National Aeronautics and Space Administration

FY24 Topic Areas Research and Technology Development (TRTD)

Microbial Pigments and Their Degradation Products as Biosignatures

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Strategic Focus Area: Preparing for returned sample investigations

Objectives: We aim to investigate the detectability of pigments, biomolecules that have diagnostic structural features that could serve as biosignatures for microbial life beyond Earth, with different spectroscopy methods. Our objective was to evaluate the detectability of carotenoids and their breakdown products in mineral matrices after irradiation under Mars-like conditions.

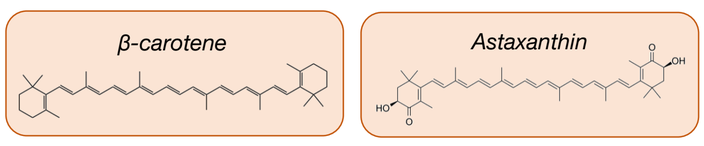


Fig. 1. Molecular structure of carotenoids examined.

Approach and Results: We first examined the Raman spectra of pure carotenoid molecules (Fig. 1), model compounds representing their end groups, and archaeal cultures producing carotenoids under different excitation wavelengths as well as UV-Vis spectroscopy to determine diagnostic features. DUV and 532 nm Raman generated different and complementary spectra, as they are sensitive to different polyene chain lengths. We identified key peaks associated with the polyene stretching modes that could serve as markers for degradation after irradiation (Fig. 2).

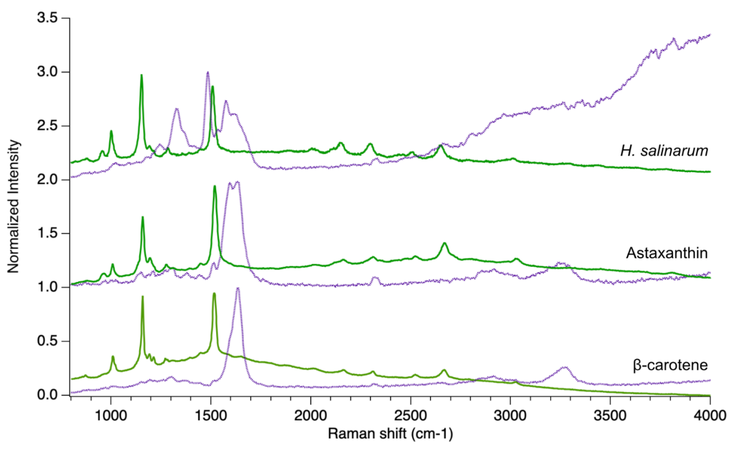


Fig. 2. Deep UV (248 nm) and 532 nm Raman spectra of *Halobacterium salinarum* NRC-1, a carotenoid-forming archeon, and powders of astaxanthin and β-carotene.

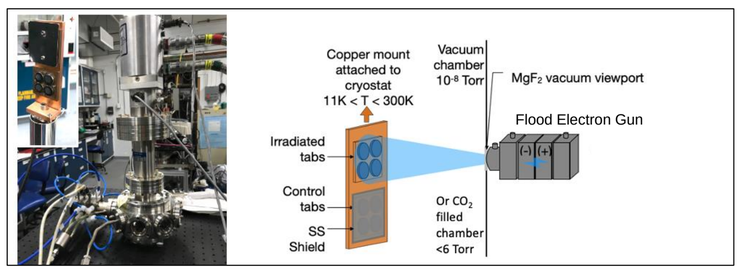
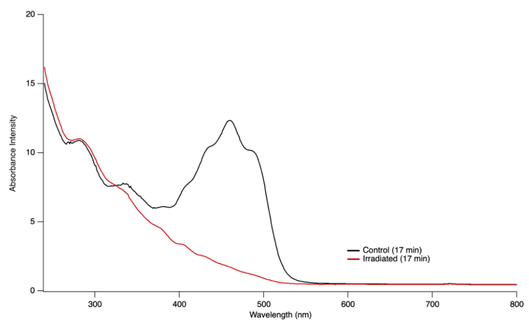


Fig. 3. Mars simulation chamber set up.

Exposure to Mars-like conditions: We then assessed the breakdown of β-carotene under Mars-like conditions using UV-Vis and Raman spectroscopy. We used an airbrush to deposit a layer of 1 mM solution of β-carotene in dichloromethane onto polished steel tabs and into the chamber (Fig. 3)

Fig. 4. β-carotene UV Vis data for control and irradiated sample tabs.



After just 17 min of exposure, we noted that the peak ~480 nm in UV-Vis was severely diminished, indicating the polyene chain was altered (Fig. 4).

We then deposited 1:10 mixtures of 1mM astaxanthin solution and different Mars-relevant minerals (NaCl, $CaSO_4$, $MgSO_4$, Na_2SO4). We put these and a control of pure astaxanthin into the chamber for 5.5-6 h, 10 keV, 33.2 muA, at 200 K.

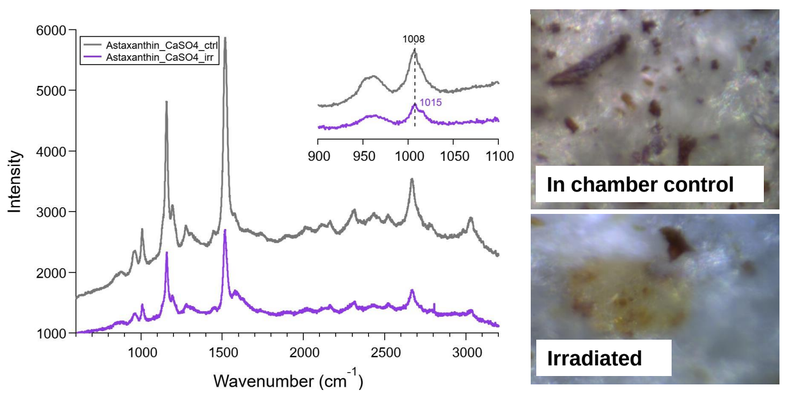


Fig. 5. Astaxanthin in CaSO4 matrix images and 532 nm Raman data of control vs. irradiated samples.

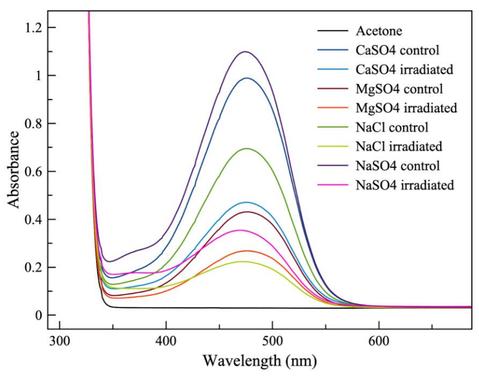


Fig. 6. UV-Vis spectra of control and irradiated mineral mixes. These were extracted with acetone.

Discussion: We did not notice changes in the Raman spectra of pigments (with or without mineral matrices) between the control and irradiated samples (Fig. 5). However, we noted changes in the UV-vis of pure pigments. (Fig. 6) This may be because the depth of radiation and depth of Raman interrogation did not overlap, or enough unaltered material remained to dominate the signal. We extracted the samples off the tabs with either dichloromethane or acetone for UV-Vis analysis. The changes that we saw in the Raman spectra were mainly peak broadening and a slight shift, suggesting that the material became more amorphous and that the polyene conjugation was broken.

We attempted GC-MS analysis to assess the range of degradation products formed. However, no diagnostic peaks were detected in major abundances, suggesting cleavage did not happen or the resulting products were not amenable to GC-MS detection.

The application of pigments and pigment-mineral mixes on the tabs as well as their extraction were technically challenging, leading to variability between samples. This led to higher uncertainty in reported results. Nevertheless, our initial analysis of the results indicates that the minerals do not appear to accelerate degradation, and in the case of $CaSO_4$, may be protective. Further work, ideally with in-chamber spectral analysis and more precise deposition methods, would be needed to support this conclusion. In addition, care should be taken not to damage the sample (which is light and air sensitive) between steps or with UV Raman.

Significance/Benefits to JPL and NASA: Our work determined that degradation of pigments and products generated were not obviously modified by different salt matrices. Thus, if carotenoids are present and protected from surface radiation, they should also be detectable in a salt matrix. Preferred techniques are DUV + green Raman combination, and UV-Vis analysis. GC-MS was not a good technique for analysis of the parent or degradation molecules. The complementary techniques should interrogate the sample differently (e.g. in situ vs. processed bulk).

Publications:

[A] Sunanda Sharma, Tuan Vu, Edith Fayolle, Carina H. Lee, Michael Malaska, Jessica Weber. “Deep Ultraviolet Raman Spectroscopy of Carotenoid Pigments: Implications for Biosignature Detection,” *In preparation*, 2024.
[B] Sunanda Sharma, Carina Lee, Edith Fayolle, Tuan Vu, William Abbey, Michael Tuite, Michael Malaska, “Microbial Pigments and their Degradation Products as Biosignatures,”Abscicon. 2024.
[C] Sunanda Sharma, Carina Lee, Michael Malaska, Edith Fayolle, Tuan Vu, Rohit Bhartia, “Detecting Pigments As Potential Biosignatures With Deep UV Raman Spectroscopy,” *Lunar and Planetary Science Conference,* #2666. 2023.
[D] Sunanda Sharma, Carina Lee, Michael Malaska, Rohit Bhartia, “Detecting Pigments with Deep UV Raman and Fluorescence Spectroscopy,” *Gordon Research Conference Geobiology*. Ventura, CA 2022.

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