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PAPER

Raman spectroscopy study of lichens using three spectrometers under different experimental conditions: analyses of the results with relevance for extraplanetary exploration

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We have carried out analyses on three extremophile lichens from the Tabernas Desert (Spain) under different experimental conditions: dry and wet samples in the laboratory and wet specimens in the field, using three spectrometers: one portable, one FT-Raman and one dispersive micro-Raman instruments. Apart from pigment characterization, the information obtained from the spectra is compared and the differences analysed. The fact that no results were achieved on dry lichens using the handheld spectrometer is of special relevance for those looking for life in hazardous environments. Since in some extreme habitats, life could be "dormant" and only activates under appropriate conditions, miniaturized instruments (such as those included for extraplanetary exploration missions) could not detect bio-markers and, then, life signals will not be noticed when, actually, life is there. Furthermore, we show in this work, using the miniaturized Raman spectrometer, that not only laser wavelength, spectral resolution and wavenumber region of the spectrum are important for the bio- or geo-marker recognition but also spot-size is of vital relevance for the unambiguous biomolecular characterization. In our opinion, mini-Raman instruments are useful for assessing *in situ* the eventual presence of bio-markers in complex natural samples, such as organisms, but are not accurate enough for precise molecular identification.

Introduction

Raman spectroscopy is a valuable technique for easier detection of organic and inorganic compounds or their mixtures under different experimental conditions. Its capability of quick unambiguous molecular *in situ* identification, without almost no sample manipulation, gives to this technique a wide range of areas of application, such as chemist, archaeological, biological and geological sciences,[1–10] pharmaceutical,[11–13] forensic,[14,15] as well as uses for medical diagnostics,[16–18] or, recently, extraplanetary exploration,[19–23] among others.

The availability nowadays of portable Raman spectrometers has the particular interest of allowing for in field analyses (excavations, caves, museums, crime scenario, police stations, *etc.*) but the necessary miniaturization that compromises the instrumental characteristics (for instance, spectral resolution, spot-size or wavenumber region) could allow identification[21,24–26] of just one compound or simple mixtures but could be detrimental to the unambiguous assignments in complex samples (for example, organisms). In spite of that, the advantages of those instruments have been proved when samples cannot be moved to the laboratory or when sampling is not possible.[26–30] An evident example where both situations come together is the extraplanetary exploration. In the last decade, the search for life in extraterrestrial bodies has increased interest and spatial research agencies (NASA and ESA) aim to investigate the possible extinct or extant signs of extraterrestrial life, particularly on the surface of Mars.[30–32] With that objective, ESA will probably include a miniaturized Raman spectrometer as part of a suite of analytical instrumentation, which is expected to be launched in 2018 (ExoMars mission).[33] The evaluation of small Raman spectrometers is a previous and necessary requirement for the precise interpretation of bio- and geo-marker signatures.[34–37] The Raman spectra that Martian rovers will send to Earth will not be, probably, as accurate as those we acquire here and an exhaustive knowledge of miniaturized Raman spectrometers response will be of vital importance for assessing the presence of life signals from the spectra.

If life existed once on Mars surface, it had to adapt to its extreme ambience since the conditions hardened as geological Martian history evolved. For understanding those adaptative mechanisms, we have to study extremophile organisms which appear here, on Earth, in as much hazardous situations as

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possible since, in each daring habitat, organisms adopt different protective strategies. Organisms have developed geo-strategies that are useful to withstand distress conditions. Mineral mobilization, mineralogical transformations or the colonization of the interior of porous rocks and cracks[38–42] protect micro-organisms against, for example, UV radiation or desiccation; these mechanisms can be discerned by the study of the Raman spectrum and are described as geo-markers. Furthermore, the production of a wide range of defensive biomolecules is one of the most common strategies used by life to survive under dangerous environments, such as, for example, high UV radiation, freezing, high or low extreme temperatures, low photosynthetically active radiation (PAR), high acidic or basic levels, desiccation, salinity, *etc.* Those bio-molecules are also detected and could be identified by their Raman spectrum; they are of great importance as biomarkers for the interpretation of survival mechanisms.

In this work, we analyse three lichen species collected from the Tabernas Desert (southern of Spain); those lichens are recorded as extremophile organisms since they live in a desert and are one of the first colonizers of the soil. They produce different protective biomolecules against desiccation or heat that can be detected and identified by their Raman signatures. We tried to identify those biomolecules in two experimental conditions: when lichens were dry, after a long period of drought, and when they were moist, after a period of heavy rain in the desert; in both cases we use a portable spectrometer. For laboratory experiments with benchtop spectrometers, we kept the lichen wet, spraying water over the samples until analyses.

We also survey the capability of the three different Raman spectrometers (one of them a portable handheld instrument) to detect biomolecular pigments and the possibility of the unambiguous compound identification. The results obtained when spectra were acquired using the handled instrument are of particular relevance since miniaturized instruments are being developed for in-field studies as well as for planetary exploration.

Experimental

Raman spectroscopy

Raman analyses were carried out using three spectrometers (portable handled Delta-nu Inspector Raman, Bruker FT-Raman FRA/106S and Bruker Senterra micro-Raman spectrometers) under different experimental situations. In a first step, we analysed dry lichens in the laboratory using the portable Raman instrument. Since the results were not as good as expected, the specimens were moistened and, after 10–15 minutes, were again analysed under the same instrumental conditions; surprisingly, Raman bands were, in most of the spectra, clearer.

The third sets of analyses were carried out directly on the field. For that purpose, we moved the portable Raman spectrometer to the Tabernas Desert, but, unfortunately, it was raining during the whole field trip and, for protecting the spectrometer and computer from water, the analyses had to be achieved under a roof, at around 10 km from the original situation and without significant altitude variation; nevertheless, all climatic conditions, such as wind, humidity, cold or even sun (when it appeared!), were the same than in the original field area.

The miniaturized handheld spectrometer was a commercial Deltanu Inspector Raman, working with a 785 nm wavelength diode laser. Spectra were achieved using a Nuscope microscope attachment, with 100× magnification. For their study, samples were placed on a manually mobile platine and no optical fibre was used. The highest laser power was 120 mW at source, the instrumental intensity possibilities ranged from very low, low, medium, high to very high; several tests were achieved using different powers but, for avoiding sample damage, we carried out our spectra using from very low to low laser intensity; although there is no specification in the user's technical manual about what those levels mean, supposing that each step has the same value, we must have been working with a power intensity of 24 or 48 mW at source. The spot size was of approximately 35 μm.

The most adequate exposure time–accumulation ratio was also studied through comparison between the Raman spectrum quality working with different parameter ranges; analysing our samples with our portable Raman spectrometer, we found that a higher exposure time was not directly related to the acquisition of a better spectrum, because of that, for collecting data, we finally decided to use between 1 and 5 seconds exposure time. The increase in the number of accumulations improved the spectrum quality but not in a linear relation and not for all spots analysed; several spectra in each point were collected with different accumulations for choosing the spectrum with the best signal-to-noise ratio.

The spectral resolution ranges from 8, 10 to 12 cm^{-1} in the Delta-nu Inspector Raman spectrometer; we have compared the spectral signatures using all resolution possibilities for the best identification of the mixtures. The spectral resolution of 8 cm^{-1} is a bit low for natural samples, which are a mixture of more than one organic and/or inorganic compound, such as in our lichen samples, and makes bio- and geo-marker identification more difficult. Wavenumber range extended from 100 to 2000 cm^{-1}.

The fourth set of analyses were carried out in the laboratory of the University of Málaga (Spain), using a Bruker FT-Raman FRA/106S Raman spectrometer, working with a Nd-YAG laser at 1064 nm wavelength. Microanalyses were achieved using a ×40 objective; 2000 accumulations were recorded at 1, 2 or 20 mW laser power at source. For dispersive micro-Raman analyses, a Bruker Senterra micro-Raman spectrometer with ×20 and ×40 magnifications was used. Laser excitations used for micro-Raman analyses were 785, 633 and 532 nm, with a laser power lower than 2 mW. A spectral range between 200 and 1800 cm^{-1} was recorded. Spot size on the sample was of 1 μm.

Samples

Three lichen species were collected from the Tabernas Desert: *Squamarina lentigera*, *Diplochistes diacapsis* and *Lepraria crassissima* (Fig. 1). The altitude was 250 m and the average annual T is 17.9 °C, with a maximum of 45 °C and a minimum of −4.5 °C. Annual rainfall is 235 mm. These lichens are the second colonizer of the ground, after cyanobacteria, and contribute to fixing the loose particles, preventing their mobilisation by rain or wind. Furthermore, they contribute to the soil formation since provide organic matter to the almost sterile ground.

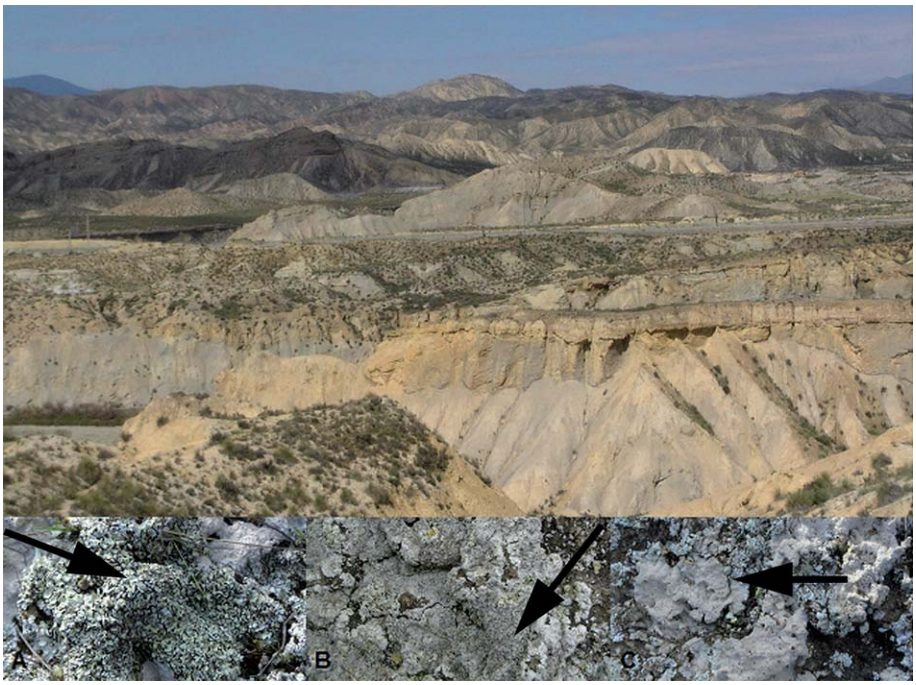

Fig. 1 Photographs of Tabernas badlands and lichens; (A) *Squamarina lentigera*; (B) *Lepraria crassissima*; (C) *Diplochistes diacapsis*.

Analytical conditions

Two sets of samples were studied under different analytical conditions. For the first analyses, dry specimens were collected from the Tabernas Desert and sent inside sample bags to the University of Burgos (Spain) for analyses in the laboratory with the portable Delta-nu Inspector Raman spectrometer, first over dry lichens and, later, after wetting them. The second set of samples were collected and analysed on the field and, afterwards, sent to the University of Málaga for analyses using laboratory Raman spectrometers. Although our first goal was to study lichens under desertic-dry environmental conditions, rain forces us to "change our mind" and we analysed wet lichens on the field. The main difference was that in drought periods, lichens are photosynthetically active only when relative humidity is close to dew point and sunlight is present, such a situation happens early in the morning or during raining periods. During the whole period of our field trip, our lichens were very active because of the heavy rain; this was clearly observable because of changes in colour intensity (green or brown were much more intense). We collected those lichens and stored them near a window, inside transparent plastic Petri dishes, allowing air penetration and keeping moisture level constant by spraying water over the specimens every few days to avoid desiccation processes.

Results and discussion

When the analyses were achieved on dry samples, using the portable analytical instrument, spectra collected showed just fluorescence background. This experimental result is very interesting because, as has been described previously, some lichens could be inactive if environmental conditions are adverse and only when an appropriate level of PAR and hydratation are reached, such as in the early morning condensation or when it is raining, biochemical lichen activity triggers, and, only in this case, pigments are easily detectable by using the portable Raman spectrometer. This could be of special relevance for the search for life signals on hazardous environments since, in some extreme habitats, extremophile organisms could be "dormant" until the vital parameters for life developing are reached.

The best spectral resolution given by our portable Deltanu Inspector Raman spectrometer is of 8 cm^{-1}, which is quite low for mixtures of compounds that show a multi-signature spectrum. This poor spectral resolution is reflected in the spectrum for the collection of broad bands; these scatterings can then overlap with other close signatures from a given molecule or with the features from different compounds. When many close signatures overlap the resulting broader feature is neither centred at the same wavenumber of the strongest band nor at the arithmetic mean but its final position depends on the number of overlapped signatures and their relative intensities.[19,24] For a particular pigment mixture, the relative intensities of the characteristic Raman bands from different constituents change as the compound proportion changes. As a result, in each spot Raman shifts may be different in wavenumbers and, even, in the direction of the shift (*i.e.*, towards higher or lower wavenumbers). This phenomenon is magnified because of the broad spot size (35 μm). For example, for one analyzed spot (spot "A") the amount of rhizocarpic acid reached by the laser could be high whereas parietin is low and, at the same time, chlorophyll and carotenoid can also be detected; this particular mixture, together with a low spectral resolution, can shift a *n* wavenumber to the signature centre whereas on spot "B" the amount of rhizocarpic acid could be very low but parietin very high and, in this case, the shift can be different in wavenumbers and direction. This explanation could be the reason why when a series of analyses were achieved using the handled Raman instrument on a lichen, some bands shifted an appreciable range of wavenumbers from

spot to spot when analyzed over the same lichen specimen; in these cases, the right molecular identification is extremely difficult; although some attempts have been done, we cannot assess that the assignment was precise in such circumstances. Furthermore, there is no a reliable Raman database of lichen pigments, which makes even more complex the molecular characterization of their different pigments (Table 1).

It is interesting to notice that for some biomolecules, such as chlorophyll, carotenoids or calcium oxalates, bands do not shift significantly and, then, could be easily identified. This different response could be related to the relative higher proportion in lichens between these compounds and other protective molecules, such as emodin, rhizocarpic acid, parietin, lecanoric acid, *etc.*

Squamarina lentigera

Spectra achieved using the miniaturized spectrometer show bands at 1492, 1388, 1325, 1074, 980 and 914 cm^{-1} assigned to chlorophyll (Fig. 2). The presence of Raman peaks around 1521, 1153 cm^{-1} suggests a carotenoid, both signatures are related to the carotenoid backbone chain; the wavenumber position at 1521 cm^{-1} (C=C carotenoid Raman stretching mode) suggests a carotenoid with short chain-bone, probably zeaxanthine or lutein, but its definitive assignment is unclear. A second small peak, like a shoulder, at 1525 cm^{-1}, is sometimes observable in other spectra collected on the same species of lichen and could be, perhaps, related to the same characteristic Raman-active carotenoid vibration. We thus estimate that this shoulder actually belongs to a second carotenoid based on the Raman results obtained using the laboratory spectrometers. When the band at 1521 cm^{-1} is further studied in the spectra collected using the FT Raman spectrometer, which has a spectral resolution of 1 cm^{-1} (Fig. 3), and despite the large number of analyses carried out, it clearly displays a symmetric spectral pattern, with no shoulders, indicating that there is no contribution from a second carotene. This result is, in addition, corroborated by the analyses achieved on this lichen using the 532 nm laser wavelength; as known, the green and blue laser excitations induce a resonance effect on carotenoid Raman vibrations and those pigments become clearly distinguishable even if they appear in very low proportion; here, again, a single Raman feature, centred at 1520 cm^{-1}, is visible and no shoulders are appreciable. We dismiss, then, the possibility that the shoulder at 1525 cm^{-1} could be the contribution of a second carotenoid and guess that this "*nonpermanent band*" appears owing to the interaction with other compound signatures because of the lower spectral resolution of the handheld Raman spectrometer or even could also be just a noise artefact.

When spectra are collected using the miniaturized spectrometer, bands at 1488, 1466 and 504 cm^{-1} suggest the presence of whewellite (calcium oxalate monohydrate). Contrary to what happens with the carotene C=C stretching band in the FT-Raman spectrum (Fig. 3), using this last spectrometer, it is evident that the strong scattering at 1477 cm^{-1} displays a shoulder at 1489 cm^{-1} and a medium intensity signature, at 1464 cm^{-1}, also becomes clearly distinguishable. The first signature, together with those at 910 and 506 cm^{-1} (not shown in the figure), is characteristic of weddellite (calcium oxalate dihydrate), whereas Raman scatterings at 1489, 1464, 896 and 504 cm^{-1} are unambiguously assigned to whewellite. In this case, the study of the lichen using a FT-Raman laboratory spectrometer shows the presence of both calcium oxalates despite that none of the analyses achieved using the portable instrument (neither in the laboratory nor in the field) have provided spectroscopic information regarding the presence of dihydrate oxalate on *S. lentigera*. Given that both oxalates have been found in some field measurements performed on *L. crassissima*, the hypothesis that miniaturised Raman instrument laser power could have dehydrated weddellite because of a high laser power at the sample is dismissed.

Spectra collected using the portable spectrometer (inside the laboratory and on wet samples) show bands at 1587, 1540, 1466,

Table 1 Comparative table of the results obtained on different lichens under different experimental conditions. CAR = carotenoid; OX = oxalate

	Squamarina lentigera	*Diplochistes diacapsis*	*Lepraria crassissima*
Deltanu Inspector Raman dry samples	No results	No results	No results
Deltanu Inspector Raman wet samples in the laboratory	Chlorophyll Zeaxanthine/lutein (CAR) Whewellite (OX) Parietin Rhizocarpic acid	Axtaxanthine (CAR) Decapreno-betacarotene (CAR) Whewellite (OX) Usnic acid Rhizocarpic acid	Broad band with doublet Zeaxanthine/lutein (CAR) Weddellite (OX) Whewellite (OX) Fumarprotocetraric acid Calycin
Deltanu Inspector Raman wet samples in field	Chlorophyll Zeaxanthine/lutein (CAR) Whewellite (OX) Usnic acid Parietin	Chlorophyll Zeaxanthine/Lutein (CAR) Whewellite (OX) Usnic acid Rhizocarpic acid Emodin	Zeaxanthine/lutein (CAR) Weddellite (OX) Whewellite (OX) Unknown compound
FT Bruker	Chlorophyll Zeaxanthine/lutein (CAR) Weddellite (OX) Whewellite (OX) Lecanoric acid	Chlorophyll Lutein (CAR) Axtaxanthine (CAR) Decapreno-betacarotene (CAR) Whewellite (OX) Lecanoric acid Emodin	Chlorophyll Weddellite (OX) Calcite Gypsum
Dispersive Senterra Bruker	Zeaxanthine/lutein (CAR)	No results	Zeaxanthine/lutein (CAR)

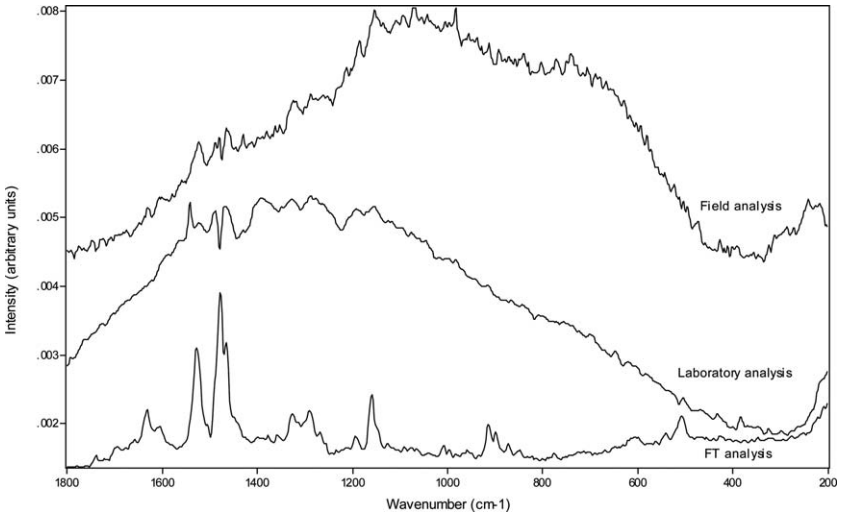

Fig. 2 Comparative Raman spectra acquired on *Squamarina lentigera* achieved using the Bruker FRA/106 FT-Raman spectrometer in the laboratory of University of Málaga (Spain). Field and laboratory analysis spectra collected using the miniaturized handheld Deltanu Inspector Raman spectrometer. The laboratory analysis spectrum using the portable instrument was collected on wet lichen.

1388, 1286, 1190, 980, 842, 620, 582, 431, 383 and 323 cm^{-1} and signatures at 1760, 1587, 521, 1487, 1466, 1340, 1286, 1250, 1190, 997, 701, 646, 620, 504 and 431 cm^{-1} that could suggest the presence of parietin and rhizocarpic acid, respectively. In the spectra acquired with the same instrument but in the field, signatures appear at 1629, 1604, 1530, 1480, 1462, 1323, 1287, 1184, 1152, 1142, 1070 and 992 cm^{-1}, which could, tentatively, be assigned to usnic acid.

Apart from carotene, chlorophyll and calcium oxalate (mono- and dihydrate) identified in the spectra collected using the Bruker FT-Raman spectrometer, the signatures at 1630, 1610, 1605, 1447, 1376, 1357, 1289, 1144, 994, 956, 872, 847, 773 and 539 cm^{-1} could be assigned to lecanoric acid.

It is difficult to fit all the Raman data collected for this lichen by using the three different spectrometers. Only the presence of carotene, chlorophyll and oxalates is clear wherever the Raman spectrometer or spectral analytical conditions have been achieved (but on dry lichens), but the identification of other pigments by the portable field instrument, such as parietin or rhizocarpic acid, is questionable, since they should give good enough FT-Raman signals to be identified in the spectrum and, yet, they are not present (Table 1).

Diplochistes diacapsis

No results were obtained when *D. diacapsis* was analysed by the dispersive micro-Raman spectrometer with any of the three laser excitations used, whereas when spectra were achieved using 1064 nm wavelength (FT-Raman spectrometer), several pigments could be identified, as well as calcium oxalate monohydrated

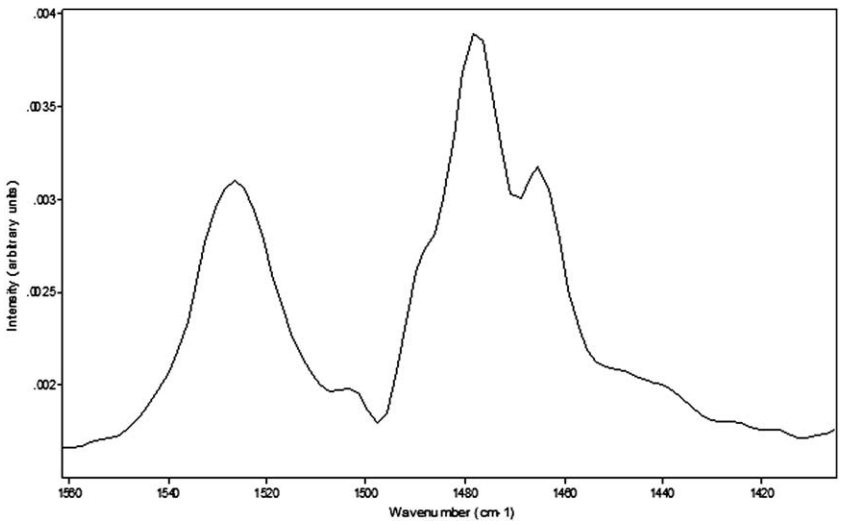

Fig. 3 Spectrum collected by the Bruker FRA/106 FT-Raman spectrometer, on *Squamarina lentigera* showing bands assigned to carotenoid (zeaxanthine/lutein 1521 cm^{-1}) and calcium oxalate mono (shoulders, 1489 and 1464 cm^{-1}) and dihydrate (1477 cm^{-1}).

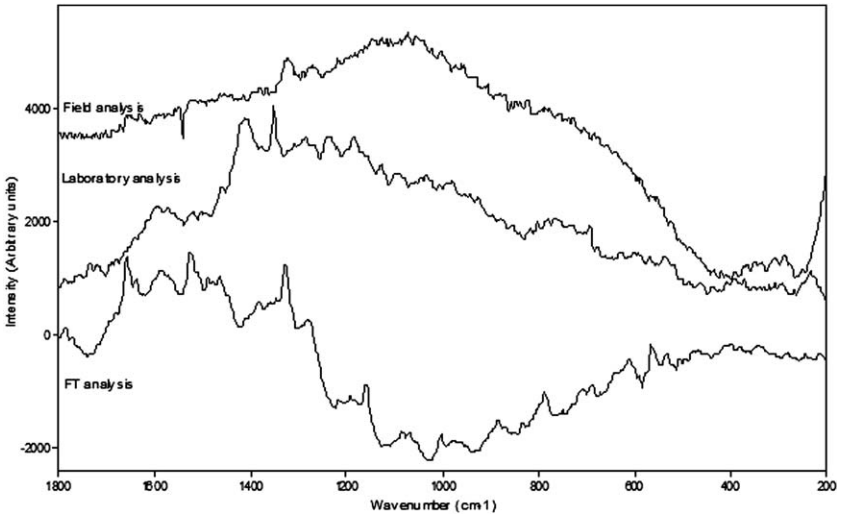

Fig. 4 Comparative Raman spectra acquired on *Diploschistes diacapsi* achieved using the Bruker FRA/106 FT-Raman spectrometer in the laboratory of the University of Málaga (Spain). Field and laboratory analysis spectra collected using the miniaturized handheld Deltanu Inspector Raman spectrometer. The laboratory analysis spectrum using the portable instrument was collected on wet lichen.

(whewellite). Apart from chlorophyll, signatures at 1657, 1637, 1602, 1573, 1489, 1463, 1383, 1298, 1282, 1196, 632, 564, 465 and 397 cm^{-1} suggest the presence of emodin whereas those at 1657, 1637, 1580, 1383, 1327, 1300, 1084, 1002, 963, 882, 788, 686, 642, 609, 564, 532, 465, 348 and 243 cm^{-1} could also be attributable to lecanoric acid (Fig. 4).

The Raman signature centred at 1525 cm^{-1} (likely lutein) shows shoulders (Fig. 5) at 1511 and 1503 cm^{-1} that could also be related to the carotenoids astaxanthin and decapreno-betacarotene, respectively, when *D. diacapsis* specimens are analysed by FT-Raman spectroscopy. De Oliveira and Castro *et al.*[43] have published an interesting work in which they compare shifts of the stretching C=C Raman band of carotenes in natural samples with the signature position when carotene is analysed from a commercial pure compound. They reported that there were significant wavenumber shifts due to several reasons which range from interaction with other nearby compounds to changes in crystallinity due to the water insolubility of carotenoids; from their studies, they conclude that the unambiguous carotenoid assignment in natural samples is extremely difficult and that all assignments may be considered as tentative.

A weak band centred at 1503 cm^{-1} could be related to the decapreno-betacarotene presence and the signature at 1505 cm^{-1} to astaxanthin; those results were obtained using our handheld spectrometer in the laboratory, whereas in the field only the signature at 1520 cm^{-1} could be ascribed to zeaxanthine/lutein. Chlorophyll is not clear in the laboratory spectra although one strong peak centred at 1322 cm^{-1} can be associated to this photosynthetic pigment in some spectra acquired in the field. Usnic acid could be identified because of the bands at 1689, 1594,

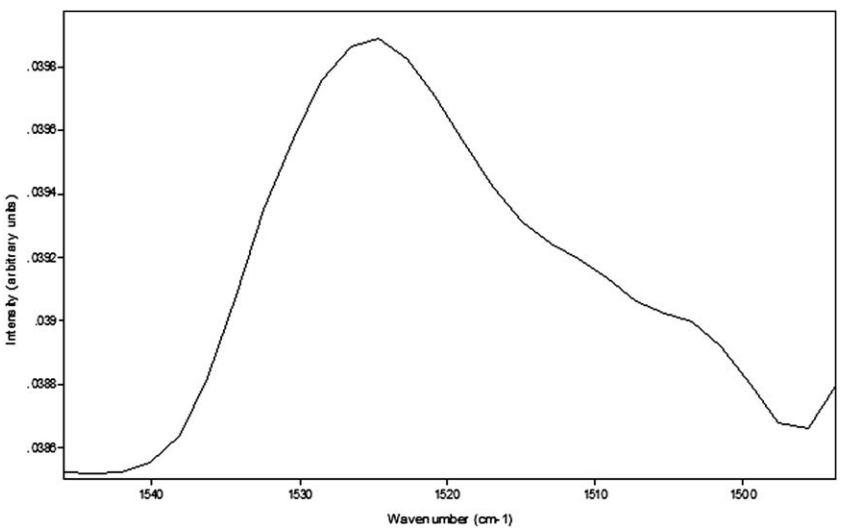

Fig. 5 Spectrum collected by the Bruker FRA/106 FT-Raman spectrometer, on *Diploschistes diacapsi* showing the signature assigned to a carotenoid (lutein) and two shoulders, at 1511 and 1503 cm^{-1}, related to axtaxanthine and decapreno-betacarotene, respectively.

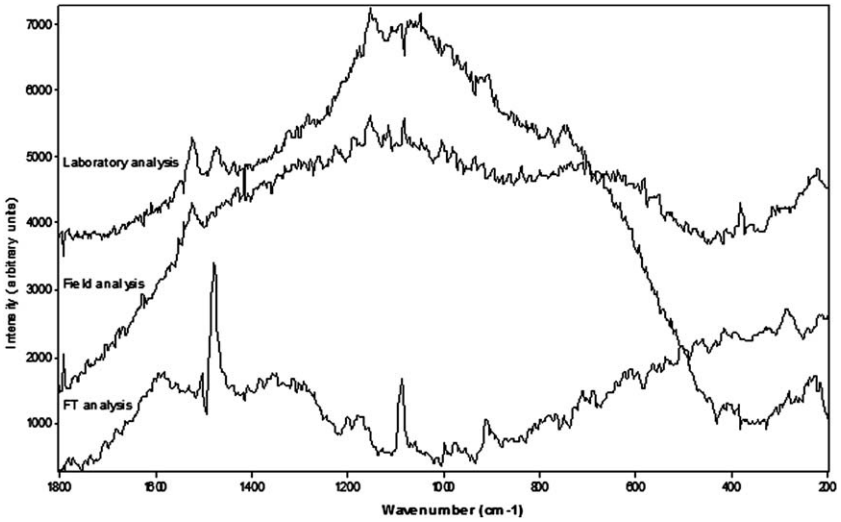

Fig. 6 Comparative Raman spectra acquired on *Lepraria crassissima* achieved using the Bruker FRA/106 FT-Raman spectrometer in the laboratory of the University of Málaga (Spain). Field and laboratory analysis spectra collected using the miniaturized handheld Deltanu Inspector Raman spectrometer. The laboratory analysis spectrum using the portable instrument was collected on wet lichen.

1460, 1351, 1284, 1184, 1127, 985, 692, 601 and 537 cm^{-1}, whereas signatures at 1786, 1735, 1680, 1594, 1503, 1351, 1284, 1184, 1033, 979, 780, 711, 780, 692, 601 cm^{-1} could be associated to rhizocarpic acid; both pigments were detected in the laboratory and in field analyses using the handheld spectrometer. Although some Raman signatures from emodin are absent in the spectra collected in the field, we attempt its presence in the bands at 1700, 1665, 1589, 1416, 1290, 1136, 1096, 939, 495 and 468 cm^{-1}.

Lepraria crassissima

A carotenoid, likely zeaxanthine/lutein (1523 cm^{-1}), was clearly distinguishable in most of the Raman spectra collected using the handheld spectrometer (Fig. 6) (either in the laboratory or in the field); however, it was unexpected that, except for a very broad band, centred at 1330 cm^{-1}, assigned to chlorophyll, no other signatures related to protective pigments appear in the spectra acquired using either FT- or dispersive Raman spectrometers.

When analyses were carried out on wet *L. crassissima* specimen using the miniaturized spectrometer, a very broad feature, split into two components at *ca* 1405 and 1351 cm^{-1}, appears, but this doublet has not been assigned yet.

The signature assignment in the spectra operating with the Inspector Raman spectrometer is even more difficult for this lichen than in the case of *S. lentigera* and *D. diacapsis* because bands are weaker. Signatures at 1753, 1713, 1676, 1380, 1315, 1287, 1135, 1082 and 1029 cm^{-1} could be related to fumarprotocetraric acid whereas bands at 1604, 1592, 1483, 1378, 1348,

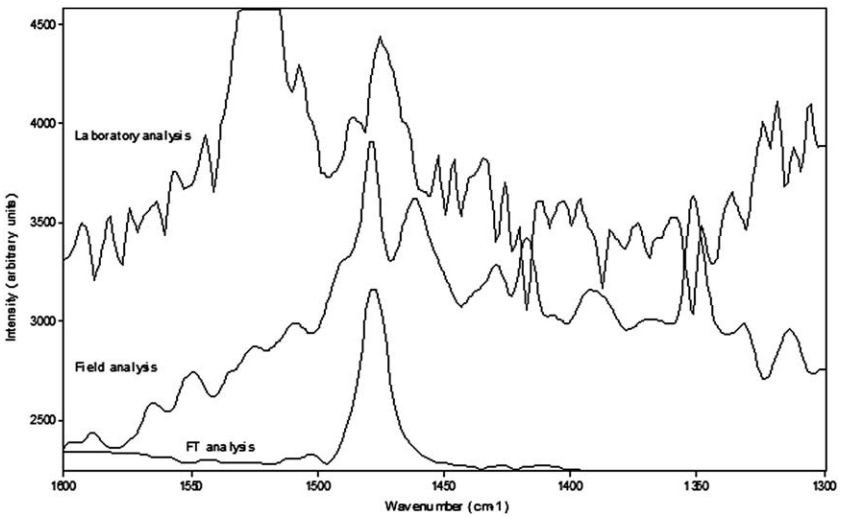

Fig. 7 Comparative Raman spectra, in the wavenumber between 1300 and 1600 cm^{-1} showing the bands of calcium oxalate mono- and dihydrate and the signature of a carotenoid on *Lepraria crassissima*, for the same experimental conditions than in Fig. 6.

1315, 1242, 1111, 1029, 1002 and 943 cm^{-1} could be associated with the presence of calycin. Some Raman spectra also show signatures of calcium oxalate dihydrate (weddellite) at 1473 and 910 cm^{-1} with shoulders of calcium oxalate monohydrate (whewellite), at 1468 and 1486 cm^{-1} (Fig. 7). The weak broad band centred at 1323 cm^{-1} could be related to chlorophyll (laboratory analyses).

In the Raman spectra field analyses, weddellite was the most abundant calcium oxalate, showing a strong band at 1473 cm^{-1} and a shoulder at 1490 cm^{-1}, this shoulder together with the signature a 1463 cm^{-1} is characteristic of whewellite; chlorophyll is unclear in any of the spectra achieved in the field, although a very weak feature centred at 1315 cm^{-1} could be associated with this pigment. However, apart from calcite (1086, 713 and 281 cm^{-1}) and gypsum (1007 and 418 cm^{-1}), only calcium oxalate dihydrate (weddellite) was identified using the FT-Raman spectrometer.

It is interesting to note that, whereas for *S. lentigera* and *D. diacapsis*, the best and most reliable identification are based upon the results obtained by the FT-Raman spectrometer, in this third case, there is almost no information from those spectra; it is possible that the enormous amount of oxalate produced by *L. crassissima* (visible at naked eye as a white dust even on the lichen surface) had physically hidden the presence of other pigments. We should have in mind that we had not manipulated in any way those specimens (neither scrape nor break them) but sprinkled water to keep them wet (simulating the rain conditions under they were collected) until being analysed in the laboratory of the University of Málaga.

Conclusions

Specimens of three different lichen species from the semi-arid Tabernas desert (Spain) have been analysed using one handled and two benchtop Raman spectrometers.

Each lichen species uses different protective strategies against heat and drought and is based on the production of different mixtures of biomolecules.

The identification of chlorophyll, carotenoids and calcium oxalates is clear regardless of the spectrometer used; however, the characterization of more specific pigments, such as parietin, rhizocarpic acid, usnic acid, emodin, *etc.*, using the handled Deltanu Inspector Raman spectrometer is less precise since bands shift significantly because of the spectral resolution and spot size.

Spot size could compromise the molecular assignment and together with laser wavelength and spectral resolution is a parameter to take into account when molecular identification in complex samples is required. This is, in our knowledge, the first time that the effect of spot size on the Raman spectrum has been reported.

An outstanding point is that using the portable instrument, life markers were detected only on active lichens. Since miniaturized spectrometers are being developed for extraplanetary exploration this observation is of crucial importance when looking for life in extreme environments since the junction of both, experimental conditions and portable instruments, could lead to the false conclusion that there are no organic signatures when, actually, life is there, but is not yet active.

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