

Did life exist on Mars? Search for organic and inorganic signatures, one of the goals for “SAM” (sample analysis at Mars)

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Abstract

Observation of Mars shows signs of a past Earth-like climate, and, in that case, there is no objection to the possible development of life, in the underground or at the surface, as in the terrestrial primitive biosphere. Sample analysis at Mars (SAM) is an experiment which may be proposed for atmospheric, ground and underground in situ measurements. One of its goals is to bring direct or indirect information on the possibility for life to have developed on Mars, and to detect traces of past or present biological activity. With this aim, it focuses on the detection of organic molecules: volatile organics are extracted from the sample by simple heating, whereas refractory molecules are made analyzable (i.e. volatile), using derivatization technique or fragmentation by pyrolysis. Gaseous mixtures thus obtained are analyzed by gas chromatography associated to mass spectrometry. Beyond organics, carbonates and other salts are associated to the dense and moist atmosphere necessary to the development of life, and might have formed and accumulated in some places on Mars. They represent another target for SAM. Heating of the samples allows the analysis of structural gases of these minerals (CO₂ from carbonates, etc.), enabling to identify them. We also show, in this paper, that it may be possible to discriminate between abiotic minerals, and minerals (shells, etc.) created by living organisms. © 2003 COSPAR. Published by Elsevier Ltd. All rights reserved.

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1. Introduction

In 2009, NASA will probably send a lander/rover to Mars (Mars Science Laboratory, MSL); the aim of the scientific payload of this mission will be, in particular, to search for the presence of extinct or extant traces of life, or prebiotic chemistry that could have existed. It will use a drilling system able to attain depths of some decimeters to meters, and, consequently, will be able to sample zones where inorganic material may be a witness of

epochs where Mars atmosphere was able to ensure the development of life, and where organic remnants might have been preserved from destruction (McKay et al., 1992; Brack et al., 1999). The SAM project that we foresee to present as a part of the scientific payload uses the knowledge that we have acquired in developing analog experiments on other space missions (to Titan, to Jupiter, to comets).

Carbonates, as observed in the SNC-Martian meteorites (Gooding, 1992), have not been fully confirmed at the surface of Mars, nevertheless some observations (Pollack et al., 1990; Lellouch et al., 2000) show that one can hope to find them in the underground (Fonti et al., 2001), even if they may have been destroyed at the

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surface under the action of solar UV. From their presence, one could assert that, in the past, Mars atmosphere was denser and moister than at the present time. Consequently, life could have developed at the surface or in the near underground. Other manifestations of a milder Martian climate may be searched for, e.g. carbonates, salts, under the form of evaporites from ancient lakes, or phyllosilicates (clays).

Organic material has not been observed by Viking in the pyrolysis-GC/MS experiment (Biemann et al., 1976; see also Klein et al., 1992). Glavin et al. (2001) have shown that this can be due, if it was effectively present, to the experimental conditions which were not adapted to detect amino-acids (destroyed by heating) and had a relatively high threshold for their decomposition products (amines). Furthermore, Viking sampled only the first centimeters in depth of the Martian ground, where the organic material has been oxidized/destroyed by atmospheric H_2O_2 (Zent, 1998) or by atomic oxygen (Kolb et al., 2003). It has been shown by Benner et al. (2000) that it may be degraded into salts of benzene-carboxylic acids, that are highly refractory. Therefore, Viking's ovens were unable to pyrolyse such molecules ($T_{\text{max}} = 500\text{ }^\circ\text{C}$), and no signal was observed. The same problem occurs with the search for relevant minerals: it appears (Kotra et al., 1982) that most of the minerals that could be witnesses of a past mild atmosphere (hydrates, carbonates, sulfates, clays) degrade and release their structural gases at temperatures that were not attained by Viking's GC/MS. One may conclude that, even in the presence of organic remnants and associated inorganic matter, it was almost impossible for Viking's pyro-GC/MS to observe any signal.

We have used our knowledge of pyrolysis and GC/MS analysis for other space experiments (Israël et al., 1997, 1999; Niemann et al., 1997; Mahaffy et al., 2000a,b) to develop the project of an experiment devoted to in situ analysis of the Martian ground and underground. This project, sample analysis at Mars (SAM) (see also Cabane et al., 2001), will be proposed in the frame of the future NASA Mars Science Laboratory, in 2009. One part of this experiment will be devoted to the study of the atmosphere (rare gases, isotopic ratios), to increase our understanding of the history of the planet, and will not be presented here. The other part will be devoted to analysis of the ground and underground, to search for indicators of prebiotic or biotic activity. We present, here, the part of SAM proposal dedicated, for this latter purpose, to the analysis of the evolved gases, after processing and heating of the samples.

Concerning the organic matter that could be present in the samples, we have improved the techniques on two points. On the one hand, we have increased the oven temperature (1100 °C) in order to analyze the gases evolved from moderately refractory molecules. On the

other hand, in the case of very fragile or refractory molecules, we foresee to proceed, in parallel with pyrolysis, to substitution reactions (derivatization) in which the target molecule gives birth to an analyzable one, while preserving the structural information: knowing the reagent one may retrieve the nature of the parent molecule.

In the case of the inorganic matter, the fact that the temperature of the oven reaches 1100 °C will permit to observe the production of structural gases for most of the relevant minerals (Kotra et al., 1982) and to compare to observations of other instruments on SAM or MSL. Furthermore, it is well known that a given mineral may exist under an abiogenic form (due to classical hydrothermal processes, for example) or under a biogenic form, its existence being linked to the development of living bodies. For example, calcium carbonate (calcite, aragonite) may be found under the form of shells or carapaces, or deposited as stromatolites, witnesses of a microbial life. Consequently, one can suppose that the temperatures at which crystals will break will be different (lower in the case of a quickly built 'biogenic' material) and that clues on the presence of a past life might be obtained from the study of inorganic matter.

2. SAM: Sample analysis at Mars

SAM is intended to analyze Martian atmosphere, ground and underground:

- (a) To determine the elementary composition of the recovered samples of ground or underground, in order to compare with the results of other experiments on the Lander and to give points of comparison for objectives (b), (f) and (g) below.
- (b) To seek, in ground and underground, for organic traces which could be witnesses of a past prebiotic chemistry, and, in the best of the cases, of an organized life, which would have developed at Mars surface or underground. The chirality of organic molecules will give clues on their origin. In parallel with the search for organic molecules, the study of isotopic ratios will bring additional information.
- (c) To obtain information on atmospheric noble gases (Ar, Xe, Kr): isotopic abundances and ratios, as well as isotopic ratios of carbon (13/12) in CO_2 , of nitrogen (15/14) in N_2 , and of D/H in H_2O . This will make it possible to correlate with other observations (on the lander, from the Earth) and to better understand crustal degassing and atmospheric escape in Mars history.
- (d) To measure isotopic ratios of carbon, nitrogen, sulfur (34/32), oxygen (16/17/18), and D/H in samples of ground (or underground), after laser ablation or heating, in order to correlate with measurements performed -on Earth- on SNCs and to compare with

atmospheric measurements (previous subparagraph).

- (e) To search for atmospheric gases (CH_4 , H_2S , etc.) whose origin could be organic, and correlate with (b).
- (f) To obtain information on the presence of hydrates, carbonates, sulfates, clays in the sample of ground (or underground); the presence of mineralogical indices of a dense and wet atmosphere will come in support to the search for organic traces in the ground (item b)). One will also differentiate minerals from biominerals (carapaces, shells, tests, etc.), by simplified differential thermal analysis.
- (g) If the drilling system allows a sampling at different depths, to obtain a stratigraphy, not only for items (a), (b), (d) and (f) above, but also for gases adsorbed on the solid phase (H_2O , CO_2 , H_2O_2 , etc.).

We will, here, focus on goals (b), (d), (f) and (g), namely the analysis of samples obtained from the ground and underground (pyro-deriv/GC–MS analysis, see below). A laser-ablation/mass-spectrometer device (LDMS, Brinckerhoff et al., 2000) will be devoted to (a), and contribute to (b) (f) and (g); a mass spectrometer MS associated to a gas chromatograph GC (Israël et al., 1997; Niemann et al., 1997; Mahaffy et al., 2000a,b) will fulfill goals (c) and (e), and be used as detectors for goals (b), (d), (f) and (g), gases being obtained after pyrolysis or derivatization of the relevant molecules present in the samples.

3. Organic matter: pyrolysis and derivatization

3.1. Pyrolysis

Organic matter may be present in the first layers of the ground under the form of complex molecules, coming from living or dead organisms. These molecules, if they are not fragile or refractory (see following paragraph) may be analyzed using the pyrolysis/GC–MS method that we use for the ACP/GC–MS experiment (Israël et al., 1997; Niemann et al., 1997) aboard the CASSINI/HUYGENS probe to Titan. The sample containing possible organic matter is heated in an oven at temperatures from the ambient to 100 °C, and gases produced by the cracking of the organic molecules are pushed (by He, for example) into chromatographic columns of the GC. The nature of the eluted molecules is then obtained from the calibration data of the columns, giving elution time vs. nature of the molecules. Using databases, the nature of the pyrolysed molecules is obtained from their fingerprint, i.e. these gas molecules they produce under cracking (Fig. 1; see also Szopa et al., 2001, 2002). The retention/elution time is deduced from the moment when the gas pulse is introduced into the column and the moment when molecules leave it. This latter moment is obtained using gas detectors at the column outlet. Two kinds of GC detectors may be used

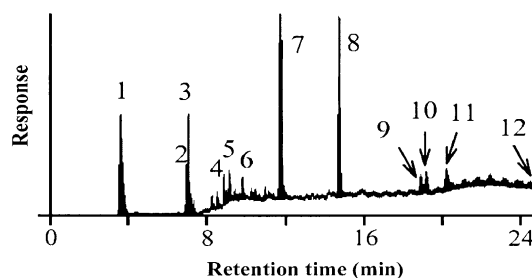


Fig. 1. Reconstructed ion gas chromatogram of the organic volatile fraction released during flash pyrolysis of a Mars' like soil from the Atacama Desert: (1) oxybis-methene; (2) 2-butene; (3) 1,2-butadiene; (4) propenenitrile; (5) 1,3-pentadiene; (6) 2-methylfuran; (7) benzene; (8) methylbenzene; (9) ethylbenzene; (10) 1,2-dimethylbenzene; (11) styrene and (12) benzenenitrile.

to detect eluted gases in the He flow: thermal conductivity detectors (down to some tens ppm) and Helium ionization detectors (down to the ppb). It may happen that some molecules are eluted at the same moment (co-elution), in this case the use of a mass spectrometer as a detector permits to identify the molecules. Moreover, the use of MS allows isotopic analysis of the gases and comparison with the current knowledge. The mass spectrometer, which can detect down to the ppb, may also be used alone, to avoid the delay time in analysis, due to the elution inside the GC columns, but identification of parent molecules is more difficult.

4. Derivatization

As quoted in Section 1, some organic molecules may be refractory, consequently insensitive to the oven's heating, or too sensitive to heating, and may be destroyed into too elementary molecules. The chemical derivatization (functionalization) permits to improve their detection. Using the reaction of target molecules with specific organic reactants (silylation, etc.), one can transform such molecules into ones that are able to be transferred to the GC-MS, while containing the structural information (Fig. 2).

Fig. 3. shows, after derivatization of amino acids, the molecules as they are identified using a chiral column (Rodier et al., 2001). Such columns will permit to obtain the percentage of L and D molecules of the same species and to show where they find their place between abiotic (L = D) and biotic (L or D) matter.

5. Inorganic matter

5.1. Minerals

At the location of the lander/rover, the ground/underground may contain inorganic minerals of

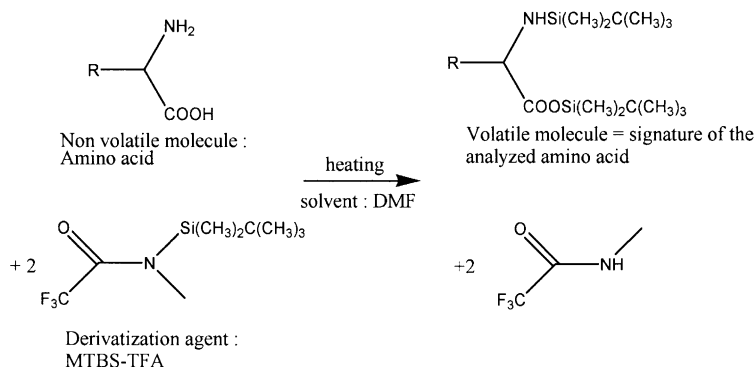


Fig. 2. Derivatization of an amino acid, using *N*-methyl,*N*-*tert*-butyl(dimethylsilyl)trifluoroacetamide (MTBS-TFA) as chemical reactant. Dimethylformamide (DMF) is used as the reaction solvent.

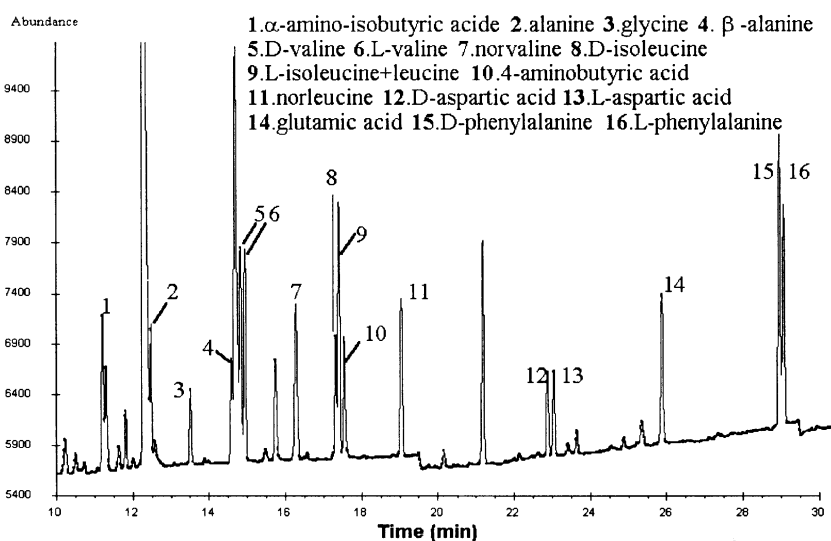


Fig. 3. GC–MS analysis of a derivatized (DMF-DMA) amino acids standard mixture (100 μ M each). Chiral column Chirasil-Dex fused-silica WCOT 10 m \times 0.25 mm. 4 $^{\circ}$ C/min from 70 to 170 $^{\circ}$ C, P = 19.5 kPa.

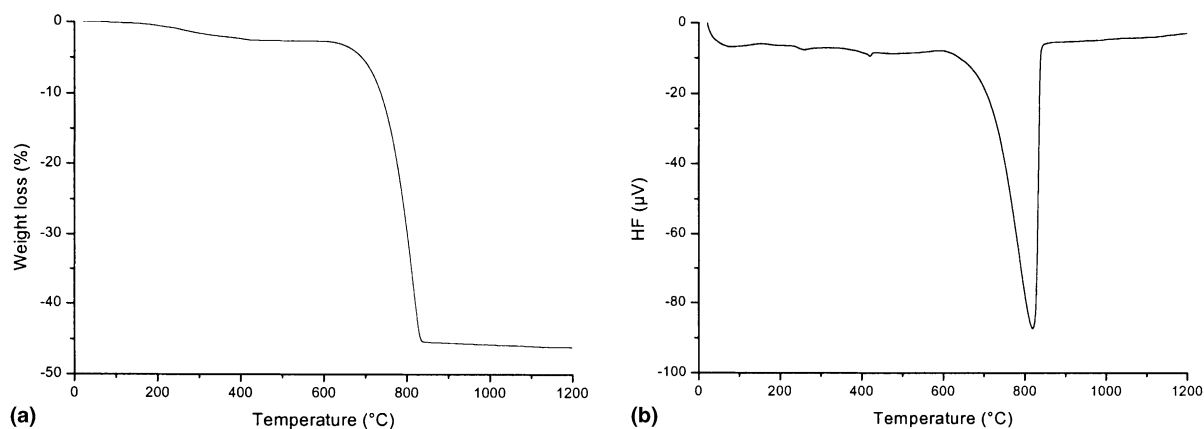


Fig. 4. Differential thermal analysis of a sample (35 mg) of biogenic aragonite CaCO_3 heated under 1 atm N_2 . (a) Weight loss following the breakdown of calcite into calcium oxide. Maximum value: $w = 46.2\%$, theoretical value: $w = 43.9\%$. (b) Thermal behavior (DTA): $T_1 = 420$ $^{\circ}$ C, aragonite to calcite; $T_2 = 825$ $^{\circ}$ C, calcite to calcium oxide.

climatologic interest: carbonates, salts, phyllosilicates, hydrates may be witnesses of a humid and dense past atmosphere. If such compounds are present in the sample that is introduced into the oven, they will break and deliver their structural gases. The knowledge of these molecules (CO_2 , H_2O , SO_2 , etc.), and of the temperatures at which they are delivered, will help to identify the mineral phase present in the sample: for example, the CO_2 emission from MgCO_3 and CaCO_3 differ of about 100°C (see Fig. 1 in Kotra et al., 1982). On Fig. 4(a) (next sub-paragraph) we show the measured mass loss of a sample of aragonite (see below, biominerals), that corresponds to the transformation of solid CaCO_3 into solid CaO , associated to the production of gaseous CO_2 , following the mass loss ratio $W = (\text{CaCO}_3 - \text{CaO})/\text{CaCO}_3 = 43.9\%$. Such analysis will be performed at Mars using the pyrolysis oven associated to the mass spectrometer, and will benefit of its high sensitivity. Emission of CO_2 or other gases will be correlated to the sample temperature, furthermore, isotopic ratios will easily be compared to the ones measured for current Martian atmospheric gases.

6. Biominerals

If a mineral is produced from the biotic activity of some organism, it will not have exactly the same properties that its non-biogenic equivalent. This comes from the fact that a shell or a skeleton is built in a rather short time, compared to a classical hydrothermal mineral, hence small defects in its structure. This fragility can be increased by the inclusion of organic molecules during the growth of the biomineral structure. To verify these assumptions, we have performed differential thermal analysis (DTA) on some tens milligrams of the crushed shell of a modern oceanic gastropod (*Gibbula Magus*), which consists in aragonite (metastable CaCO_3), and on the same mass, and grain granulometry, of hydrothermal non-biogenic aragonite. The DTA classical analysis of aragonite (Mackenzie, 1970) shows two peaks. The first one, rather weak, appears at $T_1 = 400/500^\circ\text{C}$ (depending on the experimental device and conditions), and is related to the endothermic transformation of aragonite into calcite, stable state of CaCO_3 . The second one, more intense, at $T_2 = 800/900^\circ\text{C}$, corresponds to the decomposition of calcite into solid calcium oxide (CaO) and gaseous CO_2 . If one registers the weight of the sample during the heating process, the first reaction, at T_1 occurs without any weight modification, whereas the second one, at T_2 , is associated to the weight loss W .

For these two samples, and using the same device and experimental conditions, we have registered the temperature behavior (T_1 , T_2) and the mass loss W (Fig. 4, Table 1). The general trend of the heat curve is the same, and the observed value of W is close to the theoretical

Table 1

Abiotic origin vs. biotic origin, using DTA on two samples of Aragonite CaCO_3

	Sample A (abiotic)	Sample B (biotic)
T_1 ($^\circ\text{C}$)		
Aragonite to calcite (CaCO_3)	485	420
T_2 ($^\circ\text{C}$)		
Calcite to calcium oxide (CaO)	860	825
Weight loss, W (%)	44.8	46.2

one (43.9%). When comparing heat curves, one observes (Table 1) that, in the same experimental conditions, temperatures T_1 and T_2 in the case of the biogenic mineral are lower than the ones observed for the non-biogenic one, due to the increased fragility of the biogenic carbonate.

Consequently, the temperature at which one observes the emission of CO_2 (T_2 in the present case) can give clues on the origin of the mineral. This mineral vs. biomineral dichotomy, has been observed, here, for a modern biomineral, and we think that it will necessarily appear for the other pairs mineral/biomineral that we intend to study and for possible biominerals preserved in Martian conditions. Same types of measurements could be performed aboard MSL. In this case, from the association of this analysis to elemental (e.g. from SAM-LDMS), and to mineralogical ones, it will be possible to know if the analyzed compounds behave differently from abiotic ones. In consequence, monitoring, via GC-MS measurements, the thermal degradation of a mineral sample will give information, not only on its nature, but also on the way it has been built. Associated to the organic analysis, it may permit to assert the existence of ancient life.

7. Conclusion

Pyrolysis and/or derivatization of samples of ground and underground in devoted ovens, followed by MS/GC analysis (including isotopic analysis) can demonstrate the presence of organic remnants associated to a prebiotic activity or an ancient life, down to the fraction of picomole. The chromatographic study of organic complex molecules will also be performed on chiral columns, allowing the study of enantiomeric excesses. In parallel with the search for organics, sample heating at high temperatures (up to 1100°C) allows to search for the presence of minerals (carbonates, salts, clays) associated with favorable climates, even in low quantities. Moreover, the thermal behavior of the samples helps to seek for possible inorganic biominerals (e.g. aragonite or calcite from shells, magnesium carbonate from stromatolites, etc.) that present a different thermal behavior from their pure abiotic form.

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