

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

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**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-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 [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  $\mu$ l 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 hydrocarbon 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

