effects could not be observed, but prolonged inhalation of lunar dust may possibly yield similar outcomes on astronauts to those observed on workers exposed to pathogenic dusts such as crystalline silica or asbestos. Recently, several space agencies showed a renewed interest in robotic and manned missions to the Moon (Carpenter et al., 2010; Crawford et al., 2012, and references therein) and pointed out that more reliable information is required on the nature and intensity of the damage caused by lunar dust to astronauts and equipment. On Earth, the toxicity of a given kind of particle is assessed by cell free, cellular and animal tests whose results are confirmed by epidemiological evidence. Obviously this cannot be done on lunar dust. The assessment of the hazard posed by lunar dust must rely on both earthly studies performed on lunar dust analogs, and a few conceptually simple tests that might be performed *in situ* during unmanned missions. Such an approach would allow the consequent risk analysis and management during future missions to be performed on a reliable experimental base.

The consequences of exposure to lunar dust were consistently underestimated in the Apollo missions that landed on the lunar surface (Gaier, 2005). The most alarming was mucous irritation and lung inflammation following inhalation of lunar dust. A review of medical operations during Apollo missions reported on mucous membrane irritation that afflicted many crew members (Scheuring et al., 2008). In one case, respiratory symptoms progressively worsened with marked eosinophilia.

The potential toxicity of lunar dust has been investigated in vitro and in vivo by using a sample of real lunar dust from an Apollo mission and several lunar dust simulants made of terrestrial rocks (Latch et al., 2008; Jordan et al., 2009; Cain, 2010; Loftus et al., 2010; Krisanova et al., 2013; Lam et al., 2013). The mineral composition of lunar dust, which is mainly formed by vitreous and agglutinated particles, does not explain *per se* the detrimental effects observed on Apollo's crew. In fact, very little is known about the molecular mechanisms of action of lunar dust within the human body, potential toxicity of which is likely triggered by micrometeorite impacts, solar wind, and UV irradiation. These agents may enhance the surface reactivity of the particles by promoting formation of transient reactive sites (Wallace et al., 2009) as is the case on Earth with freshly ground quartz (Vallyathan et al., 1995; Fubini and Hubbard, 2003; Schwarze et al., 2006). Furthermore, the absence of atmospheric oxygen and water at the surface of the Moon prevents the annealing of such reactive sites and thus preserves the reactivity of lunar dust at the Moon's surface (Carpenter et al., 2010). As both Apollo samples and the lunar dust simulants studied so far have been exposed to the oxidative atmosphere of Earth, the reactivity of lunar dust at the Moon's surface is expected to be higher than that measured by tests on Earth.

The nature of reactive sites and their possible involvement in toxic responses has been previously discussed on the basis of the compositional and mineralogical analogies of lunar dust with other well-characterized toxic minerals (Wallace et al., 2009) or volcanic ashes (Linnarsson et al., 2012). In these studies, the reactivity of lunar dust was mainly explained by the presence of dangling bonds and/or redox-reactive iron centers (Fubini et al., 1995b; Horwell et al., 2010). When lunar dust comes in contact with cells and tissues, these active sites may react with biomolecules, and ROS (Reactive Oxygen Species) may be generated both within cells and on the particle surface. ROS – namely, hydroxyl, hydroperoxyl, and superoxide– release is currently considered the cause of oxidative damages induced by some thoroughly investigated toxic inorganic particles and fibers, including quartz (Fubini, 1998), asbestos (Fubini et al., 1995a; Kamp and Weitzman, 1999; Turci et al., 2011), sintered indium-tin oxide (Lison et al., 2009), and hard metals (Lison et al., 1995). To

better protect the astronauts' health and to develop a more efficient cleaning procedure for extra vehicular activity (EVA), new and more informative tests about the surface reactivity of lunar micro- and nano-particulate are highly desirable. The hydroxylation of the non-fluorescent terephthalate (TA) probe into its fluorescent derivative (2-hydroxyterephthalate, TA-OH) was proposed as a feasible method to quantify *in situ* the radical-mediated oxidative potential of lunar dust during manned or unmanned missions (Wallace et al., 2009; Loftus et al., 2010). However, the chemical nature of the reactive species that activates the terephthalate assay and the mechanism of the terephthalate oxidation by lunar dust is not yet clear. To understand what the TA assay actually measures, the present study reports a detailed description of the reaction mechanisms induced by lunar dust analog on several assays and proposes some informative tests to be adopted to measure the reactivity of dust *in situ*.

Commento [FT1]: I think "are" is correct, since the subjects are the chemical nature of the reactive species AND the mechanism of.

Due to the low availability of real lunar dust, the very fine fraction (grain size < 20 μm) of the lunar dust simulant JSC-1A from NASA Johnson Space Center has been used here, which is a basaltic ash with high glass content that is chemically close to the soil of the lunar *maria* (Ray et al., 2010). Measurements concerned two complementary approaches: i) the quantitative assessment of the hydroxylation of terephthalate (TA), and ii) the qualitative/quantitative measurement of surface-induced free radicals by the EPR/spin trapping technique. To evaluate the role of molecular oxygen in the hydroxylation of TA, the assay was also run in an inert atmosphere. The outcomes of the TA assay were compared with spin-trapping results on free radicals generated by other means on aqueous suspension of JSC-1A-vf with specific biological targets and in the presence of molecular oxygen (hydrogen peroxide, formate ion, and a fatty acid). According to a previously established procedure (Turci et al., 2011; Turci et al., 2013; Le Blond et al., 2014), a reducing agent (ascorbic acid) was added in physiological concentration (Cross et al., 1994) and used to mimic the reduction of metal ions likely to occur *in vivo* when inhaled particles get in contact with the lung-lining fluid.

2 Materials and methods

2.1 Lunar Dust simulant

The lunar dust simulant JSC-1A-vf employed for this study was kindly provided by the NASA Johnson Space Center, Houston, Texas (USA) and consists of the very fine fraction (< 20 μm) of JSC 1A, a lunar simulant extracted from the Merriam Crater near Flagstaff, Arizona (AZ, US). The mineralogical and chemical composition of JSC-1A-vf is similar to that of the well-characterized JSC 1A (Hill et al., 2007; Ray et al., 2010); it is made of a basaltic tuff ash with a relatively high glassy content (ca. 50 wt. %) (Liu et al., 2008) and is chemically similar to the lunar soil of the *maria*. (Wallace et al., 2009) The chemical composition of JSC-1A-vf is very close to that of the actual lunar soil collected by Apollo 17. Particularly, the total amount of iron in both dusts is nearly the same. However, the actual lunar dust contains Fe^{2+} and metallic iron (np-Fe^0) distributed as nano-sized domains in the glassy matrix, while no iron (III) is present. JSC-1A-vf contains Fe^{2+} (ca. 76% of the total iron amount) and Fe^{3+} (ca. 24% of the total iron), but not np-Fe^0 (Wallace et al., 2009). Since iron is involved in many redox reactions, this discrepancy between simulant and actual lunar sample may possibly alter some reactivity results.

2.2 Reagents

5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Alexis (Lausen, Switzerland); Disodium terephthalate (99 % purity) was purchased from Alfa Aesar (Ward Hill, MA). The other reagents employed were from Sigma-Aldrich (St. Louis, MO – USA). In all experiments ultrapure MilliQ (Millipore, Billerica, MA – USA) water was used.

2.3 Fluorimetric determination of hydroxyl radicals by sodium terephthalate assay

Terephthalate solution was prepared by dissolving disodium terephthalate in 0.01 M phosphate-buffered saline (PBS, pH 7.4) to produce a final terephthalate concentration of 10 mM following the method proposed by Wallace et al. (2009). JSC-1A-vf was suspended in TA solution (15 mg of

dust/ml) and incubated for 30 min at 25 °C under continuous stirring. In some experiments, ascorbic acid (AA, 3 mM) was added to the reaction mixture. After incubation, the suspensions were filtered with cellulose acetate membrane (pore diameter 0.20 μm), and the fluorescence of the dust-free solutions was measured with a Horiba Jobin-Yvon Fluorolog 3 TCSPC spectrofluorimeter equipped with a 450-W Xenon lamp and a Hamamatsu R928 photomultiplier, collecting photoemission steady-state spectra by using an excitation light λ_{ex} = 324 nm and reading the maximum emission intensity at λ_{em} = ca. 425 nm.

Some experiments were carried out in an oxygen-free environment, performing all of the procedure described above in a N_2-fluxed glove bag (positive pressure of ca. 1 bar, gas purity > 99.9995%). For this experiment, the PBS solution employed to prepare the TA solution and the JSC-1A-vf dust were previously deoxygenated by vigorous bubbling N_2 for 2 h within the glove-bag.

2.4 EPR/spin trapping

The generation of free radicals was monitored by electron paramagnetic resonance (EPR) spectroscopy with a Miniscope MS 100 (Magnettech, Berlin, Germany) EPR spectrometer employing DMPO as spin trapping agent. The instrument settings were as follows: microwave power 10 mW; modulation 1000 mG; scan range 120 G; center of field approximately 3345 G. In all cases, the suspensions obtained (see below) were under continuous stirring during the experiments, and aliquots of 50 μl were withdrawn after 10, 30, and 60 minutes of incubation, and the EPR spectra were recorded on the suspensions. Blanks were performed in parallel in the absence of dust.

2.4.1 Generation of oxygen centered radicals

JSC-1A-vf was suspended (15 mg/ml) in a DMPO (30 mM) buffered solution (potassium phosphate buffer, KPB, 0.1 M pH 7.4. The experiments were performed in triplicate. As a positive control for ROS generation, a TiO_2 (Aeroxide®, Evonik Industries, Essen, Germany) suspension was irradiated

with a simulated solar light under the same experimental conditions (Corazzari et al., 2012). The irradiation of TiO_2 suspension was performed with a 500 W mercury/xenon lamp (Oriel instruments) equipped with an IR water filter. Simulated solar light was obtained by applying a 400nm cut-off filter. This filter let to pass about 5% of UV light in the UV A region.

2.4.2 Surface-driven Fenton reactivity (target molecule H_2O_2)

JSC-1A-vf was suspended in a H_2O_2 (0.04 M) buffered solution (potassium phosphate buffer, KPB 125 mM, pH 7.4) at two different doses (15 mg/ml and 45 mg/ml) in the presence of DMPO (0.075 M) as spin-trapping agent. The experiments were performed in triplicate.

2.4.3 Generation of carbon centered radicals (target molecule formate ion)

JSC-1A-vf was suspended in a sodium formate (1 M) buffered solution (KPB, 250 mM, pH 7.4) at two different doses (15 mg/ml and 45 mg/ml) in the presence of DMPO (0.075 M) as spin-trapping agent. The experiment was carried out in the same conditions adding ascorbic acid (3 mM) to the reactive mixture. The experiments were repeated at least twice. To assess the reactivity of solubilized ions, the generation of carbon centered radicals was also tested on the supernatant: 70 mg of dust was suspended in 1 ml of ascorbic acid (3 mM) buffered solution (potassium phosphate buffer, KPB 500 mM, pH 7.4) and incubated at 25 °C for 1 h under continuous stirring. The suspension was centrifuged (13 000 RPM) for 30 minutes, and the supernatant, which was collected with a syringe, was filtered (cellulose acetate filters, cut off 0.20 μm) to remove any residual dust particles. A quantity of 0.5 ml of this solution was mixed with 0.5 ml of DMPO (0.15 M) and formate (0.4 M) solution. The mixture was continuously stirred. After 1, 10, and 30 minutes of incubation at 25 °C, 50 μl of the mixture was withdrawn and the EPR spectra recorded. The number of radicals released is proportional to the intensity of the EPR signal. The signals were double integrated, and the values obtained expressed as arbitrary units. Blanks were performed in parallel in the absence of dust. All the experiments were carried out in triplicate.

2.5 Oxidative degradation of linoleic acid (TBA assay)

The TBA assay, commonly used as an index of lipoperoxidation, is based on the reaction between malondialdehyde (MDA), a colorless product of lipid peroxidation, and tiobarbituric acid (TBA) to produce a pink adduct absorbing at 535 nm. All the reagents were from Sigma-Aldrich (St. Louis, MO, USA). MilliQ ultrapure water (Millipore, Billerica, MA, USA) was used.

JSC-1A-vf (15 or 45 mg) was suspended in 2 ml of a buffered (sodium phosphate buffer 5 mM, pH 7.4) emulsion of linoleic acid (1 mM) containing the 2.5 % w/w of ethanol. The suspensions were stirred in the dark at 37 °C for 72 h. The lipid peroxidation was stopped by adding 0.1 ml of an ethanolic solution of butyl hydroxyl toluene (BHT, 0.2 % w/w) to the suspensions. The dust was removed by centrifugation and filtration (cellulose acetate filters, cut off 0.20 μm). A quantity of 2 ml of a solution of TBA (0.034 M) containing HCl (0.25 M) and trichloroacetic acid (TCA, 0.92 M) was added to 1 ml of the supernatants, and the resulting solutions were stirred for 30 minutes. After cooling in an ice bath, 3 ml of 1-butanol was added to extract the colored complexes. The absorbance at 535 nm was measured on the organic phase by means of UV/Vis spectrophotometry (Uvikon, Kontron Instruments, Inc., Everett, MA). The same experiment was carried out adding ascorbic acid (3 mM) to the reactive mixtures. All experiments were performed in triplicate.

3 Results

3.1 The role of molecular O_2 in TA assay

The commonly reported mechanism of TA hydroxylation involves oxygen-mediated addition of a hydroxyl radical yielding TA-OH. Some authors, however, support mechanisms that do not involve molecular oxygen and ROS (Youngman and Elstner, 1981; Saran et al., 2000). Results of the two parallel experiments (with and without molecular oxygen) are reported in Figure 1. JSC-1A-vf was able to hydroxylate TA in both cases, and almost identical results were obtained. The absence of any role of molecular oxygen posed the puzzling question: what is the oxidizing agent in the TA assay? The use of the spin trapping technique and EPR spectroscopy allowed further clarification of JSC-1A-vf reactivity. DMPO was added to an aqueous suspension of JSC-1A-vf as a spin-trapping reagent. Under this condition, no EPR signal was observed (Figure 2). To support the selectivity and sensitivity of the adopted approach to •OH radical, a well-known reaction to generate •OH in solution was used as previously described (Corazzari et al., 2012). A buffered suspension of TiO_2, dosed at the same concentration as JSC-1A-vf, was irradiated with a solar lamp light ($\lambda > 400$ nm; UV ca. 5%) and used as positive control. The lack of ROS in the reaction mechanism of JSC-1A-vf towards TA was further confirmed by following the reaction yield at different pH values (Figure S1, in Supporting Information). The maximum yield was observed for pH between 6 and 7, while it was almost negligible outside this narrow range. This pH-dependent behavior supports the hypothesis of a reaction driven by iron-phosphate complexes, which are thermodynamically stable in a defined range of pH values.

3.2 Radical reactivity of JSC-1A-vf toward biologically-relevant molecules

In the first test (Fenton-like reaction), H_2O_2 was allowed to interact with redox–reactive transition metal ions at the particle surface (generally, iron) and the generation of •OH radicals followed (Eqn. 1).

$$Fe^{2+}_{surf} + H_2O_2 \longrightarrow Fe^{3+}_{surf} + OH^- + \bullet OH \qquad (1)$$

The release of •OH radicals in the presence of H_2O_2 mimics the reaction that might take place when a particle is exposed to the oxidative environment of lysosomal fluid following phagocytosis by macrophages and polymorphonucleated cells (PMN) (Gazzano et al., 2007). By using DMPO as a spin trapping agent, the capability of JSC-1A-vf (15 mg/ml) to generate •OH in the presence of H_2O_2 was assessed and the representative EPR signal of [DMPO-HO]• adduct recorded and reported in Figure 3. Increasing the quantity of dust (35mg/ml) did not significantly increase the amount of generated •OH (data not shown). This is in agreement with the catalytic role of transition metal ions in driving the Fenton reaction toward H_2O_2.

The second radical reaction investigated followed the cleavage of the C-H bond in the formate ion, which can be taken as a model reaction that may occur with several molecules of biological interest such as peptides, proteins, and lipids. Such a reaction yields on JSC-1A-vf the formation of a carbon-centered radical $^{\bullet}CO_2^-$. In the presence of a reactive dust, a direct surface-assisted homolytic cleavage possibly occurs on redox-reactive transition metal (e.g. iron, copper) exposed at the particle surface. Alternatively, or even complementarily, molecular oxygen can be reduced to superoxide anion by Fe^{2+}, initiating a ROS-mediated mechanism sustained by Haber-Weiss cycle (Turci et al., 2011). The carboxyl radical is stabilized by resonance, and it is the only radical species that can practically be detected via the spin-trapping technique under such experimental conditions (i.e., relatively high concentration of HCO_2^- and neutral pH). At both low (15 mg/ml) and high (35 mg/ml) JSC-1A-vf doses, the incubation of the dust with formate anion yields the typical six line signal of [DMPO-CO_2^-]• adduct, which indicates a substantial release of •CO_2^- (Figure 4A). The same experiments were carried out in the absence of the dust (blank solution), and no EPR signal was detected at each time point (see Figure S2). The signals were double integrated and the

Commento [FT2]: I think present tense is more appropriate here, since the test is actually used to follow the cleavage of CH bond in many other different experiments.

intensity reported as the mean values (± SE) of two separated experiments. A clear time- and dose-dependent increase in the intensity of the EPR signal was observed (Figure 4B).

The potential of JSC-1A-vf to induce radical oxidative degradation of biomolecules was also assessed by evaluating its potency to induce cell-free lipid peroxidation. Linoleic acid was used as a model for polyunsaturated fatty acid that naturally composes the cell membrane. Thiobarbituric acid (TBA) assay was used to quantify the linoleic peroxidation reaction. JSC-1A-vf was suspended at low and high dose (15 and 35 mg/ml) to evaluate the dose-dependency of the reaction. The oxidative degradation of linoleic acid upon contact with JSC-1A-vf is reported in Figure 5. The amount of MDA produced evolves in a dose-dependent manner, with the higher dose being able to degrade a larger amount of linoleic acid. In our experimental conditions, MDA production was monitored by UV-Vis spectrophotometry. However, similarly to the TA assay, MDA production in the lipoperoxidation assay can also be detected by fluorescence spectroscopy (Gutteridge and Halliwell, 1990).

3.3 Effect of ascorbic acid on JSC-1A-vf reactivity

Inhaled particles primarily interact with the lung lining layer fluid, which includes surfactants, proteins, and important components of the antioxidant defenses of cells such as ascorbic acid and glutathione (Cantin et al., 1989; Bui et al., 1992). Ascorbic acid (AA) is one of several metabolites that can act as a reducing agent *in vivo*. It was previously observed that ascorbic acid, once in contact with asbestos and other toxic particulates, reduces surface Fe^{3+} to Fe^{2+}, which allows radical generation to take place (Martra et al., 2003; Turci et al., 2011). The effect of AA on JSC-1A-vf reactivity markers, including TA hydroxylation, C-H bond cleavage, and linoleic acid peroxidation, was investigated.

3.3.1 Effect of ascorbic acid on the hydroxylation of TA

The redox state of surface iron in the iron-rich minerals of JSC-1A-vf is likely oxidized, and the addition of AA to the suspension promptly promotes the reduction of surface Fe^{3+} to Fe^{2+}, which in turn could affect the hydroxylation of TA to HTA. The hydroxylation of TA quantitatively depends on the concentration of Fe^{2+} in the case of homogeneous reaction (Saran et al., 2000). The fluorescence intensity recorded on the supernatant of a suspension of JSC-1A-vf in the presence of TA and AA was compared to that obtained in the same experimental conditions but in the absence of the antioxidant (Figure 6). The fluorescence of the two blank solutions without JSC-1A-vf is also reported (blank). The results show that the hydroxylation of TA by JSC-1A-vf + AA was two-fold higher than in the absence of AA, confirming that surface-exposed Fe^{2+} is involved in the reactivity of JSC-1A-vf toward TA and its oxidation state may modulate the reaction yield.

3.3.2 Effect of ascorbic acid on the cleavage of C-H bond

Previous studies on asbestos have shown that in the presence of ascorbic acid the potency to induce a homolytic C-H rupture in the formate ion is enhanced. Surface iron sites are activated following reduction by ascorbic acid and become the active center for the release of carbon-centered radicals (Martra et al., 2003; Tomatis et al., 2010). To explore the occurrence of such redox sites in JSC-1A-vf, ascorbic acid (3 mM) was added to the buffered suspension of JSC-1A-vf containing the formate anion and the spin trapping (DMPO). Figure 7 reports the EPR signals recorded in the absence (spectrum a) or in the presence (spectrum b) of AA. A much more intense signal recorded for JSC-1A-vf + AA is reported. This result strongly suggests the presence of a reducible redox center at the mineral surface.

3.4 Effect of ascorbic acid on linoleic acid peroxidation

To give further evidence of the role of redox-centers in the reactivity of JSC-1A-vf, the dust was suspended (15 mg/ml) in a buffered linoleic acid in the presence of AA (3 mM). The results are

reported in Figure 8. After incubation, the amount of MDA produced by the oxidative reaction promoted by JSC-1A-vf was determined on the supernatant (JSC-1A-vf + AA) and compared to the data obtained in the same conditions, but in the absence of ascorbic acid (JSC-1A-vf). Autoxidation was monitored on linoleic acid suspensions (no dust) with ascorbic acid (blank + AA) and without ascorbic acid (blank). The data indicate that the amount of MDA produced when the JSC-1A-vf is in the presence of AA is two-fold higher than in its absence. This result is consistent with that observed with the formate ion, suggesting that iron centers are also involved in lipid peroxidation.

4 Discussion

The comparison of the reactivity of JSC-1A-vf towards TA performed in aerobic and anaerobic conditions clarifies the role of molecular oxygen and ROS in the reaction mechanism. When TA hydroxylation is performed in a homogeneous phase under aerobic conditions, the reaction yield is usually higher than under anaerobic conditions (Charbouillot et al., 2011), which highlights the role of molecular oxygen in the reaction mechanism. In this study, however, our twin experiments (under O_2 or N_2 atmosphere, Figure 1) show the same hydroxylation yield, which supports a molecular mechanism of the reaction of JSC-1A-vf towards TA that does not involve dissolved oxygen, likely indicating that hydroxylation of TA by JSC-1A-vf is not ROS-mediated. In fact, the generation of ROS in the absence of molecular O_2 requires a radical mechanism based on the homolytic cleavage of the O-H bond in molecular water. Such reaction requires, in turn, the use of high-energy sources, usually electromagnetic or acoustic radiation (Baldacchino, 2008 and Cravotto and Cintas, 2006), and is not compatible with our heterogeneous solid/liquid system. The experimental evidence of the absence of ROS (including •OH, $•O_2^-$ / HOO•) was provided by the spin trapping/EPR measure (Figure 2) carried out on the buffered suspension of JSC-1A-vf. If not mediated by ROS in solution, the observed oxidation of TA can likely be assisted by the formation of iron-phosphate complexes, for example, $[(P_2O_7)_2Fe^{III}(H_2O)]^{5-}$, which may act under our experimental design as an oxidizing species going to $[(P_2O_7)_2Fe^{III}(OH)]^{6-}$ via ferryl-state Fe^{IV}

(Melton and Bielski, 1990). Such complexes may form either adsorbed on the particle surface or free in solution. Our hypothesis is supported by the ability of some low-molecular inorganic complexes to hydroxylate aromatic compounds (Manevich et al., 1997). A similar mechanism, involving an iron-complex in an activated ferryl-state, was proposed in some pioneering works on the formation of radical species from the active surface sites of asbestos (Zalma et al., 1987; Pezerat, 1991). The role played by such iron species when JSC-1A-vf enters in contact with cells and tissues was investigated employing some simple models of biologically relevant molecules (i.e., H_2O_2, formate ion and linoleic acid; Figure 3, 4, and 5, respectively). The reactivity of JSC-1A-vf towards H_2O_2 was evidenced by the production of •OH in accordance with a Fenton mechanism with the iron playing a catalytic role. The reactivity of JSC-1A-vf towards the C-H bond of the formate anion occurred in a time- and dose-dependent manner, further confirming that the iron ions involved in this specific reaction are not involved in a catalytic cycle.

In the presence of reducing agents, such as ascorbic acid (AA), redox active transition metal ions (TMI) are easily reduced, and their reactivity may be enhanced. Similar to what is observed for other minerals with redox-active TMI (e.g. asbestos – Tomatis et al. 2010; Turci et al., 2013), the reactivity of JSC-1A-vf towards C-H bond in the presence of AA is largely increased with respect to experiments carried out in a non-reducing environment (phosphate buffer only). Such an increased reactivity indicates that redox-active TMI – likely iron – at the surface of JSC-1A-vf minerals are easily, reduced and their radical reactivity is enhanced. To discriminate between the role of surface-incorporated iron ions and the ions possibly leached out during the reaction, the spin trapping test was performed also on the dust-free, clear supernatant possibly containing leached ions. The lack of any EPR signal when DMPO is added to the buffered supernatant of JSC-1A-vf + AA rules out the presence of any radical species in solution (see Figure S2, in Supporting Information). This directly confirms that the reactivity of JSC-1A-vf is due to the occurrence of ions embedded on the mineral surface. The experiments with linoleic acid (TBA assay) carried out in the

reducing environment (+AA) showed the enhancement of the reactivity of the JSC-1A-vf, thus confirming the reaction mechanism evidenced by EPR-spin trapping.

In the lunar environment, where strongly reducing conditions due to the proton implantation from solar wind (Housley et al., 1974) exist, the reactivity of the dust may be increased as a consequence of this behavior, and hazardous particulate may be formed. To monitor the hazard and mitigate the exposure risk to potentially toxic lunar dust, in situ experiments, including TA and TBA assay, could be conjugated taking advantage of a shared analytical setup. A more informative and reliable simultaneous multi-technique assessment of the oxidative potential of lunar dust could thus be delivered.

5 Conclusions

Monitoring the oxidative potential of lunar dust by *in situ* experiments must be part of a comprehensive lunar exploration program. The terephthalate (TA) assay, once compared with results obtained with the EPR spin trapping technique, appears to be an easy-to-perform method to assess the overall oxidative potential of lunar dust for experiments at the Moon surface. Opposite to what has been previously reported, the mechanism of hydroxylation of the TA by JSC-1A-vf does not require the •OH radical as an intermediate. Redox centers of transition metal ions, likely iron, on the surface of the dust may be responsible for such reactivity, as indicated by the following findings: i) the molecular O_2 is not required for the hydroxylation of TA in the presence of JSC-1A-vf, ii) the Fenton-like reactivity occurs when H_2O_2 is added to JSC-1A-vf suspension, and iii) the reduction of redox centers with ascorbic acid enhances the hydroxylation of TA, the cleavage of C-H bond, and the peroxidation of linoleic acid. These results lead us to conclude that an *in situ* study may gain advantage from an integrated approach of various tests employing different molecular target models and can provide a reliable approach to the evaluation of surface reactivity-related health hazards of lunar dust.

Commento [FT3]: I think the singular form is more appropriate.

Acknowledgments

This study was conceived within the meetings in subsequent years of the Topical Team for the Toxicity of Lunar Dust (T3LD), organized by the European Space Agency (ESA) in 2010, which besides other activities gave rise to one of the paper largely cited here (Linnarson et al., 2013). The results reported in this paper were discussed among the group, thus the authors are indebted to the whole team and particularly to professor Dag Linnarson. Authors are grateful to the anonymous Reviewers for their constructive criticisms and suggestions. We are indebted to Ms. Rebecca Walker for revising the English text of this article.

Authors Disclosure Statement

The authors declare that they have no competing interests.

References

Baldacchino, G. (2008) Pulse radiolysis in water with heavy-ion beams. A short review. *Radiat Phys Chem* 77:1218-1223.

Bui, M.H., Sauty, A., Collet, F., Leuenberger, P. (1992) Dietary vitamin C intake and concentrations in the body fluids and cells of male smokers and nonsmokers. *J Nutr* 122:312-316.

Cain, J.R. (2010) Lunar dust: The Hazard and Astronaut Exposure Risks. *Earth Moon Planets* 107:107-125.

Cantin, A.M., Hubbard, R.C., Crystal, R.G. (1989) Glutathione Deficiency in the Epithelial Lining Fluid of the Lower Respiratory-Tract in Idiopathic Pulmonary Fibrosis. *Am Rev Respir Dis* 139:370-372.

Carpenter, J.D., Angerer, O., Durante, M., Linnarson, D., Pike, W.T. (2010) Life Sciences Investigations for ESA's First Lunar Lander. *Earth Moon Planets* 107:11-23.

Charbouillot, T., Brigante, M., Mailhot, G., Maddigapu, P.R., Minero, C., Vione, D. (2011) Performance and selectivity of the terephthalic acid probe for (OH)-O-center dot as a function of temperature, pH and composition of atmospherically relevant aqueous media. *J Photoch Photobiol A* 222:70-76.

Corazzari, I., Livraghi, S., Ferrero, S., Giamello, E., Fubini, B., Fenoglio, I. (2012) Inactivation of TiO2 nano-powders for the preparation of photo-stable sunscreens via carbon-based surface modification. *J Mat Chem* 22:19105-19112.

Cravotto, G., Cintas, P. (2006) Power ultrasound in organic synthesis: moving cavitational chemistry from academia to innovative and large-scale applications. *Chem Soc Rev* 35:180-196.

Crawford, I.A., Anand, M., Cockell, C.S., Falcke, H., Green, D.A., Jaumann, R., Wieczorek, M.A. (2012) Back to the Moon:The scientific rationale for resuming lunar surface exploration. *Planet Space Sci* 74:3-14.

Cross, C.E., Vandervliet, A., Oneill, C.A., Louie, S., Halliwell, B. (1994) Oxidants, Antioxidants, and Respiratory-Tract Lining Fluids. *Environ Health Persp* 102:185-191.

Fubini, B. (1998) Surface chemistry and quartz hazard. *Ann Occup Hyg* 42:521-530.

Fubini, B., Bolis, V., Cavenago, A., Volante, M. (1995a) Physicochemical properties of crystalline silica dusts and their possible implication in various biological responses. *Scand J Work Environ Health* 21 (Suppl 2):9-14.

Fubini, B., Hubbard, A. (2003) Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. *Free Radic Biol Med* 34:1507-1516.

Fubini, B., Mollo, L., Giamello, E. (1995b) Free radical generation at the solid/liquid interface in iron containing minerals. *Free Radic.Res.* 23:593-614.

Gaier, J.R. (2005) The Effects of Lunar Dust on EVA Systems During the Apollo Missions. NASA/TM—2005-213610 *http://gltrs.grc.nasa.gov*

Gazzano, E., Turci, F., Foresti, E., Putzu, M.G., Aldieri, E., Silvagno, F., Lesci, I.G., Tomatis, M., Riganti, C., Romano, C., Fubini, B., Roveri, N., Ghigo, D. (2007) Iron-loaded synthetic chrysotile: a new model solid for studying the role of iron in asbestos toxicity. *Chem Res Toxicol* 20:380-387.

Gutteridge, J.M.C., Halliwell, B. (1990) The measurement and mechanism of lipid-peroxidation in biological-systems. *Trends Biochem Sci* 15:129-135.

Hill, E., Mellin, M.J., Deane, B., Liu, Y., Taylor, L.A. (2007) Apollo sample 70051 and high- and low-Ti lunar soil simulants MLS-1A and JSC-1A: Implications for future lunar exploration. *J Geophys Res-Planet* 112 (E2).

Horwell, C.J., Stannett, G.W., Andronico, D., Bertagnini, A., Fenoglio, I., Fubini, B., Le Blond, J.S., Williamson, B.J. (2010) A physico-chemical assessment of the health hazard of Mt. Vesuvius volcanic ash. *J Volcanol Geoth Res* 191:222-232.

Housley, R.M., Cirlin, E.H., Paton, N.E., Goldberg, I.B. (1974) Solar wind and micrometeorite alteration of the lunar regolith in *5th Lunar Science Conference*. Pergamon Press, Houston, TX, pp. 2623-2642.

Jordan, J.A., Verhoff, A.M., Morgan, J.E., Fischer, D.G. (2009) Assessing the in vitro toxicity of the lunar dust environment using respiratory cells exposed to Al_2O_3 or SiO_2 fine dust particles. *In Vitro Cell Dev-An* 45:602-613.

Kamp, D.W., Weitzman, S.A. (1999) The molecular basis of asbestos induced lung injury. *Thorax* 54:638-652.

Khan-Mayberry, N. (2008) The lunar environment: Determining the health effects of exposure to moon dusts. *Acta Astronaut* 63:1006-1014.

Krisanova, N., Kasatkina, L., Sivko, R., Borysov, A., Nazarova, A., Slenzka, K., Borisova, T.(2013). Neurotoxic Potential of Lunar and Martian Dust: Influence on Em, Proton Gradient, Active Transport, and Binding of Glutamate in Rat Brain Nerve Terminals. *Astrobiology* 13:679-692.

Lam, C.W., Scully, R.R., Zhang, Y., Renne, R.A., Hunter, R.L., McCluskey, R.A., Chen, B.T., Castranova, V., Driscoll, K.E., Gardner, D.E., McClellan, R.O., Cooper, B.L., McKay, D.S., Marshall, L., James, J.T. (2013). Toxicity of lunar dust assessed in inhalation-exposed rats. *Inhal Toxicol* 25:661-678.

Latch, J.N., Hamilton, R.F., Holian, A., James, J.T., Lam, C.W. (2008). Toxicity of lunar and Martian dust simulants to alveolar macrophages isolated from human volunteers. *Inhal Toxicol* 20:157-165.

Le Blond, J.S., Tomatis, M., Horwell, C.J., Dunster, C., Murphy, F., Corazzari, I., Grendene, F., Turci, F., Gazzano, E., Ghigo, D., Williamson, B.J., Oppenheimer, C., Fubini, B. (2014) The surface reactivity and implied toxicity of ash produced from sugarcane burning. *Environ Toxicol* 29 (5):503-516.

Linnarsson, D., Carpenter, J., Fubini, B., Gerde, P., Karlsson, L.L., Loftus, D.J., Prisk, G.K., Staufer, U., Tranfield, E.M., van Westrenen, W. (2012). Toxicity of lunar dust. *Planetary Space Sci* 74 (1):57-71.

Lison, D., Carbonnelle, P., Mollo, L., Lauwerys, R., Fubini, B. (1995) Physicochemical mechanism of the interaction between cobalt metal and carbide particles to generate toxic activated oxygen species. *Chem Res Toxicol* 8:600-606.

Lison, D., Laloy, J., Corazzari, I., Muller, J., Rabolli, V., Panin, N., Huaux, F., Fenoglio, I., Fubini, B. (2009) Sintered Indium-Tin-Oxide (ITO) Particles: A New Pneumotoxic Entity. *Toxicol Sci* 108:472-481.

Liu, Y., Schnare, D.W., Eimer, B.C., Taylor, L.A. (2008) Dry separation of respirable lunar dust: Providing samples for the lunar airborne dust toxicity advisory group. *Planetary Space Sci* 56:1517-1523.

Loftus, D.J., Rask, J.C., McCrossin, C.G., Tranfield, E.M. (2010) The Chemical Reactivity of Lunar Dust:From Toxicity to Astrobiology. *Earth Moon Planets* 107:95-105.

Manevich, Y., Held, K.D., Biaglow, J.E. (1997) Coumarin-3-carboxylic acid as a detector for hydroxyl radicals generated chemically and by gamma radiation. *Radiat Res* 148:580-591.

Martra, G., Tomatis, M., Fenoglio, I., Coluccia, S., Fubini, B. (2003) Ascorbic acid modifies the surface of asbestos: possible implications in the molecular mechanisms of toxicity. *Chem Res Toxicol* 16:328-335.

Melton, J.D., Bielski, B.H.J. (1990) Studies of the kinetic, spectral and chemical-properties of Fe(IV) pyrophosphate by pulse-radiolysis. *Radiat Phys Chem* 36:725-733.

Pezerat, H. (1991) The Surface-Activity of Mineral Dusts and the Process of Oxidative Stress. *Mechanisms in Fibre Carcinogenesis* 223:387-395.

Ray, C.S., Reis, S.T., Sen, S., O'Dell, J.S. (2010). JSC-1A lunar soil simulant: Characterization, glass formation, and selected glass properties. *J Non-Cryst Solids* 356:2369-2374.

Saran, M., Michel, C., Stettmaier, K., Bors, W. (2000) Arguments against the significance of the Fenton reaction contributing to signal pathways under in vivo conditions. *Free Radical Res* 33:567-579.

Scheuring, R.A., Jones, J.A., Novak, J.D., Polk, J.D., Gillis, D.B., Schmid, J., Duncan, J.M., Davis, J.R. (2008). The Apollo Medical Operations Project: Recommendations to improve crew health and performance for future exploration missions and lunar surface operations. *Acta Astronaut* 63:980-987.

Schwarze, P.E., Ovrevik, J., Lag, M., Refsnes, M., Nafstad, P., Hetland, R.B., Dybing, E. (2006) Particulate matter properties and health effects: consistency of epidemiological and toxicological studies. *Hum Exp Toxicol* 25:559-579.

Tomatis, M., Turci, F., Ceschino, R., Riganti, C., Gazzano, E., Martra, G., Ghigo, D., Fubini, B. (2010) High aspect ratio materials: role of surface chemistry vs. length in the historical "long and short amosite asbestos fibers". *Inhalat Toxicol* 22:984-998.

Turci, F., Peira, E., Corazzari, I., Fenoglio, I., Trotta, M., Fubini, B. (2013) Crystalline Phase Modulates the Potency of Nanometric TiO_2 to Adhere to and Perturb the Stratum Corneum of Porcine Skin under Indoor Light. *Chem Res Toxicol* 26:1579-1590.

Turci, F., Tomatis, M., Lesci, I.G., Roveri, N., Fubini, B. (2011) The Iron-Related Molecular Toxicity Mechanism of Synthetic Asbestos Nanofibres: A Model Study for High-Aspect-Ratio Nanoparticles. *Chem-Eur J* 17:350-358.

Vallyathan, V., Castranova, V., Pack, D., Leonard, S., Shumaker, J., Hubbs, A.F., Shoemaker, D.A., Ramsey, D.M., Pretty, J.R., Mclaurin, J.L., Khan, A., Teass, A. (1995) Freshly Fractured Quartz Inhalation Leads to Enhanced Lung Injury and Inflammation - Potential Role of Free-Radicals. *Am J Resp Crit Care* 152:1003-1009.

Wallace, W.T., Taylor, L.A., Liu, Y., Cooper, B.L., Mckay, D.S., Chen, B., Jeevarajan, A.S. (2009) Lunar dust and lunar simulant activation' and monitoring. *Meteorit Planet Sci* 44:961-970.

Youngman, R.J., Elstner, E.F. (1981) Oxygen species in paraquat toxicity: the crypto-OH radical. *FEBS Lett* 129:265-268.

Zalma, R., Bonneau, L., Guignard, J., Pezerat, H., Jaurand, M.C. (1987) Formation of Oxy Radicals by Oxygen Reduction Arising from the Surface-Activity of Asbestos. *Can J Chem* 65:2338-2341.

Figures

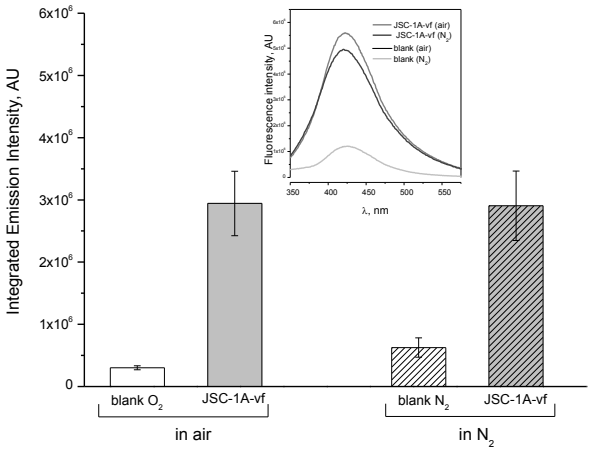

FIG. 1. JSC-1A-vf-driven hydroxylation of TA: the role of molecular O_2. Fluorescence intensities ± SD recorded on the supernatant of JSC-1A-vf suspensions in a buffered (PBS) solution of disodium terephthalate (10 mM) after 30 minutes of incubation in air or N_2. The concentration of the dust suspension was 15 mg/ml. In the inset, the fluorescence spectra for representative experiments are reported.

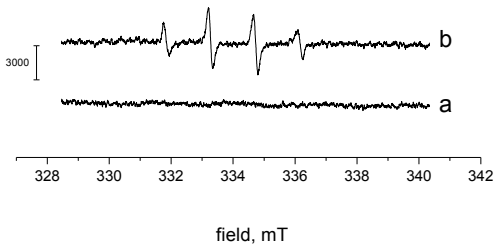

FIG. 2. ROS generation in JSC-1A-vf aqueous suspension. EPR spectrum recorded on a suspension of (a) JSC-1A-vf and (b) UV-irradiated TiO_2 (positive control) in a buffered solution (KPB, pH 7.4) in the presence of DMPO as spin trapping agent. The concentration of the dust suspension was 15 mg/ml. Splitting constants of the signal recorded: a_N = 1.47 mT, a_H = 1.42 mT.

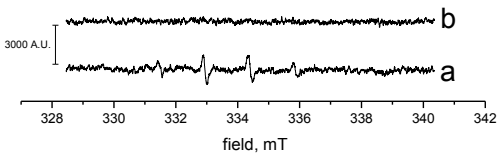

FIG. 3. Fenton-like JSC-1A-vf-induced generation of •OH radical. (a) Representative EPR spectrum recorded on a suspension of JSC-1A-vf (15 mg/ml) in a buffered solution (KPB, pH 7.4) of H_2O_2 in the presence of DMPO as spin trapping agent. Blank (b) experiment was carried out in the same conditions without JSC-1A-vf. Splitting constants of the signal recorded: a_N = 1.47 mT, a_H = 1.42 mT

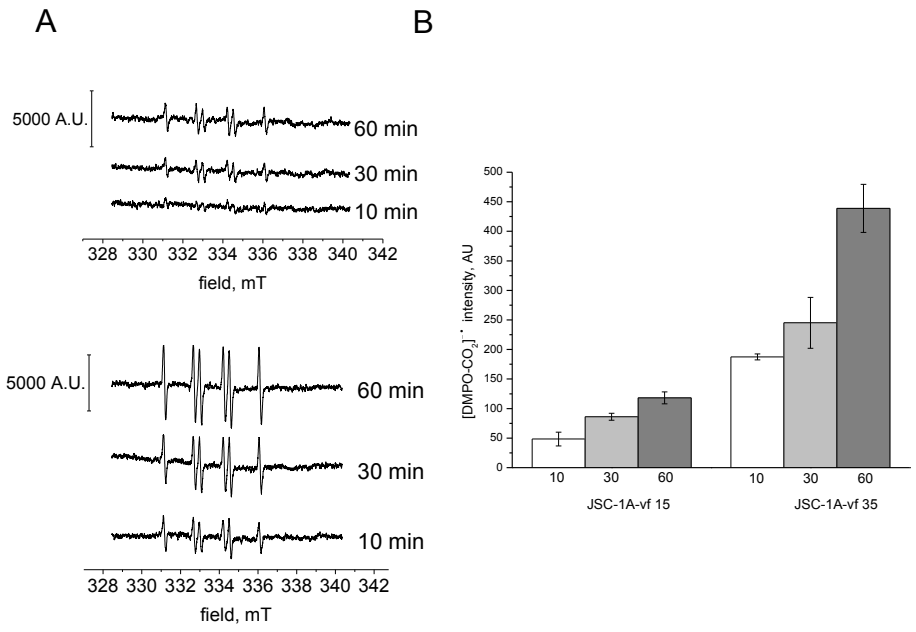

FIG. 4. JSC-1A-vf-induced generation of •COO⁻ radical. (A) EPR spectra recorded on suspensions of JSC-1A-vf in a sodium formate buffered solution at low dose (15 mg/ml, upper spectra) and high dose (35 mg/ml, lower spectra) of powder in the presence of DMPO as spin trapping agent. Splitting constants of the signal recorded: a_N = 1.56 mT and a_H = 1.87 mT. (B) Average spectra signal intensity from double integration of the EPR spectra recorded on JSC-1A-vf suspensions at two different doses (15 mg/ml and 35 mg/ml).

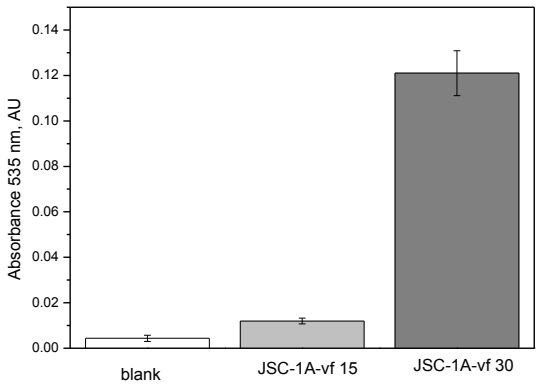

FIG. 5. JSC-1A-vf-induced lipid peroxidation. Malondialdehyde (MDA) production after 72 h of incubation measured on the supernatant of JSC-1A-vf suspensions at two different doses: 15 mg/ml (the JSC-1A-vf 15 column) and 35 mg/ml (JSC-1A-vf 35 column) in a buffered suspension of linoleic acid. The same experiment was also carried out in the absence of the dust (blank).

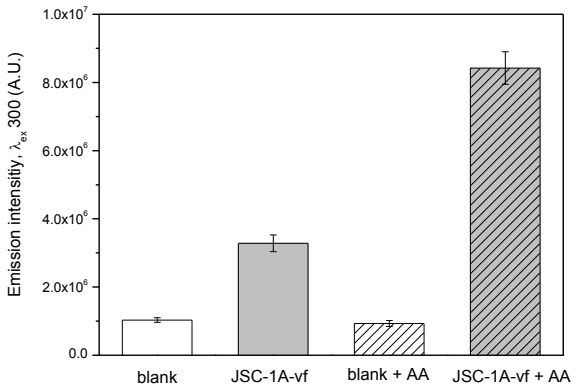

FIG. 6. Effect of ascorbic acid on the hydroxylation of TA. Fluorescence intensities ± SD recorded on the supernatant of JSC-1A-vf suspensions (15 mg/ml) in a buffered (PBS) TA solution (10 mM) after 30 minutes of incubation.

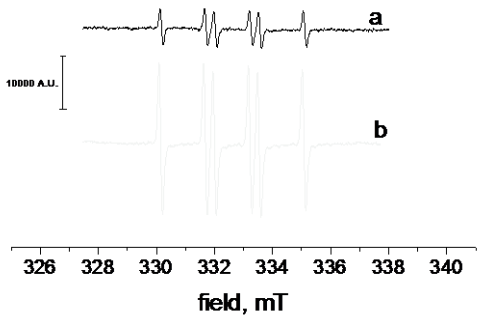

FIG. 7. Effect of ascorbic acid on JSC-1A-vf-induced generation of $\bullet CO_2^-$ radical. EPR spectra recorded on suspensions of JSC-1A-vf in a buffered solution of sodium formate (1 M) employing DMPO as spin trapping in the absence (spectrum a) and in the presence (spectrum b) of ascorbic acid (3 mM). Splitting constants of the signal recorded: aN = 1.56 mT and aH = 1.87 mT.

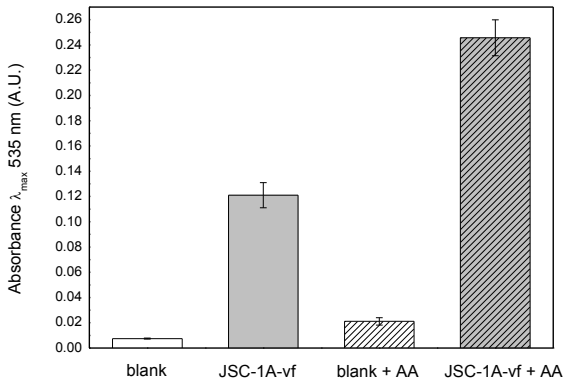

FIG. 8. Effect of ascorbic acid on JSC-1A-vf induced lipid peroxidation. MDA production after 72 h of incubation on the supernatant of JSC-1A-vf suspended (35 mg/ml) in a buffered suspension of linoleic acid in the presence and in the absence of ascorbic acid.

Free-radical chemistry as a means to evaluate lunar dust health hazard in view of future missions to the Moon

Francesco Turci[1,2,3,#], Ingrid Corazzari[1,2,#], Gabriele Alberto[1,3], Gianmario Martra[1,2,3], Bice Fubini[1,2,3, *]

[1] Dip. Chimica, University of Torino, via P. Giuria 7, 10125, Torino, Italy.

[2] "G. Scansetti" Interdepartmental Center, University of Torino, via P. Giuria 9, 10125, Torino, Italy.

[3] NIS Excellence Center, University of Torino, via Quarello 11, 10135, Torino, Italy.

[#] These authors equally contributed to the manuscript.

[*] Corresponding author at the Department of Chemistry, University of Torino, via P. Giuria 7, 10125, Torino, phone number: +390116707566, e-mail address: bice.fubini@unito.it

6 Supporting information

6.1 TA hydroxylation: dependence from pH

The work of Saran et al. (2000) evidenced that the hydroxylation of TA caused by HO• free in solution (for instance, HO• generated by a Fenton reaction) is not dependent on the pH. At the opposite, when the hydroxylation of TA is not caused by free ROS in solution, the amount of TA-OH produced was strictly dependent on the pH of the solution, reaching the maximum of fluorescence emission intensity at ca. 6.5 pH units. This finding supports the hypothesis of a reactions driven by a Fe^{2+}-phosphate complex, which stability is maximized at a given pH value.

To consolidate our EPR/spin trapping findings, which seems to exclude the involvement of ROS radicals in JC1-A reactivity towards TA, we performed the experiment suspending JSC-1A-vf (15 mg/ml) in buffered solutions at different pH values, obtained by mixing K_2HPO_4 and KH_2PO_4 in different proportions. Figure S1 reports the results obtained when fluorescence intensity is measured at different values in the pH range 4.5-9 pH values. The amount of TA-OH reached the highest value at pH 6-7, while the reaction yield becomes almost negligible outside this narrow range. This pH-dependent behavior consolidates the proposed mechanism for TA hydroxylation, which is not related in the present case to free ROS in solution.

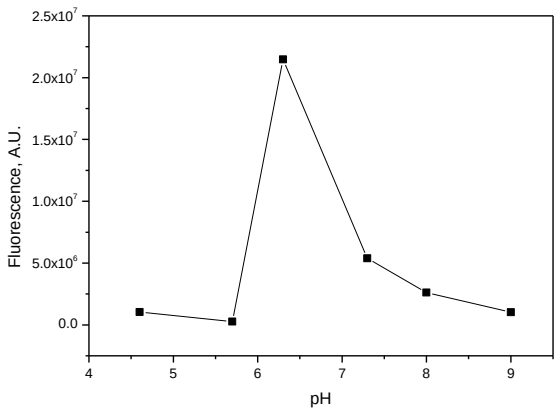

Figure S1. Effect of pH on TA hydroxylation. Fluorescence intensities ± SD recorded on the supernatant of JSC-1A-vf suspensions (15 mg/ml) in phosphate buffer solutions of TA (10 mM) after 30 minutes of incubation. The pH was adjusted point by point by mixing K_2HPO_4 and KH_2PO_4 in different proportions.

6.2 EPR spectra of the negative controls (blanks)

To clearly point out any radical reactions possibly occurring during the spin-trapping experiments, negative controls (i.e., the target molecules + DMPO, without the dust) were run simultaneously with every test. The spectra are reported in Figure S2 and show an undetectable production of radical for the generation of •OH in the presence of H_2O_2 (spectrum *a*), and the generation of $•CO_2^-$ in the presence of formate anion (HCO_2^-, spectrum *b*). A negligible amount of ascorbyl radical (AA•, g = 2.0054) and $•CO_2^-$ radical with respect to the signal detected in the experiments run with the dust (Figure 7) is recorded for the spectrum c only, when the autoxidation of AA induces the formation of ascorbyl radical (•AA), which eventually reacts with formate anion to form $•CO_2^-$ radical.

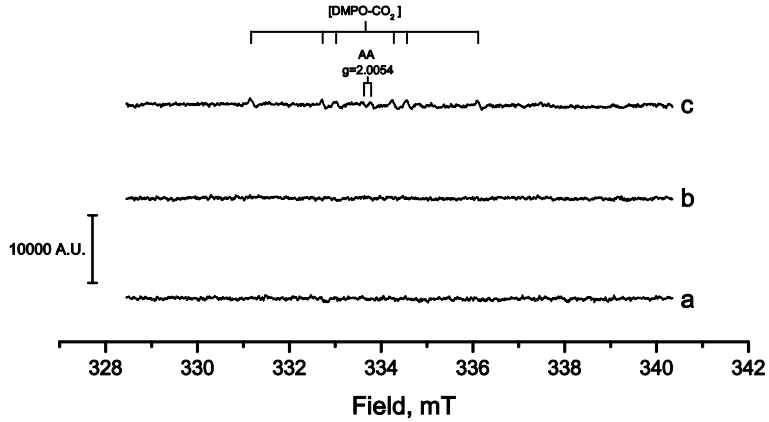

Figure S2. Representative EPR spectra collected after 60 min of reaction for the negative control (DMPO+target molecule, without dust) of a) •OH generation in the presence of H_2O_2, b) $•CO_2^-$ generation in the presence of formate anion (HCO_2^-), and c) $•CO_2^-$ generation in the presence of formate anion (HCO_2^-) and ascorbic acid (AA) as reducing agent. The autoxoidation of AA with time induces the formation of a negligible amount of ascorbyl radical (AA•, g = 2.0054) and $•CO_2^-$.

6.3 Reactivity of soluble redox active ions

To assess the involvement of soluble ions in the reactivity of JSC-1A-vf, the sample (15 mg/ml) was contacted for 60 minutes with a buffered solution (KPB, 250 mM, pH 7.4) containing sodium formate (1 M) and ascorbic acid (3 mM) as reducing agent. The supernatant was separated from the dust by centrifuging and DMPO (75 mM) as spin-trapping agent was added to the supernatant. The EPR spectrum recorded right after the addition of DMPO did not evidence any $\bullet CO_2^-$ radicals, while a weak signal of the ascorbyl radical was observed (asterisk). These result indicates that soluble redox active metal ions do not contribute to the overall reactivity measured with JSC-1A-vf.

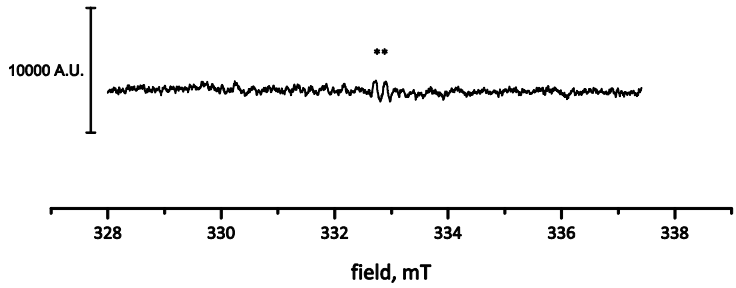

Figure S3. EPR spectrum recorded after the addition of DMPO to the clear supernatant of a JSC-1A-vf suspension (15 mg/ml) incubated for 1 h in a buffered solution (KPB 0.25 M) of sodium formate (1M) with ascorbic acid (3mM) as reducing agent.