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Raman spectroscopy of the Dukhan sabkha: identification of geological and biogeological molecules in an extreme environment

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The characterization of minerals and biogeological deposits in a terrestrial Arabian sabkha has a direct relevance for the exploration of Mars since the discovery by the NASA rovers *Spirit* and *Opportunity* of evaporate minerals on Mars that could have arisen from aquifers and subsurface water movement. The recognition of carbonates and sulphates in Gusev Crater has afforded an additional impetus to these studies, as relict or extant microbial extremophilic organisms could have colonized these geological matrices, as has been recorded on Earth. Here, we describe the Raman spectroscopic analysis of specimens of evaporitic materials sampled from the Dukhan sabkha, the largest inland sabkha in the Persian Gulf. With daily temperatures reaching in excess of 60°C and extreme salinity, we have identified the characteristic Raman signatures of key biomolecular compounds in association with evaporitic minerals and geological carbonate and sulphate matrices, which indicate that extremophilic cyanobacterial colonies are existent there. This evidence, the first to be acquired spectroscopically from such a region, establishes a platform for further studies using remote, portable Raman instrumentation that will inform the potential of detection of similar systems on the Martian surface or subsurface in future space missions. A comparison is made between the results from this study and the previous analysis of a gypsum/halite sabkha where the extremophilic molecular signatures were better preserved.

Keywords: Raman spectroscopy; sabkha; evaporites; cyanobacteria; extremophiles

1. Introduction

The successful deployment of the NASA Mars rovers *Spirit* and *Opportunity* on the planetary surface has resulted in the provision of much novel information about the mineral composition of the planet's surface; the presence of sulphates

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and hydrates related to terrestrial compounds of calcium, magnesium and iron coupled with the discovery of subsurface water makes an investigation of analogous systems on Earth a high priority, especially for the evaluation of analytical instrumentation that will constitute future exploratory missions to the Martian surface. In the forthcoming ExoMars mission for the detection of extant or extinct life on Mars (Edwards 2007), a miniaturized Raman spectrometer will form part of the Pasteur suite seeking spectral signatures of key biomolecules in the Martian geological record in addition to the characterization of the host mineral matrices. Several sites on Earth will bear investigation in this respect and have been labelled as Mars analogue sites; an example of these is provided by salterns such as the *sabkhas*, a generic term for a salt-encrusted deflation surface (Shearman 1963).

Originally applied to describe the origin of evaporite minerals such as gypsum and anhydrite in carbonate matrices, the sabkha cycle involves rock facies that have been deposited in the supratidal as well as the subtidal and intertidal zones of shallow-water carbonated systems under arid conditions (Kendall & Skipwith 1968, 1969; Kendall *et al.* 2002). Sabkhas occur in extreme environmental regions of high temperature and aridity and are characterized by extensive surface deposits or crusts of saline material that nevertheless support life in the form of extremophilic or extremotrophic colonies of cyanobacterial mats in the intertidal zone and salt-tolerant extremophiles within the subsurface minerals. It is important to realize that a sabkha is a dynamic system in which the subsurface water resource is critical for the establishment of the precipitation of mineral solutes such as halite at the surface and for retrograde minerals such as anhydrite, calcite, dolomite and gypsum in the subsurface capillary zones. Despite their importance geologically and special relevance to Martian exploration, the only previous Raman spectroscopic study of a sabkha was carried out recently in the Rhub al Khali or Empty Quarter of the Arabian Desert by Edwards *et al.* (2006) and the survival strategy enunciated through the Raman spectroscopic identification of the key biomolecular signatures of protective biochemicals synthesized by the extremophile colonies to minimize the effects of intense low-wavelength radiation insolation, extreme temperatures, desiccation and salt stress. The stratigraphy of the biological colonization in the host mineral matrix was revealing in that it demonstrated that the biological activity registered by the presence of scytonemin and carotenoids was located under the surface, comprising crystalline halite and gypsum in an area rich in dolomitized calcite.

The opportunity was afforded recently to sample the Dukhan sabkha in Qatar, which represents a different type of sabkha from that which was analysed previously using Raman spectroscopy. The Dukhan sabkha is situated in the central western part of Qatar and lies to the south of Zikrit Gulf; the sabkha covers an area of about $73\,\mathrm{km}^2$, with a length of 20 km and a width of between 2 and 4 km and a surface between ± 6 and 7 (m).a.s.l. (Al-Youssef *et al.* 2006). The sabkha is described as a depression continuation of the Zikrit Gulf filled with aggregation sediments, evaporites and accumulation of aeolian sediments. The negative topography has an impact upon the nature of the sediments found in the sabkha, and these differ significantly from those found in the Abu Dhabi sabkha in the Rhub al Khali. Here, the surface sediments of our study area are composed of hard, dry crusts of halite about 2 cm thick, covering brownish-grey

Figure 1. Surface crust of the Dukhan sabkha showing the friable nature of the mixture of sand, carbonates and halite.

aeolian sand (figure 1). In some parts, these sand deposits are intercalated with gypsum; in the central part of the sabkha depression, the halite crust covers a 2 m thick bed of gypsum (Perthuisot 1977). Chemical analysis (Illing *et al.* 1965; Illing & Taylor 1993; Al-Youssef *et al.* 2006) of these deposits indicates that they comprise 33 per cent anhydrite, 19 per cent halite, 10 per cent gypsum and 14 per cent dolomite, with quartz as the main clastic material at 18 per cent.

In the present work, we have sampled the Qatar sabkha to provide a scenario for Raman spectroscopic data evaluation in only the second location of this type terrestrially. The discovery of extensive halite and gypsum deposits on Mars, therefore, makes this an attractive terrestrial analogue for the detection of extremophilic biochemicals for which there has been but little evidence provided hitherto.

2. Experimental

Raman spectra were recorded using a Renishaw InVia Raman microspectrometer with 785 nm laser excitation and objective lenses of 50× and 100× magnification that provided a spectral footprint of between 2 and 5 μm at the specimen. Laser powers were kept to a minimum to avoid radiation damage to sensitive biochemicals encased in their host mineral matrices and were typically several microwatts. Individual spectral scans of 10 s duration were accumulated up to 10 times and signal averaged to provide improved signal-to-noise ratios at each sample point over a typical wavenumber shift range of 100–3200 cm^{-1} with a spectral resolution of 2 cm^{-1}. The range was sufficient to encompass the spectral signatures of most inorganic molecular ions and biological materials; the spectral selection rules in Raman spectroscopy are such that monatomic ions are inactive, so the halite species are invisible. Each specimen was analysed at several different sampling points in replicate to account for sample inhomogeneities. The spectra shown as examples of those obtained have not been subjected to background subtraction or spectral manipulation.

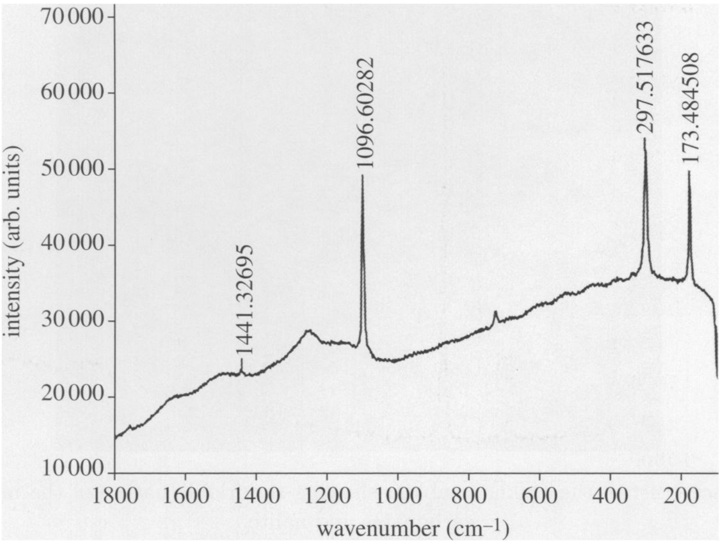

Figure 2. Raman spectrum of sample 3 from the Zikrit sabkha, showing evidence for dolomite mineral and organic components.

(*a*) *Samples*

Eleven specimens of the Zikrit sabkha encrustations were collected from two sites, both of which showed evidence of cyanobacterial colonization and mineral efflorescence. A major difference that distinguished the sampling procedures at this site compared with that of the Rhub al Khali sabkha was the friability of the specimens encountered here; the surface mineralization in the former was powdery compared with large crystalline composites and a well-developed stratigraphy in the latter site. All specimens from the Zikrit sabkha were beige-brown in colour with darker brownish-black regions as shown in the photograph in figure 1.

3. Results

Generally, the Raman spectra obtained from the Zikrit sabkha specimens showed evidence of minerals and organic compounds in admixture; a good example of this is shown in figure 2, which demonstrates the characteristic spectral features of dolomite, $CaMg(CO_3)_2$, with Raman bands at 1096, 750, 298 and 173 cm^{-1} and the presence of broad features owing to organic molecules at 1690, 1645, 1520, 1441 and 1250 cm^{-1}. The latter bands clearly show evidence of degradation from their large bandwidths (Edwards *et al.* 2008).

In figure 3, sample 1, the spectra indicate that both the organic and inorganic components are different, the former being assignable to amorphous carbon with broad Raman bands at 1580 and 1320 cm^{-1} (data not shown) and the latter to gypsum, $CaSO_4{\cdot}2H_2O$, with characteristic features at 1135, 1006, 668, 616, 491, 411 and 176 cm^{-1}. Here, it can be concluded that the organic component has suffered extreme degradation to carbon.

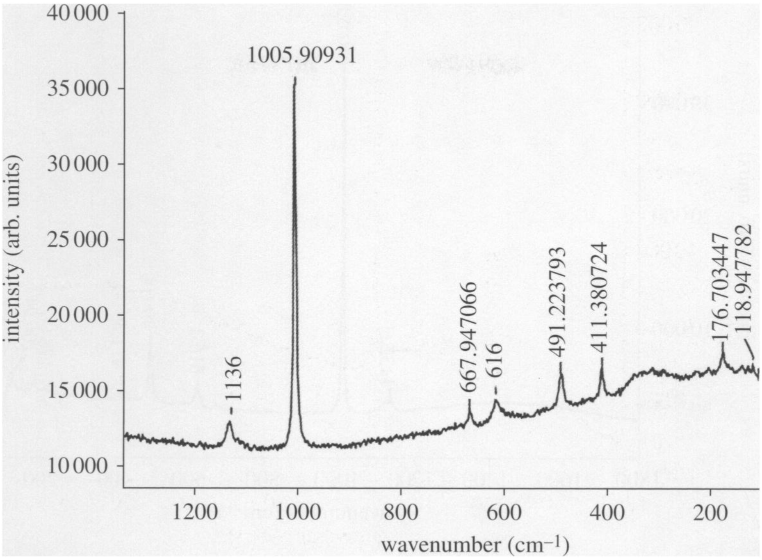

Figure 3. Raman spectrum of sample 1 showing the presence of gypsum.

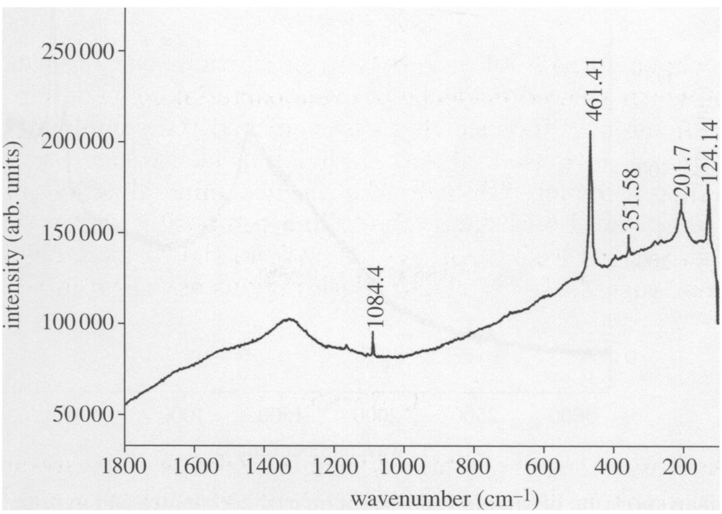

Figure 4. Raman spectrum of sample 4 with aragonite and quartz as minerals and organic components identified as carbon with possibly chlorophyll.

Figure 4 provides another example of the sabkha inhomogeneity in that now the minerals are identified as aragonite (Raman bands at 1085, 202 and 124 cm^{-1}) and quartz (band at 461 cm^{-1}) and the organic components as chlorophyll and carbon. Other differences can be noted in the spectrum of sample 6 (figure 5), in which the features of gypsum and carbon are clearly seen, and sample 7,

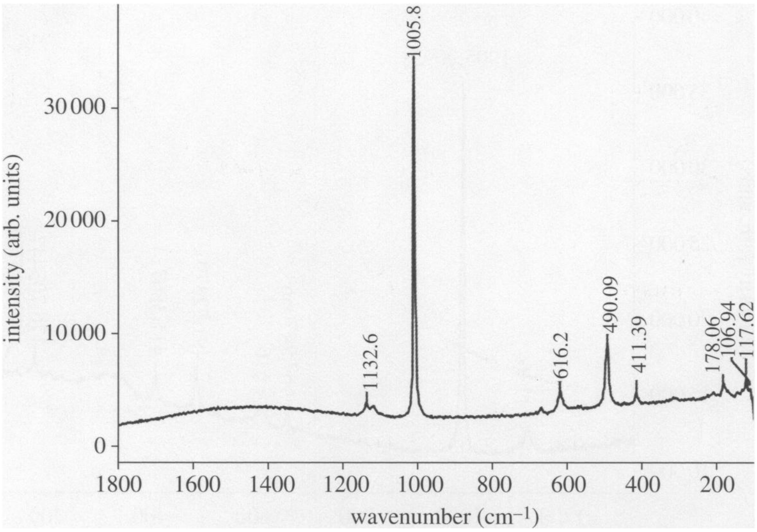

Figure 5. Raman spectrum of sample 6, showing the presence of gypsum and carbon.

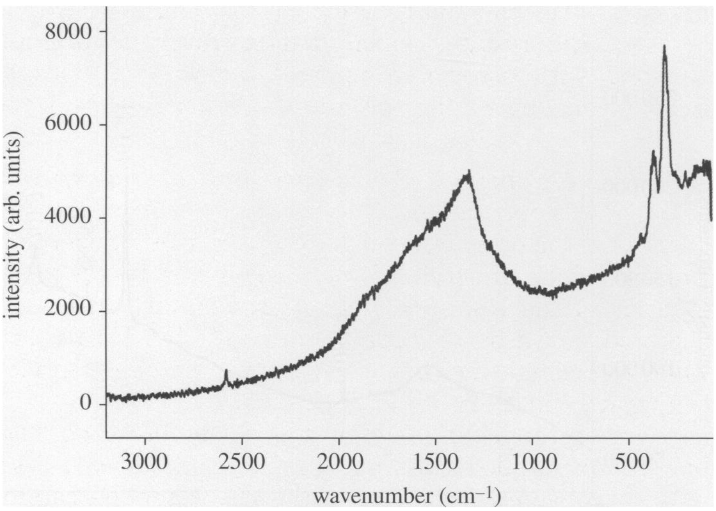

Figure 6. A Raman spectrum of sample 7, devoid of quartz, carbonates and gypsum, but with clear signatures of carbon and an unidentified mineral species with bands at 387 and 333 cm^{-1}.

taken nearby (figure 6), which gives a different composition, being carbon- and gypsum-free, but now we have a new, as yet unidentified, mineral with bands at 332 and 382 cm^{-1}.

Sample 11 is unique in that the Raman spectral data (figure 7) show no mineral features at all (remembering that the presence of halite cannot be excluded), but there are clear characteristic bands owing to scytonemin, the cyanobacterial radiation protectant biomolecule.

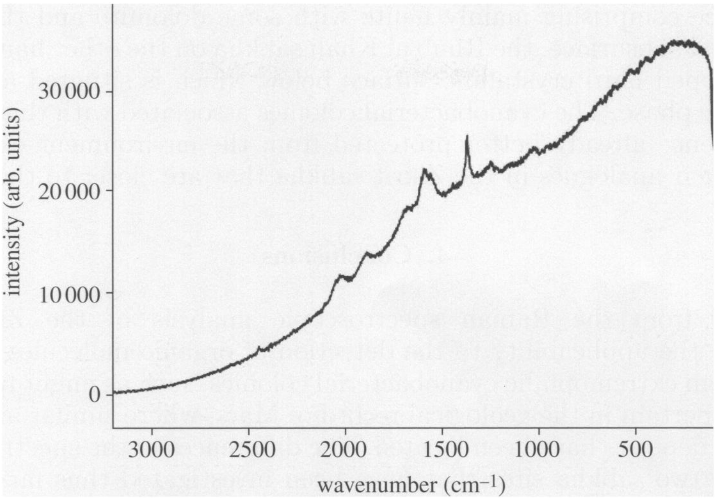

Figure 7. Raman spectrum of sample 11, showing the characteristic features of scytonemin, the cyanobacterial radiation protectant pigment.

In comparison with the only previous sabkha studied using Raman spectroscopy, from the results, which demonstrate the presence of organic biomolecules along with associated mineral deposits in both cases, we can conclude that the following similarities and differences can be identified.

— In both cases, whereas the association of dolomite with organic biomolecules is apparent, the Zikrit sabkha features significantly higher degradation of the organics, which can be attributed to the lower integrity and fragility of the mineralized surface compared with the well-developed stratigraphy of the Rhub al Khali specimens in which the biosignatures were indicative of a better protection being afforded to the organic guest molecules and colonies associated with the dolomitized calcite substructure of the gypsum and halite crystalline surface.
— A much wider range of minerals has been identified here, comprising aragonite, gypsum, dolomite, quartz and so on. It is tempting to suggest that the cyanobacterial colonies in the Zikrit sabkha cannot cope as well with this admixture as can their counterparts in the Rhub al Khali sabkha where a simpler mineral composition is omnipresent. This hypothesis requires further comprehensive studies of other sabkhas to be undertaken to evaluate the successful survival strategies of cyanobacteria in hot desert and halite-stressed conditions. In only one sample from the Zikrit sabkha has the survival strategy been successful, namely sample 11, and, in all other cases, the protection afforded has been met to varying degrees; in some cases, the degradation has proceeded with complete loss of chemical functionality to carbon.
— It is possible that the obvious differences in mineral disposition between the two sabkha sites explain the very different response of the cyanobacterial colonies in both cases; whereas the Zikrit sabkha has a friable, weak

surface comprising mainly halite with some dolomite and the gypsum is located subsurface, the Rhub al Khali sabkha on the other hand has a well-developed hard crystalline surface below which is situated a dolomitized calcite phase. The cyanobacterial colonies associated with this latter phase are hence already better protected from the environment exposure than are their analogues in the Zikrit sabkha that are closer to the surface.

4. Conclusions

The results from the Raman spectroscopic analysis of the Zikrit sabkha demonstrate the applicability to the detection of organic molecules of biological relevance from extremophilic cyanobacterial colonies, such as might be reasonably expected to pertain in the geological record of Mars, where similar areas of halite and gypsum deposits have been located. The differences in the spectral signatures between the two sabkha sites that have been investigated thus far indicate the need to access further sites of this type to better understand the survival strategies being adopted by extremophiles under some of the harshest desert conditions experienced terrestrially.

Clearly, the cyanobacterial colonization of the Zikrit sabkha is a much more difficult survival task than appears from the initial observations made there of vast tracts of cyanobacterial mats at or near the surface; this is in contrast to the Rhub al Khali sabkha, where there is no evidence at all of surface biological colonization. In the Antarctic, cyanobacterial mats are a surface feature of several glaciers and streams, and in these we have shown that a well-developed survival strategy exists that gives recognizable biosignatures for the key protectant molecules such as carotenoids and scytonemin. The relative absence of such features from the Zikrit sabkha demonstrates the greater difficulty that such cyanobacterial mats have to survive there; possible explanations of this could be higher temperatures or larger radiation insolation exposure.

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