LABORATORY SIMULATIONS OF MARTIAN CONDITIONS: HINTS FOR DETECTION OF MOLECULAR BIOMARKERS ON MARS

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1. Preparation of Mars soil analogues doped with biomarkers

- Biomarkers of extant life: AMP and UMP
- Minerals: forsterite, natrolite, labradorite, hematite, apatite, lizardite, antigorite

2. Characterization of Mars soil analogues

- Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)
- Confocal Raman Imaging Spectroscopy (CRIS) $h\nu$

3. UV irradiation processing

- Terrestrial ambient conditions
- Martian simulation chamber

2. CHARACTERIZATION OF MARS SOIL ANALOGUES

Raman and Infrared spectroscopies are very sensitive complementary techniques to investigate molecule-mineral interactions and for detection of biomarkers features.
3. UV IRRADIATION INSIDE MARTIAN CHAMBER

Comparison of the catalytic/protective properties of a variety of minerals relevant to Mars mineralogy:

- Develop models for degradation of biomarkers
- Predict the mineral deposits with the highest preservation potential
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Scientific Relevance → Laboratory Studies → Summary of the Results → Acknowledgements
Characterization of Mars soil analogues through various techniques:

- Assay the sensitivity of different laboratory instruments to detect diagnostic features of molecular biomarkers
- Support development of flight instruments and life detection methods
- Interpretation of data obtained by remote sensing
Study of the transformation of potential biomarkers adsorbed on a variety of mineral matrices under Martian-like conditions:

- Establish habitability of the planet
- Identify potential biomarkers
- Select landing sites
- Interpret data collected on the ground
1. Preparation of Mars soil analogues doped with biomarkers
   - Equilibrium adsorption
   - Water deposition

2. Characterization of Mars soil analogues
   - Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)
   - Confocal Raman Imaging Spectroscopy (CRIS)

3. UV irradiation processing
   - Terrestrial ambient conditions
   - Martian simulation chamber

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1. PREPARATION OF MARS SOIL ANALOGUES

Systems under study

- Biomarkers of extant life: nucleotides adenosine and uridine monophosphate **AMP** and **UMP**

- Minerals: **forsterite** \([\text{Mg}_2\text{SiO}_4]\), **natrolite** \([\text{Na}_2\text{Al}_2\text{Si}_3\text{O}_{10} \cdot 2\text{H}_2\text{O}]\), **labradorite** \([(\text{Ca},\text{Na})(\text{Al},\text{Si})_4\text{O}_8]\), **hematite** \([\text{Fe}_2\text{O}_3]\), **apatite** \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\), **lizardite** and **antigorite** \([(\text{Mg,Fe})_3\text{Si}_2\text{O}_5(\text{OH})_4]\)
1. PREPARATION OF MARS SOIL ANALOGUES

Photoprotective properties of nucleobases: ultrafast internal conversion

1. PREPARATION OF MARS SOIL ANALOGUES

Mineral treatments to remove possible organic and biological contaminations:

- Crush and grind rock samples with a Planetary Ball Mill
- Sieve mineral powder in different grain size ranges - 500, 250, 100, 50, <20 μm – using a Vibratory Sieve Shaker
- Sterilization in autoclave
- Washing cycles with polar/non-polar solvents (e.g. chloroform, toluene, acetonitrile, methanol, water) and sonication
- H₂O₂ oxidation
- Heating at 550°C for 3 hours
1. Preparation of Mars Soil Analogues

**Equilibrium Adsorption Procedure**

- Mineral (specific mass $m$)
  - Adsorption on a rotator until steady state equilibrium
  - Centrifugation & supernatant separation
  - Supernatant stored for further analysis...

- Biomarker Aqueous Stock Solution
  - Dissolution of the biomarker of molecular weight $MW$ in a specific volume $V$ of milli-Q water
  - Measurement of concentration ($C_{stock}$) by UV-vis spectrophotometry

- Molecules Adsorbed on the Mineral in Equilibrium with the Liquid Phase
  - Calculation of adsorbed biomarker percentage:
    \[
    \% \text{ adsorbed biomarker} = \left( \frac{C_{stock} - C_{eq}}{C_{stock}} \right) \times \frac{V \times MW_{biomarker}}{m_{\text{mineral}}} \times 100
    \]

- Pellet stored for further analysis...

- Supernatant
  - Measurement of analytical concentration ($C_{eq}$) by UV-vis spectrophotometry

**Incipient Wetness Impregnation**

- Mineral (specific mass $m$)
  - Dry in oven at 50°C

- Biomarker Aqueous Stock Solution
  - Dissolution of the biomarker in a specific volume of milli-Q water corresponding to the mineral porous volume
  - Addition to the mineral until "incipient wetness"

- Spiked Sample stored for further analysis...
Water deposition vs. Equilibrium adsorption

- Control of the amount of molecules adsorbed on the solid support
- Control on experimental conditions and degree of coverage
- Study of physico-chemical interactions establishing between molecules and mineral surface
- Simulated environment on Mars

Evaporation of warm little ponds of liquid water / desiccation of liquid water bodies

Adsorption processes in dilute aqueous environments like those presumably present on Noachian Mars
Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

2. CHARACTERIZATION OF MARS SOIL ANALOGUES

Assignment of vibrational modes: ν = stretching; δ = bending; sciss = scissoring.
Δ indicates the shift of vibrational frequencies of AMP adsorbed on the mineral with respect to pure AMP.

➢ Red-shift of NH₂ scissoring indicates that AMP molecules interact with the mineral surface through the nucleobase amino group
➢ No new bands indicative of formation of covalent bonds through the nucleobase amino group (maybe only physical interactions)

Splitting of phosphate stretching vibration, decrease of intensity for stretching of lizardite hydroxyl group, and appearance of new bands in the phosphate and hydroxyl stretching regions, indicate that AMP molecules interact mainly through the phosphate group, presumably forming covalent bonds with metal-hydroxyls of lizardite.
Red-shifts of the nucleobase vibrational modes indicate the involvement of the adenine moiety in the interaction with the mineral surface.

No significant Raman bands for the stretching motion of the phosphate group (maybe only electrostatically-mediated interaction).
UV source
Xenon lamp (200-930 nm), flux $2.75 \times 10^{17}$ photons s$^{-1}$ cm$^{-2}$ in 200-400 nm spectral range

**DRIFTS analysis**

*In situ* during UV irradiation

**Degradation Kinetics**

\[
\frac{N(t)}{N_0} = B e^{-\beta t} + c
\]

$\beta$ degradation rate
$t_{1/2}$ half-lifetime
$\sigma$ cross section
$\Phi_{\text{tot}}$ UV flux
$A_0$ sample irradiated area
No remarkable changes for pure AMP

Significant changes and appearance of new peaks for pure UMP, likely formation of cis-syn cyclobutane dimer (CBD)

$\sigma_{\text{pure AMP / UMP}} \approx 10^{-22} \text{ cm}^2$ and $t_{1/2} \approx 30$ Martian days
AMP peak at 1600 cm\(^{-1}\) (\(\delta_{\text{sciss}}\) NH\(_2\), \(\nu\) C=N, \(\nu\) C=C)

Pure AMP:
\[\sigma = (2.1 \pm 0.2) \times 10^{-22} \text{ cm}^2\]
\[t_{1/2} = 27 \pm 3 \text{ Martian days}\]

AMP adsorbed on Lizardite:
\[\sigma = (2.2 \pm 0.6) \times 10^{-22} \text{ cm}^2\]
\[t_{1/2} = 26 \pm 8 \text{ Martian days}\]

Similar degradation kinetics \(\Rightarrow\) lizardite does NOT behave as a catalyst

Appearance of the peak at 2164 cm\(^{-1}\) for AMP on Lizardite

**Formation of a new peak at 2164 cm\(^{-1}\), likely corresponding to a cyanate OCN- fragment (\(\sigma_f \approx 10^{-22} \text{ cm}^2\))**

**UV source** Xenon lamp (180-900 nm), flux $5.42 \times 10^{18}$ photons s$^{-1}$ cm$^{-2}$ in 200-400 nm spectral range

**DRIFTS analysis** ex situ pre- and post- 40 h irradiation

- Slower degradation than terrestrial ambient conditions: $\sigma_{\text{pure AMP / UMP}} \approx 10^{-25}$ cm$^2$, $t_{1/2} \approx 50000$ Martian days

- **No** formation of new peaks for AMP

- Likely formation of CBD for UMP

- **No** appearance of the peak at 2160 cm$^{-1}$ for AMP and UMP adsorbed on Lizardite

- Current Martian conditions favour preservation of “building block of life” such as nucleotides

3. **Ex situ UV Irradiation inside Martian Chamber (T≈-20°C, 6 mbar CO2)**

Comparison of different minerals

- Labradorite and natrolite minerals feature the greatest catalytic activity
- Forsterite and Hematite have an intermediate behavior
- Apatite, lizardite and antigorite do not show any significant catalytic effect

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Raman and IR spectroscopies are sensitive complementary techniques to investigate molecule-mineral interactions and for detection of biomarkers features.

Martian conditions are more favorable for preservation than terrestrial conditions.

Minerals can act as photocatalysts or photoprotectors. Thus, in high-UV irradiation environments like Mars and early Earth, minerals are primarily involved in the processes of preservation/transformation of organic matter.

The capability of apatite and serpentines to protect important molecular biomarkers of extant life such as nucleotides against damaging UV radiations is encouraging for detecting biomarkers, while feldspars and zeolites should not be targeted for the detection of biomarkers in future life detection missions to Mars.
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