**Experimental Extraction Procedures For Detection Of the Chemical Composition Of Biomarkers In Martian Soil Simulant**

Sonya D. Barner_, Dr. Wesley Stites_, _Jackson State University, Biology, School of Science and Technology, 1400 J.R. Lynch St. Jackson, MS 39217. _University of Arkansas at Fayetteville, Biochemistry, J. Williams Fulbright College of Arts and Sciences, 525 Old Main Ave. Fayetteville, AR 72701

**Introduction:** Controversial issues as the result of fossil evidence that suggest the possibility of past or present Martian life exhibit the common interest that we share about whether there is extraterrestrial life in places other than Earth.[3] The finding of chemical fossils has an impact on the types and allotment of molecules.[5] In order for us to propose solid evidence of present or past life on Mars its important that efficient methods of detecting the chemical evidence present in Martian soil be found.[2] Searching for more techniques may allow us to build on more sensitive methods of exposure, prepare future explorations for identification of signs of life, and give proposals about the evolutionary development of the surface of mars over time. Experimentation is initiated with the theory that life does exist on Mars and is somewhat similar to that on Earth. Similar in the aspect that Martian beings are made of cellular machinery such as nucleic acids, proteins, and carbohydrates. Previous experiments have estimated the likelihood that organismal endurance exists on Mars under a range of modern environmental factors. The nature of Mars is quite unlike that of the Earth in that it is high in carbon monoxide, low in oxygen, and dry.[3] The central aim of this research is to locate successful methods for testing the biomarkers or chemical fossils present under dreadful conditions of Martian atmosphere with the assumption that life possibly existed millions of years ago.

**Experimental:** A simulant soil from the Johnson Space Center(JSC) was used in all of the samples that were deliberated into glass test tubes. *E. coli* was added to half of the samples in a 0.5% bacteria to soil ratio while only a buffer was added to the others as a control. The control and experimental soil samples were separated into mars atmospheric and earth atmospheric samples.

The Mars atmosphere entailed the samples be exposed to a nitrogen environment or oxygen free by being placed in a dessicator which was tightly sealed and hooked to a t-valve that vacuumed oxygen out and pumped nitrogen in. The sole purpose was to transfer out water and oxygen thereby creating a "Mars atmosphere". Earth and Mars sample tubes were covered with foil and placed in a 150° C oven for the same amount of time to produce an accelerated aging process.

*Initial Extraction Procedure:* The samples of soil were removed from the oven and an extraction was performed after 1 _ weeks. Each of the sample tubes containing 10 g of soil were removed from the oven, cooled, and placed in a beaker with 50 ml of deionized water for 20 to 30 minutes. A vacuum pump, buckner funnel, side arm flask and filter paper system was constructed. After a filtration was performed, it was executed again on a Nalgene disposable filter which was able to capture the smaller solid particles that may have escaped through the first filtration process. The liquid sample that was accumulated from the filtration of the soil was then pulled into a plastic syringe and pushed through a solid phase extraction column attached to the end of the syringe. The molecules collected in the column were eluted with methanol and gathered in a 2ml glass tube to be used on high performance liquid chromatography (HPLC). Analysis of all samples was performed using the Bruker Esquire Ion Trap MS. The liquid sample was injected onto the HPLC column then introduced in series into a UV detector and then the mass spectrometer, for mass analysis using electrospray ionization. The solvent flow rate was 0.7/min. A water/methanol solvent gradient was used to perform HPLC separation of the components during the runs. The mass spectrometer was scanned from 50 - 2000 daltons. The methanol elute was run through a mass spectrometer for the detection of the electron ionization.

One sample of soil containing *E. coli* and another one containing just the buffer were analyzed on HPLC prior to that of the aged samples just to see how much earthly contaminants were present do to previous research.

*Second Extraction Procedure* Under another method of extraction, 10g soil samples were saturated in ethyl acetate solution for 30 minutes. The solid was separated by vacuum filtration using the buckner funnel setup and then a roto-vap was used to remove any water. This works by evaporating water from a sample in a round bottom flask while it is rotating over a heating water source. Following the removal of all of the liquid sample, HPLC grade methanol is suspended in the dry flask to confine any specimens in the and analyzed on the HPLC. These procedures were exercised as a continuation of a previous technique that was used and demonstrated to be fairly competent but not necessarily preeminent.

**Results:** Three samples containing larger amounts of soil (100g)were rinsed several times with deionized water and filtered with the buckner funnel setup. These samples which were kept in the oven at Earth's atmosphere, in the long run were induced with bacteria, extracted organically and run on the HPLC. Fortunately the multiple rinses proved to beneficial obtaining a cleaner soil sample thereby making it much easier to acquire what we were looking for as far as chemical detection. Pre-extracting the soil with deionized water several times can effectively clean out a lot the background noise that may be present in the simulant. Cleaner soil makes it a lot easier to

distinguish the differences in the control and experimental peaks present on the chromatograms which otherwise would exhibit a vast amount of undesirable compositons. The experimental chromatogram did show a small difference from that of the control. There were two peaks present in fig. 2 but it is imprecise as to what they are. It is possible that they could be a hydrocarbon that cannot be ionized on the HPLC/UV ion chromatogram. Other than that information, there is no explicit account for what these two peaks represent.

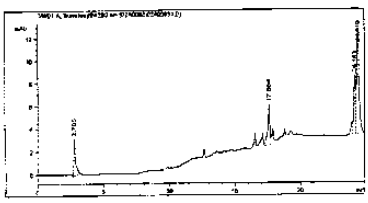

**Fig 1:** HPLC chromatogram of absorbance units vs. time for control sample.

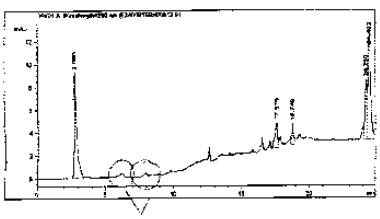

**Fig. 2:** HPLC Chromatogram of absorbance units vs. time for experimental sample.

**Discussion:** The soil used in this experiment was sufficient enough for the tests conducted in that it replicates the mineral composition of the soil that is found in Martian soil. *E. coli* was chosen as the bacteria of choice on the rationale that it is easily accessible even though it may not have lived on Mars in the past. This project faces the exact intricacies in that "Our task is difficult as we are searching for Martian biomarkers on the basis of what we know about life on Earth."[3] In efforts to age Martian samples, they were put into a 150° oven for at least 2 weeks. A representation known as the time-temperature index functions as a clear-cut prediction in determining aging rates. This heating of the *E. coli* along with the time span serve to age the samples to a small degree similar to what they would be in the atmospheric elements of millions of years.[1] The objective is to ascertain if a greater amount of degradation in the samples takes place as the age is amplified and to discover a pattern for this rate of degradation. Exposing the samples to nitrogen functions to keep the Martian samples free of oxygen and water. This was done by applying a vacuum pump to a dessicator by exposing the samples to considerable volumes of nitrogen and eliminating water. A solid phase extraction(SPE) column used in the first extraction procedure contained 18 carbon long molecules as the solid phase. The water extraction of the initial extraction was used to elute only polar particles. The organic removal of the second extraction was done to elute hydrophobic composites from the solution.[1] The purpose of the SPE column is to accumulate and purify the amount of hydrophobic compounds in the soil sample and eradicate all of the unwanted ones. Using methanol attracts the preferred carbon chain molecules that are caught in the column and pulled into a solution testable on HPLC. HPLC was chosen for the method of analysis because of its availability and universality. Analytical performance is based on the product from concentration proportions between samples.

This in conjunction with the mass spectrometer helps to separate the compounds and identify them by molecular weight even though the mass spectrometer is not crucial in analyzing the data. The focal point of the research is to compare variation in chromatograms amid the range of sample types and not determine the concrete structure of the samples. The conclusions for this project are based on the ratios of concentrations given by the UV chromatograms.

**Conclusions:** Samples were left to degrade in the oven for various periods of time. The decision to use the 2nd extraction came about with the notion that if more care was taken to elute definite compounds from the samples, then the chromatograms would produce a more pleasing outcome. With either extraction, the chromatograms of the control samples do not differ much from those of the experimental samples. Future steps to progress this project would be to get samples formulated with much better care toward this type of research in mind. The JSC simulant used in this project was much cleaner than the JPL simulant used in the preceding study. More efficient extraction levels could also serve to enhance the experiment. It would be beneficial to start looking for more definite target compounds that are characteristics of life. Once there is a better method to separate samples for analysis, additional steps need to taken to uncover the identities of the chromatographic peaks.

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**References:** [1] Hartsfield, Faith. "*Studies Into The Extraction Process For And Chemical Make-Up Of Biomakers In Martian Soil Simulant Compared To Earth Soil.*" Univ. of Arkansas . Dept of Biochemistrty . 2002 [2] Philip RP. *Fossil Fuel Biomarkers* 1985, Elsevier [3] Davis WL, McKay CP. Origins of Life: a comparison of theories and applications to Mars. *Orig Life Evol Biosph* 1996, 26, 61-73.[4] Harvey RP, McSween HY jr. A possible high temperature origin for the carbonates in the martian meteorite ALH84001. *Nature* 1996,382,49-51. [5] Buczko CM, Vas L. Effect of climate on chemical compostion of fossil bones. *Nature* 1977,269,792-793.