DETECTING PIGMENTS AS POTENTIAL BIOSIGNATURES WITH DEEP UV RAMAN SPECTROSCOPY. S. Sharma[1], C. Lee[2], M. Malaska[1], E. Fayolle[1], T. H. Vu[1], R. Bhartia[3]
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Introduction: Biological pigments are complex organic molecules that are produced by a wide variety of microorganisms, including bacteria, archaea, cyanobacteria, and algae. They are of significant interest as biomarkers and have shown resilience in some extreme environments relevant to planetary surface conditions that may be encountered on life detection missions[1]. These complex molecules are not produced abiotically and have therefore been proposed as a molecular biosignature with a high likelihood of biogenicity. Pigments and their derivatives are detectable by different types of spectroscopy, including Raman spectroscopy[2], which is currently a technique used by two instruments on the Mars 2020 Perseverance Rover and proposed for future missions[3].

The majority of previous Raman studies on pigments utilize visible excitation wavelengths, which can lead to strong fluorescence that occludes Raman signals. Furthermore, it is likely that pigments found as biosignatures *in situ* may not be pristine and may be thoroughly altered, meaning that the known resonance enhancement that occurs under certain visible excitation wavelengths may not come into effect. It is possible that deep UV (DUV: <250 nm) Raman is particularly useful to detect pigments as biosignatures, as there is spectral separation between Raman and fluorescence regions and different possible resonance enhancement effects, especially as many pigments absorb UV light. In this study, we compared the detection of a variety of carotenoids (zeaxanthin, beta carotene, beta cryptoxanthin, lycopene, lutein, xanthophyll, and astaxanthin) and a carotenoid-forming microorganism (*H. salinarum*) with two Raman spectrometers that employ different excitation wavelengths: DUV (248.6 nm) and green (532 nm). Here, we present data for one key pigment, beta carotene (Fig. 1), which has been extensively examined with visible excitation Raman spectroscopy [4,5] but rarely with UV.

Materials & Methods: Beta carotene (synthetic 99%, Sigma Aldrich, powder) was analyzed on a stainless steel chip, and as a saturated solution in *n*-hexane within a UV-permissive cuvette. 532 nm Raman data were collected using a HORIBA LabRAM spectrometer, and 248.6 nm Raman and fluorescence data were collected on a custom laboratory breadboard (MOBIUS). MOBIUS is an analog to the SHERLOC instrument on the Perseverance rover, and features a NeCu laser, liquid nitrogen cooled detector, and tunable optical setup that can acquire both Raman and fluorescence spectra of organic and inorganic samples. The data collected on MOBIUS was processed to normalize and recalibrate with a custom Python script. Data from both instruments was analyzed using the Multipeak Fitting Package in Igor64 (WaveMetrics).

Preliminary Results: Beta carotene in *n*-hexane was analyzed under 532 nm (visible) and 248.6 nm (DUV) excitation. The spectrum under visible excitation (Fig. 2) aligns well with expected values from literature[7]. There is a visible rise in baseline due to fluorescence interference, though peaks corresponding to the ν_1, ν_2, and ν_3 vibrational modes are clearly identifiable at ~1516 cm^{-1}, 1157 cm^{-1}, and 1007 cm^{-1}, respectively.

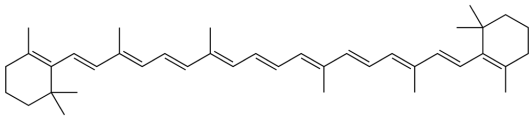

Fig 1*. Beta carotene structure.*

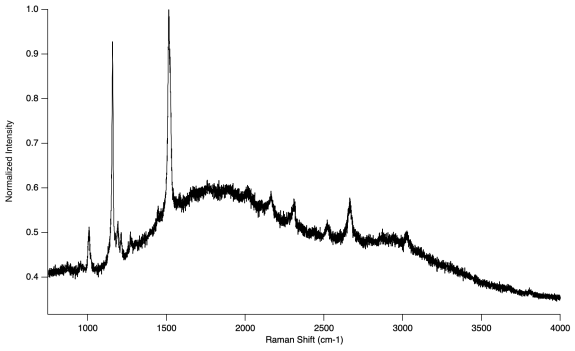

Fig 2*. Beta carotene in* n*-hexane (ex: 532 nm).*

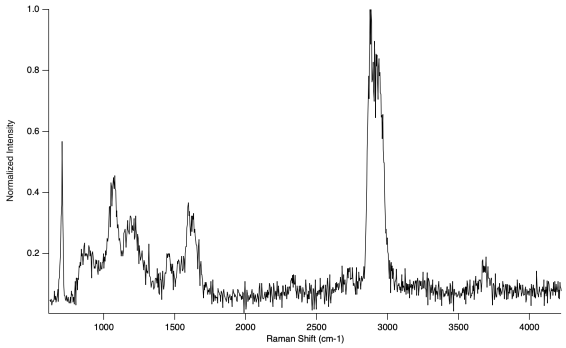

Fig 3*. Beta carotene in* n*-hexane (ex: 248.6 nm).*

Under DUV excitation, however, the spectrum is significantly different (Fig. 3). The expected v_1 peak is not clearly detectable, possibly due to an overlap with neighboring peaks. Similarly, the v_2 and v_3 peaks are not clearly identifiable. There is a clear contribution from the *n*-hexane in the solution under DUV excitation (Fig. 4, blue), most clearly seen in the 2700-3000 cm^{-1} region. In both the solution and powder form, peaks were detected between ~1580-1630 cm^{-1} and at ~2328 cm^{-1}.

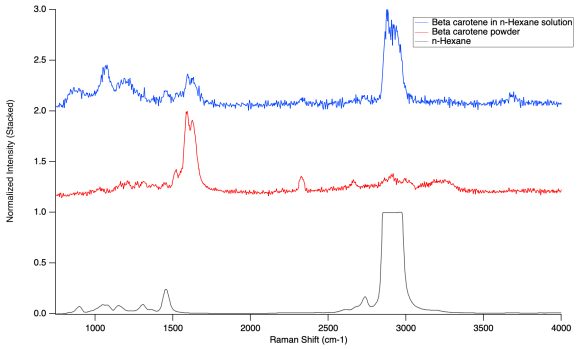

Fig 4. *Beta carotene in* n*-hexane solution, in powder, and* n*-hexane standard under 248.6 nm excitation. All spectra normalized to the maximum intensity of the powder spectrum; y-axis is stacked.*

Discussion: The discrepancy between spectra acquired under DUV versus visible excitation could be due to several reasons: (1) different resonance effects are generated under DUV than visible excitation; (2) the sample contains other trace compounds that are detected with DUV; or (3) alteration, such as photo- or thermal degradation, under DUV excitation in ambient conditions.

In the first case, the most prominent peaks under 532 nm excitation are due to stretching modes of the C-C and C=C bonds in the polyene chain. Prior studies have reported a shift in v_1 position based on excitation wavelength, but this is expected to be <5 cm^{-1}[6]. However, beta carotene absorbs UV light <300 nm[7] and may have other modes that are resonantly enhanced under 248.6 nm excitation. In the second case, trace contamination in the standard could confound both the solution and powder data; however, the compound would need to be highly resonant. In the final case, it is possible that alteration may have occurred with exposure to ambient light, air, and to UV. Prior studies have demonstrated that beta carotene in organic solutions without antioxidants is readily damaged in a matter of hours under UV and fluorescent light[8]. This observation opens an opportunity to investigate the patterns of alteration due to different factors, such as temperature, short wavelength light, and oxidation.

Future Work: The next stage of this research is to conduct further experiments to investigate the possibilities listed above regarding the discrepancy between beta carotene spectra under visible versus DUV excitation. This includes repeated DUV scans under different parameters, using visible excitation after DUV to interrogate any changes, acquiring alternative high purity standards, and utilizing different preparation and solutions to minimize solvent signal. Importantly, we aim to expand this study to include other pigments, including carotenoids with different functional groups that also absorb UV light. We aim to contribute a new dataset that expands the capabilities of Raman spectroscopy in detecting this important class of molecular biosignatures, with relevance to existing and future flight instrumentation for life detection missions.

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