ANALYSIS OF CARBONACEOUS BIOMARKERS WITH THE MARS ORGANIC ANALYZER MICROCHIP CAPILLARY ELECTROPHORESIS SYSTEM: ALDEHYDES, KETONES, AND CARBOXYLIC ACIDS. Amanda M. Stockton, Caroline Chandra Tjin, Grace L. Huang, Merwan Benhabib, Thomas N. Chiesl, and Richard A. Mathies, Department of Chemistry, University of California, Berkeley, CA 94720, ramathies@berkeley.edu.

Introduction: The Mars Organic Analyzer (MOA) has enabled the sensitive detection of a range of organic molecules indicative of extinct and/or extant life, including amino acids,[1-2] amino acid chirality,[3] bioamines,[4] and PAHs[5] in laboratory standards and samples of environmental and astrobiological interest. The MOA has been field tested in the Panoche Valley, CA,[2] and the Acatama Desert, Chile.[6] However, in the search for organic biosignatures in extraterrestrial targets, it is important to have analytical capability for a broad base of target analyte classes. For *in situ* analysis on Mars, in particular, it is important to analyze for oxidized organic molecules, based on interpretation of Viking's data as being indicative of a strong oxidant in the regolith[7] and Phoenix's measurement of the oxidant perchlorate.[8]

Here, we use the fluorescent probe Cascade Blue hydrazide to expand the MOA capability to achieve highly sensitive analysis of the critical oxidized classes of organic molecules: aldehydes, ketones, and carboxylic acids.

Results: We determined the optimal pH (pH 5-6) for the hydrazone-formation labeling reaction between Cascade Blue hydrazide and aldehydes and ketones. The optimum analysis pH was determined to occur at pH 9.5. A carbonyl standard was developed based on the C_1-C_4 aldehydes and C_3-C_5 ketones detected in the Murchison meteorite[9] and is shown in Figure 1. Limits of detection were determined for representative molecules: formaldehyde 70 ± 30 pM, acetaldehyde 700 ± 200 pM, acetone 5 ± 2 nM, and benzophenone 1.5 ± 0.6 μM. This analysis was verified for complex samples via the analysis of several fermented beverages (Figure 2).

We also determined the optimal pH (pH 3) for the 1-ethyl-3[3-dimethylaminopropyl]carbodiimide (EDC) activated reaction of carboxylic acids with Cascade Blue hydrazide to form an amide-like linkage. A carboxylic acid standard was developed using aliphatic straight-chain acids C_1 to C_8 (Figure 3B). Additionally, mellitic acid was labeled, resulting in the expected charge ladder from multiple labeling (Figure 3A).

Conclusions: The successful analysis of aldehydes, ketones, and carboxylic acids expands the MOA's capabilities in organic biomarker detection to these important classes of oxidized organic molecules. Limits of detection are determined for aldehydes and ketones to the pM range (high pptr to ppb when used with subcritical water extraction[10]). Due to the water solubility and stability of both the fluorescent probe and the target analytes, this analysis requires no organic solvents. This analytical method and instrumentation therefore has direct applicability to the development of *in situ* instrumentation for extraterrestrial analysis and provides highly sensitive analysis of the oxidized organic targets expected on Mars.

Additional Information: For more information on the MOA, see http://astrobiology.berkeley.edu.

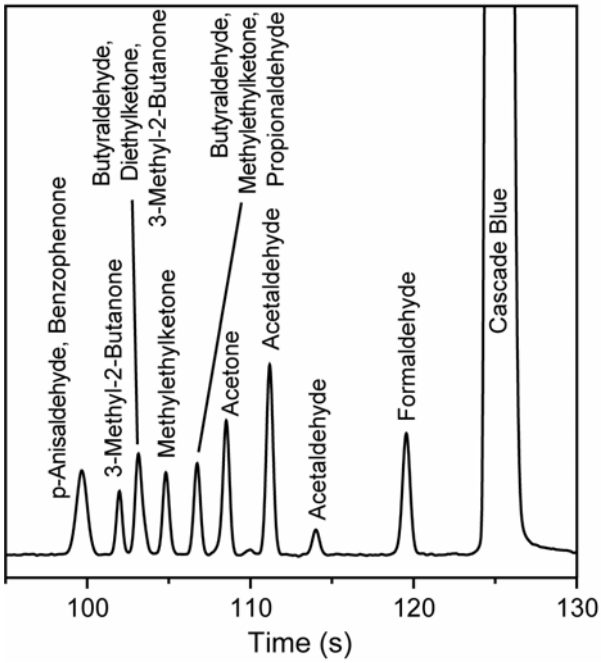

Figure 1. The optimized MOA CE separation of the carbonyl standard. Separation was conducted using 30 mM borate buffer, pH 9.5. Concentrations are 1.6 μM formaldehyde, 3.2 μM acetaldehyde, propionaldehyde, and butyraldehyde, 16 μM acetone, p-anisaldehyde, and benzophenone, and 32 μM methylethylketone, diethylketone, and 3-methyl-2-butanone. Aldehydes and ketones with asymmetry about the carbonyl group are resolved into two peaks, due to slow isomerization between the cis-like and trans-like isomers at the hydrazone linkage.

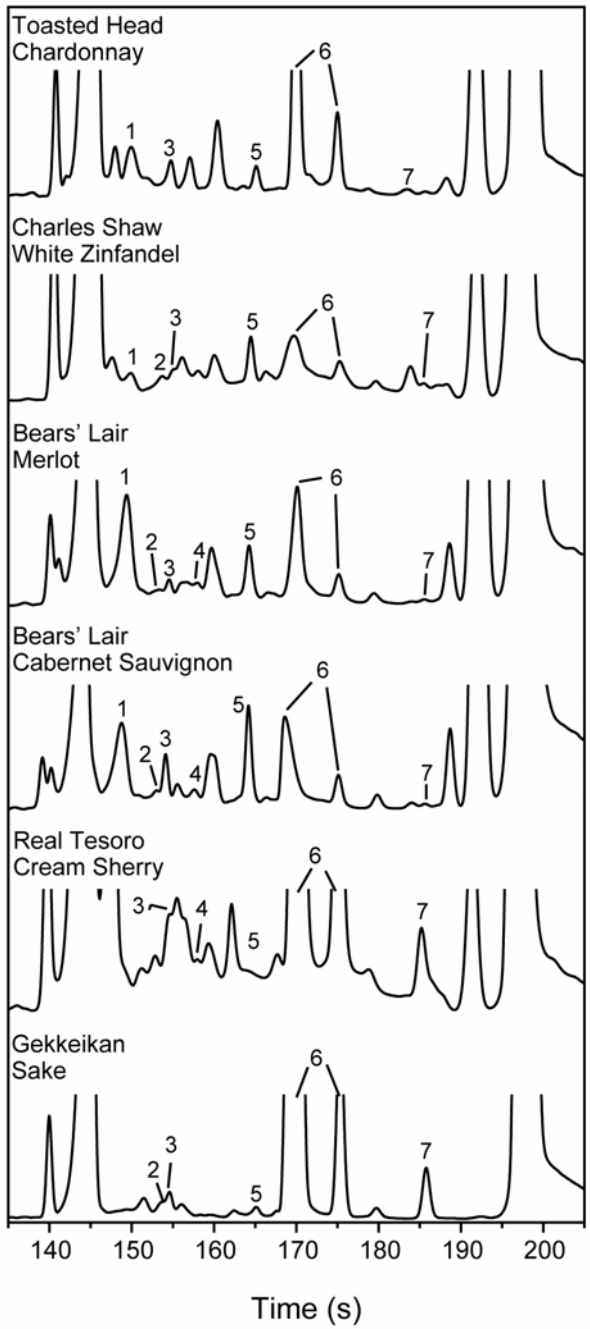

Figure 2. Analysis of Cascade Blue labeled fermented beverages. The indicated species are benzophenone and p-anisaldehyde (1), 3-methyl-2-butanone (2), acetoin (3), methylethylketone (4), diacetyl (5), acetaldehyde (6), and formaldehyde (7). All electropherograms are of samples of equal beverage volumes and are displayed offset on the same scale. Samples are labeled at pH 5, then diluted to pH 9.5 for analysis on the MOA.

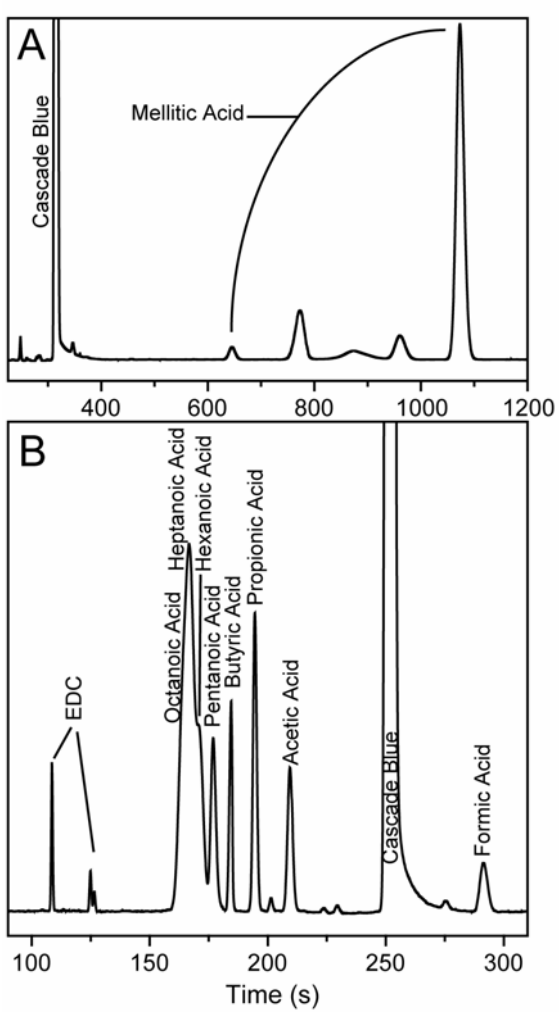

Figure 3. The MOA CE separation of (A) mellitic acid and (B) the carboxylic acid standard. Separation was conducted using 30 mM borate buffer, pH 9.5. Concentrations are 11 μM formic and heptanoic acids, 5 μM acetic, propionic, butyric, pentanoic, and hexanoic acids, 16 μM octanoic acid, and 800 μM mellitic acid.

References:

(1) Chiesl, T. N., *et al. Anal. Chem.* **2009**, *81*, 2537.
(2) Skelley, A. M., *et al. Proc. Natl. Acad. of Sci. U. S. A.* **2005**, *102*, 1041.
(3) Skelley, A. M.; Mathies, R. A. *J. Chroma. A* **2003**, *1021*, 191.
(4) Skelley, A. M., *et al. Astrobiology* **2006**, *6*, 824.
(5) Stockton, A. M., *et al. Anal. Chem.* **2009**, *81*, 790.
(6) Skelley, A. M., *et al. JGR-Biogeosciences* **2007**, *112*.
(7) Benner, S. A., *et al. Proc. Natl. Acad. Sci.U. S. A.* **2000**, *97*, 2425.
(8) Hecht, M. H., *et al. Science* **2009**, *325*, 64.
(9) Botta, O.; Bada, J. L. *Surveys in Geophysics* **2002**, *23*, 411.
(10) Amashukeli, X., *et al. JGR-Biogeosciences* **2007**, *112*.