LIPID DETECTION IN FE(III)-DOMINATED SAMPLES TO PREPARE FOR THE TETRAMETHYLAMMONIUM HYDROXYDE (TMAH) WET CHEMISTRY EXPERIMENT ON THE SAM

INSTRUMENT SUITE. A.J. Williams¹, J.Eigenbrode², M. Floyd², A. McAdam², C. Freissinet², D. Glavin², P. Mahaffy², and the SAM Science Team, ¹University of Maryland Baltimore County/ CRESST/ NASA Goddard Space Flight Center, Planetary Environments Laboratory, Code 699, Greenbelt, MD 20771 (amy.j.williams@nasa.gov), ²NASA Goddard Space Flight Center, Planetary Environments Laboratory, Code 699, Greenbelt, MD 20771

Introduction: The first two years of the Mars Science Laboratory (MSL) mission in Gale Crater has revolutionized our view of Mars. Underneath the oxidized surface is a rich record of past conditions hosted by sediments of variable redox state. The rocks of Yellowknife Bay revealed evidence of an ancient habitable lake, having sustained organic molecules [1], water, and a source of chemical energy for microbial life [2]. Now at the base of Mt. Sharp in Gale Crater, MSL will explore stratigraphic packages of rocks for further evidence of habitability and search more for organic compounds using the Sample Analysis at Mars (SAM) instrument suite. SAM includes a gas chromatograph mass spectrometer (GCMS) and the ability to perform evolved gas analysis mass spectrometry, all connected by a gas processing system that includes sample pyrolysis ovens. SAM also has the capability of performing wet chemistry experiments, either by N-methyl-Ntertbutyldimethylsilyltrifluoracetamide (MTBSTFA) derivatization or tetramethylammonium hydroxide (TMAH) thermally assisted hydrolysis/methylation (THM). Coupled with wet chemistry experiments, the GCMS is capable of detecting large carboxylic acids and other hydrocarbons (e.g. lipid biosignatures). If present, these molecules may be bound into large macromolecules (e.g. biomolecules or kerogen) as they are often on Earth. On Mars, SAM will use the TMAH experiment onboard MSL to hydrolyze molecules, releasing them from their host macromolecules, and then methylate those molecules so they are sufficiently volatile for detection by SAM's GCMS [3]. Two of the nine wet chemistry cups on SAM contain the TMAH reagent; the other seven contain MTBSTFA [3]. Each TMAH cup contains an outer reservoir filled with ~ 500 µl of a 25% solution of TMAH in methanol (1:3v) with 25 nmol pyrene and 34 nmol 1-fluoronaphthalene in solution. Inside is a second reservoir filled with ~12 nmol nonanoic acid that serves as the internal calibration standard. These TMAH cups have been reserved for use at Mt. Sharp where MSL now explores. This experiment is especially useful for hydrated samples where MTBSTFA derivatization can be compromised.

This research optimizes the TMAH experiment for the SAM instrument. This procedure enhances detection of trace amounts of lipids in natural terrestrial Fe(III)-dominated rock samples—analogs of the martian rocks known to host iron-oxides, like those in the lower mound of Mt. Sharp. Organic carbon is thermo-

dynamically unstable in the presence of Fe(III) [4], however, organic carbon may be preserved depending on how organic molecules are hosted in mineralogically diverse sediments [5,6]. This research explores the preservation of organics in a select suite of iron dominated rocks (PS5G, PS5P, PS17 from the Iron Mountain, CA, massive sulfide deposit [7]) and iron metabolizing microbial communities (ES1, ES2 are cultured Fe-oxidizing organisms [8]; IFS6, IFS7 are environmental samples from an iron seep) to determine the optimal procedure to detect organics in those samples using the TMAH experiment on SAM-like instruments. Applications involving TMAH to meteorites, soil humic substances, kerogens, and other natural samples have been used for decades [9,10]. However, TMAH can cause degradation of acids due to its high alkalinity [11,12]. A reagent similar to TMAH, TMSH (trimethylsulfonium hydroxide), can serve as a more efficient THM agent than TMAH in the methylation of carboxylic acids at lower temperatures [11]. TMSH is not present in SAM but will help the optimization of the TMAH procedure development. The direct THM of biologic samples with TMSH has been previously explored [11], although never applied in Fe(III)dominated samples. THM with TMSH was also utilized in this study to assess how the optimized TMAH experiment compares to other THM methods for hvdrocarbon detection.

Optimization of the TMAH experiment on SAM for these samples will inform the sample selection for this experiment, analysis protocols, and data interpretation, ultimately improving the chances of successful detection of macromolecularly bound hydrocarbons, including lipid biosignatures, if they are present.

Methods: Biologic samples were collected in organically clean glass vials either from culture or the environment. Aliquots were sampled with a solvent-washed syringe. Rocks were sampled under organically clean conditions. Rock samples were broken open on organically clean ultra-high vacuum (UHV) foil with a rock hammer wrapped in UHV foil to access the uncompromised rock interior. The interior of the rock was sampled with a solvent-cleaned drill bit into ashed glass vials. Rock samples were ground to a fine powder in an ashed mortar and pestle and parsed into aliquots with a solvent-washed scoop in a hood.

Lipid Hydrolysis/Methylation: Both biologic and rock samples underwent direct THM or pyrolysis at

either a 1:9 ratio with TMSH or a 1:1 ratio with TMAH. All direct THM samples were left to diffuse into the sample pore spaces for at least 6 hours. Modifications to the TMH technique to improve lipid yield included determining the optimal ratio of rock sample to TMAH reagent, time sample is left to react with the TMAH, and the benefits and disadvantages of lyophilizing the samples prior to TMAH reaction [5].

GCMS of lipid-containing samples: The hydrocarbon fractions were analyzed by GCMS on Agilent GCMS instruments coupled to a Frontier pyrolyzer or a Gerstel thermal desorption unit for direct THM.

Results & Interpretations: Some fatty acid methyl esters (FAMEs) and steranes were identified from a selection of iron-rich biologic and rock samples with varying degrees of success using both TMSH and TMAH protocols with both direct THM and pyrolysis (Table 1). Fatty acids identified included $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, and cis- $C_{18:1\omega 9}$. All of these fatty acids are widely found in bacteria and eukaryotes [13], and the $\omega 9$ unsaturation in $C_{18:1\omega 9}$ is more specific to gram positive bacteria [13] and fungi [14]. Steroids, characteristic of eukaryotes, were identified by the presence of m/z = 217, 218 and 231 [15]. FAMEs in ES1 and ES2 were identified in [8] and those results agree with our study's findings. The preliminary results indicate that the direct THM TMSH protocol produced the greatest number of positive results for lipid identification. Direct THM with TMAH thus far has yielded inconsistent results, with individual experiment results ranging from no lipid peaks to the presence of several FAMEs (Fig. 1). Pyrolysis with

ES1 TMSH + + + + + + + + + + [8]	Table 1. Diagnostic lipid biomarkers in Fe(III)-dominated biologic and rock samples. + = identified = not identified.											
ES1 TMAH				n-C10:0	n-C12:0	n-C14:0	n-C15:0	n-C16:0	<i>n</i> -C16:1	n-C17:0	n-C18:0	cis -C18:1 ω 9
S S S S S S S S S S	Biologic Samples	ES1	TMSH	+	+	+	+	+	+	+	+	+
IFS7			TMAH	-	<u>-</u>	<u>-</u>	<u>-</u>	+	_		+	-
IFS7			[8]	-	+	+	-	+	+	-	+	+
IFS7		ES2	TMSH	+	-	+	-	+	-		+	+
IFS7			TMAH	-	-	-	-	+	-	-	-	-
IFS7			[8]	-	+	+	-	+	+	-	+	+
IFS7		IFS6	TMSH	+	+	+	-	+	-	-	+	+
PS5G TMAH + + + + + +			TMAH	+	-	+	+	+	-	-	+	+
PS5G TMAH + + + + + + + + + + + + + +		IFS7	TMSH	+	-	-	-	+		-	+	+
PS5G TMAH + - + - + - PYRO TMAH + - + - + - + - + - + - + - +			TMAH	-	-	-	-	+	-	-	-	-
	Iron-Oxide Rocks	PS5G -	TMSH	+	+	+	-	+	-	-	+	+
			TMAH	-	-	-	-	+	-	-	+	-
7 /			PYRO	-							,	
			TMAH		-	-	-	+	-	-	+	-
7 /		PS5P	TMSH	+	+	+	-	+	-	-	+	+
7 /			TMAH	-	-	-	-	+	-	-	-	-
7 /		PS17 -	TMSH	+	+	+	-	+	-	-	+	+
TMAH			TMAH	-	-	-	-	-	-	-	-	-

TMAH produced lipid peaks equivalent to direct THM with TMAH, and fewer peaks than direct THM with TMSH. The disparity in direct THM results may be due to the temperature of the oven during THM, such that the two methods may require different values. The TMAH method must be further refined to improve the lipid yield for both terrestrial samples and martian sample analysis with SAM.

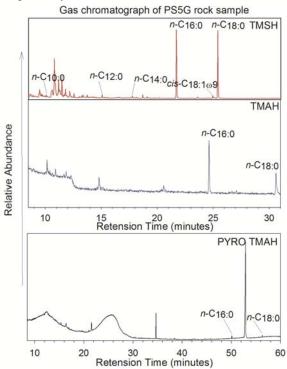


Figure 1. Gas chromatograph of PS5G rock sample with direct THM TMSH, THM TMAH, and pyrolysis TMAH methods.

Conclusions: An optimized TMAH method will elucidate the potential for hydrocarbon release from Fe(III)-dominated rocks, which may typify the mineralogies encountered in the lower mound of Mt. Sharp. This methodology is also be applicable to a diverse suite of lithologies, as lipids are difficult to extract from Fe(III)-bearing rocks, but may be easier to extract from other rock types. Future TMAH optimization experiments will include MTBSTFA to understand the any effects of MTBSTFA chemistry on thermochemolysis and the interpretation of the data. This research enhances the ability of SAM to characterize organic compounds in representative iron-oxides and identify potential chemical biosignatures in Gale Crater.

References: [1] Freissinet, C., et al (2014) 8th Intl conf Mars 1349. [2] Grotzinger, J.P., et al (2013) Sci Express 343, 1242771-1242714. [3] Mahaffy P.R. et al (2012) Space Sci Rev, 401-478. [4] Sumner D.Y. (2004) JGR 109, E12007. [5] Parenteau M.N. et al (2014) Astrobiology 14, 502-521. [6] Lalonde K. et al (2012) Nature 483, 198-200. [7] Williams, A.J. et al (2015) Astrobiology in review. [8] Emerson D. (2013) Frontiers Microbiol 4, 1-17. [9] Nierop K.G. and Filley T.R. (2007) Org Geochem 38, 551-565. [10] Remusat L. et al (2005) GCA 69, 3919-3932. [11] Akoto L. et al (2005) J Anal App Pyrol 73, 69-75. [12] Blokker P. et al (2002) J Chromatogr A 959, 191-201. [13] Zelles L. (1999) Biol. Fert. Soils 29, 111-129. [14] Vestal J.R. and White D.C. (1989) BioScience 39, 535-541. [15] Ruo T.C.S. et al (1977) Symp Oil Sand Oil Shale, 26-47.