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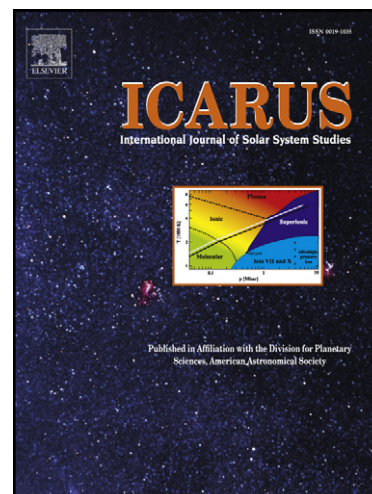
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1 A spectroscopic method for identifying
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35 **Abstract**

36 Searching for traces of extinct and/or extant life on Mars is one of the major objectives for remote-
37 sensing and *in-situ* exploration of the planet. In previous laboratory works we have investigated the
38 infrared spectral modifications induced by thermal processing on different carbonate samples, in the
39 form of fresh shells and fossils of different ages, whose biotic origin is easily recognisable. The goal
40 was to discriminate them from their abiotic counterparts. In general, it is difficult to identify biotic
41 signatures, especially when the organisms inducing the carbonate precipitation have low
42 fossilization potential (i.e. microbes, bacteria, archaea). A wide variety of microorganisms are
43 implicated in carbonate genesis, and their direct characterization is very difficult to evaluate by
44 traditional methods, both in ancient sedimentary systems and even in recent environments.
45 In the present work we apply our analysis to problematic carbonate samples, in which there is no
46 clear evidence of controlled or induced biomineralization. This analysis indicates a very likely
47 biotic origin of the aragonite samples under study, in agreement with the conclusion previously
48 reported by Guido et al. (2007) who followed a completely different approach based on a complex
49 set of sedimentary, petrographic, geochemical and biochemical analyses. We show that our method
50 is reliable for discriminating between biotic and abiotic carbonates, and therefore it is a powerful
51 tool in the search for life on Mars in the next generation of space missions to the planet.

52

53 **Keywords:** Exobiology; Mars; Spectroscopy

54 **1. Introduction**

55 Mars, with its arid and cold climate, is now apparently an inhospitable world for sustaining any
56 form of life. Because of its present temperature and pressure conditions, liquid water is totally
57 absent from the surface. However, spacecraft observations showed us a wealth of geomorphologic
58 features that could be entirely consistent with precipitation and surface runoff (e.g. Craddock &
59 Howard, 2002; Mangold et al., 2004; Ansan and Mangold, 2006). In fact, a warm, wet early climate
60 capable of supporting rainfall and surface runoff is a very plausible scenario for explaining some
61 geologic features in the Martian cratered highlands (Erkeling et al., 2010). Observations performed
62 during several missions (both by orbiters and landers) strongly suggest that Mars was once warm
63 and wet enough to allow the presence of abundant liquid water on the surface (e.g. Mars Pathfinder
64 Rover team, 1997; Phillips et al., 2001; Craddock and Howard, 2002; Paige, 2005), due to a thicker
65 atmosphere providing a strong greenhouse effect. This suggestion is supported by the results of a
66 number of climatic models (Pollack et al., 1987; Forget and Pierrehumbert, 1997; Yung et al., 1997;
67 Forget et al., 2004), even if this issue is not completely settled (Colaprete and Toon, 2003; Tian et
68 al., 2010).

69 More recently the study of the mineralogy of Mars has independently demonstrated the role of
70 water in the past. Mineralogical data, recovered by instruments operating in orbit and on the surface
71 of Mars, show clear evidence for the presence of sulphates (Bibring et al., 2005; Gendrin et al.,
72 2005), whose formation depends on the presence of liquid water (Christensen et al., 2004;
73 Klingelhöfer et al., 2004; Squyres et al., 2004). The Mars Express spacecraft detected, together with
74 sulfates, also phyllosilicates (clay minerals) which can be produced by the alteration of mafic or
75 ultramafic rocks in presence of liquid water (Poulet et al., 2005; Bibring et al., 2006; Loizeau et al.,
76 2007). The diversity of phyllosilicate mineralogy has been expanded by the observations made with
77 the CRISM (Compact Reconnaissance Imaging Spectrometer for Mars) instrument on board of the
78 Mars Reconnaissance Orbiter (MRO) with the identification of various hydrated silicates (Mustard
79 et al., 2008).

80 New recent investigations with CRISM of the Noachian southern highlands of Mars have revealed
81 both sulfates and phyllosilicates sometimes in intimate association (Wray et al., 2009). These
82 assemblages are suggestive of a greater diversity of aqueous environments on ancient Mars than has
83 previously been recognized, and their distribution implies that during the Noachian Period aqueous
84 alteration was not restricted to a few locations but globally widespread (Wray et al., 2009).

85 Additional spectral and geological evidence consistent with impact-induced hydrothermal activity
86 (Marzo et al., 2010), suggest post-Noachian formation of hydrated silicates, bringing into question
87 models indicating that the formation of hydrated silicates is limited to the early part of the geologic
88 record of the planet (Bibring et al., 2006).

89 Mars orbital spectral data have provided evidence also for carbonates (Bandfield et al., 2003;
90 Ehlmann et al., 2008; Palomba et al., 2009; Murchie et al., 2009) while Morris et al. (2010), using
91 the rover Spirit have recently identified outcrops rich in Mg-Fe carbonate (16 to 34 wt.%) in the
92 Columbia Hills of Gusev crater. Interestingly, its composition approximates the average
93 composition of the carbonate globules in Martian meteorite ALH84001. The presence of
94 carbonates, probably precipitated from carbonate-bearing solutions, imply that neutral-to alkaline
95 waters existed at the time of their formation.

96 All the above mineralogical evidence suggests that several distinct habitable niches may thus have
97 been present on ancient Mars, providing a range of opportunities for the origin of life, including
98 places of impact-induced hydrothermal systems (Newsom, 1980; Gulick and Baker, 1989, 1990;
99 Gulick, 1998, Marzo et al., 2010). This hypothesis provides not only an interesting example of post-
100 Noachian aqueous alteration on Mars, but suggests also further sites of astrobiological potential.

101 Searching for traces of extinct or extant life is a major objective of Mars exploration, even though
102 the detection of a biosignature is not a straightforward process (e.g. Marzo et al., 2007). However,
103 the probability of finding traces of biological activity would certainly be higher if the search is
104 directed towards materials whose origin can be traced back to some form of life. This is the case
105 with some terrestrial living organisms which are able to produce mineral matrices in the so called

106 biomineralization process (Lowenstam, 1981; Mann, 1983, 2001). Nevertheless the problem of
107 discriminating between biominerals and their abiotic counterparts is far from trivial. However, by
108 means of thermal processing, it is possible to distinguish, using differential thermal analysis
109 (Cabane et al., 2004; Stalport et al., 2005, 2007) or infrared (IR) spectroscopy (Orofino et al., 2007),
110 abiotic calcium carbonate minerals (CaCO_3 i.e. aragonite or calcite) from the corresponding
111 biominerals. In a series of recent papers we have developed and applied our method (D'Elia et al.,
112 2007; Orofino et al., 2007, 2009, 2010) to different carbonate samples in the form of fresh shells
113 and fossils of different ages found in different places and easily recognizable as of biotic origin. In
114 this work, applying our analysis to problematic carbonate samples, in which there is no clear
115 evidence of controlled or induced biomineralization, we show that the method is reliable, allowing
116 for the discrimination of biotic and abiotic carbonates.

117

118 **2. Previous work and method**

119 The polymorphs of CaCO_3 , aragonite and calcite, are interesting because on Earth these compounds
120 are produced by abiotic processes as well as by biological activity. According to Mackenzie (1970),
121 when aragonite (a metastable form of CaCO_3 present in almost all mollusc shells) undergoes heat
122 treatment, an endothermic transformation into calcite (the stable state of CaCO_3) occurs. Then, after
123 further heating, calcite decomposes into solid calcium oxide (CaO) and gaseous carbon dioxide
124 (CO_2). The latter transformation is complete at 800 - 1000 °C (Stalport et al., 2005, 2007), but it
125 begins at a lower temperature which depends on the biotic or abiotic origin of the sample. In the
126 case of recent biominerals, heating at 485 °C for 3.5 hours is sufficient to start altering their
127 chemical and/or physical properties, while for minerals of abiotic origin higher temperatures are
128 necessary (see Orofino et al., 2007, 2009, for details). As described in previous work, all the
129 samples were ground for approximately 10 minutes in a mechanical mortar grinder in order to
130 obtain fine powders suitable for IR spectral analyses. The thermal processing of the samples was
131 performed by means of a furnace under vacuum.

132 To study the above mentioned transformations we performed IR transmission spectroscopy in the
133 range 2000 - 370 cm^{-1} , focusing our attention only on the last part of the spectra (700 - 370 cm^{-1}).
134 This allows to monitor the decomposition process undergone by our samples giving, at the same
135 time, the advantage of using only one type of matrix (potassium bromide, KBr) for the IR
136 transmission spectroscopy in the range 2000 – 370 cm^{-1} (Orofino et al. 2009). For all the samples
137 we measured the spectra before and after the heating treatment at 485 °C for 3.5 hours. In Fig. 1 we
138 compare, as an example, the spectra obtained before and after such a treatment for a fresh shell
139 (*Tellina exigua*).

140 Figure 1

141 Analysing the solid curve in Fig. 1, it is possible to infer that the sample is composed of aragonite,
142 due to the presence of the relevant features at 1478 cm^{-1} (6.8 μm), 1083 cm^{-1} (9.2 μm), 859 cm^{-1}
143 (11.6 μm) and a double peak around 700 cm^{-1} (14.3 μm), characteristic of this mineral (White,
144 1974). The dotted line shows the spectrum obtained for the sample thermally processed at 485 °C.
145 The transformation from the metastable form of aragonite to the more stable form of calcite is
146 indicated by the loss of the feature at 1083 cm^{-1} and of the doublet at 700 cm^{-1} , where we observe,
147 instead, a single transmittance minimum. In addition, the ongoing decomposition of CaCO_3 into
148 CaO (and CO_2) starts to appear, since the bands at 1083 and 859 cm^{-1} become weaker and the
149 transmittance below 700 cm^{-1} starts to decrease considerably due to the onset of the broad band
150 centered at 330 cm^{-1} (30.3 μm) characteristic of CaO (Farmer, 1974), partially shown in Fig. 1. For
151 comparison in Fig. 2 we report the transmission spectrum of a sample of CaO grains (supplied by
152 Sigma–Aldrich Co.) obtained in our laboratory. In the same figure are reported the reference spectra
153 of particulate samples of calcite and aragonite obtained by Salisbury et al. (1991).

154 Figure 2

155 For every spectrum of each processed and unprocessed sample, we have evaluated the transmittance
156 ratios, R_p and R_u respectively:

157

158 $R_p = T_p(v_2) / T_p(v_1)$

159 $R_u = T_u(v_2) / T_u(v_1)$

160

161 where $v_1 = 650 \text{ cm}^{-1}$ (15.4 μm) and $v_2 = 510 \text{ cm}^{-1}$ (19.6 μm) are two wavenumbers chosen in the
162 spectral range of interest where the CaO band begins to develop (the subscripts p and u refer to
163 processed and unprocessed samples respectively).

164 Taking into account all the spectra relative to a given sample, we have averaged the values of R_u
165 and R_p and, from these two mean ratios $\langle R_u \rangle$ and $\langle R_p \rangle$, we have obtained for each sample a slope
166 variation index:

167

168 $D = \langle R_p \rangle / \langle R_u \rangle$.

169

170 The index D estimates the slope variation of the spectra obtained after heating at 485 °C with
171 respect to that measured for the unprocessed sample. Due to the fact that the spectral slope is always
172 steeper in the case of the processed sample, $D \leq 1$ for all the samples. We note that as D increases,
173 the spectral slope of the processed sample becomes more similar to that of the unprocessed one,
174 meaning that the thermal processing is less effective in the transformation of CaCO_3 into CaO.

175 Taking into account the samples described in Orofino et al. (2010), i.e. recent shells (*Tellina exigua*,
176 *Cardites antiquata*), fossils of various geologic time from the Holocene to the Mesoproterozoic Era
177 (*Pecten* sp., *Ostrea* sp., *Myriapora truncata*, *Clypeaster* sp., *Haliotis lamellosa*, rudist bivalves,
178 *Ortoceras* sp., fossil stromatolites) and aragonite and calcite minerals, we found a correlation
179 between the age of a biotic carbonate sample and the relative spectral slope, in the range 700 - 370
180 cm^{-1} , of the heated sample with respect to that of the unprocessed one.

181 The D index is plotted in Fig. 3 versus the logarithm of the stratigraphic age of each sample. The
182 beginning and the end of each period/epoch are those established by the International Commission
183 on Stratigraphy (ICS) devoted to the terrestrial stratigraphy on a global scale (Gradstein et al., 2004;

184 Ogg et al., 2008). In this respect we have chosen the central age of the period the sample dates
185 from, with an uncertainty equal to $\pm \Delta/2$, where Δ is the duration of each period. As far as the error
186 on the D index is concerned, all the vertical error bars reported in Fig. 3 refer to the larger
187 uncertainty between the statistical dispersion, derived from the error analysis of the various
188 measurements performed on the same sample, and the instrumental accuracy of the transmittance
189 spectra (typically 1%).

190 *Figure 3*

191 The linear trend shown in Fig. 3 is reasonably well fitted by the function:

192

$$193 \quad D = a \log t + b$$

194

195 where t is the age of the sample (in Myr), $a = 0.0125$ and $b = 0.8666$; we find a correlation
196 coefficient $r \cong 0.95$ (Taylor, 1982).

197 This correlation implies that the recent fossils maintain the characteristic “fragility” of fresh
198 biominerals – see Orofino et al. (2007) for details – while for more ancient fossils the spectroscopic
199 behavior, after heat treatment, becomes increasingly similar to that of abiotic minerals and the D
200 index gets closer to 1. Therefore D could be seen as an index of degradation of the fossil, in the
201 sense that $D = 1$ implies the impossibility of discriminating between biotic and abiotic carbonate
202 samples. The straight line represents therefore the place where the D index values of “normal”
203 biotic carbonates fall as a function of the age, at least up to about 1 Gyr in terrestrial environments.

204 A deviation from this linear trend may indicate a particular situation and/or a different evolution of
205 the biotic carbonate sample under analysis. In this context, we recently found that a group of
206 terrestrial fossils embedded in a layer of clays exhibit values of D smaller than their coeval fossils
207 found in other mineralogical environments. We interpreted such behavior as the result of a
208 favourable condition for preserving their biomineral characteristics (Orofino et al., 2010). An

209 interesting consequence of this result is that the phyllosilicates regions recently discovered on Mars
210 may represent very interesting environments that can provide conditions favourable to preserve
211 evidence of biomarkers. Therefore they can be regarded as good candidate locations for
212 biosignature detection.

213 As far as very old biotic samples are concerned we have found that on Earth a billion years is long
214 enough to produce a complete degradation of the fossil structure, giving rise to post-processed
215 spectral behaviors that are very similar to those of unprocessed samples ($D \cong 1$).

216 It is worthwhile to stress the importance of the heating of the samples as a powerful means to
217 evaluate their level of degradation, since samples which experienced different degrees of
218 degradation react in different way to thermal processing probably due to the different arrangement
219 of atoms into the structure of the fossil. The IR spectroscopy of the sample after heating at 485 °C
220 allows discriminating its origin, by means of the evaluation of the D parameter, as previously
221 described.

222

223 **3. The new carbonate rock samples**

224 Mineral deposits resulting from microbially-induced mineralization are called microbialites (Burne
225 and Moore, 1987). Other terms such as microbolite (Riding, 1991), automicrite (e.g. Wolf, 1965;
226 Reitner et al., 1995) or organomicrite (Reitner et al., 1995) are also used. Most microbialites can be
227 classified into one of three main categories based on their macroscopic features (Riding, 1991;
228 Dupraz and Strasser, 1999): stromatolites, showing a laminated macrofabric (Monty, 1977;
229 Semikhatov et al., 1979), thrombolites, displaying a clotted (mesoclots) macrofabric (Aitken, 1967;
230 Kennard and James, 1986; Turner et al., 2000; Shapiro, 2000), and leiolites, without well defined
231 macrofabric (Braga et al., 1995). These three types of microbialites can display a wide range of
232 microstructures including micropeloidal, densely micritic, or agglutinated microfabrics (Riding,
233 1991; Dupraz and Strasser, 1999). One of the major problems is the characterization of the micrite
234 component. The term micrite, introduced by Folk (1959), is the abbreviation of “microcrystalline

235 calcite” and is commonly used to describe very fine carbonate particles less than 4 microns in size.
236 Particularly important is the distinction between biotic and abiotic micrite. Micromorphological
237 observations can furnish an initial contribution to understand the origin of the micrites but in some
238 cases they do not show any diagnostic features even if they are biotic in origin. For this reason the
239 recognition of microbial activity is very difficult in ancient carbonates.

240 As already mentioned in Section 1, in this work we present the analysis and the measurements
241 performed with our method on some carbonate samples in which there are no macroscopic
242 evidences of controlled or induced biomineralization.

243 The carbonate samples used in our study come from the Calcare di Base (CB) formation, which
244 crops out in the Rossano Basin (Northern Calabria, Italy) and formed in the Late Miocene
245 (Messinian, ~ 6 Myr – see below). In the study area the CB succession consists of two massive fine-
246 grained calcareous beds interlayered with decimetric laminated marls. In such geological settings
247 the CB formation is massive, without any evidence of evaporitic minerals, and is preserved as
248 aragonite (Guido et al., 2007; Rao et al., 2006).

249 .

250

251 ***3.1 Origin and properties***

252 The six bulk CB samples, labelled CB1-CB6, were collected near the village of Cropalati and
253 selected according to their position in the stratigraphic succession (see panel A in Fig. 4) and to the
254 lithological features observed in the field (see panel B in Fig. 4). These samples were previously
255 studied and characterized through molecular fossils and other organic markers (Guido et al., 2007).

256 Figure 4

257 The origin of these deposits (biotic *versus* abiotic) is not clear, due to the absence of fossils and
258 well-defined sedimentary structures. While several authors interpreted their genesis as evaporitic
259 and/or diagenetic (Rouchy and Caruso, 2006), recently Guido et al. (2007) demonstrated that the
260 CB carbonates are preserved in their original aragonitic mineralogical phase and are composed of

261 small crystals organized as peloids forming clots with an antigravitative fabric (i. e. an arrangement
262 that is not grain supported and with large interclot areas showing a fabric contrary to what dictated
263 by gravity) The bright UV fluorescence indicates that peloidal micrite is rich in organic matter.
264 Guido et al. (2007), using an organic matter study performed through Rock-Eval pyrolysis analyses,
265 palynofacies observations and molecular-fossil recognition, demonstrated that carbonate
266 precipitation was induced by bacterial ammonification of amino acids in aerobic conditions.
267 Therefore these deposits are the results of biotic mineralization in a marine depositional scenario
268 influenced by freshwater input rather than evaporitic concentration. These problematic carbonates
269 seem to represent a good sample to validate the applicability of the IR transmission spectroscopy in
270 the discrimination between biotic and abiotic carbonates.

271

272 ***3.2 Analysis and measurements***

273 The samples were ground to obtain fine powders and analysed by means of IR transmission
274 spectroscopy before and after thermal processing at 485 °C for 3.5 hours. All of them exhibit the
275 same trend and, as examples, we show in Figs. 5a and 5b the spectra of samples CB1 and CB4 (see
276 panel A in Fig. 4 for their location). As it can be clearly seen the transmission spectra before
277 thermal processing (solid lines in Figs. 5a and 5b) exhibit the typical absorption bands of aragonite
278 at 1476 cm⁻¹ (6.8 μm), 1083 cm⁻¹ (9.2 μm), 856 cm⁻¹ (11.7 μm) and a double peak around 700 cm⁻¹
279 (14.3 μm), characteristic of this mineral (White, 1974). The position of the main bands in the
280 spectra after thermal processing reveals that the transformation to calcite has occurred while the
281 changes in the slope beyond 700 cm⁻¹ indicates that the conversion to CaO also began (for the
282 details see Orofino et al., 2007, 2009). The presence in the spectra, both before and after thermal
283 processing, of other features in the 1200 – 1000 cm⁻¹ range and the doublet around 630 cm⁻¹,
284 indicates the existence of some sulphate, most probably celestine (SrSO₄) as suggested also by our
285 laboratory EDX (Energy Dispersive X-ray) analysis of the samples.

286 *Figure 5a and 5b*

287 The evaluation of the slope variation index D for the six samples and the knowledge of their
288 geologic age, allow their positioning in the plot of Fig. 3, where we report the average value of this
289 parameter along with its error bar, due to uncertainty on the single samples.

290 The age of the samples (~ 6 Myr) has been inferred from their stratigraphic position while its
291 uncertainty, as usual, has been assumed equal to half of the duration of the Messinian period,
292 according to Gradstein et al. (2004)

293 As it can be seen in Fig. 3, the point representing our samples, in what we can define as the “biotic
294 carbonate diagram”, clearly indicates a very likely biotic origin of the aragonite under analysis. This
295 conclusion is in good agreement with that obtained by Guido et al. (2007) using a completely
296 different approach based on complex geochemical techniques.

297

298 **4. Discussion and conclusions**

299 In the framework of the search for life on Mars, a method able to discriminate between biotic and
300 abiotic origin of carbonate samples is certainly of valuable importance for the next generation of
301 space missions to the planet. In previous work (D’Elia et al., 2007; Orofino et al., 2007, 2009,
302 2010) we have developed a method to distinguish, by means of IR spectroscopy, abiotic calcium
303 carbonate minerals from the corresponding biominerals in form of fresh shells and fossils found in
304 different places and easily recognizable as of biotic origin. The IR spectroscopy of the sample after
305 heating at $485\text{ }^{\circ}\text{C}$ allows discriminating its origin, by means of the evaluation of the D parameter
306 (see Section 2).

307 The linear correlation found between the D index and the logarithm of the age of known biotic
308 samples, which we named “biotic carbonate diagram”, can be used to:

- 309 a) discriminate between biotic and abiotic origin of other carbonate samples;
- 310 b) individuate particular groups of carbonate fossils;
- 311 c) estimate the age of terrestrial samples of known biotic origin.

312 The evaluation of the age of the samples (point (c)) can be easily understood and applied.
313 Concerning point (b), the deviation from the linear trend has been already evidenced for a group of
314 terrestrial fossils embedded in a layer of clays, with important implications in the search for
315 biosignatures on Mars (Orofino et al., 2010).

316 As far as point (a) is concerned, in this work we have applied our analysis to carbonate samples, in
317 which there is no clear evidence of controlled or induced biomineralization, and we have shown that
318 the method is reliable and useful to discriminate between biotic and abiotic carbonates, even in the
319 absence of fossils and well-defined sedimentary structures. Indeed, as it can be seen from Fig. 3, the
320 mean position of these samples in the “biotic carbonate diagram”, clearly indicates a very likely
321 biotic origin of the aragonite samples under analysis. This is in agreement with the conclusion
322 reported by Guido et al. (2007) who followed a completely different approach based on a complex
323 set of sedimentary, petrographic, geochemical and biochemical analyses.

324 It is worthwhile to note the relative simplicity of our method compared to the sophisticated
325 geobiological investigations already applied and discussed by Guido et al. (2007). On the other
326 hand, we acknowledge a possible drawback of our method due to the relatively small number of
327 samples used to validate it. For this reason future work will be devoted to increase the number of
328 analysed samples. In addition, it is necessary to recall that our samples are, on the geological scale,
329 relatively young (less than 1 Gyr old), while the hypothetical Martian fossils are expected to be
330 older. In fact it is likely that the initial conditions favorable to life, with a thick atmosphere able to
331 allow abundant liquid water on the surface of the planet, lasted only 1 Gyr or less (Pollack et al.,
332 1987), so it is possible that we would not find fossils, if any, younger than 3.5 Gyr old. These
333 fossils could be so degraded that they would be indistinguishable from abiotic carbonates.

334 It is important to note, however, that in the last 3.5 Gyr the geologic and climate conditions on
335 Mars have been quite different from those present on Earth. In particular, while in the terrestrial
336 environment the circulation of water in the ground has chemically altered the nature of biotic
337 materials, the likely absence of such conditions on Mars for most of its history may have preserved

338 some biotic signatures even for samples as old as 3.5 Gyr. Such possibility is also reported and
339 discussed by Stalport et al. (2005), leading to the same conclusions. Due to the very different
340 evolution of the environmental conditions on Mars and Earth, it is not unreasonable to think that the
341 ancient Martian biotic carbonates, if they exist, may have experienced a degradation process much
342 slower than that on Earth and therefore they could have a degradation level comparable to that of
343 much younger terrestrial analogues. If so, such Martian biotic carbonates could be detected by
344 means of IR spectral analyses, making the “biotic carbonate diagram” a powerful tool for the search
345 for life on Mars. This means that experimental projects designed as *in-situ* or sample-return
346 analyses and based on IR spectroscopy, could be useful as diagnostic technique complementary
347 and/or alternative to the DTA analysis already proposed for the same purpose (Cabane et al., 2004;
348 Stalport et al., 2005, 2007). The choice of the more suitable procedure may obviously be dictated by
349 several considerations and opportunities which at the moment are beyond the aim of the present
350 work.

351 As mentioned before, in the future we plan to increase the number of analysed fossils in order to
352 improve the statistical significance of the “biotic carbonate diagram”. The analysis of fossils older
353 than those studied up to now, will be also useful in order to investigate the spectral reaction to heat
354 processing of less preserved biological structures. Furthermore, as already pointed out, it will be
355 possible to apply our method to discriminate between biotic and abiotic origin of other carbonate
356 samples coming from different localities (e.g. Brown et al., 2010).

357

358

359

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585 **Figure captions**

586

587 Figure 1. IR spectra of *Tellina exigua* fresh shell obtained before (solid line) and after (dotted line)

588 thermal processing for 3.5 hours at 485 °C.

589

590 Figure 2. Reference spectra of aragonite and calcite (data from Salisbury et al., 1991) and pure CaO

591 grains analyzed in our laboratory. The spectra have been arbitrarily scaled for sake of clarity.

592

593 Figure 3. Values of the slope variation index D , calculated for all the samples (from #1 to #12)594 analysed by Orofino et al. (2010) and plotted versus the age of the samples. The D values

595 determined for minerals of abiotic origin (samples #11 and #12) have to be considered outside the

596 chronological range. The arrow indicates the average value (#13) for the CB samples studied in the

597 present work (see text, Sect. 3).

598

599 Figure 4. Study section (panel A) and general view of the Rossano Basin (panel B) showing the

600 position where the six samples of Calcare di Base have been collected. The black box in B indicates

601 the location shown in A.

602

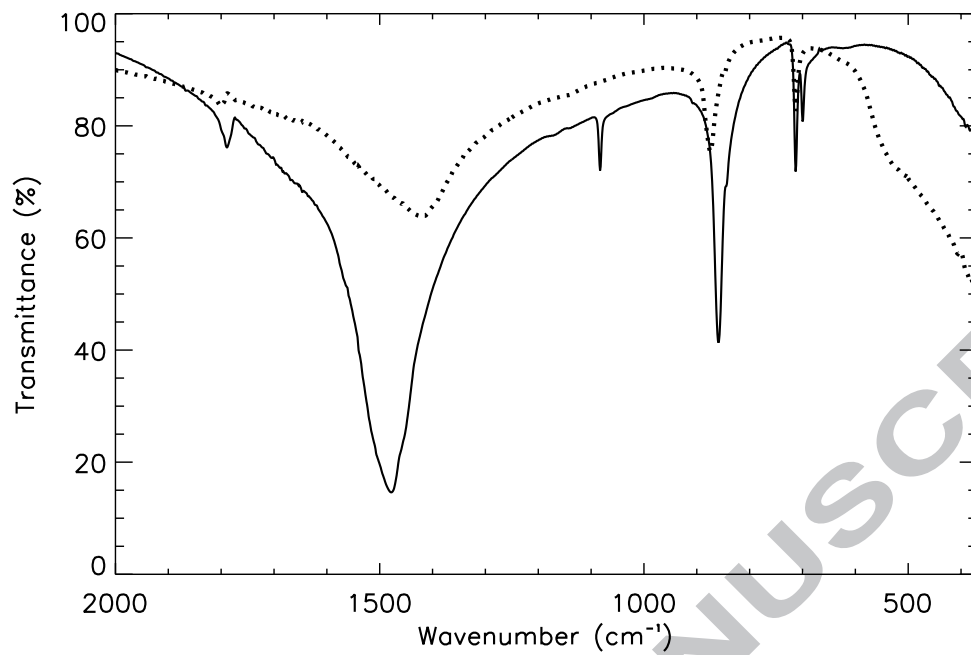
603 Figure 5a. IR transmission spectra of sample CB1 before (solid curve) and after (dotted curve)

604 thermal processing for 3.5 hours at 485 °C.

605

606 Figure 5b. Same as Fig. 5a, but for sample CB4.

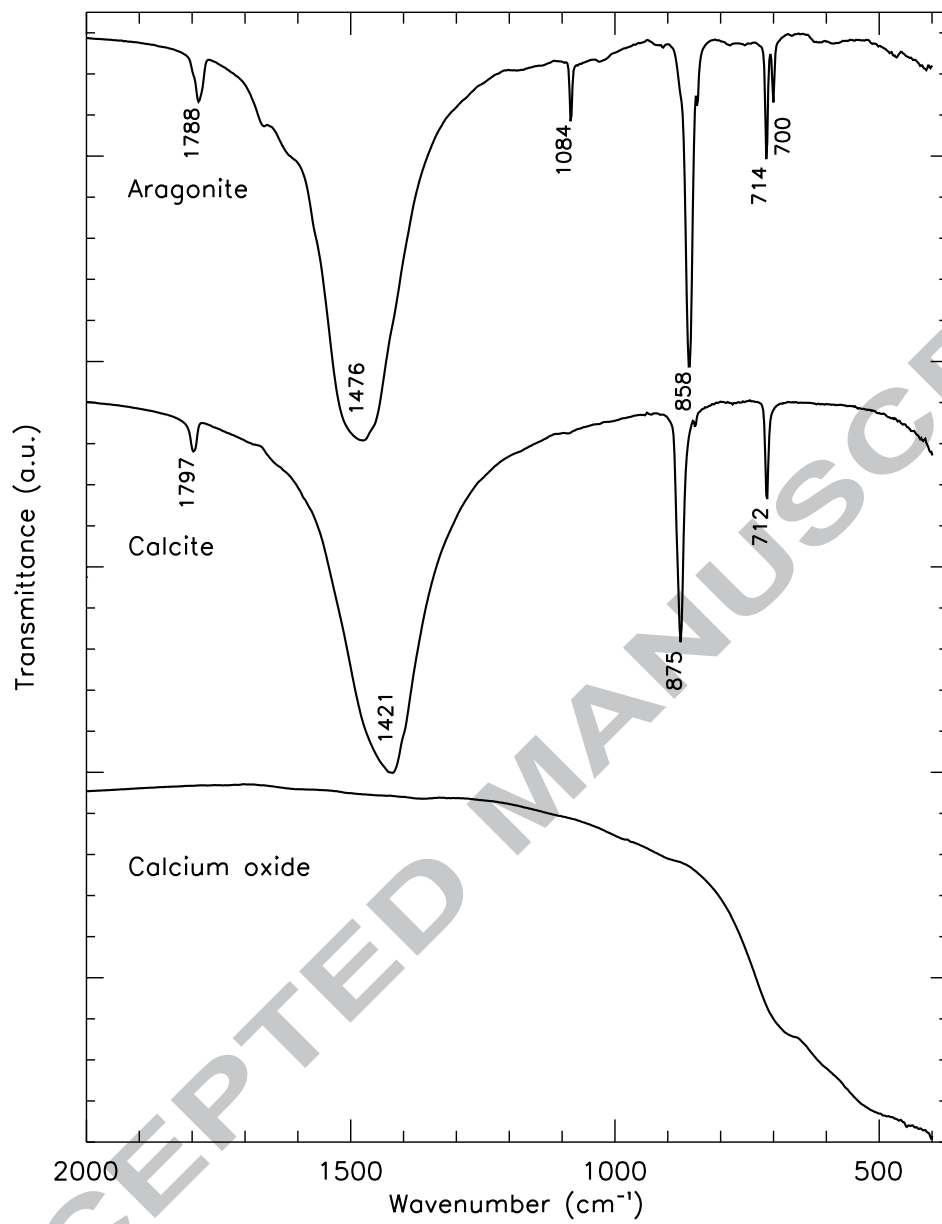
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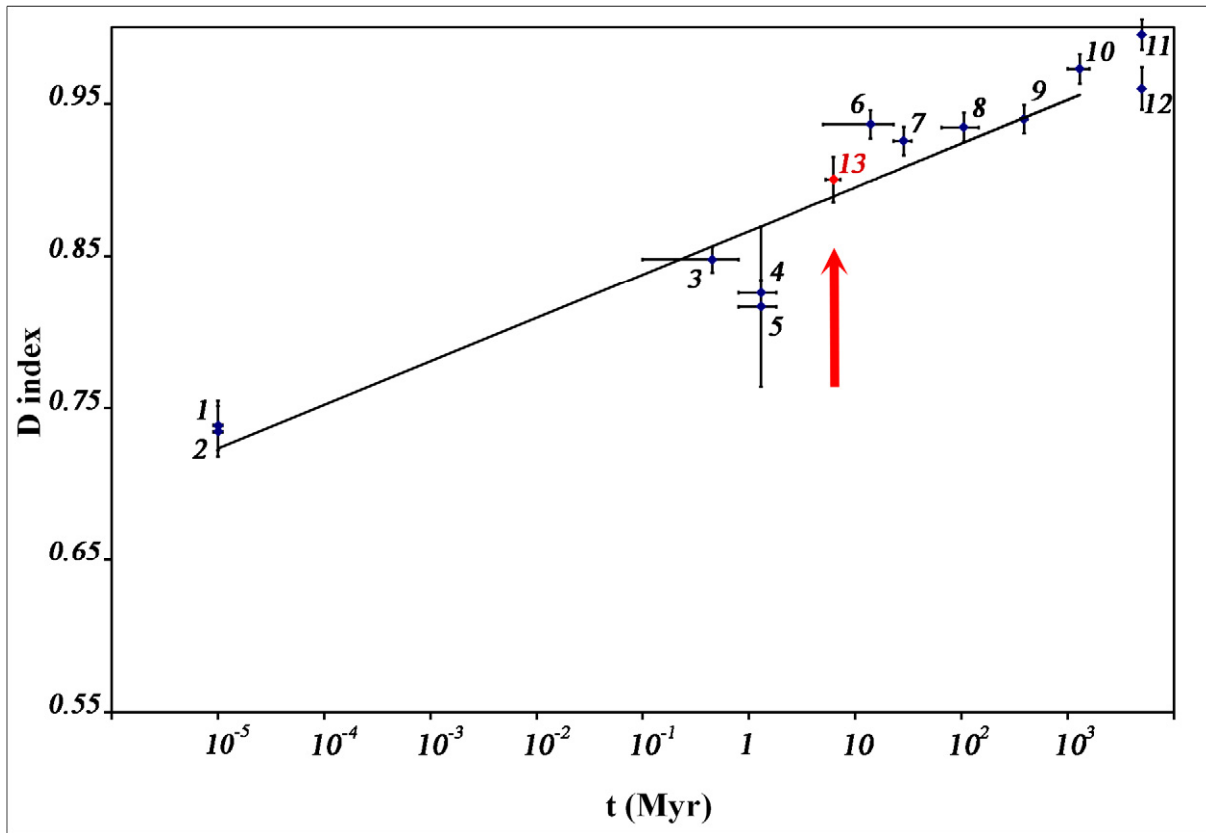
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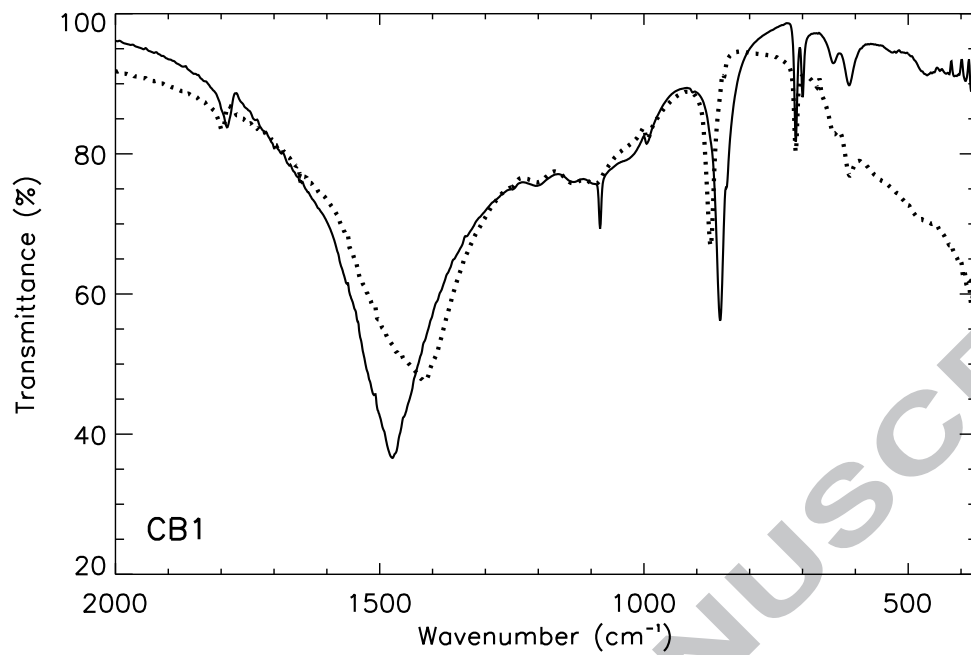
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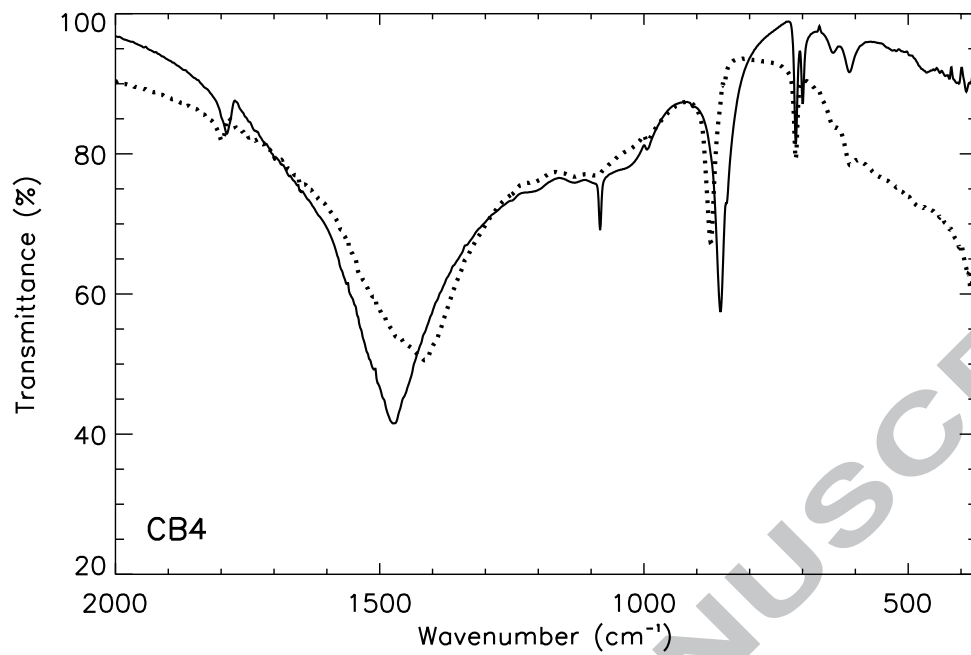
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617 > We present a spectroscopic method to discriminate biotic from abiotic carbonates. > Infrared
618 spectral modifications induced by thermal processing are investigated. > We analyse carbonate
619 samples with no evidences of biomineralization. > Our method is a powerful tool for the search of
620 life on Mars.

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