EXTRACTION OF ORGANIC MOLECULES FROM TERRESTRIAL MATERIAL: QUANTITATIVE
YIELDS FROM HEAT AND WATER EXTRACTIONS. L.W. Beegle, W. A. Abbey, A. T. Tsapin., D. Dragoi,
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Introduction: In the robotic search for life on Mars, different proposed missions will analyze the chemical and biological signatures of life using different platforms. The analysis of samples via analytical instrumentation on the surface of Mars has thus far only been attempted by the two Viking missions. Robotic arms scooped relogith material into a pyrolysis oven attached to a GC/MS. No trace of organic material was found on any of the two different samples at either of the two different landing sites [1]. This null result puts an upper limit on the amount of organics that might be present in Martian soil/rocks, although the level of detection for each individual molecular species is still debated [2].

Determining the absolute limit of detection for each analytical instrument is essential so that null results can be understood. This includes investigating the trade off of using pyrolysis versus liquid solvent extraction to release organic materials (in terms of extraction efficiencies and the complexity of the sample extraction process.)

Extraction of organics from field samples can be accomplished by a variety of methods such utilizing various solvents including HCl, pure water, supercritical fluid and Soxhelt extraction. Utilizing 6N HCl is one of the most commonly used method and frequently utilized for extraction of organics from meteorites [3,4,5] but it is probably infeasible for robotic exploration due to difficulty of storage and transport. Extraction utilizing H2O is promising, but it could be less efficient than 6N HCl. Both supercritical fluid and Soxhelt extraction methods require bulky hardware and require complex steps, inappropriate for inclusion on rover spacecraft.

This investigation reports the efficiencies of pyrolysis and solvent extraction methods for amino acids for different terrestrial samples. The samples studied here, initially created in aqueous environments, are sedimentary in nature. These particular samples were chosen because they possibly represent one of the best terrestrial analogs of Mars [6] and they represent one of the absolute best case scenarios for finding organic molecules on the Martian surface.

Rock Samples: The four samples we have studied in this work includes a sample of tufa obtained at Mono Lake which possesses two discernable regions. One of the regions is a crusted formation that most likely consists of dead organisms, while the other region does not have this crusted formation. The third sample is a limestone (chalk) sample acquired from Ward's Scientific (#47 E 4663) which was obtained in Oktibbeha County, Mississippi. The fourth sample was collected as part of an expedition in the Mojave Desert.

Each of the samples was crushed using a pestle and mortar and the crushed sample was analyzed. No effort was made to make the sample size uniform, which should be analogous to what is expected aboard a lander such as Mars Science Laboratory (MSL). We estimated the average size of the particles to be less than 250 µm, with some chips as large as 1 mm.

Samples 1 and 2 are the non-crustal and crustal part of the tufa sample, respectively. Tufa is a travertine (calcium carbonate) deposit formed by precipitation when calcium-rich fresh water flows into carbonate-rich lake water. This process is both chemical and biochemical and frequently occurs in association with algal colonies like those at Mono Lake (i.e. the crust in sample 2).

Sample 3 is a particular variety of limestone, chalk, forms by the accumulation of fine scale organic debris in a marine sedimentary environment. The debris is composed predominantly of microscopic shells and skeletons of sea animals.

Sample 4 is tremolite which is a metamorphic mineral that occurs in both contact and regionally metamorphosed rocks. It commonly occurs when impure dolomites, in association with quartz or silica, experience low grade, thermal metamorphism. Dolomite occurs in marine sedimentary environments as a secondary mineral, formed from ordinary limestone by replacement of Ca with Mg.

Each of these four samples represent a particular time evolution of minerals formed originally in aqueous environments under the influence of biochemical processes. Organics exist naturally created by both biotic and abiotic processes, however amino acids have a chirality that can only be created through biotic processes, and hence makes them a potentially powerful biomarker. Extracting the amino acids while preserving these biomarkers would be the goal of any instrument package.

Heat extraction: For heat extraction of organics from the aforementioned samples we used a small volume 2.1 cm³ Knudsen cell (KC) in which 100 mg of samples were placed. The cell was then heated to 100°C,
200°C, and 300°C for 1 hour. Al foil was placed above the KC and held at room temperature which froze the sample on its surface. The samples were then dissolved in H2O and introduced into the HPLC.

**Liquid Extraction:** Liquid extractions of amino acids and other organics were done for all four samples using both 6N HCl and ultra high purity H2O. 100 mg (dry weight) of each sample was placed in a sterilized screw cap glass tube (baked at 500°C overnight) and treated with 1 mL of 6N HCl. A duplicate set was treated with 1 mL of ultra pure H2O (de-ionized and filter sterilized).

**High Pressure Liquid Chromatography (HPLC) Analysis:** In order to remove any potential instrument bias from the analysis of samples, all samples were analyzed on the same HPLC, with the same settings applied. Amino acid analyses were carried out by HPLC separation of fluorescent diastereomeric derivatives [4,7]. The derivatives separated and identified by reverse-phase HPLC using a C18 column (Phenomenex) with 50 mM sodium acetate and methanol as the solvent system. Eluting amino acid derivatives were detected using a fluorescence detector, with $\varepsilon_{ex} = 340$ nm and $\varepsilon_{em} = 450$ nm. Aspartic acid standards were used to calibrate retention times and detector response.

**Conclusions:** Figure 1 shows the comparison between heat (bottom) H2O (middle) and HCl (top) extraction of Tufa (without crust) found at Mono Lake Ca. **NOTE:** The Intensity of H2O, and HCl was reduced by 10.

Fig 1- Heat (Bottom), H2O (middle), and HCl (Top) extraction of Tufa (without crust) found at Mono Lake Ca.

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The metamorphic mineral found in the Mojave desert (Fig. 2) while, showing the same increase in number of species present, does not have the same increase in intensity of the peak intensities. Several amino acids, Aspartic acid (asp), Glycine (gly) etc., have substantially increased peaks intensities with the solvent extraction over heat extraction. For many species, including Serine (ser) and Isoleucine (ile). There is no detectable trace of these molecules in the heat only sample. This could be a direct result either from not-sublimating the molecules, or due to de-carboxilication of the species, which is a known result of heating amino acids [8].

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