

# RAMAN SPECTROSCOPIC PROTOCOL FOR THE MOLECULAR RECOGNITION OF KEY BIOMARKERS IN ASTROBIOLOGICAL EXPLORATION

HOWELL G. M. EDWARDS

*Department of Chemical and Forensic Sciences, University of Bradford, Bradford BD7 1DP, U.K.  
(e-mail: h.g.m.edwards@bradford.ac.uk, phone: 00 44 1274 233787, fax: 00 44 1274 235350)*

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**Abstract.** Raman spectroscopy is proposed as novel instrumentation for the remote, robotic exploration of planetary surfaces, especially Mars. In recent years, information about the chemicals produced by organisms at the terrestrial limits of life, such as those surviving in Antarctic habitats, has facilitated the assembly of a spectral database of key biomarkers. In addition biogeological modifications which are essential for the survival strategies of environmentally stressed organisms have been identified. In this paper, the requirements for Raman spectroscopic instrumental detection of key bio – and bio-geological markers are outlined and a preliminary protocol established for the molecular spectral recognition of biological signatures in remote astrobiological exploration.

**Keywords:** astrobiological instrumentation, biogeological modification, molecular recognition, Raman spectroscopy, remote sensing, spectral biomarkers

## 1. Introduction

The search for extant or extinct life on Mars is a prime objective for planned space missions and for the robotic exploration of the Martian regolith surface and subsurface. In addition to the selection of targets which promise to house terrains and geology considered to be suitable for the storage of relict fossil information, the identification of extremophiles in the terrestrial record in hostile environments such as fumaroles, brine lakes and salt evaporates have extended the search patterns for organisms which are considered to exist 'at the limits of life' (Farmer, 1998; Davis and McKay, 1996; Blankenship, 1992; Hartman, 1998).

The protocols for the selection criteria for Martian exobiological instrumental analysis are dependent on two critical themes:

- The detection of key biomolecular signatures of extant or relict organisms which are capable of surviving current and past environmental conditions on the Martian surface or subsurface, and
- The recognition of geological environments which are suspected of harbouring traces of life, in the fossil record or through biogeological modifications.

The detection of these biomolecular markers in the rocks or subsurface regolith of Mars is a primary goal for astrobiology (Varman *et al.*, 1998; Schwartz *et al.*,



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1992). However, the molecular biocharacteristics of Martian material cannot be assumed to be identical to terrestrial Earth analogues, despite the evidence for a common carbon-based organic nature for biomolecular precursors from carbonaceous chondrite meteorites and interplanetary dust (Gibson *et al.*, 2001; Ehrenfreund and Charnley, 2000). The evolutionary pressure of environmental stress on early Mars, such as high levels of UVB and UVC radiation, low temperatures, extreme desiccation and hypersalinity would have demanded appropriate protective strategies to promote the origin, survival and evolution of microbial life (Newson *et al.*, 1999; Cockell, 2000). Although the protection afforded to organisms by iron (III) oxide in the regolith acting as a UV-filter (Benton Clark, 1998) has been proposed as a key factor for the existence of biomolecules in the Martian planetary subsurface, a serious setback is the presence of peroxides and hydroxyl radicals generated in the surface regolith by UV-insolation. Hence, the longevity of survival of complex biomolecules, such as proteins, nucleic acids (DNA and RNA) and lipids, would be compromised by oxidation and radical attack; in such cases, the expected pattern of biomarkers would be drastically changed and the diagnosis, catagenesis, metagenesis and biodegradation of terrestrial plant biomolecules to give the 'molecular fossils' recognised in ancient terrestrial geological specimens might not be expected to pertain to Mars. The complex chemical mixtures which comprise oils, bitumens, kerogens and macerals found in terrestrial lithology can provide valuable information about their source processes, e.g. steranes in carbonaceous rocks and gammacerane from hypersaline evaporates. Additionally, under anoxic conditions, planetary euxinic sedimentation can occur from the action of sulfate-reducing bacteria and hydrogen sulfide (Doran *et al.*, 1998).

Much of the Martian surface may preserve a chemical directory in rocks from the Noachian era, an age which overlaps the earliest Archaean geological history on Earth, ca. 3.8 Gy. Early life on Earth is believed to have utilized phosphate and carbon in the presence of liquid water and an energy source; the identification of tracers of this early life in the form of chemofossils (Mojzsis and Arrhenius, 1998) would most certainly need to be included in a biomolecular database of relevance to Martian exploration. A typical example of this type of key mineral marker is carbonated hydroxyfluoroapatite of general formulation,  $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{F},\text{OH})_{2+x}$ ; the low fluoride, high-hydroxy form of this mineral class is believed to be both biologically induced and controlled (Krajewski *et al.*, 1994) and is found as micro fossils terrestrially in gunflint chert from Ontario, Canada dating from ca. 2.1 Gy.

The environmentally hostile conditions prevailing in the Antarctic have forced organisms to adopt special strategies for survival in locations where the humidity is very low, ambient temperatures reach  $-30^\circ\text{C}$  and where exposure to strongly ionising UVB and UVC radiation at the terrestrial surface is exacerbated by atmospheric ozone depletion at higher latitudes (Edwards and Wynn-Williams, 2002). Epilithic lichen communities, which are the prime precursors of soil formation, cannot survive without adaptation and production of key molecular chemical barriers

to combat the extreme radiation, desiccation and cryogenic stresses imposed on cellular integrity. Despite this, in some exposed parts of Antarctica lichen communities have exploited fissures in geological substrata (chasmoliths) or have colonised strata within rock formations (endoliths) (Holder *et al.*, 2000).

An example of demonstrable success in the survival strategy of organisms exposed to these Antarctic extremes is provided by *Nostoc commune* benthic mats, which grow profusely on the shores of Antarctic lakes, such as Lake Wanda in Victoria Land. The survival of these communities is directly related to the production of the UV-radiation protectant molecule scytonemin in their outer sheaths (Garcia-Pichel, 1998). A recent study, encompassing an experimental two-year field exposure of *Xanthoria* lichens at Leonie Island has demonstrated the complexity of the chemical response which is engendered by adjacent communities which are exposed to ambient environmental conditions compared with similar communities which are protected using perspex cloches (Edwards *et al.*, to be published, 2003).

The application of FT-Raman spectroscopy with an excitation wavelength of 1064 nm to the study of Antarctic lichen and cyanobacterial communities has resulted in novel information relating to the biogeochemical strategic response of these organisms to stressed environmental conditions being provided. Spectroscopic work over many years has contributed to the assemblage of a key molecular biomarker database of protective chemicals produced by lichens and cyanobacteria to minimise the effects of radiation exposure, desiccation and cryogenic cellular damage. Some of the important chemicals identified in lichen and cyanobacterial Antarctic systems are trehalose, carotenoids, scytonemin, chlorophylls, phycocyanins and aromatic quinones. The Raman spectra have also indicated how these geological systems have been adapted for biological colonisation, incorporating calcite, calcium oxalate monohydrate and dihydrate (whewellite and weddellite, respectively) and iron (III) oxide (haematite and goethite).

The drive to miniaturise Raman spectrometers for space missions involving micro-instrumentation suitable for the remote sensing of planetary surfaces from robotic landers has stimulated the construction of Raman spectral databases of key molecular signatures; these comprise two main themes of investigative study, namely, geological materials with visible wavelength excitation and biological material with near infra-red wavelength excitation (Edwards and Newton, 1999).

## 2. Raman Spectroscopic Criteria for Exobiological Prospecting

The criteria for the Raman spectroscopic detection of geological signatures which have been modified by organisms existing, or which once existed, at the limits of life can be summarised as follows:

- Instrumentation which is small and of sufficiently low mass to be suitable for a space mission and lander, such as Beagle, Vanguard or Mars Express; in

practice, this means an operational mass of the spectrometer approaching 1 kg or less.

- The instrumentation must be of robust construction and able to withstand the low temperatures, abrasive winds and reactive chemical dust (peroxides) of the Martian planetary surface.
- The use of remote probe sensing with the possibility of a confocal microscope attachment to bring the laser excitation to the specimen and to collect the scattered Raman radiation is essential.
- Subsurface sampling with remote sensing apparatus capable of penetrations of the Martian regolith to a depth of several metres; a ‘side-on’ sample illumination device which would facilitate the acquisition of spectral data during drilling or MOLE penetration operations would be a desirable attachment.
- Spectral recognition packages for the detection of key molecular entities based on established spectral databases of likely Martian geological and biological residence markers.

There are now several prototype portable Raman spectrometers which are in various stages of adoption or evaluation for space exploration; these have a common feature with regard to robustness and small size, but they also have a selection of operating excitation wavelengths, all based on rapid CCD detection and diode-laser technology (Dickensheets *et al.*, 2000). Whilst spectral sensitivity and operating wavenumber ranges have been assessed for a wide range of geological materials such as feldspars, basalts, silicates and carbonates, relatively little attention has been directed hitherto in comparison to the biologically functional molecules which may be expected to exist in protected sites in the Martian regolith. A possible explanation of this anomaly is that the visible-laser diodes used for geological characterisation are often unsuitable for biological marker work as they can stimulate fluorescence, which is several orders of magnitude stronger than the Raman spectral response.

### 3. Results

In recent papers (Wynn-Williams and Edwards *et al.*, 2002a and b) we have addressed the issue of variable wavelength Raman excitation for the detection of biomolecules in lichens and cyanobacteria under environmental stress; in only a few cases of those examined did visible excitation (488, 515 and 633 nm) provide adequate information on the biomolecular composition of the organisms. The best quality information came from 1064 nm excitation in the near infrared region; even at 758 and 852 nm, prototype and commercial spectrometers did not prove completely successful. This raises an important issue for the future adoption of Raman spectroscopic instrumentation for exobiological prospecting, in that a single excitation wavelength for both biological and geological spectral data acquisition has yet to be identified.

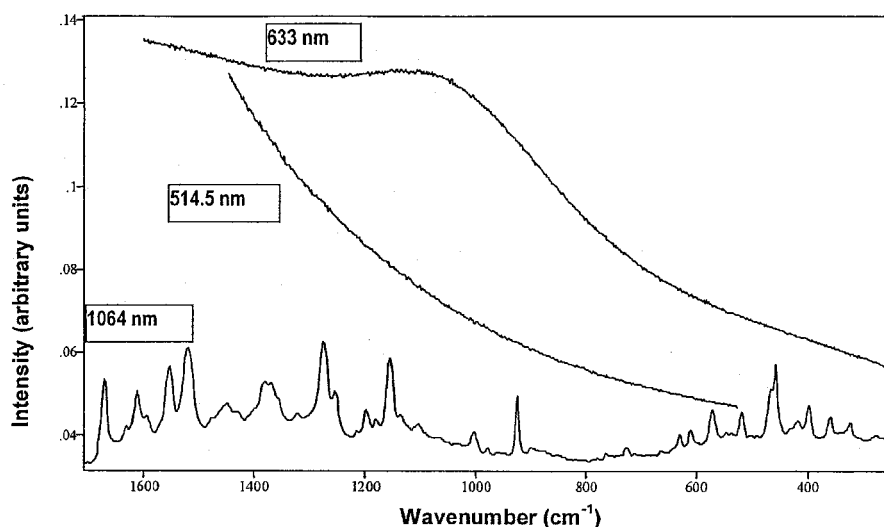


Figure 1. Raman spectral stack-plot of an Antarctic epilithic lichen, *Caloplaca saxicola*, with laser excitation at 514.5 (green) 633 (red) and 1064 nm (near infrared). Clearly, the vibrational band information quality is better with excitation in the near infrared, whilst onset of fluorescence emission swamps the Raman spectral bands in the visible.

A summary of the Raman spectral data for some biological and geological systems of relevance to Mars is given in Figures 1–5. Figure 1 shows a comparison stack-plot of Raman spectra of an Antarctic epilithic lichen *Caloplaca saxicola* recorded using excitation wavelengths of 514.5, 633 and 1064 nm. Figures 2 and 3 show similar spectral stackplot for a ‘limit of life’ system, exemplified by a cryptoendolithic colony, existing in a subsurface lithic environment; this is normally associated with the most extreme conditions under which life can still be maintained. Even more extreme environments are thought not to tolerate life at all and then we can at best expect fossil residues only of previous limiting life organisms existing when the terrestrial conditions were more favourable.

Figure 4 shows a stack plot of an Antarctic lichen, *Xanthoria elegans*, which produces paretin as a UV-radiation protectant biomolecule; in the lower spectrum the Raman spectrum of paretin is shown, with nineteen matches to bands in the living lichen system spectrum. Figure 5 shows a similar comparison for a specimen of one of the known SNC meteorites believed to be of Martian origin – the Nakhla meteorite fell as a fragmentary shower in 1911. Hence, the spectra in Figure 5 recorded using 785 nm wavelength excitation and a micro-Raman arrangement are clearly indicative of the complex geological composition of this meteorite – and individual Raman bands of plagioclase, clinopyroxene and olivine are clearly identifiable. A Raman spectrum of Nakhla recorded with 1064 nm excitation gives information of an inferior quality to that obtained at 785 nm; whereas, both excitations are formally classified as being in the near-infrared region of the

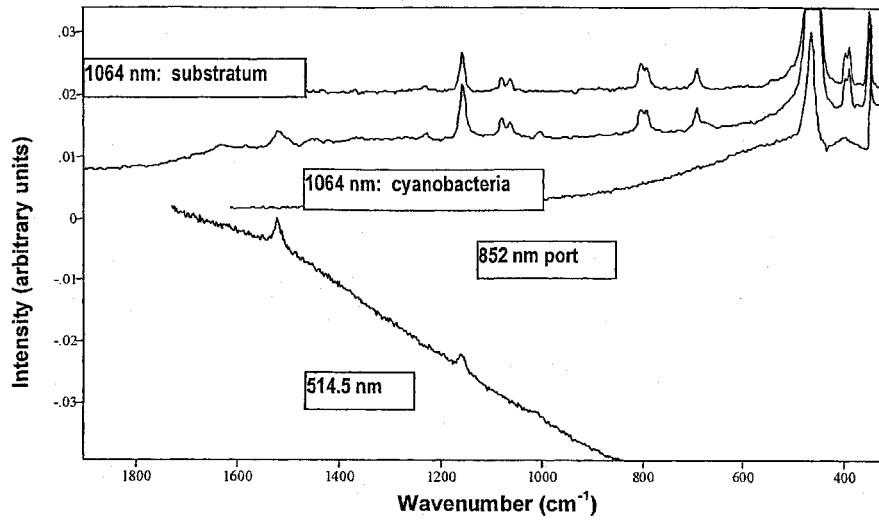


Figure 2. Stack-plot Raman spectra of *Chroococidiopsis* collected using laser excitation of 514.5, 852 (portable instrument) and 1064 nm; the 1064 nm spectra are of the substratum (upper) and cyanobacterial layer (lower spectrum). The 852 nm excitation spectrum gives reasonable response at low wavenumbers (geological component) but little at higher wavenumbers). Hence, some Raman bands can be identified weakly at 514.5 nm on the baseline of the fluorescence emission.

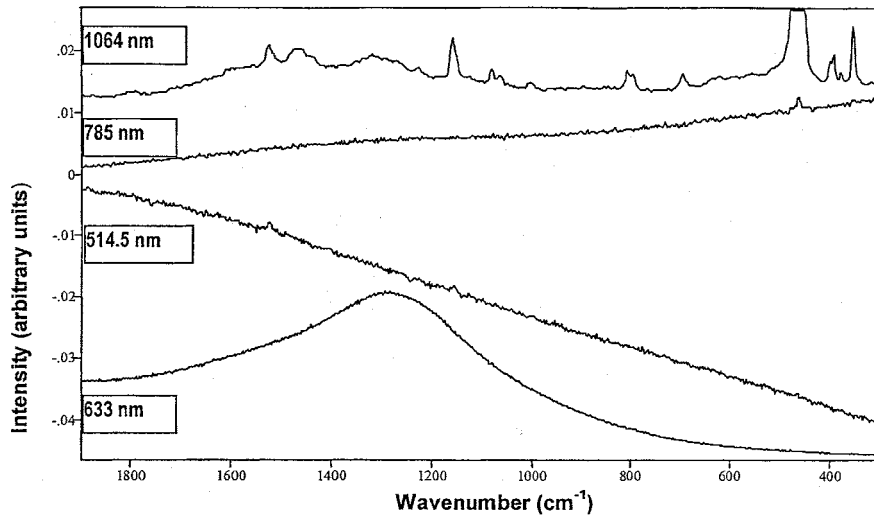


Figure 3. Stack-plot Raman spectra of endolithic green algal layer in cryptoendolithic colonisation of Beacon Sandstone, Mars Oasis, Antarctica. Again, the 514.5, 633 and 785 nm excitation fail to demonstrate the presence of the Raman bands of the biomolecular system shown in the top spectrum excited at 1064 nm.

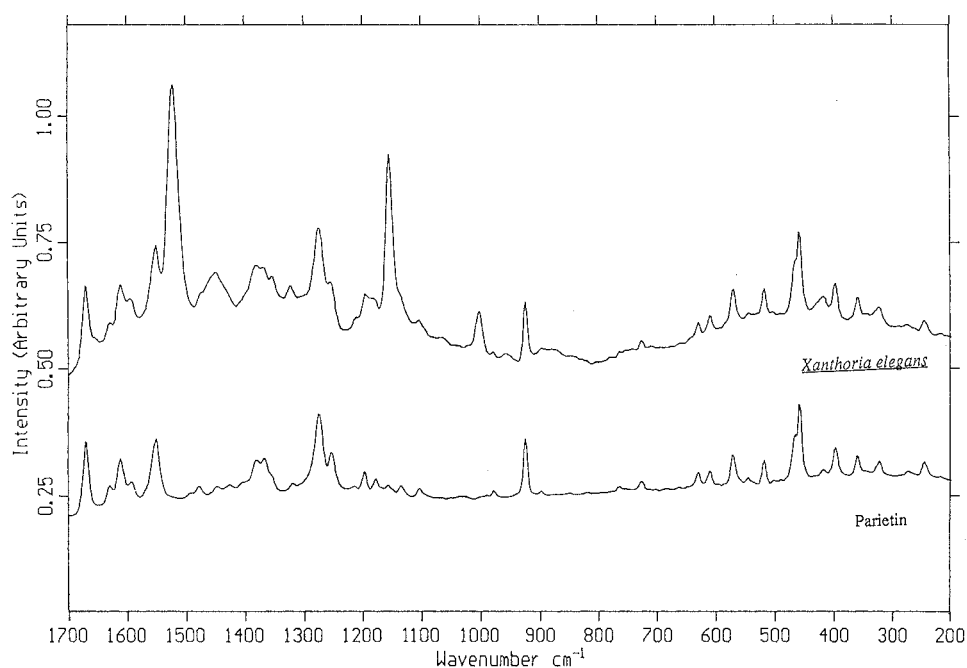


Figure 4. Raman spectra excited at 1065 nm of an Antarctic epilithic colony (upper) of *Xanthoria elegans*, which exists in exposed environments at the 'limit of life' by production of the UV-irradiation protectant biomolecule parietin (lower spectrum). Spectral matching between upper and lower spectra indicate the significant presence of parietin in the lichen specimen.

electromagnetic spectrum, but the 785 nm excitation is obviously much closer to visible red wavelengths, and a superior geological picture is given.

#### 4. Conclusions

Much work needs to be done in the near future on the establishment of databases of geological and biological systems of relevance to Martian exobiology and, in particular, in the optimisation of the Raman instrumentation for coverage of the biogeological envelope. This will involve not only the micro-miniaturisation of the Raman spectrometers and associated sample illumination devices, but also the excitation wavelengths and detectors need to be fully assessed.

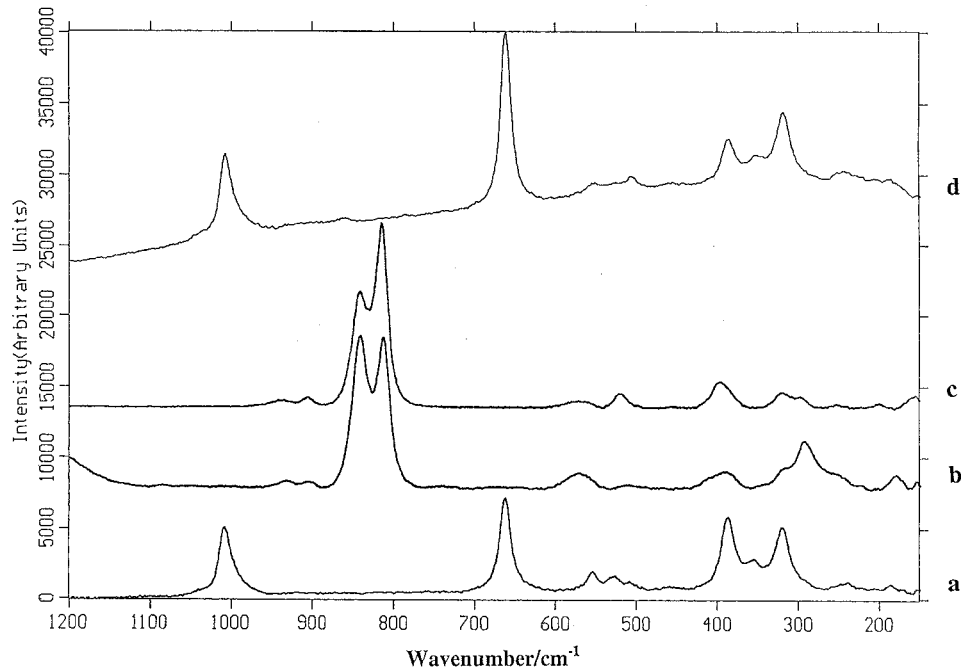


Figure 5. Raman microscope spectrum excited using 785 nm radiation of the Nakhla meteorite; (a) black particle; (b) white particle; (c) brown, glassy particle; (d) clear, glassy particle. The spectral 'footprint' is  $\sim 2 \mu\text{m}$  and the specimen geological heterogeneity should be noted. Bands at 320, 350, 386, 662 and  $1009 \text{ cm}^{-1}$  are characteristic of clinopyroxene, those at 815,  $841 \text{ cm}^{-1}$  of olivine and 508,  $520 \text{ cm}^{-1}$  of plagioclase. An extremely weak feature at  $1086 \text{ cm}^{-1}$  in spectrum (b) (just visible here) is characteristic of calcium carbonate. No biomolecular bands are identified

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