Vibratinal Spectroscopic Identification of Beta-Carotene in Usnic Acid and PAHs as a Potential Martian Analogue

A. I. Alajtal, H. G. M. Edwards, M. A. Elbagermi

Abstract—Raman spectroscopy is currently a part of the instrumentation suite of the ESA ExoMars mission for the remote detection of life signatures in the Martian surface and subsurface. Terrestrial analogues of Martian sites have been identified and the biogeological modifications incurred as a result of extremophilic activity have been studied. Analytical instrumentation protocols for the unequivocal detection of biomarkers in suitable geological matrices are critical for future unmanned explorations, including the forthcoming ESA ExoMars mission to search for life on Mars scheduled for 2018 and Raman spectroscopy is currently a part of the Pasteur instrumentation suite of this mission. Here, Raman spectroscopy using 785nm excitation was evaluated for determining various concentrations of beta-carotene in admixture with polyaromatic hydrocarbons and usnic acid have been investigated by Raman microspectrometry to determine the lowest levels detectable in simulation of their potential identification remotely in geobiological conditions in Martian scenarios. Information from this study will be important for the development of a miniaturized Raman instrument for targetting Martian sites where the biosignatures of relict or extant life could remain in the geological record.

Keywords—Raman spectroscopy, Mars-analog, Beta-carotene, PAHs.

I. INTRODUCTION

THE quest for evidence of life, both extant and extinct, especially on Mars, is a basic goal of current space exploration. In the case of Mars, the detection of extant or recent life and the search for chemical tracers left by past life have been summarized in an excellent report [1]. In addition to the geosignatures (e.g., morphological features, erosion consistent with aquifer processes) life can leave traces of biosignatures (e.g., organic macromolecules) that can be attributed to previous or current biological activity [2], allowing us to detect its past and present occurrence. On Earth, molecular biomarkers derive from biochemical precursors through reduction or oxidation processes and generally include lipids and pigments, their derivatives and degradation products [3], [4]. Within the model payload of the forthcoming instruments currently being developed by ESA and NASA for future missions on Mars, Raman spectroscopy is considered as a fundamental instrument for characterizing

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mineralogical and organic material (separately or in combination with LIBS or fluorescence techniques).

Therefore, it is necessary to perform a series of Earth-based analyses on Martian-analogues, to evaluate the possibilities of Raman spectroscopy in this context and subsequently to facilitate future in-situ measurements. Raman spectroscopy has hitherto been proved to be a useful method for characterizing organic minerals derived from biological activity in the geological record [5]-[8].

Studies of terrestrial environments [9] show that in cases where the external conditions do not allow for the existence of surface growths, rocks become the refuge for microorganisms that need additional protection from hostile environmental conditions. Within the Martian context, any organic matter trapped as intracrystalline inclusions within sulfates, for example would be expected to be resistant to the oxidizing conditions present on the surface of Mars [9].

Beta-carotene is one of the most widespread natural molecules, exhibiting several biological functions in a variety of organisms from bacteria and plants. Beta-carotene has two strong Raman bands at 1515 and $1157cm^{-1}$ due to in-phase $v_1(C=C)$ and $v_2(C–C)$ stretching vibrations. A feature of medium intensity also occurs at $1008cm^{-1}$, corresponding to the in-plane rocking modes of the $(C-CH_3)$ group in phenylalanine (Fig. 1) [10], [11]. Marshall et al. [12] have reported the identification of carotenoids in halophilic archaea by resonance Raman spectroscopy using the 514.5nm excitation wavelength. This excitation wavelength has proved to be useful when analyzing carotenoids due to its coincidence with an electronic transition in carotenoids resulting in a resonance Raman effect, thus enhancing significantly the intensity of the Raman signal for example [10]-[12]. However, considering the need for other organic biomolecular species to be analyzed within astrobiological missions, other wavelengths must be considered as possible excitation sources.

In this paper, Raman micro-spectroscopy was used as a non-destructive method of determining the presence of chemically pure beta-carotene in experimentally prepared mixtures with powdered usnic acid, phenanthrene and triphenylene. The aim of this work is to evaluate the discriminatory ability of the technique and to determine the lowest proportion of beta-carotene that is still possible to detect in the selected compounds—thereby simulating the analysis of beta-carotene incorporated inside organic acids and polyaromatic hydrocarbons (PAHs) as residues from extinct

life. Our samples were analyzed using 785nm excitation to test this more universal excitation source, which may be of importance from the astrobiological point of view. Various concentrations of beta-carotene in organic acids and PAHs were investigated to determine the ability of the Raman micro-spectroscopic method to detect this biomarker species.

II. MATERIALS AND METHODS

A. Materials

Beta-carotene, usnic acid, phenanthrene and triphenylene were supplied by Sigma-Aldrich (UK) and AA pin Chemicals Limited (UK), and were used as received. The mixtures were ground and homogenized in an agate mortar. Powders of five different concentrations of beta-carotene in usnic acid, phenanthrene and triphenylene were prepared representing 0.25, 0.50, 1, 5, and 10 mg kg^{-1}. Spectra of the powdered mixtures were obtained directly from the surface of the specimen.

B. Raman Spectroscopy

Raman spectra of the mixtures were obtained using an In Via Raman microscope (Renishaw plc.) with 785nm stabilized diode laser excitation. The laser beam was focused on the sample using a 5x objective lens, resulting in a laser spot footprint of approximately 10μm diameter. Spectra were obtained for 5 accumulations, each of 10s exposure of the CCD detector, in the wavenumber range 100-3200cm^{-1} using the extended scanning mode of the instrument. The total acquisition time of the spectrum of each mixture was about eight minutes. Spectral acquisition, presentation, and analysis were performed with the Renishaw WIRE 2 (Renishaw plc) and GRAMS AI version 8 (Galactic Industries, Salem, NH) software.

III. RESULT AND DISCUSSION

A. Calculations of I/σ Ratio

The I/σ ratios were measured for the all the Raman bands in the spectra of the compounds to differentiate between true Raman bands and noise in the spectra (where I is intensity of band and σ is the standard deviation).When this ratio is more than 3, the Raman band is considered definitive and whenever it less than 3, it is relegated to noise in the spectra [25]. The results are shown in Table II.

B. Beta-carotene in Organic Acid and PAHs

Beta-carotene is part of a family of chemicals called the carotenoids. It has the molecular formula $C_{40}H_{56}$ (M.W 536.87g/mol). The Raman spectrum of beta-carotene is shown in Fig. 1. The three vibrational signals identified above are situated in the wavenumber region between 1600 and 1050 cm^{-1}. The strong bands at 1513 and 1156cm^{-1} correspond to the in-phase ν (C=C) and ν (C-C) stretching vibrations, respectively, and the feature at 1008cm^{-1} is due to the in-plane rocking modes of the (C-CH$_3$) group.

Various concentrations of beta-carotene in usnic acid, phenanthrene and triphenylene have been investigated to determine the detection capability of the three selected key molecular features. The use of a 785nm excitation wavelength was considered to be a good choice to avoid the problem of fluorescence assigned with organic molecules and visible excitation. Raman band wavenumber positions and corresponding assignments of beta-carotene, usnic acid, phenanthrene and triphenylene are listed in Table I. The assignments of phenanthrene, triphenylene, and usnic acid have been taken from previous literature assignments [7], [10], [11], [13]-[24].
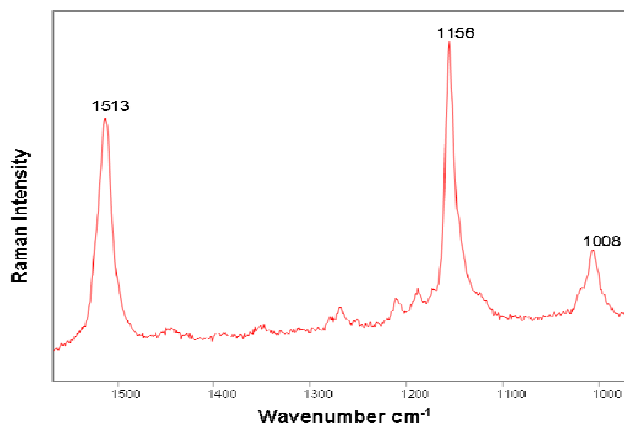

Fig. 1 Raman spectrum of beta carotene with three selected key features at 785nm
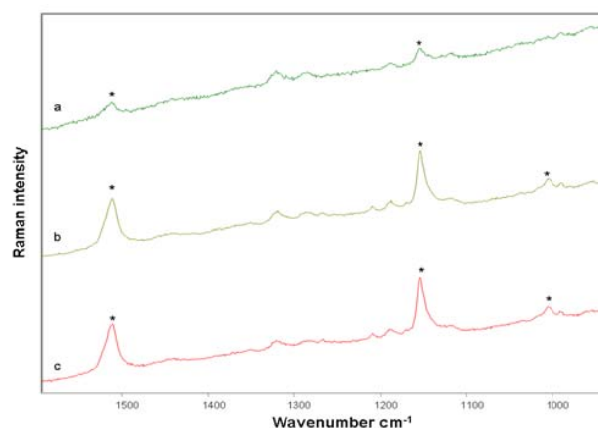

Fig. 2 Raman spectra of beta-carotene in admixture with usnic acid (Asterisks indicate beta-carotene bands): (a) 0.25mg kg^{-1} (b) 0.50mg kg^{-1} (c) 1mg kg^{-1}

TABLE I
RAMAN BANDS POSITIONS (IN WAVENUMBERS, CM^{-1}) AND CORRESPONDING ASSIGNMENT OF BETA-CAROTENE, USNIC ACID, PHENANTHRENE AND TRIPHENYLENE

Beta-carotene	Usnic acid	Phenanthrene	Triphenylene
1513	1693	3071	1615
in-phase ν (C=C)	ν(C=O) conjugated cyclic ketone	CH stretching	C-C stretching
1156	1629	3055	1604
ν (C-C) Stretching vibrations	ν(C=O)aromatic ketone	CH stretching	C-C stretching
1008	1608	1622	1458
in-plane rocking modes of (C-CH$_3$)	Quadrant ring stretch	C=C stretching vibration	C-C stretching
	1322	1523	1340
	Ring stretch	C-C stretching	C-C stretching
	1289	1440	1228
	ν (COC) as aryl alkyl ether	C-C stretching, HCC bending	C-H in plane bending
		1349	1162
		C-C stretching, HCC bending	
		1245	1061
		HCC bending	C-H in plane bending
		1200	698
		C-C stretching, HCC bending	C-H out of plane bending
		1168	418
		C-C stretching	C-C-C out of plane bending
		1036	
		C-C stretching, HCC bending	
		710	
		HCCC out of plane bending	
		547	
		CCC bending	
		410	
		CCC bending	

C. Beta-Carotene in Usnic Acid

The Raman spectra of beta-carotene in usnic acid at different concentrations are shown in Fig. 2. At the concentration level of 0.25mg kg^{-1}, only two weak Raman bands at 1513 and 1156cm^{-1} assigned to the in-phase ν (C=C) and ν (C-C) stretching vibrations, respectively, are seen in the spectra when accumulating 5 scans of 10s each , using a 5x objective lens, resulting in a laser ″footprint″ of approximately 10 μm diameter. (Table II).

Usnic acid features at 1322 and 1289cm^{-1} which are assigned to a ring stretch and a ν (COC) of an aryl alkyl ether, respectively, clearly appear in the spectra at a concentration level of beta-carotene of 1mg kg^{-1}, as seen in Fig. 3, compared with the usnic acid features.

TABLE II
I/Σ RATIO OF BETA-CAROTENE BANDS AT VARIOUS CONCENTRATIONS IN USNIC ACID, PHENANTHRENE AND TRIPHENYLENE MIXTURES

mg kg^{-1}	1513 cm^{-1}	1156 cm^{-1}	1008 cm^{-1}	Organic compounds
0.25	13.84	46.09	2.23	Usnic acid
0.50	26.93	60.72	47.78	
01	56.42	95.33	71.60	
05	159.69	180.90	78.75	
10	164.72	211.69	106.81	
0.25	13.44	17.11	2.58	Phenanthrene
0.50	41.63	58.02	19.69	
01	167.30	187.44	69.36	
05	196.61	245.44	107.47	
10	217.97	249.91	117.75	
0.25	15.59	35.25	2.01	Triphenylene
0.50	29.17	49.34	1843	
01	61.40	85.65	28.75	
05	213.96	244.28	103.03	
10	218.25	249.59	107.38	

Limit of detection at 99% confidence assgned to I/σ >3 [25]

D. Beta-Carotene in Phenanthrene

The Raman spectra of beta-carotene in phenanthrene show the beta-carotene Raman band at 1513cm^{-1} assigned to the in-phase ν (C=C) which appeared as a doublet bands at 0. 50mg kg^{-1} and as a shoulder at 0.25mg kg^{-1} with the phenanthrene Raman band at 1523cm^{-1}, also assigned to C-C stretching. Furthermore, the beta-carotene Raman band at 1156cm^{-1} assigned to the ν (C-C) stretching vibration was observed at 0.50mg kg^{-1} and appeared as a triplet band at 0.25mg kg^{-1} concentration. Also, the beta-carotene Raman band, at 1008cm^{-1} which is assigned to the in-plane rocking modes of (C-CH$_3$) was observed in the spectra at the concentration level equal to or higher than 0.50mg kg^{-1}; however, at the concentration level of 0.25mg kg^{-1} this band was not observed (Fig. 4 and Table II).

E. Beta-Carotene in Triphenylene

Measurements using the 785nm excitation wavelength allowed the detection of three characteristic bands of beta-carotene (1513, 1156 and 1008cm^{-1}) corresponding to the in-phase ν (C=C) and ν (C-C) stretching vibrations and in-plane rocking modes of (C-CH$_3$), respectively) at a concentration level equal to or higher than 0.50mg kg^{-1} (Fig. 4). Only two weak Raman bands of beta-carotene are seen in the spectra at the concentration level of 0.25mg kg^{-1} (Table II). Therefore, the beta-carotene Raman band at 1156cm^{-1} assigned to the ν (C-C) stretching vibration is seen as a doublet at 0.25 and 0.50mg kg^{-1} concentration levels along with the triphenylene Raman band at 1162cm^{-1} as shown in Fig. 5.
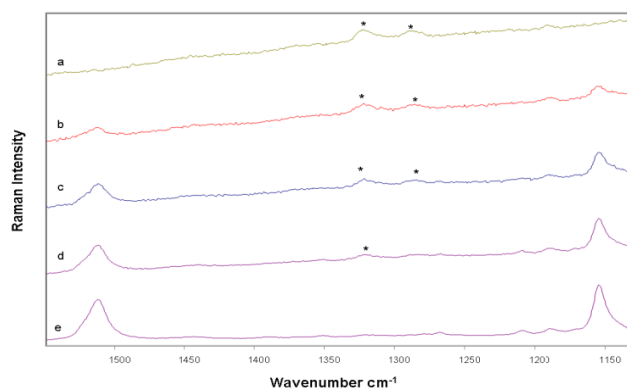
Fig. 3 Raman spectra of beta-carotene in admixture with usnic acid (Asterisks indicate usnic acid bands): (a) usnic acid (b) 0.25mg kg^{-1} (c) 0.50mg kg^{-1} (d) 1mg kg^{-1} (d) 5mg kg^{-1}
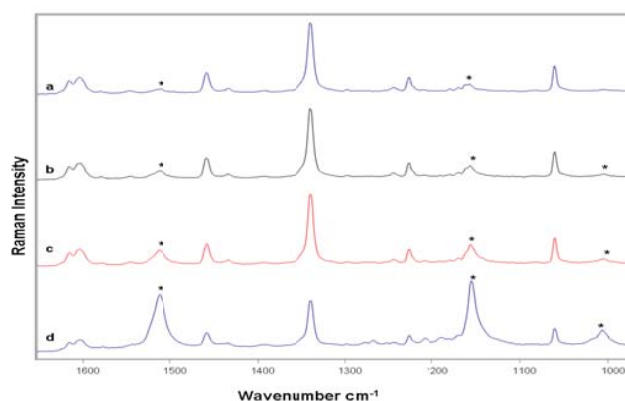

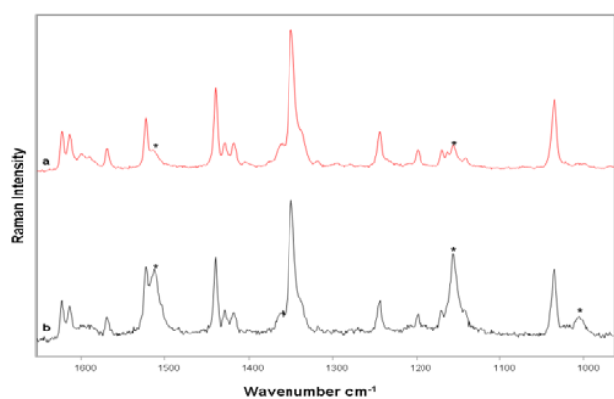
Fig. 4 Raman spectra of beta-carotene with phenanthrene mixture (Asterisks indicate beta-carotene bands): (a) 0.25 mg kg^{-1} (b) 0.50mg kg^{-1}

IV. CONCLUSION

Raman spectra obtained in this study confirm that by using a 785nm excitation wavelength it is possible to determine the presence of beta-carotene, a potential biomarker, in experimentally prepared organic mixtures, which are considered to be potential scenarios on Mars. In addition, this potential biomarker- organic mixture system demonstrates the ability of Raman spectroscopy to detect key individual components mixture of organic compounds. In a previous study, polyaromatic hydrocarbons (PAHs) were investigated as biomarkers in a mineral matrix system, and the result demonstrated that the number of observed Raman bands in PAHs differed depending on the particular mineral, the excitation wavelength and the concentrations deployed [26]. In this study, we found that the minimum detectable concentration of beta-carotene is 0.25mg kg^{-1} (i.e. 250 ppb) for the organic compound mixtures. The results obtained have significant implications for planned in situ robotic Raman spectroscopic measurements on Mars or elsewhere.

Fig. 5 Raman spectra of beta-carotene in admixture with triphenylene (Asterisks indicate beta-carotene bands) (a) 0.25mg kg^{-1} (b) 0.50mg kg^{-1} (c) 1mg kg^{-1} (d) 5mg kg^{-1}

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