

FATTY ACID PRESERVATION IN MARS-ANALOGOUS ROCK SAMPLES AND DETECTION WITH THE TMAH WET CHEMISTRY EXPERIMENT ON THE SAMPLE ANALYSIS AT MARS (SAM) INSTRUMENT. A.J. Williams^{1,2}, J.L. Eigenbrode², S.S. Johnson³, K.L. Craft⁴, M.B. Wilhelm⁵, S.S. O'Reilly⁶, J.M.T. Lewis², R. Williams², A. McAdam², C.A. Knudson², M. Millan^{2,3}, A. Buch⁷, C. Freissinet⁷, D. Glavin², R.E. Summons⁶, K.C. Benison⁸, R. Navarro-González⁹, P. Mahaffy², ¹Dept. Physics, Astronomy, & Geosciences, 8000 York Rd, Towson University (ajwilliams@towson.edu), ²NASA Goddard Space Flight Center, ³Georgetown University, ⁴Johns Hopkins University Applied Physics Laboratory, ⁵NASA Ames Research Center, ⁶Massachusetts Institute of Technology, ⁷CNRS - LATMOS, ⁸West Virginia University, ⁹Universidad Nacional Autónoma de México

	C ₈	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C _{16:1}	C ₁₇	C ₁₈	C _{18:1}	C _{18:2}	C ₂₀	%TOC
Iron Oxides	Modern acidic	--	x	--	x	--	--	x	x	--	--	--	--	--	0.97%
	Older acidic	x	x	--	x	--	--	x	x	--	x	x	--	--	0.05%
	Sub-Eocene acidic	x	x	--	x	--	--	--	--	--	--	--	--	--	0.81%
	Eocene neutral	x	x	x	x	x	x	x	x	--	--	x	--	--	0.07%
		x	x	x	x	x	x	x	x	--	x	x	--	--	x
Siliceous Sinter	Active Vent	x	x	--	x	x	x	x	x	x	x	x	x	x	0.24%
	Inactive Vent	x	x	--	x	--	--	--	--	--	x	--	--	--	0.02%
	Relict Vent	x	x	--	x	--	x	x	x	x	x	x	x	x	nd
Carbonate		x	--	--	x	--	x	--	--	--	--	--	--	--	2.10%
		x	x	--	x	x	x	x	x	x	x	x	--	--	x
Shale		x	x	x	x	x	x	x	--	x	x	--	--	--	x
		x	x	x	x	x	x	x	x	x	x	x	x	x	32.57%

Table 1. FAME detection in Mars-analogous samples with variable mineralogies. 'X' = FAME detected, '--' = FAME absent. 'SAM-like 35°C/min pyro ramp analyses' = dark gray cells. '500°C flash pyrolysis analyses' = light gray cells. TOC = total organic carbon determined via loss-on-ignition ± 0.06%.

Introduction: The Mars Curiosity rover is ascending Mt. Sharp, in Gale Crater, exploring stratigraphic packages of rocks for evidence of habitability [1] and searching for organic compounds using the Sample Analysis at Mars (SAM) instrument suite. SAM includes a gas chromatograph mass spectrometer (GCMS) and sample pyrolysis ovens. SAM also has the capability of performing wet chemistry experiments, either by N-methyl-N-tertbutyldimethylsilyltrifluoroacetamide (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 [2]. Two of the nine wet chemistry cups on SAM contain the TMAH reagent; the other seven contain MTBSTFA [2]. 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. This project determines the effectiveness of the SAM-like TMAH experiment to liberate organics from a variety of Mars-analogous rock samples.

Background: Rock samples were collected from a variety of Mars analogue environments. Samples include *iron oxides* from a modern mineral precipitate at Iron Mountain, CA, the older surface gossan (oxidized massive sulfide deposit) at Iron Mountain, and Sub-Eocene age acid salt lake sediments and Eocene age circumneutral lake sediments from Western Australia; *siliceous sinter* from Icelandic hot spring vents at an active deposit, recently inactive deposit, and relict deposit; *modern carbonate ooids* from Pigeon Cay, The Bahamas, and *organic-rich shale* from Messel, Germany.

Methods: Samples collected using organically-clean techniques underwent pyrolysis at a 1mg:1µL ratio with TMAH. The fatty acids were analyzed by pyro-GCMS using either a SAM-like pyrolysis oven ramp (35 °C/min) or an "optimized" flash pyrolysis at 500 °C on a Frontier pyrolyzer coupled to an Agilent GCMS instrument.

Results: The SAM-like and optimized methods are compared below.

SAM-like Pyrolysis. Using the SAM-like pyrolysis oven ramp, FAMES were detected in all samples but a shallow sub-Eocene acidic iron oxide sample (data not shown). Low molecular weight (LMW) (C₈-C₁₀) FAMES were present in all other samples, medium MW (MMW) (C₁₁-C_{18.2}) FAMES were present in select samples (Table 1), and high MW (HMW) (C₂₀-C₃₀) FAMES were detected in the shale sample only (not all data shown).

Optimized Flash Pyrolysis. Using the optimized flash pyrolysis method, FAME detection was improved in all samples over the SAM-like method. Low and medium molecular weight FAMES were present in all samples, and high molecular weight FAMES were detected in the circumneutral Western Australia lake sediment, active and relict Icelandic hot springs, modern carbonate ooids, and the shale sample (not all data shown).

Discussion: In general, the flash pyrolysis method yielded more FAMES than the SAM-like oven ramp method for all samples. Interpretations of note are discussed below:

Iron Oxides. The number of FAMES detected in the modern acidic iron oxide sample was lower than expected for a modern microbial community, which may indicate pyrolytic interactions with the iron oxides that decreased FAME yield. The low FAME detection in the older acidic Iron Mountain iron oxide may be due to a decreased microbial population within the rocks, or a reduction in FAME preservation due to the presence of iron oxides [3]. Within the Western Australia lake deposits, which also host phyllosilicates, the acidic sample yielded fewer FAMES than the circumneutral sample with both pyrolysis methods. The circumneutral sample had much better preservation of FAMES over the acidic sample, suggesting that lipid preservation is improved in circumneutral iron oxides + clay environments than in acidic iron oxide + clay environments. In addition, HMW FAMES (C₂₀-C₂₇) were also detected in the circumneutral lake sediments, indicating a terrigenous plant or algal origin [4, 5].

Siliceous Sinter. The active hot spring contained 12 to 15 low, medium, and high MW FAMES depending on pyrolysis method. A subsurface sample collected 4 cm below the surface (data not shown) contained 9 to 10 low, medium, and high MW FAMES depending on pyrolysis method. Mono- and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) were sometimes detected in the subsurface, indicating improved preservation and/or lack of early degradation of the FAMES.

The inactive hot spring contained 5 to 11 low, medium, and high MW FAMES depending on pyrolysis method. A subsurface sample collected 7 cm below the surface (data not shown) contained 2 to 9 low, medium, and high MW FAMES depending on pyrolysis method. MUFAs and PUFAs were not detected in the subsurface, indicating rapid degradation of the unsaturated FAMES, either via microbial heterotrophy or diagenesis [6].

The relict hot spring contained 11 to 19 low, medium, and high MW FAMES depending on pyrolysis method. A subsurface sample collected 6 cm below the surface (data not shown) contained 9 low, medium, and high MW FAMES with the SAM-like pyrolysis method. MUFAs and PUFAs were not detected in the subsurface, indicating again rapid degradation of the unsaturated FAMES [6]. The high number of FAMES detected in the relict surface sample relative to the active and inactive spring samples, and comparable numbers of FAMES detected in the surface and subsurface relict samples, suggesting that microbial (re)colonization has overprinted the original FAME profile. More FAMES were detected in the active vs inactive sinter, indicating a modern microbial community with degraded preservation over time in inactive sinter.

Carbonate. The modern ooids contained 4 to 15 low, medium, and high MW FAMES depending on pyrolysis method. The SAM-like detection represented a very small fraction of the lipids present in these ooids [7].

Shale. The Messel shale contained 22 to 35 low, medium, and high MW FAMES depending on pyrolysis method, including 11 HMW FAMES. HMW FAMES are most consistent with plant and algal origins [7], and the lipids in the Messel Shale have a demonstrated algal origin and strong lipid signal [8].

Conclusions: These experiments demonstrate that TMAH thermochemolysis with a SAM-like pyro-GCMS is effective, and FAMES have been detected in Mars-analog samples that vary in mineralogy, age, and microbial community input. HMW FAMES are not detected in iron-dominated samples, and may not be detectable at low concentrations, but fatty acids are detectable and possibly preferentially preserved in iron oxide + clay mixed mineralogies. Similar mineralogies are anticipated to be encountered in the “phyllosilicate trough” south of the Vera Rubin (Hematite) Ridge on Mt. Sharp. Laboratory experiments using the other wet chemistry reagent available on SAM, MTBSTFA, are ongoing on the same set of martian analogs. This method will allow us to compare the number of fatty acids detectable with MTBSTFA and TMAH to better understand the efficiency of both reagents. Future high fidelity experiments will include MTBSTFA to understand any effects of MTBSTFA chemistry on thermochemolysis and the interpretation of the data. This research enhances the ability of SAM to characterize organic compounds and identify potential organic biosignatures in Gale Crater.

References: [1] Grotzinger, J.P., et al. (2013) *Sci Express* 343, 1242771-1242714. [2] Mahaffy, P.R., et al. (2012) *Space Sci Rev*, 401-478. [3] Sumner, D.Y., (2004) *JGR* 109, E12007. [4] Volkman, J., et al. (1989) *J. Exp. Mar. Bio. Ecol.*, 128. [5] Wilhelm, M., et al. (2017) *Org. Geochem.* 103, 97-104. [6] Sun, M.-Y., et al. (1997) *Geochim. Cosmochim. Acta* 61, 341-355. [7] O'Reilly, S., et al. (2017) *Geobiology*, 15, 112-130. [8] Goth, K., et al. (1988) *Nature*, 336, 759-761.