The search for $sp^2$-bonded carbonaceous material is one of the major life detection strategies of the astrobiological exploration programmes of National Aeronautics and Space Administration and European Space Agency (ESA). The ESA ExoMars rover scheduled for launch in 2018 will include a Raman spectrometer with the goal of detecting $sp^2$-bonded carbonaceous material as potential evidence of ancient life. However, $sp^2$-bonded carbonaceous material will yield the same Raman spectra of well-developed G and D bands whether they are synthesized biologically or non-biologically. Therefore, the origin and source of $sp^2$-bonded carbonaceous material cannot be elucidated by Raman spectroscopy alone. Here, we report the combined approach of Raman spectroscopy and gas chromatography–mass spectrometry biomarker analysis to Precambrian sedimentary rocks, which taken together, provides a promising new methodology for readily detecting and rapidly screening samples for immature organic material amenable to successful biomarker analysis.

1. Introduction

The search for reduced carbon is one of the major goals of astrobiological exploration programmes of the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) as part of life detection strategies (e.g. [1]). The upcoming ExoMars mission, slated to fly in 2018, will include a miniaturized Raman spectrometer [2] that will be used to detect various analytes, in particular reduced carbon [1]. Reduced carbon, more properly referred to as carbonaceous $sp^2$-bonded network solids, are substances of which the major component is carbon. In addition, these materials can contain varying quantities of substituents...
for example, hydrogen and heteroatoms such as oxygen, nitrogen, sulfur and phosphorous contingent upon the source and grade of metamorphism that these materials have experienced. From a molecular perspective, the degree of structural organization of carbonaceous \( sp^2 \)-bonded network solids can be variable, ranging from well crystalline, consisting of flat stacked layers of \( sp^2 \)-bonded carbon in sixfold rings, to poorly crystalline with short, curved or discontinuous layers of \( sp^2 \)-bonded carbon in sixfold rings that lack long-range continuity, to nanocrystalline carbon, which consists of nanometre-sized crystallites of \( sp^2 \)-bonded carbon.

The carbonaceous \( sp^2 \)-bonded network solids can be formed from carbonaceous material of biological or non-biological origin. It is worth noting that biological processes in and of themselves cannot form this material, although biologically produced organic material in sedimentary rocks can be thermally altered to form carbonaceous \( sp^2 \)-bonded network solids. By contrast, carbonaceous \( sp^2 \)-bonded network solids can be formed by high-temperature heating of inorganic compounds [3,4], inorganic precipitation from hydrothermal fluids [5–7] and redox reactions during serpentinization [8,9], all of which are carbonaceous material of non-biological origin. Despite this abiotic pathway of formation, the Raman signal acquired from carbonaceous \( sp^2 \)-bonded network solids has been erroneously postulated by some (e.g. [10–13]) as evidence of the thermal alteration of biological remains, and thus evidence of the presence of life. However others [3,14,15] have clearly demonstrated that Raman signals acquired from biologically and non-biologically synthesized carbonaceous \( sp^2 \)-bonded network solids are indistinguishable. Similarly, it has been proposed that the presence of a shoulder on the low-frequency side of the D band of carbonaceous \( sp^2 \)-bonded network solids should be used as a definitive biosignature [10]. However, carbonaceous \( sp^2 \)-bonded network solids synthesized non-biologically such as nanostructured carbons (e.g. multi-walled carbon nanotubes) and inorganic chars can also display this low-frequency shoulder on the D band [16,17].

Despite these limitations, Raman spectroscopy can be useful to prospect for biosignatures, but only if used in conjunction with other approaches, rather than as definitive proof [18–21]. For example, gas chromatography–mass spectrometry (GC–MS) is an analytical technique used for identifying biomarker compounds in thermally immature rocks. These compounds are derived exclusively from formerly living organisms. Biomarkers are complex compounds that are composed of carbon, hydrogen and heteroatoms. Structurally, they show little or no change from their parent organic molecules in living organisms (e.g. [22]). Biomarkers originate from many biological sources that are discernible from their diagnostic structural characteristics. Contributions from bacteria, algae, vascular plants and animals have been recognized among the complex assemblage of molecular species present in sediments and sedimentary rocks (e.g. [23]).

Here, we illustrate the utility of Raman spectroscopy as a screening tool for the elucidation of promising samples that may yield organic molecules of unequivocal biological origin upon subsequent analysis with mass spectrometric techniques.

2. Material and methods

(a) Samples

Samples were collected from Precambrian glacial sedimentary rocks from the Vazante Group of Brazil. The Vazante Basin is located in the northwestern part of Minas Gerais, on the São Francisco Craton. The craton is composed of Archean and Paleoproterozoic basement rocks covered by Mesozoic and Neo-proterozoic sedimentary sequences. Sedimentary rocks of the Vazante Group outcrop over ca. 10 000 km², but are generally too highly metamorphosed for organic geochemical studies [24]. However, some subsurface samples have been drilled, which exhibit only slight metamorphism [25,26]. Seven separate formations are recognized within the Vazante Group (from oldest to youngest): Santo Antônio do Bonito, Rocina, Lagamar, Serra do Garrote, Serra do Poço Verde, Morro do Calcário and Lapa. The Vazante Group was first recognized as glaciogenic [27], although its age has long been contentious. On the basis of lithology and chemostratigraphy,
many researchers identify the sediments as Neoproterozoic \[24,28\], although some have proposed an older age, ca 1000 Ma, on the basis of stromatolite biostratigraphy \[29\] and Re-Os chronometry \[30\]. Regardless of age, the Lapa Formation is recognized as a post-glacial, cap carbonate, while the Serra do Garrote, Serra do Poço Verde and Morro do Calcário formations are all synglacial and contain glaciogenic lithologies such as diamicite and limestones.

In 2002, the authors sampled the Serra do Poço Verde Formation from core MAF 42–88, originally recovered in 2000 at 17°29’ 49” S, 46°49’49” W by the Brazilian mining company Votorantim. Samples were collected from a black shale unit, as well as the flanking diamictic and carbonate marl units. Thin sections of samples from the black shale unit reveal finely disseminated solid crystalline carbonaceous material, as well as abundant pyrite and glendonite, a pseudomorph of the hydrated low-temperature carbonate ikaite. Glendonite is viewed as an indication that fresh organic material was buried, just as the formation of ikaite is thought to require both cold temperatures and the rapid remineralization of organic matter to elevate dissolved inorganic carbon \[30\]. By contrast, thin sections from the carbonate units flanking the black shale contain little carbonaceous material or pyrite.

\(\text{(b) Elemental H/C analysis}\)

Carbonaceous \(sp^2\)-bonded network solids were isolated by agitation in HCl and HF for 2 days and ca 2 mg were weighed into a tin sample boat. The elemental H and C contents were determined using a Perkin–Elmer 2400 Elemental Analyzer (the H/C ratio values were measured by the commercial laboratory Baseline Resolution, Shenandoah, TX, USA).

\(\text{(c) Raman spectroscopy}\)

Standard petrographic thin sections were analysed using Raman spectroscopy. Spectra were collected at several different locations in multiple thin sections with a Renishaw InVia Reflex Raman Microprobe (Renishaw plc, Wotton-under-Edge, UK), equipped with a Peltier cooled charge-coupled device (CCD) camera (1024 × 256 pixels). The collection optics are based on a Leica DMLM microscope. A refractive glass ×100 (0.9 NA) objective lens was used to focus the laser to a 1 μm spot to collect backscattered radiation. The Raman light was dispersed by a diffraction grating with 2400 mm per line, and the signal was analysed with a Peltier cooled CCD camera at room temperature (1024 × 256 pixels). The 514.5 nm line of a 5 W Ar\(^+\) laser (Spectra-Physics Stabilite 2017 laser) orientated normal to the sample and to the laminations in the shale was used to excite the sample. Calibration of the Raman shift is achieved by recording the Raman spectrum of the silicon F\(_{1g}\) mode for one accumulation and 10 s. If necessary, an offset correction is performed to ensure that the position of the F\(_{1g}\) band is at 520.50 ± 0.10 cm\(^{-1}\). Surface laser power settings of 1.0–1.5 mW were used to minimize laser induced heating of the carbonaceous \(sp^2\)-bonded network solids. Such heating can be readily detected as a downward shift in the G band to 1565 cm\(^{-1}\), which was not observed for any of the spectra acquired. Each spectrum was acquired using 10 scans and an accumulation time of 30 s, which gave good signal-to-noise ratio. Scan ranges were 1000–1800 cm\(^{-1}\) in the carbon first-order region, and whole spectral region scans 100–4000 cm\(^{-1}\) were also collected. Multiple analyses were performed on each thin section on carbon clots a few millimetre away from one another.

\(\text{(d) Biomarker analysis}\)

Samples for biomarker analysis were processed one at a time to avoid cross contamination. Approximately 30 g of rock were washed in distilled water. Samples were air-dried at room temperature and crushed to less than 5 cm pieces with a jaw-type rock crusher whose exposed surfaces had been cleaned \((4×)\) with acetone and then dichloromethane. The pieces were sonicated in 9 : 1 dichloromethane and methanol for 2 min and crushed again to less than 1 cm with a smaller rock crusher cleaned as above. The ultrasonic extraction was repeated, and the
sample was powdered in a shatterbox that had been cleaned using grinding quartz sand followed by acetone and dichloromethane rinses ($4\times$). The powdered rock was extracted in a microwave accelerated reaction system (MARS Express, CEM Corp.) as follows: 20 g rock powder were split equally between five clean Teflon vessels; 25 ml of 9 : 1 dichloromethane and methanol was added to each, and the samples were extracted at 100°C for 15 min with stirring. Extracts were filtered through combusted glass fibre filters to remove particulates and the solvent was evaporated to ca 30 ml under N$_2$ at 35°C, taking care not to allow them to completely dry out. Elemental S was removed by filtration through activated Cu and the S$^0$-free extract was evaporated to near dryness under N$_2$. Silica gel column chromatography was used to separate the extracts into aliphatic and aromatic fractions. The aliphatic fraction was transferred to a vial with hexane; the solvent volume was reduced under N$_2$ to 50 μl and the fraction analysed using GC–MS. Hexadecanoic acid isobutyl ester was added to all samples as internal standard. Extracts from the two intermediate stages of crushing were prepared and analysed following the same procedures. All samples were analysed with a ThermoFinnigan Trace GC-DSQ quadrupole MS equipped with a DB-5MS column (30 m × 0.25 mm, 0.25 μm film thickness). Aliquots (1 μl) were injected using a PTV injector (35°C for 3 min, 14.5°C s$^{-1}$ ramp to 200°C, then 12°C s$^{-1}$ ramp to 350°C (held 3 min). The column oven was programmed at 20°C min$^{-1}$ ramp to 130°C, then 5°C min$^{-1}$ ramp to 320°C (held 20 min).

Laboratory blanks for all materials used in the laboratory procedures, including the solvents, Cu, silica gel and MARS vessels, were analysed using GC–MS. No hydrocarbons, oil residues or unresolved complex mixtures were observed. In addition, a block of basalt was baked overnight at 450°C and spiked with a standard hydrocarbon solution and subjected to the entire analytical procedure, no contaminants again being detected. Biomarker yields were confirmed with replicate extractions of duplicate samples.

3. Results

(a) H/C values

The atomic hydrogen to carbon (H/C) ratios of the samples, 805, 812 and 816 (not determined) m from the black shale, and 783 m from the carbonate marl varies from 0.48, 0.68 and 0.29, respectively.

(b) Raman spectroscopic analyses

A collection of representative Raman spectra measured in the range from 100 to 4000 cm$^{-1}$ acquired from the samples is displayed in figure 1. Samples from the black shale unit at 805, 812 and 816 m, and one from the carbonate marl unit (783 m) exhibit Raman spectra that are quite different from one another (figure 1).

The Raman spectrum acquired from the carbonate marl sample at 783 m shows five broad bands at ca 1350, 1600, 2720, 2935 and 3240 cm$^{-1}$. These bands are due to first- and second-order phonon modes of carbonaceous $sp^2$-bonded network solids. The first-order bands at ca 1350 cm$^{-1}$ is assigned to the D1 band which is a totally symmetric radial breathing mode of a $sp^2$-bonded carbon sixfold ring mode ($A_{1g}$ symmetry) that becomes Raman-allowed when defects are introduced into the lattice or decreasing dimension of crystallites, and the band at ca 1600 cm$^{-1}$ is assigned to the G band which is due to a doubly degenerate in-plane C–C stretching mode ($E_{2g}$ symmetry). Second-order bands result from overtone scattering ($2 \times 1360 = 2720$ cm$^{-1}$, the most intense, and $2 \times 1620 = 3240$ cm$^{-1}$, a weak band) and combination scattering ($1620 + 830 = 2450$ cm$^{-1}$, $1580 + 1355 = 2935$ cm$^{-1}$). The appearance of carbon second-order bands indicates three-dimensional (triperiodic) ordering in carbonaceous $sp^2$-bonded network solids. On the molecular level, the spectra indicate that the carbonaceous $sp^2$-bonded network solid is composed of isolated polyaromatic layers that are becoming progressively stacked to form coherent nanometric domains.
Figure 1. Representative Raman spectra measured in the range from 100 to 4000 cm$^{-1}$ acquired from samples from the black shale unit at 805, 812 and 816 m, and one from the carbonate marl unit (783 m).

Generally, the characterization of the molecular structure of carbonaceous $sp^2$-bonded network solids are based on the dimensions and organization of these domains, that is, layer size ($L_a$) and stacking extension ($L_c$). Raman spectroscopy can be used to elucidate $L_a$ which is derived from the intensity ratio of D and G bands given by: $L_a = 44[I_D/I_G]^{-1}$ (nm) [31]. The $I_D/I_G$ ratio obtained from the spectrum acquired from the carbonaceous $sp^2$-bonded network solid in the carbonate marl sample is 1.68 which substituted into the above equation calculates a polyaromatic domain layer size of 26 nm. Given that the diameter of a benzene ring is ca 0.28 nm, a polyaromatic layer size of 26 nm consists of ca 93 fused aromatic rings.

By contrast, the Raman spectra acquired from samples 805, 812 and 816 m from the black shale unit show vibration modes associated with organic functional groups. These spectra contain a moderate broad band at 2700–3000 cm$^{-1}$, assigned to aliphatic C–H stretching modes, a band at 1650 cm$^{-1}$, assigned to ν (C=O) stretching mode, and a band between 1470 and 1435 cm$^{-1}$ due to the aliphatic deformational mode δ (C–H). Interestingly, the sample from a depth of 816 m from the black shale unit contains minor G and D bands in addition to the functional group modes, indicating that this functionalized organic material is undergoing carbonization and leading to an enriched carbon composition with various degrees of condensation, aromatization and two-dimensional (biperiodic) structural ordering.

(c) Gas chromatography–mass spectrometry analyses

Total ion chromatograms (TICs) for all the samples are presented in figure 2. The most abundant compounds in all the extracts are long chain $n$-alkanes, based on their retention time, distribution and fragmentograms ($m/z$ 85). However, the distribution of $n$-alkanes varies for the samples analysed (figure 2). While the extraction procedure does not recover $n$-alkanes lighter than ca $C_{17}$, samples from within black shale units, 805, 812 and 816 m are dominated by low molecular weight (LMW) compounds, with the most abundant $n$-alkane less than $n$-$C_{26}$. By contrast, samples extracted from carbonate marls (783 m) are dominated by higher molecular weight (HMW) $n$-alkanes, with the most abundant at ca $n$-$C_{30}$.

Significantly, figure 3 shows that hopanes from $C_{29}$ to $C_{32}$, dominated by the $C_{29αβ}$ and $C_{30αβ}$ hopanes, were detected in all the black shale samples (805, 812 and 816 m), but not in samples from the from carbonate marls (783 m). Similarly, figure 4 shows that $C_{27}$–$C_{29}$ steranes, dominated by the $C_{27}$ 20R $aaa$ sterane, were detected in all the black shale samples but not in the carbonate marl sample.
Figure 2. TICs for all the samples from the black shale unit at 805, 812 and 816 m, and one from the carbonate marl unit (783 m). The internal standard is marked by a black dot and the most abundant compounds in all the extracts are long chain \(n\)-alkanes, assignment is based on retention time, distribution and fragmentograms (\(m/z\) 85).

Figure 3. Selected-ion GC–MS chromatograms from black shale units and carbonate marl unit. Partial \(m/z\) 191 (hopanes) trace from samples from the black shale unit at 805, 812 and 816 m, and one from the carbonate marl unit (783 m), shown at the same scale. The S and R peak assignments define the stereochemistry at C-22, Ts denotes the \(C_{27} 18\alpha\)(H),22,29,30-trisnorhopane, Tm denotes the \(C_{27} 17\alpha\)(H),22,29,30-trisnorhopane.
Figure 4. Selected-ion GC–MS chromatograms from black shale units and carbonate marl unit. Partial m/z 217 (steranes) trace from samples from the black shale unit at 805, 812 and 816 m, and one from the carbonate marl unit (783 m), shown at the same scale. The S and R peak assignments define the stereochemistry at C-20, while βα, ααα, αββ denote 13β(H), 17α(H)-diasteranes, 5α(H),14α(H),17α(H)-steranes and 5α(H),14β(H),17β(H)-steranes, respectively.

4. Discussion

(a) Elemental H/C

The H/C ratio of carbonaceous sp²-bonded network solids is often used to ascertain the thermal maturity of a sample, as increasing thermal alteration leads to dehydrogenation, condensation and aromatization reactions which removes H and thus leads to lower H/C values (e.g. [32]). This elemental ratio can be used as a potential screening for samples likely to contain biomarkers however, this technique is laborious and moreover, affords no information on molecular structure. Here, we show that Raman spectroscopy can be a rapid, successful screening methodology that can delineate which samples are useful for subsequent biomarker analyses and additionally, this technique provides information on molecular structure.

(b) Raman spectra

The Raman spectra acquired from the carbonate marl at 783 m indicate thermally over-mature carbonaceous material. Both the presence of evolved D and G bands, indicating carbonaceous sp²-bonded network solids, and additionally, the presence of overtone bands is consistent with this conclusion. The lineshape of the carbon first-order region are similar to those of other over-mature samples that have undergone amphibolite facies metamorphic alteration in the temperature range of 500–700°C (e.g. [33,34]). By contrast, the spectra acquired from the black shale unit contain aliphatic hydrocarbon and carbonyl functional group vibrational modes indicating this material is a solid crystalline aliphatic macromolecular hydrocarbon. While the samples at 816 m also contains D and G bands in the spectra, they also have a band at 1650 cm⁻¹, indicative of C=O functional groups. This functional group chemistry is suggestive that the
sample has not undergone a high degree of thermal alteration, as C=O moieties are not preserved at high temperature (e.g. [35]). Alternatively, the presence of D and G Raman bands in spectra acquired from these samples could arise from an immature carbonaceous material with an unusual aromatic nature containing C=O and C–H functional groups, rather than from a lack of thermal alteration.

(c) Extractable biomarkers

The distribution of \( n \)-alkanes is quite different between the two black shale samples and the carbonate marl sample (figure 2). The black shale samples are dominated by LMW \( n \)-alkanes, whereas the carbonate marl sample is dominated by HMW \( n \)-alkanes. However, the high maturity indicated for this sample by the low H/C value of 0.29 and Raman spectra of well-developed D and G bands is inconsistent with the preservation of even HMW \( n \)-alkanes. Therefore, it is possible that the HMW \( n \)-alkane envelope in the carbonate marl sample represents trace contamination of geologically younger material, a common problem for Precambrian samples [36]. The \( n \)-alkanes are not considered to be biomarkers as they can be synthesized biologically or non-biologically. For example, \( n \)-alkanes can be synthesized non-biologically via a process known as Fischer–Tropsch synthesis. The \( n \)-alkanes are formed during this abiotic process by the catalyzed addition of single carbons, with no obvious patterns in distribution, chain lengths and carbon number [37].

Bacteriohopanepolyols are pentacyclic organic compounds, whereas sterols are tetracyclic organic compounds (figure 5). Exclusively, biological processes form both these classes of compounds and thus these molecules can be used as target biosignatures for unequivocal extant life detection. Figure 5 shows that bacteriohopanepolyols and sterol molecules generally contain one or more oxygen atoms and often have carbon–carbon double bonds. During diagenesis, a low-temperature relatively shallow burial process occurring after the death of an organism, the oxygen atoms are cleaved from the molecule and the doubly bonded carbons are hydrogenated to produce the saturated-hydrocarbon geolipid form of the biomarkers (figure 5). In most cases, the reactions outlined above that occur during diagenesis have little or no effect on the skeletal part of the molecule. Therefore, the geologically derived hopanes and steranes can also be used as target biosignatures for unequivocal ancient fossil life detection.

Hopanes and steranes were detected above the background signal only in samples from black shale units (i.e. those containing LMW \( n \)-alkanes) and producing Raman spectra indicative of the thermally immature macromolecular hydrocarbon. Unlike \( n \)-alkanes, which are ubiquitous and not indicative of a specific biologic input, hopanes and steranes are indicative of bacterial and algal inputs, respectively [38,39]. Like the \( n \)-alkanes, these compounds are generally not preserved above \( ca 200^\circ C \) and have never been reliably detected in rocks reaching greenschist facies metamorphism [35].

(d) Significance for screening techniques and sample return missions

This work demonstrates that carbonaceous \( sp^2 \)-bonded network solids and thermally immature macromolecular hydrocarbon solids are readily and rapidly detected by Raman spectroscopy. We show that Raman spectra acquired from samples displaying well-developed G and D bands and additionally second-order bands are indicative of samples too thermally mature to contain any evidence of biologically derived organic material. Therefore, the source or origin of this material is impossible to elucidate by Raman spectroscopy alone. However, we demonstrate that Raman spectra acquired from samples showing bands due to vibrational modes from skeletal hydrocarbon backbones, and carbon–oxygen functional groups might be promising for subsequent analysis by GC–MS that could potentially reveal organic compounds of bona fide biological origin. Furthermore, these biomarker results obtained by GC–MS on ancient sedimentary material can afford source information such as bacterial and micro-algae inputs by the presence of hopanes and steranes, respectively.
Figure 5. Molecular structure of bacteriohopanepolyols and sterols and their geologically derived saturated-hydrocarbon biomarkers.

The major goal of the ESA 2018 mission to Mars, ExoMars, is the search for traces of past or present life [40]. The instrument payload has been purported to contain a drill for obtaining samples from depths of up to 2 m, PanCam a tool for observing rock outcrops, HR camera and microscope to investigate rock/core surfaces and variety of chemical analytical instruments for analysing mineralogy and organics (IR and Raman spectroscopies, GC– and laser desorption–MS) [40]. Raman spectroscopic analyses of thermally over-mature carbonaceous \( sp^2 \)-bonded network solids and thermally immature macromolecular hydrocarbon solids is not sufficient to determine the biogenecity of these organic materials. However, we show that Raman spectroscopy provides a promising new approach in astrobiological prospecting for rapidly screening samples containing thermally immature macromolecular hydrocarbon materials amenable to potentially successful biomarker analysis by GC–MS.

5. Conclusion

Here, we have demonstrated that Raman spectroscopy can be a valuable approach for delineating thermally immature geological organic matter as an effective methodology for rapid screening of samples prior to laborious biomarker analysis by GC–MS. This is of great significance for astrobiological prospecting on Mars during the ExoMars 2018 mission in the search for traces of past or present life. Promising samples that have a Raman spectrum indicative of low thermally mature functionalized hydrocarbon that are acquired in a relatively short period of time can then be selected for more intensive GC–MS analysis that is amenable for the detection of organic matter of an unequivocal biological origin.
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