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# Testing the Limits of Biosignature Detection in Calcium Sulphate Mixtures Through a Simulated Martian Environment

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## Abstract

Mixtures of cyanobacterial microbial mat and Ca-sulphate minerals were exposed to a simulated Martian environment via the Thales Alenia Space's "MARSBalloon" Project in partnership with St Bernard's High School via the Orbyts science inclusivity program. FTIR spectroscopic analyses identified that at 50% Ca-sulphate, most microbial mat biosignatures were obscured under Earth conditions, except for the C–O stretch and polysaccharide O–H vibrational modes. At 75% Ca-sulphate concentrations, no spectral biosignatures were observed. However, after exposure to a simulated stratospheric Martian environment, partial dehydration of the Ca-sulphate occurred to reveal the spectral biosignatures. This has implications in the search for life on Mars, particularly within evaporite-rich environments similar to those being investigated by the Curiosity rover.

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## 1. Introduction

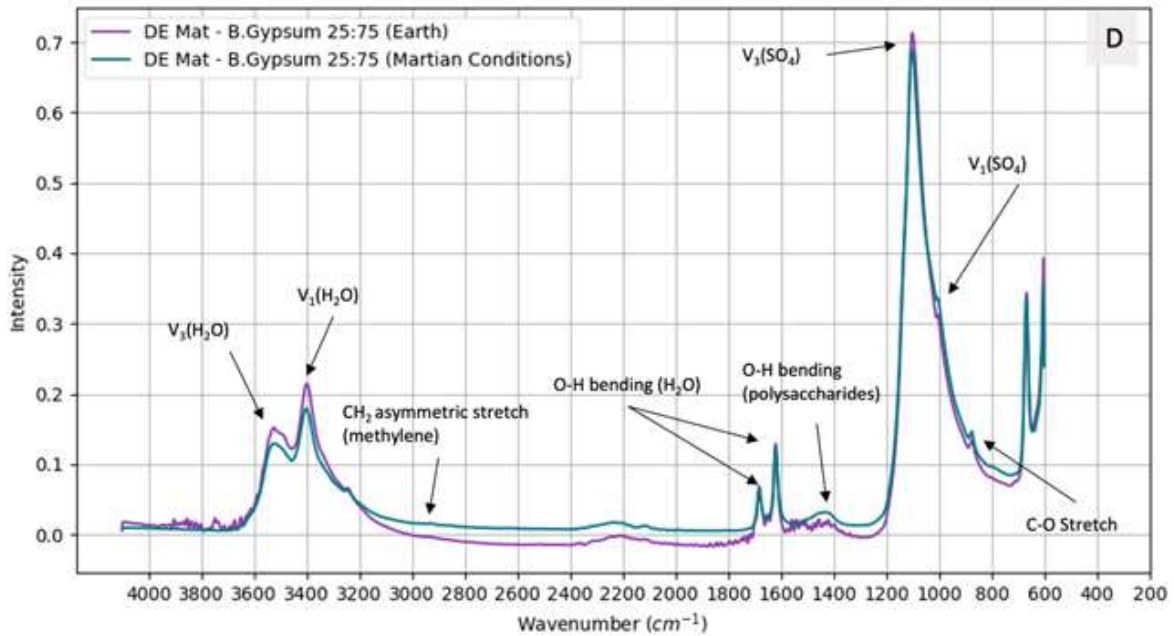
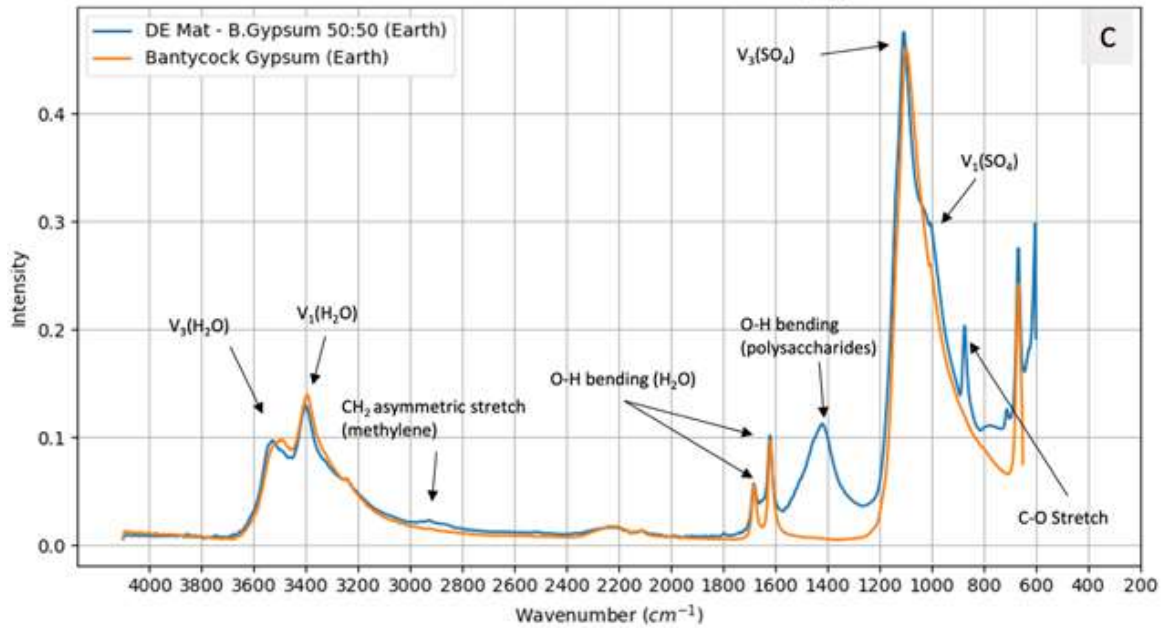
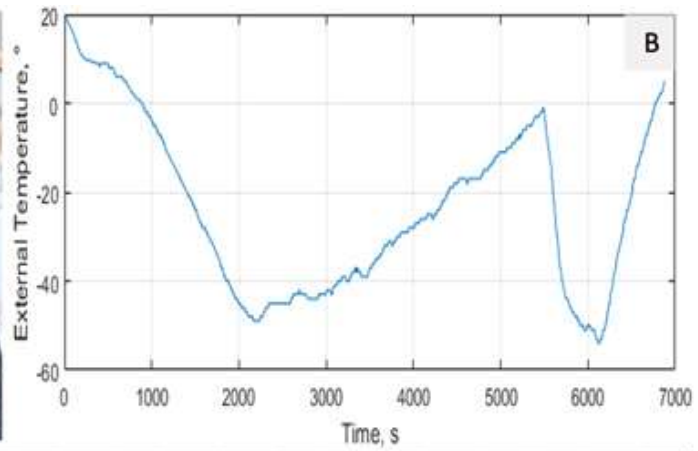
Multiple missions have been sent to Mars searching for evidence of water and carbon-based organic molecules (Rizzo et al. 2021 and references therein). Yet, the detection of life's organic signatures (biosignatures) on Mars is challenging due to poor biosignature stability with time and extreme environmental conditions (Hays et al. 2017), in particular, the radiation environment (Dartnell & Patel 2014). Consequently, the accurate detection of Martian organics relies on a comprehensive understanding of their degradation via terrestrial analog studies.

## 2. Method

As part of the Orbyts program ([www.orbyts.org](http://www.orbyts.org)) which widens access to science and education through student involvement in scientific research (Edwards et al. 2020; Francis et al. 2020; Niculescu-Duvaz et al. 2023), we created, together with St Bernard's High School, a suite of microbe-mineral mixtures to ascertain the impact of a stratospheric simulated Martian environment on biosignature detection limits.

We used a herbarium cyanobacterial mat, sample BM001062584, from the NHM (DE-mat), collected during the 1902-1903 Discovery Expedition (Fritsch 1912), as microbial mats are vertically stratified assemblages of microorganisms that have been prevalent throughout Earth's history (Almela et al. 2019). Sulphate deposits are common on Mars (Vantiman et al. 2017; Simon et al. 2023) and are known to preserve biosignatures (Preston et al. 2020), therefore DE-mat was mixed with Ca-sulphate from the Bantycok opencast gypsum mine, UK (B-Gypsum).

Samples were analyzed at MSSL using an Agilent 4300 Handheld FTIR spectrometer with diamond ATR attachment, a non-invasive technique for studying bacterial cell components (Naumann et al. 1991). We identified relative changes in DE-mat and B-Gypsum absorption band intensities pre- and post-exposure to simulated Martian conditions during the Thales Alenia Space "MARSBalloon" flight. Launched by a Hwoyee 1600 He weather balloon to the mid-stratosphere (~25–35 km), the 200+ experiments experienced temperatures of ~-50°C (Figure 1(B)), pressures of ~10 mbar (Brown & Williams 1971), and extremely dry air (Houghton 2002), emulating the Martian environment. The balloon ascended for 91 minutes before bursting at 33.04 km (flight time ~122 minutes) with position data and temperature recorded by an external sensor.



**Figure 1.** (A), the MARSBalloon capsules. (B), temperature profile. The balloon ascended through temperature regions close to  $-50^{\circ}\text{C}$  and continued to a warmer region ( $\sim 0^{\circ}\text{C}$ ), associated with ozone formation, where it burst and rapidly descended. (C), FTIR spectra of the DE-Mat and B-Gypsum mixture (50:50) and pure B-Gypsum at Earth conditions. (D), FTIR spectra of DE-mat—B-Gypsum mixture (25:75) at Earth and simulated Martian conditions.

We present results from experiment A12303 (25:75 DE-mat—B-Gypsum mixture) containing 0.085 g of powdered mat and 0.225 g of Bantycok Gypsum placed inside a  $4.5 \times 4.5 \times 6.5$  cm plastic capsule for flight. FTIR spectra were obtained of pure B-Gypsum, a 50:50 mixture, and pre- and post-flight 25:75 DE-mat—B-Gypsum mixtures, to compare the spectral profiles and biosignature preservation.

### 3. Results

Figure 1 shows the resulting spectra from samples under solely terrestrial conditions (Figure 1(C)) and contrasts these with those exposed to the simulated Martian conditions via the MARSBalloon (Figure 1(D)). The pure B-Gypsum spectrum shows typical Ca-sulphate absorptions at  $1004\text{ cm}^{-1}$  ( $V_1\text{ SO}_4$ ) and  $1104\text{ cm}^{-1}$  ( $V_3\text{ SO}_4$ ), with strong O–H bending vibrations ( $1622\text{ cm}^{-1}$  and  $1685\text{ cm}^{-1}$ ), including O–H stretching and bending vibrational modes at  $3400\text{ cm}^{-1}$  ( $V_1\text{ H}_2\text{O}$ ) and  $3526\text{ cm}^{-1}$  ( $V_3\text{ H}_2\text{O}$ ).

The DE-mat and B-Gypsum mixture (50:50) shows unique features belonging to the mat, most notably at  $876\text{ cm}^{-1}$  assigned to a C–O stretch band, and at  $1419\text{ cm}^{-1}$  assigned to the O–H bending vibrational mode of polysaccharides. A minor absorption at  $2930\text{ cm}^{-1}$  is assigned to the asymmetric stretch vibration of  $\text{CH}_2$  (methylene) (Figure 1(C)).

The spectrum of the 25:75 DE-mat—B-Gypsum mixture that remained under Earth conditions demonstrates typical Ca-sulphate absorption bands. However, the intensity of the C–O stretch ( $876\text{ cm}^{-1}$ ), O–H bending ( $1622\text{ cm}^{-1}$ ,  $1685\text{ cm}^{-1}$ ) and  $\text{CH}_2$  asymmetric stretch ( $2930\text{ cm}^{-1}$ ) microbial mat bands are greatly reduced by the gypsum.

After exposure to Martian conditions, sample hydration appears reduced, reflected by lowered relative intensities of absorption bands at  $3400\text{ cm}^{-1}$  ( $V_1\text{ H}_2\text{O}$ ) and  $3526\text{ cm}^{-1}$  ( $V_3\text{ H}_2\text{O}$ ). This enhances visibility of key microbial mat biosignatures, such as the  $\text{CH}_2$  asymmetric stretch ( $2930\text{ cm}^{-1}$ ) and the O–H bending (polysaccharide) band at  $1419\text{ cm}^{-1}$ , the latter forming a clear asymmetric peak with greater intensity than observed in the sample under Earth conditions (Figure 1(D)).

## 4. Discussion

Our aim was to investigate the difficulty of detecting biosignatures within Ca-sulphate mixtures, despite their tendency to preserve organics and biosignatures in terrestrial deposits. We show that within the 50:50 DE-Mat—B-Gypsum mixture, sulphate absorption bands mask a number of organic signatures, such as the CH<sub>2</sub> asymmetric stretch (2930 cm<sup>-1</sup>). However, organic features such as the O–H bending (1419 cm<sup>-1</sup>) and C–O stretch (876 cm<sup>-1</sup>), clearly attributable to the DE mat, remain detectable.

In the 25:75 DE-Mat—B-Gypsum mixture, we approach the detection limit, with many of the organic signatures assigned to the DE-mat masked by the gypsum mineralogy. Post-exposure to simulated Martian conditions, in particular the increased radiation, low temperatures, reduced pressures, and dry air (Houghton 2002), sample mixtures show partial dehydration, increasing our ability to detect the asymmetric O–H polysaccharide peak (1419 cm<sup>-1</sup>) (Figure 1(D)). We posit that despite Ca-sulphate overprinting of key biosignatures, the removal of a currently unknown volume of water from organic-rich Ca-sulphate mixtures, due to exposure to Mars-like conditions, may aid organic matter detection.

## 5. Conclusion

Ca-sulphate deposits are common on Mars and are targets for astrobiological investigation due to their biosignature preservation potential. However, their spectral intensity and band positions have the potential to obscure absorptions created by biosignatures. In this preliminary study, we show that exposure to a stratospheric simulated Martian environment, even for relatively short periods, can cause partial dehydration of the sample and highlight the spectral fingerprints of key biosignatures.

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