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# Characterization of fossil organic matter with Fourier-Transform Infrared (FTIR) Spectroscopy: an attempt to record extraterrestrial life

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**Abstract.** The characterization of the insoluble macromolecular fraction of organic matter preserved in carbonate sediments allows the identification of fossil organisms otherwise not recognizable. This approach represents a new tool for the detection of extraterrestrial traces of life even in their primitive form, one of the primary goals of exobiological studies on Mars. Considering the deep connection between carbonate sediments and biological activities on Earth, we checked if it was possible to characterize the organic matter remains dispersed within these rocks, with Fourier transform infrared (FTIR) spectroscopy. The experiment was performed on ancient and well preserved carbonates, belonging to the Calcare di Base Formation outcropping in Northern Calabria (Rossano Basin). The origin of these Miocene deposits is problematic, due to the absence of skeletal fossils. The results demonstrate that FTIR is a reliable method for the characterization of the organic matter preserved in carbonates, and generally in all types of sedimentary rocks.

**Key words.** Carbonates – Organic matter – Biosignatures – Spectroscopy

## 1. Introduction

Biosignatures in early terrestrial rocks are highly relevant in the search for traces of life on Mars because the early geological environments of the two planets were, in many aspects, similar and the potential habitats for early life forms were comparable (Westall, 2008). However, the identification and interpretation of biosignatures in ancient terrestrial rocks has proven contentious over the last few years.

In this paper we present a new approach for lipid detection and characterization via FTIR. It allows the identification of ancient life forms otherwise not recognizable, such as microbial communities that could have flourished in extreme habitats (temperature, pressure, pH, salinity, and nutrient availability), like those of early Earth and Mars.

The main goal of this study was to verify the applicability of the FTIR analysis for the geochemical characterization of carbonate, concerning their organic matter content and

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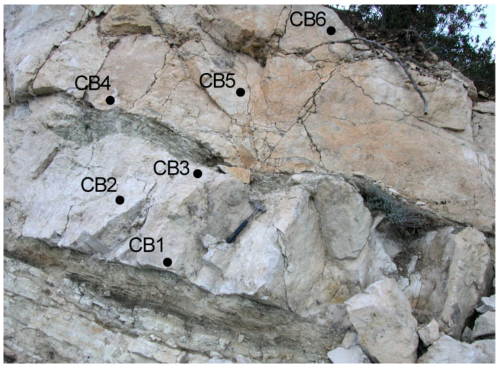


**Fig. 1.** View of the Calcare di Base carbonate beds with the location of the studied samples.

thermal evolution. To tests this approach we chose the Calcare di Base (CdB), whose origin was uncertain for the absence of fossils and sedimentary structures (Fig. 1). In particular it was unclear whether they formed abiotically, in hypersaline environments, or biotically, in normal marine settings. Guido et al. (2007), with a thorough study on organic matter remains, demonstrated that these deposits precipitated biotically *via* microbial induced mineralization. In the present paper these data are utilized to validate the FTIR analysis for discriminating between abiotic and biotic origin of these carbonates.

## 2. Fourier Transform Infrared analyses

Small fragments of carbonate samples, visibly free of alteration, were hand ground in an agate mortar and three grams of powder were ultrasonically treated three times with a mixture of dichloromethane/methanol (1:1). Samples were centrifuged after each treatment and the supernatant was collected. To concentrate the organic compounds, the solution was evaporated and the dry residue was resuspended in a few millilitres of dichloromethane/methanol. Several drops of this extract was placed onto the crystal of the ATR (Attenued total reflectance) apparatus and dried under a nitrogen flow.

The spectroscopic analyses on extracted organic matter from six different samples (CB1-CB6, see Fig. 1) were performed in the mid infrared range (4000-400 $cm^{-1}$) by accumulating 256 scans. A Perkin Elmer Spectrum 100 spectrophotometer equipped with a Universal ATR accessory, a KBr beamsplitter and a $LiTaO_3$ detector as been used at a resolution of 4 $cm^{-1}$. Spectral bands were assigned by reference to the literature (Painter et al., 1981; Solomon and Carangelo, 1988; Wang and Griffiths, 1985; Sobkowiak and Painter, 1992).

The following absorption bands of organic compounds were recorded: stretching of aromatic C-H groups in the region from 3100 to 3000 $cm^{-1}$, stretching of aliphatic C-H groups in the region from 3000 to 2800 $cm^{-1}$, bending of oxygenated and aromatic/olefinic groups in the region from 1550 to 1750 $cm^{-1}$, bending modes of $CH_2$ and $CH_3$ groups at 1450 $cm^{-1}$, absorption bands of $CH_3$ groups at 1375 $cm^{-1}$, and aromatic out-of-plane C-H deformation region from 700 to 900 $cm^{-1}$ (see Fig. 2 and Fig. 3A).

Considering that the absorption bands of organic matter overlap in some region of the spectrum, we used Fourier self-deconvolution (Kauppinen et al., 1981a, b; Wang and Griffiths, 1985) to semiquantitatively determine the content of functional groups (Starsinic et al., 1984; Solomon and Carangelo, 1988; Landais and Rochdi, 1990; Kister et al., 1990; Sobkowiak and Painter, 1992; Ruau et al., 1997). Fourier self-deconvolution resolved the region around 700 $cm^{-1}$, discriminating a peak at 720 $cm^{-1}$ due to the skeletal vibration of more than four methylene groups [$\gamma(CH_2)_4$]. The aliphatic C-H stretching region (3000-2800 $cm^{-1}$) was resolved into three spectral bands at 2950 (asymmetrical $CH_3$ stretching), 2920 (asymmetrical $CH_2$ stretching), and 2850 $cm^{-1}$ (symmetrical $CH_2$ stretching). The $CH_2/CH_3$ ratio was calculated using the asymmetrical stretching of these bands (2920/2950 $cm^{-1}$) following Lin and Ritz (1993a). Fourier self-deconvolution revealed methyl ($\delta CH_3$; 1370 $cm^{-1}$) and methyl-methylene [$\delta(CH_2 + CH_3)$; 1460 $cm^{-1}$] groups (Fig. 2).

The spectra displayed the band assigned to carbonyl and/or carboxyl groups ($\nu$C=O; 1740

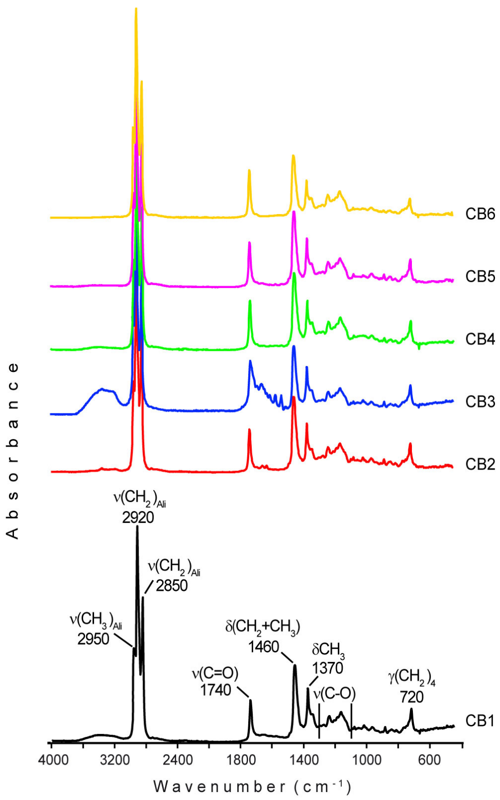


**Fig. 2.** Infrared spectra of the six different samples of extracted organic matter. Note the uniformity of the molecular compounds indicating the homogeneity of the carbonate precipitation processes.

cm$^{-1}$). The $\nu$C-O vibration of organic matter appeared between 1300 and 1100 cm$^{-1}$.

## 3. Discussion and conclusions

The characterization of recent organic molecules with FTIR has been already reported in the literature, in this paper we illustrate the efficacy of FTIR for the detection and classification of fossil organic matter preserved in ancient carbonates.

The infrared spectra obtained from powdered carbonates and thin sections, due to the interferences with the carbonate functional groups, did not yield useful information on organic molecules. The soundness of the FTIR became clear when the analysis was performed on organic matter extracted from the carbonate phase *via* acidic separation.

In the Calcare di Base samples the $CH_2/CH_3$ ratio was determined using Fourier self-deconvolution of the 2800-3000 cm$^{-1}$ aliphatic-stretching region (Painter et al., 1981; Lin and Ritz 1993). The value of this ratio ranges from 2 to 2.5 and confirms the thermal immaturity of the organic compounds. Immaturity previously deduced from biomarker data and maximum hydrocarbon-generation temperature (Tmax).

We think it is possible to characterize the kerogen type through the infrared spectra analysis. This is reasonable because kerogens (lacustrine, marine and continental) are distinguishable using their different H/C and O/C ratios (Peters et al., 2005).

To test the FTIR analyses consistency we compared the carbonyl/aliphatic ratio ($\nu$C=O/$\nu$CH$_{ali}$) with the Rock-Eval data (hydrogen index and oxygen index). Actually the carbonyl groups, being formed mainly of C=O bonds, can be correlated to the oxygen index, while the aliphatic groups, being formed mainly of C-H bonds, to the hydrogen index. In the studied samples the $\nu$C=O/$\nu$CH$_{ali}$ ratio shows the same trend of hydrogen and oxygen index that Guido et al. (2007) attributed to periodic increases in the deposition of continental organic matter.

FTIR spectra of kerogens with increasing maturity exhibit an increasing of absorption in the aromatic regions (3000-3100 cm$^{-1}$, ~1600 cm$^{-1}$ and 700-900 cm$^{-1}$), a decreasing of absorption in the aliphatic regions (at 1375, 1450, and 2800-3000 cm$^{-1}$), and a decreasing of absorption in the carbonyl/carboxyl region (at 1700 cm$^{-1}$) (Lis et al., 2005). In our samples the low thermal evolution of organic matter is confirmed by the absence of peaks in the three aromatic regions (Fig. 3). This interpretation agrees with the Rock-Eval pyrolysis and gas chromatography-mass spectrometry data that put in evidence the general immaturity of the organic remains.

The results permit to consider the FTIR a useful tool to distinguish between biotic and abiotic materials. Orofino et al. (2007, 2009, 2010) studied the infrared (IR) spectral modi-

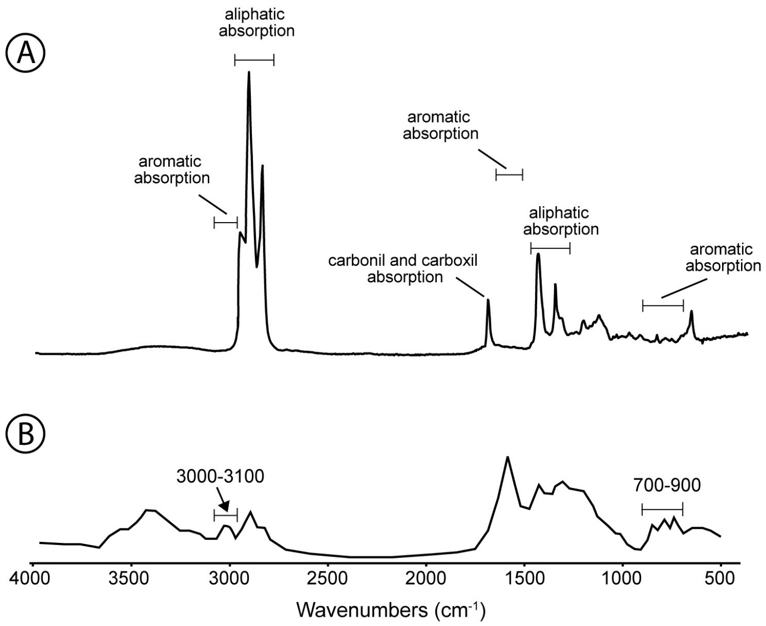


**Fig. 3.** . A) Functional groups of the organic compounds extracted from the mineral matrix of the sample CB1. B) Data from Lis et al. (2005) regarding the thermal maturity of type-II kerogens from Devonian black shales. Note the difference between our spectrum and Lis et al. (2005) data regarding the spectra of organic compounds with higher thermal maturity.

fications induced by thermal processing on various calcium carbonate fossils, in order to discriminate skeletal carbonates from their abiotic counterparts. These authors analyzing the mineral fraction of the carbonate did not consider the diagenetic processes that could have altered the original biominerals. The original micro-nanomorphologies of the biominerals could have been changed or lost during the neomorphic processes. Carbonate sediments are frequently affected by diagenetic processes that alter mineralogical composition and/or crystal shapes and fabrics (Folk 1965). The term *neomorphism* (Folk 1965) refers to the mineral transformations taking place in the presence of fluids even at atmospheric pressure and temperature. This includes processes of *replacement* (the dissolution of one mineral and simultaneous formation of another mineral), *inversion* (the replacement of a mineral by its polymorph), and *recrystallization* (changes in crystal size, shape and crystal lattice without a change in mineralogy).

On the contrary the organic matter tends to remain stable during the diagenetic processes due to insolubility of the lipidic fraction in water (Peters et al., 2005). These considerations suggest that biomarkers can preserve better the biological signal than the mineralogical counterparts. In addition the discrimination between biotic and abiotic carbonate samples appears to be more reliable analyzing organic remains with FTIR methodology. The possibility to analyze the organic matter extracted from any kind of mineral matrix permits to apply our methodology to a wide spectrum of materials collected on Earth or Martian surface.

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