Microbial Pigments and Their Degradation Products as Biosignatures

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Introduction: Carotenoids are a class of vibrant biological pigments that have a characteristic chemical structure centered around a polyene core (Lu et al. 2018). Carotenoids and their derivatives are candidate biosignatures because they can persist in the terrestrial geologic record for up to 1.73 billion years (Vinnichenko et al. 2020), have specific structures that are likely the result of complex pathways, mediate the survival of many microorganisms in Mars and Ocean Worlds analog environments, and are detectable with multiple techniques, including Raman spectroscopy. In this project, we aim to investigate the detectability of carotenoid pigments with different spectroscopic methods to inform future instrument selection. We compare the spectra of five unaltered carotenoids, two model compounds, and carotenoid-forming archaeon with visible and deep UV Raman spectroscopy and UV-Vis absorption spectrophotometry. We then use one model pigment, beta-carotene, to evaluate the likelihood that unique spectral properties of carotenoids, or their refractory byproducts, would be preserved and detectable on a remote planetary surface by exposing it to simulated conditions for Mars.

Methods: *Sample Acquisition*. Pigments beta-carotene, lutein, zeaxanthin, astaxanthin, and lycopene were purchased from Sigma Aldrich. *Halobacterium salinarum* NRC-1 was acquired from Carlina Biological and grown in Halobacterium media. Mineral salts including sodium sulfate, sodium carbonate, and halite were used to form matrices in which the beta-carotene was embedded before exposure. Pigment-mineral mixes were at a 1:10 ratio in water.

Analytical Techniques. Deep UV Raman data were collected on a custom laboratory mapping spectrometer called MOBIUS (Mineral and Organic Based Investigations using Ultraviolet Spectroscopy), which is an analog to the SHERLOC instrument on the Mars 2020 *Perseverance* rover (Bhartia et al. 2021). It features a 248.56 nm NeCu pulsed laser, liquid nitrogen-cooled detector, and tunable optical setup. Visible Raman data were collected using a Horiba Jobin Yvon LabRam HR spectrometer with a frequency-doubled Nd:YAG laser (532 nm) and a HeNe laser (633 nm). A VWR 6300 PC UV/Visible Spectrophotometer was used to collect absorption data for carotenoid solutions, model compounds, and solvents in UV-permissible capped cuvettes. Data were collected from 190-1100 nm at 1 nm increments. All spectral data were analyzed using Igor Pro 9 (Wavemetrics).

Irradiation. We used a vacuum chamber equipped with a cryostat and a flood electron gun to simulate Martian surface temperatures, low pressures, and ionizing radiation (10keV, 10µA for 6h at 200K for our initial tests). The samples were prepared by drying the pigment-mineral mix onto polished metal tabs, then mounted on the cryostat for processing. Samples were then analyzed directly on the tabs after exposure.

Results: In comparing the visible and deep UV Raman spectra of unaltered pigments, we found that they differed drastically. Carotenoids are often studied with visible Raman and typically have peaks at 1525 cm^{-1} and 1157 cm^{-1}, due to the stretching of the C=C and C-C bonds in the polyene structure. However, in deep UV, the strongest feature is at ~1630 cm-1 and is broad, possibly indicating that multiple peaks are forming this feature. This stark difference is likely due to different pre-resonant enhancement effects. The UV-Vis results show that there is an absorption band in the deep UV <300 nm, which supports the hypothesis that the 248.6 nm excitation is interrogating another aspect of carotenoids than visible Raman. Our preliminary exposure tests indicated that pigments – even without minerals present - were largely unaltered in the applied conditions, with only a slight broadening in the primary polyene peaks apparent in the visible Raman data.

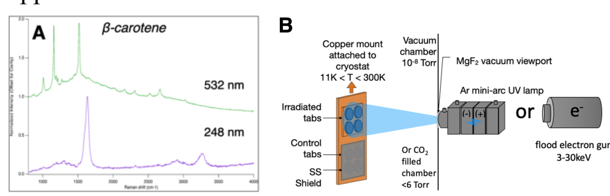

Figure 1. A) Visible vs. deep UV Raman spectra of unaltered beta carotene. B) Schematic of exposure.

Conclusions: Our results to date indicate that deep UV and visible Raman spectroscopy, both techniques with planetary mission heritage from Mars 2020 (Wiens et al. 2021, Bhartia et al. 2021), may be used in a complementary manner to observe carotenoids. In addition, we find that beta-carotene is largely resistant to our current exposure conditions, though there may be some amount of amorphization of the material which could cause the broadening of the peaks at 1525 and 1157 cm^{-1}. As a next step, we aim to increase the dosage and duration of exposure to observe degradation of the parent pigment, possibly add UV as a factor via an Ar mini-arc UV lamp and use GC-MS to characterize possible degradation products.

References:

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