Biomarkers and their Raman spectroscopic signatures: a spectral challenge for analytical astrobiology

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1. Introduction

The remote robotic exploration of extraterrestrial scenarios for evidence of biological colonization in ‘search for life’ missions using Raman spectroscopy is critically dependent on two major factors: firstly, the Raman spectral recognition of characteristic biochemical spectral signatures in the presence of mineral matrix features; and secondly, the positive unambiguous identification of molecular biomaterials which are indicative of extinct or extant life. Both of these factors are considered here: the most important criterion is the clear definition of which biochemicals truly represent biomarkers, whose presence in the planetary geological record from an analytical astrobiological standpoint will unambiguously be indicative of life as recognized from its remote instrumental interrogation. Also discussed in this paper are chemical compounds which are associated with living systems, including biominerals, which may not in themselves be definitive signatures of life processes and origins but whose presence provides an indicator of potential life-bearing matrices.

The application of Raman spectroscopic techniques to the characterization of the protective biochemicals used...
in the survival strategies of extremophilic organisms in terrestrially stressed environments [1,2], coupled with the palaeogeological recognition that early Mars and Earth had maintained similar environments under which Archaean cyanobacteria could have developed [3], has driven the proposal for the adoption of Raman spectroscopy as novel analytical instrumentation for planetary exploration [4–6]. The announcement by the European Space Agency that a miniaturized Raman spectrometer would form part of the Pasteur analytical life-detection protocol in the ExoMars mission scheduled to be launched in 2018 for the search for traces of life on Mars in the AURORA programme, with responsibility for the first-pass analytical interrogation of specimens from the Martian surface and subsurface, has confirmed that Raman spectroscopy will perform a key role for the molecular analytical protocols aboard the ExoMars rover. After several modifications, the ExoMars rover vehicle with European and Russian instruments, equipped with a drill capable of penetrating 2 m below the Martian surface regolith and housing a cache storage unit for the recovery of specimens in a later mission, will be deployed on the surface of Mars and will for the first time contain novel instrumentation to specifically seek biominerals and biological molecules arising from extinct or extant planetary life.

It is undeniable that the most important scientific discovery in a future space mission would be the furnishing of indisputable evidence for life signatures on another planet, whether they have arisen from extant or extinct sources; however, this statement itself generates two very important philosophical questions, namely, how do we define life and how would we then recognize it or its residues in the planetary geological record? We must also address the possibility that any extraterrestrial organism identified in space exploration could have originated on the Earth and have been transported to our planetary neighbours either by our own intervention, reinforcing the need for planetary protection protocols for our spacecraft and landers, or through panspermia processes, which could include the deposition of chemical building bricks from elsewhere through delivery by meteorites, comets and asteroids. The precise definition of life is actually rather elusive and many attempts to do so have been eventually deemed unsatisfactory [7–10]; the NASA definition of life as ‘a self-sustaining system capable of Darwinian evolution’ incorporates a molecular genesis with replicative procedures and avoids several pitfalls of alternative definitions based upon the ability of the system to reproduce [11].

2. Astrobiology versus exobiology

There has been much confusion in the literature between the terms astrobiology and exobiology with a conclusion that exobiology is the more archaic; in fact, as two elegant articles demonstrate [12,13], astrobiology was the earlier of the two by some 20 years. Although both are related to the study of extraterrestrial life, astrobiology was first used by Lafleur [14], an article which was largely ignored and not followed up until Tikhov’s book in 1953 [15], itself an enlargement from his use of the term ‘astrobotany’ in 1949 to describe his belief that vegetation existed on Mars and Venus. Tikhov was the first to suggest that spectral signatures from other planets could be used to assess their biotic potential, which today lies at the heart of remote space exploration. Strughold [16] also used the term ‘astrobiology’ in 1953, but thereafter it fell into disuse until 1983 [17] which prefaced its revival in the 1990s with the foundation of the NASA Astrobiology Institute and the worldwide excitement and discourse generated by the discovery of the Allen Hills SNC Martian meteorite, ALH 84001, in Antarctica and the observation of possible biotic morphological signatures therein [18]. As both Cockell and Martinez-Frias and Hochberg explain, exobiology was coined first by Lederberg [19] to specifically describe life beyond the Earth; in this context, therefore, astrobiology is a significantly wider term extending beyond extraterrestrial biology (exobiology) and addresses the origins, evolution, distribution and future of life in the universe, and indeed includes these same parameters terrestrially. This concept has been reinforced in a paper by Soffen [20] who described astrobiology in an exobiology journal (!) as ‘the study of the chemistry, physics and adaptations that influence the origin, evolution and destiny of life’.

Fundamentally, the three basic questions of astrobiology are: how did life begin and evolve, does life exist elsewhere and what is the future of life on the Earth and beyond? It is also clear from
the above analysis that astrobiology has a very different brief from astrochemistry, astrogeology and astrobotany; it is, therefore, the function of analytical astrobiology to apply the principles of chemical, biomolecular, morphological and microbiological analysis to the three baseline questions as outlined above (here, we consider analytical astrobiology to be the consideration of spectroscopic signatures rather than the morphological examination of structures).

In this paper, we endeavour to examine the terrestrial criteria for chemical biomolecular analyses associated with living organisms and apply these to extraterrestrial exploration envisaged by the inclusion of analytical instrumentation on remote planetary spacecraft and landers. The key question here, of course, is what biochemical species truly define the presence of extinct or extant life, be this terrestrial or extraterrestrial and whose signature recognition therefore unquestionably provides evidence of the presence of past or present life?

It is equally appropriate to consider some of the scientific parameters that will need to be evaluated for the detection of life using remote robotic analytical instrumentation, specifically of relevance to the forthcoming ExoMars project; primarily, the selection criteria for an analytical astrobiological mission such as ExoMars need to consider the following questions:

— What organisms could have existed and possibly survived the current and past extremes of environment on Mars?
— What types of geological niches are to be found which may conceal the traces of relict or extant life on Mars?
— What signatures would these organisms have left in such environments as indicators of their presence and how are we going to recognize them?
— What molecules could be considered as constituting a proof of life on Mars?
— Are there terrestrial scenarios or putative Mars analogue sites which could be used as ‘models’ or test beds for the evaluation of these scientific questions?

3. The historical Mars

From the birth of our Earth 4.6 Ga, the terrestrial geological record suggests that microbial autotrophic ecosystems already existed on the Earth from 3.5 to 3.8 Ga. There is now much evidence that early Earth and early Mars were indeed very similar in their physico-chemical composition; as Mars is significantly smaller than the Earth, it is very likely that planetary cooling occurred more rapidly and that Mars was able to sustain an aqueous environment on its surface earlier than was possible on the Earth. In the Epoch I (ca 4.65–3 Ga) period of Mars proposed by McKay [3], the planet was probably more temperate and wet and since there is geological evidence that life had already started on the Earth during this period, it seems reasonable to conclude that life had also started on Mars too; by Epoch IV (ca 1.5 Ga to the present), however, catastrophic changes on Mars would have compromised the survival of organisms on the Martian surface and it is possible that the Martian analogues of terrestrial extremophiles would have been the last survivors of life on Mars through their environmental adaptation only in Martian geological niche sites.

(a) Analytical astrobiology of Mars

The detection of biomolecular markers in geological substrates or the subsurface regolith of Mars is a primary goal for astrobiology [21,22]; however, the evolutionary pressure of environmental stress on Mars, especially the high levels of low-wavelength ultraviolet radiation insolation, low temperatures, extreme desiccation and hypersalinity would have demanded appropriately severe protective strategies to promote the origin, survival and evolution of microbial life [23]. The ultraviolet radiation protection afforded to subsurface organisms by the iron(III) oxide surface regolith acting as a filter has been proposed as a key factor for the maintenance of biomolecular activity at the Martian surface [24], but the same ultraviolet and low-wavelength electromagnetic radiation insolation generates hydroxyl radicals and peroxides in the surface regolith which
would certainly inhibit the survival of complex biomolecules in the surface oxidation zone. In this respect, the diagnosis, catagenesis and biodegradation of terrestrial Mars analogues to give the fossils recognizable in our own geological record would not be transposable to a Martian scenario. Hence, the complex chemical systems comprising terrestrial soils, bitumens and kerogens found in our own planetary lithology [25–27] and which provide much valuable information about their sourcing processes would not be expected to occur to the same extent on Mars.

However, it is believed that Mars might still preserve a chemical record of early life in rocks from the Noachian era, which overlaps the terrestrial Archaean geological history, from about 3.8 Gya. The search for extinct or extant life on Mars must therefore centre upon the identification and recognition of specially protected niche geological sites in which the biomolecular signatures would be preserved; the fundamental approach must then consider the detection of key molecular biomarkers, probably within rocks and certainly subsurface, perhaps even in ancient lacustrine sediments [28,29], which will necessitate the deployment of remote analytical sensors with preset protocols and an established database recognition strategy for minerals, biologically modified geological strata and biomolecular residues. Examples from terrestrial analogue sites could therefore include carbonates, carbonated hydroxyfluoroapatite, gypsum, calcium oxalates, porphyrins, carotenoids, scytotetam and anthraquinones [30,31].

Clearly, the identification and selection of terrestrial Mars analogue sites [28,32] will be a critical step in the development of an analytical astrobiology for Mars in two respects: firstly, the understanding of the type of geological formations that have been colonized by extremophilic organisms in terrestrial ‘limits of life’ situations and habitats; and secondly, the deployment of novel analytical instrumentation which can reveal the presence of the key signatures of extinct and extant life in micro-niches in the geological record [28–35]. The gathering of data from such terrestrial Mars analogue sites is therefore the first step to be taken in the search for extraterrestrial life signatures; in this, the application of Raman spectroscopic techniques has been successful through the direct characterization of the molecular and molecular ionic signatures of biomolecules and their modified structures situated in the geological record which does not involve either the physical or chemical separation of the organic and inorganic components. Some of these terrestrial Mars analogue sites are described below and the data obtained using laboratory-based Raman spectroscopic techniques have advanced our understanding of extremophile behaviour significantly.

In a recent special issue of the Philosophical Transactions of the Royal Society, in a year that celebrated its 350th anniversary as the longest running scientific journal, several articles highlighted the role of Raman spectroscopy in the characterization of biosignatures of extremophilic colonization of geological substrates in a range of stressed terrestrial environments [25,30,36–45]; these articles in the same issue which address the detection of geological and biogeological spectral markers that are relevant to space missions give a very good appreciation of the spectroscopic requirements that will be essential for the construction of a relevant spectral database [46] for the ExoMars and forthcoming space missions which have a Raman spectrometer aboard their rover vehicles. Some selected examples of the data which can be provided by the Raman spectroscopic interrogation of terrestrial Mars analogue sites will be highlighted.

4. Biomarkers

A biomarker can be defined as a chemical species or topographical pattern which is *uniquely* derived from living organisms; thus, from the standpoint of analytical spectroscopy, it is not sufficient to simply embrace an organic or bioinorganic molecule that is used generically or synthesized by life forms, as several of these can also be synthesized abiotically. Examples of the latter include amino acids and proteins. A further complication arises in that organic molecules degrade under stressed environmental conditions, forming derivatives and eventually carbon. The realization that methane, calcium carbonate, carbon and polyaromatic hydrocarbons can be synthesized by geological processes as well as through the degradation of biomolecules means that these molecular carbonaceous materials are not in themselves strictly suitable for
classification as biomarkers—even though the detection of methane, carbon or polyaromatic hydrocarbons on Mars would be an exciting and novel discovery in itself, this would not result in the unambiguous conclusion that life did once exist or may even still be extant on Mars! Ambiguous biomarkers: initially appear to be good candidates for biomarkers but they are not clearly definable as such since they can all be synthesized abiotically or in an interstellar medium: n-alkanes, polyaromatic hydrocarbons, N-heterocycles, amino acids, proteins, kerogen, urea and carbon. It is crucial, therefore, that we can identify a suite of molecular biomarkers, whose detection would positively and unambiguously indicate the presence of extinct or extant life; here, we need to also narrowly permit the definition of life as cyanobacterial, which represents the earliest identifiable Archaean life forms on the emerging planetary and oceanic Earth, some 3.8 Gya. From spectroscopic and microbiological analytical studies of terrestrial cyanobacteria, it has been possible to isolate several biomolecules which truly can be considered as key spectroscopic biomarkers, from which we can safely construct a Raman spectroscopic database that will act as a true standard of assessment for the presence of life in the biogeological record. Such a definitive list is provided in table 1.

It is interesting to compare this list of potential biomarkers with those already mentioned in two key literature publications which have also proposed biomarkers: firstly, a paper by Perry which set out to define biominerals and organominerals as direct and indirect indicators of life [47]. Secondly, in the same year, another paper [48] described a comprehensive list of biomarkers for study as selective targets for the ExoMars mission specifically addressing the antibody requirements for the Life Marker Chip instrumentation to be carried on that mission at that time; this instrument was unfortunately deselected later. The first comment is that the range of biomarkers selected in these two papers is large and needs necessarily perhaps to be focused on more definitive molecular species. Secondly, a detailed consideration of the organic molecules in these lists, although undoubtedly synthesized by living systems, casts a doubt on their authenticity and suitability per se for life-detection experiments in analytical astrobiology as they can also be synthesized abiotically, which surely renders them inadmissible as true biomarker candidate molecules. We should also address the potential longevity of survival of the selected biomarkers in the geological record as this will effectively dictate the usefulness of any selected biomarker target for remote astrobiological analysis; this is by no means an easy issue to face—although much work is now being carried out to artificially age molecules in extreme environments to visualize and evaluate the problem of degradation of biomolecular signatures on their exposure to reactive chemicals, radiation and desiccation [49].

A very important requirement fulfilled by Raman spectroscopy in the analysis of biomarkers is the ability to differentiate between the key identified molecular species on the basis of their characteristic spectral signatures; this is not only manifest in the discrimination between the relevant organic components of the complex protective biochemicals comprising the stressed biological colonies and the minerals of the geological host matrix but also the identification of

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**Table 1. Biomarkers for analytical astrobiology.**

<table>
<thead>
<tr>
<th>Bioorganic molecules</th>
<th>Bioinorganic molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>scytonemin</td>
<td>whewellite</td>
</tr>
<tr>
<td>carotenoids</td>
<td>weddellite</td>
</tr>
<tr>
<td>carotanes</td>
<td>aragonite</td>
</tr>
<tr>
<td>chlorophyll</td>
<td>vaterite</td>
</tr>
<tr>
<td>trehalose</td>
<td>mellite</td>
</tr>
<tr>
<td>phycocyanins</td>
<td></td>
</tr>
<tr>
<td>hopanoids</td>
<td></td>
</tr>
<tr>
<td>oxalates</td>
<td></td>
</tr>
</tbody>
</table>
the different types of biomarker, such as those specified in table 1. A summary of the important
and designatory Raman bands which serve in the identification and discrimination of these key
biomarkers and their mineralogical host matrices is given in table 2.

The major objective in any future planned robotic mission to our neighbouring planets and
their satellites in a search for extinct or extant life is the unequivocal analytical recognition
of the appropriate indicators; whereas biological and microbiological measurements will rely
upon visual morphologies and test stimuli from antibodies, from a chemical standpoint the
assessment of current or former life presence will need to be on the basis of identification of
suitable biomolecular signatures. In this respect, the remote chemical analysis of extraterrestrial
scenarios will be dependent on the adoption of the following protocol.

— A database of molecular signatures that are uniquely and truly biomolecular, i.e.
molecules that are synthesized from living systems and which, therefore, necessarily
exclude other potential candidate molecules which, although seemingly biomolecular
in origin, actually can be created artificially and abiotically in extreme environments.
This may seem obvious, but as will become apparent there are several target molecules
whose presence if detected in an extraterrestrial environment will not constitute
irrevocable proof of life. Not all organic molecules, even those of some complexity,
fall into this category and classic examples include methane, amino acids, porphyrins,
carbon, polyaromatic hydrocarbons and sugars. In 1828, Wohler first made an organic
biomolecule, urea, by the thermal conversion of an inorganic species, ammonium
cyanate, thereby dispelling the current idea held at that time that organic biomolecules
contained some aethereal and indefinable life force.

— Having identified an acceptable list of biomolecular targets which can be considered as
life biomarkers, the selection of appropriate analytical techniques suitable for operation
on space missions must be undertaken. The discriminatory ability of selected techniques
for these targets in the presence of abiotic and host matrix material, which may be
complex organics or minerals, is essential; this means that the respective biomarker
signatures should be differentiated from each other in admixture and also from a wide
range of potential interference data from their environments, generally expected to
comprise geological niche environments.

— The possibility of differentiating between extant and extinct life must be considered and
whether or not this can be ascertained from the biomarker signatures. Knowledge about
the longevity of biomarker survival in hostile environments on the planetary surface
is a vital requirement for assessing the prediction of extant or extinct life recognition;
in this aspect, terrestrial experiments in chambers constructed to simulate planetary
atmospheric conditions and potential habitats especially with regard to desiccation, high
energy UV solar insolation, temperature ranges and chemical toxicity are mandatory.

— Finally, as the biological colonization of extreme environments is undertaken terrestrially
by extremophilic bacteria through their adaptation of their geological host matrices,
a survey needs to be made of the appropriate changes in the biomineralogy which
result from this adaptation and which, if recognized as such, could be used as
potential indicators of extinct life wherein the biomarker molecular traces of life have
been eradicated and become undetectable to the \textit{in situ} analytical interrogation being
performed.

5. Role of Raman spectroscopy in biomarker detection

Raman spectroscopy has been identified as a prime molecular analytical technique for
mineralogical differentiation on remote robotic space missions to planetary surfaces and
subsurfaces; because the Raman spectrum consists of laser excitation giving rise to a series
of characteristic bands in the wavenumber range 100–3500 cm$^{-1}$ the molecular signatures of
minerals and biomolecules occur in the same analytical interrogation process. Hence, it is
<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Formula</th>
<th>Bands (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>CaCO₃</td>
<td>1086 712 282</td>
</tr>
<tr>
<td>Aragonite</td>
<td>CaCO₃</td>
<td>1086 704 208</td>
</tr>
<tr>
<td>Dolomite</td>
<td>CaMg(CO₃)₂</td>
<td>1098 725 300</td>
</tr>
<tr>
<td>Magnesite</td>
<td>MgCO₃</td>
<td>1094 738 330</td>
</tr>
<tr>
<td>Hydromagnesite</td>
<td>Mg₃(CO₃)₄(OH)₂·4H₂O</td>
<td>1119 728 326</td>
</tr>
<tr>
<td>Gypsum</td>
<td>CaSO₄·2H₂O</td>
<td>1133 1007 669 628</td>
</tr>
<tr>
<td>Anhydrite</td>
<td>CaSO₄</td>
<td>1015 674 628 500 416</td>
</tr>
<tr>
<td>Quartz</td>
<td>SiO₂</td>
<td>1081 1064 808 796 696 500 416</td>
</tr>
<tr>
<td>Haematite</td>
<td>Fe₂O₃</td>
<td>610 500 411 293 245 226</td>
</tr>
<tr>
<td>Limonite</td>
<td>FeO(OH)·nH₂O</td>
<td>693 555 481 393 299 203</td>
</tr>
<tr>
<td>Apatite</td>
<td>Ca₅(PO₄)₃(F,Cl,OH)</td>
<td>1034 963 586</td>
</tr>
<tr>
<td>Weddelite</td>
<td>Ca₃(C₂O₄)·2H₂O</td>
<td>1630 1475 1411</td>
</tr>
<tr>
<td>Whewellite</td>
<td>Ca₅(C₂O₄)H₂O</td>
<td>1629 1490 1463 1396 942 896 865 596 521 504 223 207</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>C₅₀H₇₂O₅N₄Mg</td>
<td>1438 1387 1326 1287 1067 1048</td>
</tr>
<tr>
<td>C-phycocyanin</td>
<td>C₃₆H₃₈O₆N₄</td>
<td>1655 1638 1582 1463 1369 1338 1272 1241 1109 1054</td>
</tr>
<tr>
<td>β-carotene</td>
<td>C₂₀H₁₆O₈</td>
<td>1515 1155 1006</td>
</tr>
<tr>
<td>Rhizocarpic acid</td>
<td>C₃₀H₂₅O₆</td>
<td>1665 1610 1595 1518 1496 1477 1347 1303 1002 944 902 768 448</td>
</tr>
<tr>
<td>Scoytenmin</td>
<td>C₃₆H₃₈N₂O₄</td>
<td>1605 1590 1549 1444 1323 1283 1245 1172 1163 984 752 675 574 270</td>
</tr>
<tr>
<td>Calycin</td>
<td>C₁₈H₁₄O₅</td>
<td>1653 1635 1611 1595 1380 1344 1240 1155 1034 960 878 498 484</td>
</tr>
<tr>
<td>Paretin</td>
<td>C₁₈H₁₂O₇</td>
<td>1671 1631 1613 1153 1387 1370 1277 1255 926 571 519 467 458</td>
</tr>
<tr>
<td>Usnic acid</td>
<td>C₁₈H₁₆O₇</td>
<td>1694 1627 1607 1322 1289 1192 1119 992 959 846 602 540</td>
</tr>
<tr>
<td>Emodin</td>
<td>C₁₈H₁₂O₇</td>
<td>1659 1607 1577 157 1298 1281 942 565 467</td>
</tr>
<tr>
<td>Atranorin</td>
<td>C₁₉H₁₈O₈</td>
<td>1666 1658 1632 1303 1294 1266 588</td>
</tr>
<tr>
<td>Pulvinic dillactone</td>
<td>C₁₈H₁₀O₄</td>
<td>1672 1603 1455 1405 1311 981 504</td>
</tr>
<tr>
<td>Gyrophoric acid</td>
<td>C₂₄H₁₀O₁₀</td>
<td>1662 1628 1612 1334 1304 1291 1235 1138 561</td>
</tr>
</tbody>
</table>

Table 2. Raman bands of the most common geo- and biomarkers in extremophile exemplars and their chemical formulae (cm⁻¹ with 532 nm excitation). Corroborative bands appear in bold.
apparently admirably suited to the analysis of the mineralogy, biogeology and biomolecules which may occur in heterogeneous specimens of colonized rocks. However, several salient points need to be appreciated for the correct interpretation of Raman spectroscopic data obtained from such complex systems.

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Each molecular species, whether mineral or biological in origin, possesses a number of characteristic bands which can be used for their identification; these bands may overlap each other depending upon the laser illumination and operating conditions of the spectrometer. Components of mixtures may have slightly different wavenumber values compared with pure materials and laboratory standards arising from the different electronic environments in which the target molecules are placed.

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With a few notable exceptions, such as the sp\(^3\) hybridized carbon in diamond, Raman spectra of minerals and biomolecules generally possess more than a single band and individual band intensities may be strong or weak depending upon the molecular scattering factors, crystal orientations and the molar fraction of the species present; this means that Raman spectroscopy may not detect species at low levels of concentration, unless special laser illumination which may excite resonance effects is invoked.

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The Raman spectrum of a given molecular species is generally much simpler than its infrared absorption counterpart, is relatively insensitive to water and can interrogate lower wavenumbers which are generally not accessible in the infrared without special moisture purging techniques. Hence, mineral differentiation of compounds which are not easily determined using elemental techniques such as scanning electron microscopy, such as the polymorphic forms of titanium(IV) oxide (anatase, rutile and brookite), lead(II) oxide (massicot and litharge) and calcium(II) carbonate (calcite, aragonite and vaterite) can all be recognized using their distinctive Raman bands. Despite this, it must be realized that in many cases a single Raman band wavenumber is insufficient to unambiguously identify a particular material: a classic example is provided by the two polymorphs of calcium(II) carbonate, calcite and aragonite, which both have a major intensity feature assigned to C–O symmetric stretching of the CO\(_2\)^\(^-\) at 1086 cm\(^{-1}\)—discrimination can be effected between the two possibilities using their subsidiary, but weaker intensity, bands occurring at 712, 703 and 281, 203 cm\(^{-1}\), respectively. This can be useful for the relative analytical quantification of the two polymorphs when these occur together in natural specimens such as corals and stalactites.

Not all the members of a family of minerals or biomolecules will have identical Raman spectral band wavenumbers; hence, the strongest intensity band in carotenoids, the C=C stretching vibration, can occur in the range 1500–1535 cm\(^{-1}\) dependent on the length of C=C–C conjugation and type of terminal groupings. Whereas this can be of diagnostic value for the differentiation of individual carotenoids and indeed often in admixture, such as astaxanthin, \(\beta\)-carotene and lutein, it can pose a significant problem for generic automatic database recognition programmes.

The identification of unique biomolecules and the interpretation as indicators of extant or extinct life in the geological environment is a prime advantage of diagnostic Raman spectroscopy; however, the assumption that the longevity of survival of these biomarkers in hostile, stressed environments is by no means assured. We must therefore consider the likelihood that these identified biomarker molecules for life in stressed environments may be degraded; this raises a very important point for analytical interrogation—does the data acquisition reveal the true presence of biomolecular entities or degradation products of these? Also, as the Raman spectra of the biomarkers cannot differentiate between biomolecules from living or extinct colonies in the geological matrix, it would be wrong to simplistically label Raman spectroscopy as a life detector as has been done in the literature.

An assessment needs to be made of both the longevity in the geological record and the degradation products of key biomolecules; several statements have been forthcoming about the former and much debate has taken place on the latter. A major philosophical problem in
the debate centres on degradation products simply because on degradation the biomolecular uniqueness of the biomarkers is relegated to simpler organic molecules which themselves cannot possibly occupy the same classification, and indeed, now become confused with abiotic congeners. Suitable illustrative examples are provided by the degradation of chlorophylls to complex corrins and thence to heterocyclics and finally carbon, and proteins to amino acids.

It is now appropriate to consider in more detail some types of potential key biomarker molecules which could be critical for the identification of the presence of extraterrestrial life.

(a) Carotenoids

Because the Raman spectral signature wavenumbers of any molecule or molecular ion are dependent upon the electronic and geometric composition of the particular bonds giving rise to the molecular scattering of radiation, the wavenumbers observed in the spectra of a group of closely related compounds such as carotenoids are found to occur over a wavenumber range rather than at a single, precise wavenumber. For example, the biogenetic synthesis of carotenes from the parent lycopene structure involves cyclization, producing $\alpha$- and $\beta$-carotenes, and further hydroxylation in the aliphatic rings gives lutein and zeaxanthin [50]; despite the latter both being centrosymmetric molecules and having a conjugated 11-ene system with three methyl groups in each ring and a further four pendant methyls situated along the unsaturated $\equiv C=C\equiv C$ backbone chain, these very similar structures can normally be readily differentiated by Raman spectroscopy. More troublesome, however, is the observation of wavenumber-shifted fundamental bands that occur because of external molecular environmental changes—and these are more difficult to quantify; hence, the importance of constructing reference databases of biomolecules in admixture particularly with minerals. Finally, it is not merely sufficient to identify the Raman spectral signatures of biomarkers that also have parallels for abiotic organic compounds as this will in itself create an ambiguous interpretation of the spectral data from a remote planetary interrogation; hence, the observation of CH stretching Raman wavenumbers associated with aliphatic organic compounds in the 2800–3000 cm$^{-1}$ region on the ExoMars mission would not be exclusively indicative per se of life signatures on Mars as this functionality also occurs widely in abiotic organic compounds, although this result would be the first direct evidence that organic compounds can survive the stressed Martian environment—itself a major step forward in current knowledge [51,52].

(b) Carbon

The degradation of biomolecules in stressed geological environments eventually produces carbon [40,53,54], whose signature(s) in the Raman spectrum have been well described in the laboratory. The idea that it is possible to differentiate between biotic and abiotic carbon formation from the shape of the so-called D and G Raman bands, characteristic of sp$^3$ and sp$^2$ hybridized carbon, effectively represented by structures typical of diamond and graphite, has had a long and rather controversial history in the literature which is still ongoing today; an elegant summary of the comprehensive and extensive literature on this rather controversial subject has been provided [26], in which it has been attempted to define a possible methodology for rigorously discriminating between different carbon sources. However, if we consider the requirements of such finely tuned spectroscopic work that has to be undertaken in the field using portable miniaturized instrumentation with all the sacrifices that have had to be made from laboratory versions, additionally performed under extremely hostile conditions that are mirrored nowhere else terrestrially, then one must pose the question whether it is possible with current remote robotic and miniaturized instrumentation to be able to differentiate between abiotic and biotic carbon. The result then is that the observation of characteristic carbon signatures does not constitute itself the presence of extinct life, so according to our definition, therefore, carbon cannot be a true biomarker. The same analogy applies to a host of other biochemicals, which have been synthesized abiotically in the laboratory under planetary atmospheric and environmental
conditions, such as amino acids, sugars, porphyrins and proteins. This is the reason that these molecules are not presented as true biomarkers in Table 1. An interesting example of this problem is found in the recent Cassini mission to the surface of Titan, a planetary moon, where pools of liquid hydrocarbons were detected by the analytical probe at the surface; however, a biotic source can probably be ruled out as its passage through the Titan atmosphere revealed signals of a range of organic chemicals such as hydrocyanic acid, cyanogen, hydrocarbons and ammonia which can be ascribed most probably to atmospheric abiotic syntheses involving electrical storm gradients.

6. Conclusion

It is likely that a definitive list of biomarkers is now emerging for discussion and eventual acceptance in the scientific community relating to the unambiguous identification of spectroscopic signatures in the search for extinct or extant life in proposed analytical astrobiological missions. The materials discussed here will provide a starting concept that will naturally be amplified and modified by future terrestrial experiments so that a definitive idea of the identity of unambiguous biomarkers will be forthcoming for analytical astrobiological space probes.

References


