OPTIMIZING THE PYROLYSIS TEMPERATURE FOR TMSH THERMOCHEMOLYSIS FOR IMPROVED BIOSIGNATURE DETECTION IN ACIDIC MARS-ANALOG SAMPLES. L. Kivrak¹ and A.J. Williams¹ ¹Department of Geological Sciences, University of Florida, 241 Williamson Hall, PO Box 112120 Gainesville, FL 32611 (kivrak@ufl.edu).

Introduction: Gas chromatography-mass spectrometry (GC-MS) is a powerful technique that has been used in planetary missions to search for the presence of life via organic biosignatures [1]. GC-MS has been used in space in multiple missions, including the Viking landers, Phoenix lander, Curiosity rover, and the upcoming Rosalind Franklin rover [2]. The Sample Analysis at Mars (SAM) instrument on the Curiosity rover has revolutionized our understanding of organic matter on Mars [3, 4, 5, 6]. SAM detects and identifies different molecular species by separating the molecules in a chromatographic column into a time sequence and analyzing the unique mass spectra of each species [2]. Samples can either be directly heated to break apart the molecules in a pyrolysis oven, or subjected to a thermochemolysis procedure – a wet chemistry experiment that liberates the organics from larger macromolecules to improve organic matter yield [7]. Thermochemolysis is crucial for turning polar organic molecules into detectable volatile derivatives [8]. SAM contains two reagents for use in thermochemolysis: MTBSTFA (N,N-methylter-butyl-dimethylsilyl trifluoroacetamide) and TMAH (tetramethylammonium hydroxide). While it has not been used in a planetary mission, the additional thermochemolysis reagent TMSH (trimethylsulfonium hydroxide) has been assessed as a promising alternative [9, 10].

TMSH works via a similar mechanism to TMAH but can achieve methylation at lower temperatures [11]. Like TMAH, it performs well with aqueous samples, as water is a by-product of the reaction. However, TMSH and TMAH are not as effective in highly acidic samples due to their alkalinity [12]. Determining the optimal conditions for TMSH thermochemolysis with GC-MS is crucial in determining its utility for future space missions.

Previous work on nucleobase standards [10] indicates that the ideal pyrolysis temperature for TMSH thermochemolysis is lower than that for TMAH thermochemolysis. Fatty acids are another excellent biosignature due to their importance as a building block in cellular structures in all known life and preservation potential over long timescales [13]. TMAH and TMSH thermochemolysis results in the methylation of fatty acids to form fatty acid methyl esters (FAMEs), which are detectable by GC-MS. Here we show the results of different pyrolysis temperatures with TMAH and TMSH thermochemolysis for FAME detection in Mars-analog samples.

Methods: The samples used in this work include opal-A from a modern hydrothermal system in Iceland (IC9I), and a modern microbiially precipitated schwertmannite (sulfate-bearing hydrous ferric oxide) from Iron Mountain, CA (SS12). They were collected in an organically clean manner [14] for previous FAME detection studies, including work testing the effectiveness of TMAH as a thermochemolysis reagent [15]. Samples were homogenized in an ashed (500°C for 8 hours) mortar and pestle. A Frontier Multi-Shot (EGA/PY-303D) pyrolyzer and Agilent 7890B GC-5975C XL inert MSD GCMS were used for pyrolysis-GCMS analyses of analytes evolved from thermal pyrolysis and thermochemolysis. 3-5 µg of powdered sample was placed into solvent-washed sample cups with 1.5 µL of CI9 as an internal standard. TMAH or TMSH was added to the sample at a ratio of 1 µL reagent to 1 mg sample. Samples were pyrolyzed for 0.5 min at four different temperatures: 350°C, 400°C, 500°C, and 600°C. The oven program ramped from 50°C to 300°C at 20°C/min with a 10-minute hold. Molecules were identified using ChemStation software.

Results and Interpretations:

Iceland Sinter. TMAH thermochemolysis of opal-A resulted in the detection of the fatty acids C₈, C₉, C₁₀, C₁₂, C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₂₀, and C₂₁. All of these FAMEs were detected at all four temperatures except for C₁₅, which was detected in very small quantities at 600°C only, and C₂₀ and C₂₁ – which were not detected at 350°C. The 600°C pyrolysis temperature also produced the greatest abundance of short chain FAMEs, including C₇ – C₁₄. A pyrolysis temperature of 350°C resulted in the highest yields of FAMEs C₁₆ and C₁₈, but 600°C also resulted in a high abundance of C₁₈. Overall, a pyrolysis temperature of 600°C resulted in a relatively high yield across all detected FAMEs. (Figure 1).

TMSH thermochemolysis of the opal-A sample resulted in the detection of C₉, C₁₂, C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₂₀, and C₂₁. C₁₅ and C₂₁ were detected at all pyrolysis temperatures except 350°C, and C₂₁ was only detected at 500°C – otherwise, all FAMEs were detected at all four temperatures in varying quantities. A pyrolysis temperature of 500°C resulted in the greatest yield of nearly all of these fatty acids, with 350°C and 400°C also resulting in the detection of significant quantities of fatty acids. In contrast to TMAH
Thermochemolysis, a pyrolysis temperature of 600°C resulted in a significantly lower quantities of FAMEs detected. (Figure 2).

**Figure 2:** FAME detection in opal-A silica sinter via TMSH thermochemolysis at 350°, 400°, 500°, and 600°C

Schwertmannite. TMAH thermochemolysis of SS12 resulted in the detection of a large quantities of FAMEs at all temperatures, including C10, C12, C13, C14, C15, C16, C17, and C18, in addition to unsaturations of C15:1, C16:1, C17:1, and C18:1. Methyl-branched FAMEs were also detected in this sample. It was expected that TMAH thermochemolysis of this sample would perform much better compared to TMSH as TMSH thermochemolysis is known to perform poorly with acidic samples [12].

TMSH thermochemolysis of the Iron Mountain sample resulted in the detection of C8, C9, C12, C14, C16, and C18 at all four temperatures. While TMSH thermochemolysis is not ideal for use in acidic samples, the FAME yield was greatly increased at 500°C compared to 600°C. (Figure 3).

**Figure 3:** FAME detection in schwertmannite Mars-analog sample via TMAH thermochemolysis at four pyrolysis temperatures.

**Conclusion:** In two Mars-analog samples with very different mineralogies, the ideal pyrolysis temperature of TMSH was lower than that of TMAH. While TMSH is not an ideal thermochemolysis reagent for detecting FAMEs in acidic samples, the FAME yield with TMSH thermochemolysis can be improved by using a pyrolysis temperature lower than 600°C. Parallel work involves analysis of more analog samples under different pyrolysis temperatures as well as on fatty acid standards.

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**References:**