TRIMETHYLSULFONIUM HYDROXIDE (TMSH) THERMOCHEMOLYSIS WITH PY-GC-MS AS A METHOD OF ORGANIC BIOSIGNATURE DETECTION: OPTIMIZATION FOR NUCLEOBASE DETECTION

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Introduction: The on-going search for life beyond of Earth is a high priority for NASA and the fields of astrobiology and planetary science [1]. The detection of organic biosignatures will be a powerful method for this going forwards, as characteristic patterns of molecules such as nucleotides, amino acids, fatty acids, and saccharides can provide evidence for the presence of life [2]. Additionally, nucleobases are biologically essential and are indicative of extant or recently extinct life due to their short preservation timescales in the rock record [3]. Improving techniques for detecting nucleobases and other organic biomarkers is therefore an important avenue towards the ultimate goal of life detection.

A technique that has been used in space missions to detect organic molecules is gas-chromatography – mass spectrometry (GC-MS). GC-MS was first used on Mars by the Viking landers, and has since flown on the Phoenix lander and Curiosity rover, and will be present on the upcoming Rosalind Franklin rover. The Sample Analysis at Mars instrument (SAM) on the Curiosity rover was responsible for the first detection of organic molecules on Mars [4]. SAM detects and identifies different molecular species by separating the molecules in a column into a time sequence and analyzing the unique mass spectra of each species [5]. On SAM, samples are pyrolyzed to break apart molecules prior to separation via GC. SAM can also perform wet chemistry experiments. Thermochemolysis can liberate organics from larger macromolecules to improve organic matter yield [6] and is crucial for turning polar organic molecules into detectable volatile derivatives [7]. SAM contains two wet chemistry reagents: MTBSTFA (N,N-methyltert-butyl-dimethylsilyltrifluoroacetamide) and TMAH (tetramethylammonium hydroxide). However, MTBSTFA is not amenable to working with aqueous samples, and TMAH can degrade GC columns at a rapid rate, producing molecules that overwhelm the detector and make product identification difficult [8]. The reagent trimethylsulfonium hydroxide (TMSH) works via a similar mechanism to TMAH but can achieve methylation at lower temperatures [9]. Like TMAH, it performs well with aqueous samples, as water is a by-product of the reaction. TMSH has been studied as a thermochemolysis reagent for fatty acid analysis in modern algal environments and cultures [9] as well as in Mars analog environments [10]. Determining the optimal conditions for the use of TMSH thermochemolysis with GC-MS, such as optimal pyrolysis temperatures, will be important for its future use on analog samples and ultimately in potential space-flight missions. Here, we examine the results of TMSH thermochemolysis of seven nucleobases (separate and as a mixture) under a range of pyrolysis temperatures.

Methods: Solutions of adenine, thymine, cytosine, guanine, uracil, xanthine, and hypoxanthine were prepared at concentrations of 0.25 mol/L in TMSH. A mixture of these seven nucleobases was also prepared at 0.25 mol/L. 1 µL of the sample was pipetted into solvent washed sample cups along with 3 µL of TMSH and 1.5 µL of naphthalene-d8 as an internal standard. Samples were analyzed in triplicate and pyrolyzed at 400°, 500°, and 600°C for thirty seconds each. The oven program began at 50°C with a 5 minute hold and was then ramped to 240°C at a 6°C/min, and then ramped to 300° at 10°C/min, following the methods of [3]. A Frontier Multi-Shot (EGA/PY-303D) pyrolyzer and Agilent 7890B GC-5975C XL inert MSD GCMS were used for direct pyrolysis-GC-MS analyses of analytes evolved from thermal pyrolysis and thermochemolysis. Molecules were identified using ChemStation software. The main peaks of each nucleobase were chosen as targets for the selected ion chromatographs (z = 177, 154, 140, 153, 194, 194) [3].

Results and Interpretations: The results from this study were compared qualitatively with those from [3] which performed similar analyses on the optimal pyrolysis temperature of TMAH thermochemolysis of nucleobases.

Adenine: Trimethyl and dimethyl adenine were present in detectable quantities at all three temperatures. Single methylated adenine was absent in the TMSH analyses in comparison to the TMAH analyses in [3].

Cytosine: The TMSH analyses produced 1-amino-2-methoxy-pyrimidine, dimethyl cytosine, trimethyl cytosine, and 2-methoxy-5-methyl-4-pyrimidinamine. These derivatives were the same as those detected in the TMAH analyses [3], but the TMSH results showed improved yield at 400° and 500°C.

Thymine: TMAH and TMSH thermochemolysis of thymine results in a single methylated derivative: dimethyl thymine. The TMSH analyses showed marked improvement at lower temperatures.

Guanine: Caffeine (trimethyl xanthine), dimethyl guanine, trimethyl guanine, and tetramethyl guanine, were detected in the TMSH analysis, although caffeine was only detectable at a pyrolysis temperature of 500ºC. In comparison to the TMAH analyses of [3], the TMSH analyses produced higher quality spectra with clearer peaks, especially at higher retention times. With the exception of caffeine, yield was consistent across all temperatures.

Uracil: Dimethyl uracil was identifiable at all three temperatures. Yield increased with temperature.

Xanthine: Caffeine was the sole methylated derivative of xanthine and was detected at all three temperatures in comparable amounts *(Figure 1).*

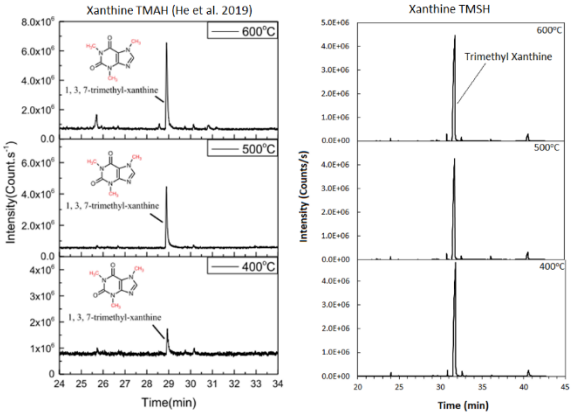

Figure 1: Comparison of TMAH and TMSH analyses of xanthine.

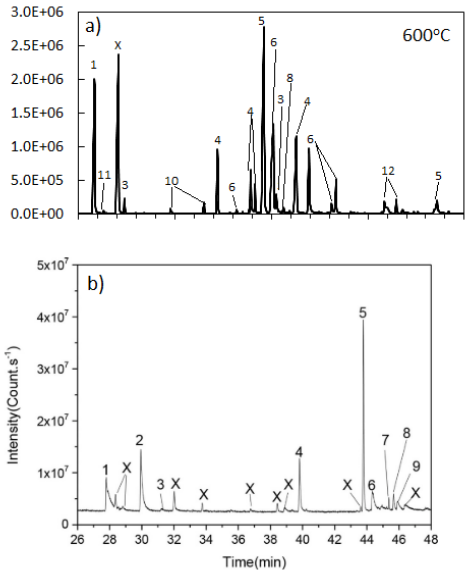

Figure 2: Selected ion chromatograms of a mixture of seven nucleobases reacted with a) TMSH and b) TMAH, adapted from [3]. Peak 1: dimethyl uracil; Peak 2: dimethyl-thymine, Peak 3: trimethyl cytosine, Peak 4: trimethyl adenine, Peak 5: trimethyl xanthine (caffeine), Peak 6: dimethyl hypoxanthine, Peak 7: methyl ester hexadecenoic acid, Peak 8: trimethyl guanine, Peak 9: trimethyl adenine, Peak 10: dimethyl adenine, Peak 11: dimethyl cytosine, Peak 12: guanine derivatives (trimethyl and tetramethyl guanine). X indicates molecules not associated with nucleobase derivatives.

Hypoxanthine: Dimethyl hypoxanthine was detected at all temperatures, but with increased yield at increased temperatures. In contrast with TMAH analyses from [3], dimethyl hypoxanthine was detected at 400ºC, and at a greater number of retention times.

Mixture: 600ºC pyrolysis with TMSH thermochemolysis produced more identifiable derivatives than with TMAH, including dimethyl cytosine, dimethyl adenine and guanine derivatives. Methylated thymine was absent in the TMSH analyses *(Figure 2).*

Overall, the results show that TMSH performs well as a thermochemolysis reagent on nucleobases at a wide range of pyrolysis temperatures. While TMSH thermochemolysis yield did improve with increased temperature, especially on adenine, uracil and hypoxanthine, significant quantities of identifiable nucleobases were present at all temperatures. At lower temperatures, TMSH was a distinct improvement over TMAH.

Conclusions: TMSH is an exciting and understudied potential thermochemolysis reagent that may be useful in the planning of future space-flight missions. TMSH allowed for nucleobase detection at a wide range of pyrolysis temperatures and was able to consistently methylate nucleobases at temperatures lower than TMAH. The ability to perform thermochemolysis at lower temperatures could extend the lifetime of the GC column and greatly assist with the detection and identification of organic biosignatures in space-flight missions. Future work will involve optimizing the pyrolysis temperature for TMSH thermochemolysis on other classes of organic molecules, and on planetary analog samples.

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