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Extraterrestrial material analysis: influence of the acid hydrolysis on the mtbstfa derivatization

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1. Introduction

1.1 Interest of Mars exploration

Sources of organics on Mars: Sources of endogenous organic matter (OM) could have existed; these sources include **(a)** abiotic production *via* hydrothermal, igneous or atmospheric synthesis and **(b)** biotic synthesis. Currently, proven sources of exogenous organic compounds delivered to Mars are known: carbon-rich meteorites, micrometeorites, comets and interstellar dust particles.

MOMA experiment, onboard the ExoMars mission, will search for **"**signs of life" molecules (large, non-volatile organic or biological molecules that suggest existing or prior biosynthetic activity) on the Martian surface and near subsurface. MOMA will also be able to distinguish between the endogenous and exogenous sources of Martian organics. MOMA's main instrument is a gas chromatograph coupled with a mass spectrometer (GC-MS), which provides the unique capability to characterize a broad range of compounds, including both volatile and non-volatile species. Nevertheless, an additional derivatization step is necessary for non-volatile species.

***Organic compounds targeted*.** To target molecules which can be still present on Mars, we have to determine the resistance of some particular molecules against environment stress. We also have to take in account the organic matters detected in Martian meteorites related and the molecules related with earth life. Among the molecules, we identify **(1)** the carboxylic acid which is found to be resistant to radiation and **(2)** organic molecules such as amino acids (AAs), nucleic bases and lipids. These molecules have the advantage of being detected in the Martian meteorite ALH 84001, to be predominant in terrestrial organisms (i.e. these molecules represent respectively 50 %, 25% and 10% of Escherichia coli bacteria (*1*) and finally to expose two chiral forms (homochirality is a strong indicator of biological trace).

However, most of these molecules are not volatile, and thus are not directly analysable by GC-MS. This analytical technique requires the compounds to be gaseous or volatilisable.

1.2 Extending the range of application of GC-MS

To extend the capabilities of GC-MS, it is possible to manipulate two parameters: the mass of the molecule and its polarity. The issue with high molecular mass molecules is that their temperature of volatilization is lower that their temperature of fragmentation. Polar molecules show sites where intermolecular interaction can be formed which makes them difficult to volatilize. To modify physicochemical proprieties, Erwin Kaal (7), three techniques can be employed: **(A)** Pyrolysis involves exposition of compounds at temperatures leading to thermal fragmentation, therefore the masses of molecules is reduced. **(B)** Derivatization replace labile hydrogens (e.g. present in a group -OH, -COOH, =NH, -NH2) by apolar groups (Fig. 1, derivatization of glycine by MTBSFTA) thereby reducing the polarity of the molecule. MTBSTFA (N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide) is a derivatization reagent present in MSL and ExoMars missions. **(C)** Thermochemolysis is a technique that combines pyrolysis and derivatization: it reduces the mass and polarity of the compounds. Indeed, the molecules are thermally fragmented before their acidic hydrogen is substituted by a apolar group from the thermochemolysis agent (TMAH). High temperature acid hydrolysis is applied to fragment the macromolecules (e.g. the proteins breaks into amino acids) and solubilizes mineral matrices which can contain OM.

Aim of this study: **(1)** Define the influence of the pH on the MTBSTFA derivation reaction **(2)** Determine if the quantity of protons delivered during hydrolysis has an influence on the derivatization of amino acids **(3)** Identify the cause of the variation of detection of amino acids according to proton concentration (pH)

2. Experiments and methods

To determine the influence of pH on the derivatization, silylation with MTBSTFA of amino acids is carried out at several pH values. The solution of amino acids is a standard solution of Sigma Aldrich and it is composed of

17 amino acids including L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine. The concentration of amino acids is 2.5 µmol/mL in a 0.1 M HCl solution except for L-cystine which is 1.25 µmol/L. Firstly, 10 µL of amino acids are dried out under a stream of argon. Subsequently, 0.4 mL of a basic solution (NaOH) or acidic (HCl) is added to set the pH at a given value. We studied two types of sample: samples with pH between 1 to 13 and sample with excess of HCl (6 and 12 M of HCl). It is then dried again under argon. The derivatization step is carried out on the solid residue via a solution of MTBSTFA/DMF (2:1). We added 30 µL of this solution to the sample and heated to 75 °C for 15 min. To allow the quantification of amino acids, 4µL of an internal standard (methyl laurate (8.4.10–3 M) in DMF) was added. The derivatized amino acids solutions are analysed by a commercial GC-MS equipped with an RTX-5 column (30m x 0.25 x 0.25µm).

3. Results and Discussion:

During the analysis, we detected 15 of the 17 amino acids: alanine and arginine are not found in our study conditions. In order to compare quantitatively the different analytes, we normalize the areas of amino acids obtained by the area of the internal standard. The results are shown in Fig. 2, and represent the ratio of the peak areas of few amino acids (Gly, Val, Leu, Pro and Met) at several proton concentrations.

In a basic environment, for pH 12 and 13, no amino acid is highlighted: the quantity of derivatized amino acids is lower than the detection limit.

In the pH range from 3 to 11, the results show a chemical stability of amino acid concentrations as a function of pH.

Under conditions of hydrolysis (12 M HCl and 6 M HCl), the concentration of proton does not influence the behavior of the amino acids except for serine and threonine. The results suggest that for these two compounds, the amounts detected are lower than the concentration of the reference sample and the concentration in the stable area (pH = 3-11).

Finally, the quantity of valine, leucine and isoleucine at pH 1 and 2 is higher than the other concentration measured for other pH.

4. Figures

Figure 1: example of MTBSTFA derivatization of glycine

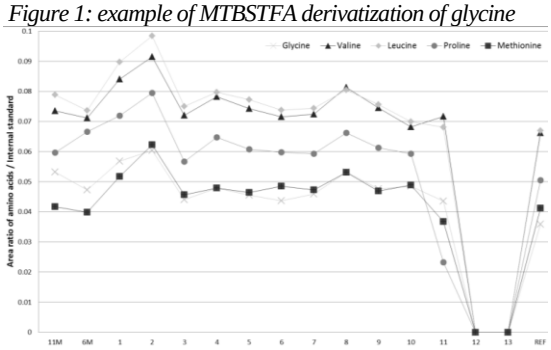

Figure 2: Influence of the pH on the MTBSTFA derivatization yield.

5. Conclusion

The effect of pH on the amount of amino acids may come from two phenomena:

- The amino acid is not in the same form according to the pH of the solution: they can be subject to deprotonation of the carboxyl and amine group. In fact, below a pH of 11, all of the amino acids have under-gone deprotonation and this explains the absence of amino acid in strongly basic solutions.
- The second hypothesis suggests that the acid or base used interacted with the derivatization agent.

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