Astrobionibbler: Microfluidic Subcritical Water Extraction of Organics from Planetary Samples.

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Introduction: Searching for trace levels of organic molecules on Mars or other rocky bodies presents a formidable challenge for robotic instrumentation. Many organic molecules of specific interest, including potential biomarkers such as amino acids, are not ideally suited for identification via gas chromatography and mass spectrometry (GC/MS) the current robotic state of the art. Wet chemical methods such as liquid chromatography (LC) and capillary electrophoresis (CE) are typically used for analysis on Earth, and because of this automated microfluidic versions of LC and CE capable of separation and sensitive detection of a broad variety of organic molecules are already demonstrated or under development [1, 2].

What is currently lacking however, is a reliable, small sample extraction and concentration instrument which can deliver the appropriate liquid extracts to microfluidic devices. The Astrobionibbler instrument (ABN) [3, 4], meets these needs and can be conceptually broken into three functional sections: 1. Sample acceptance and slurry preparation 2. Extraction of organics from the soil via subcritical water extraction (SCWE) performed on chip. 3. Organic concentration via a laminar flow diffusion interface (H-Cell).

Background: An ideal organic extraction method will efficiently extract a wide range of organic molecules from solid matrices without degradation. The development of SCWE for this purpose is based on the fact that the dielectric constant of water changes dramatically with temperature making it a polar solvent at room temperature but becoming increasing nonpolar with increasing temperature, especially above 100°C [5]. Furthermore at the increased pressure necessary to keep water subcritical at temperatures above its boiling point, the hot pressurized fluid will be able to penetrate pores in solid matrices to more efficiently extract the material within. Both amino acids and polycyclic aromatic hydrocarbons have been extracted from Atacama soils using SCWE [5, 6].

Given the likelihood of trace levels of many organic compounds in accessible samples a concentration step after extraction will greatly increase the probability of down stream detection. Laminar flow diffusion interfaces (LFDI) take advantage of the lack of turbulent mixing in side by side laminar flows to allow diffusion of an analyte from one flow to another. Microfluidic H-Cells easily create LFDIs that can be tuned for the desired properties by design of the microfluidic chip [7, 8].

Instrument Design and Preliminary Results: First prototypes of each of the three functional portions of ABN have been developed and tested.

1. Sample acceptance and slurry preparation: Small prototype sample cups designed to accept solid powder samples and water to create homogenously mixed slurries were designed. Mechanical mixing using a stir bar created homogenous slurries, but in a reusable cup a noncontact method of mixing is preferable. Therefore small cups with piezoelectric actuators on the bottom were designed to allow mixing via sonication.

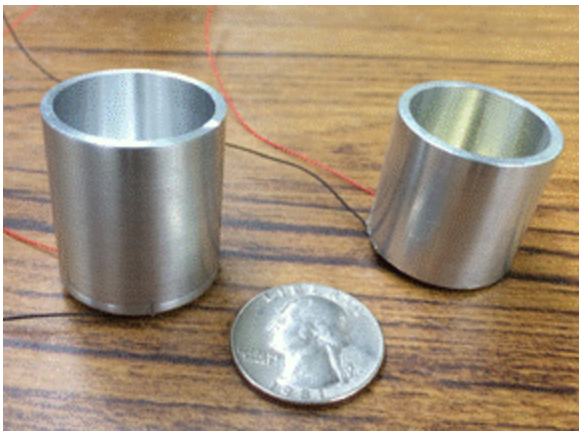

Figure 1 Prototype sample cups with piezoelectric actuators

Using a peristaltic pump it was possible to reproducibly and homogenously move slurries up to 20% weight from the sample cups to another device.

2. Microfluidic SCWE extraction chip: A significant challenge in creating a SCWE instrument is withstanding the pressures and temperatures required during extraction. A bonded glass chip of sufficient thickness has the inherent strength, but creating pressurized seals at the interfaces that are easily automatable is non-trivial. The ABN prototype extraction chip overcomes this by using ice plugs in the microfluidic channels as a sealing mechanism while ultrasonication is used to locally heat a SCWE extraction chamber defined on the chip. The ice plugs are formed reversibly via TEC coolers attached to the exterior glass surface.

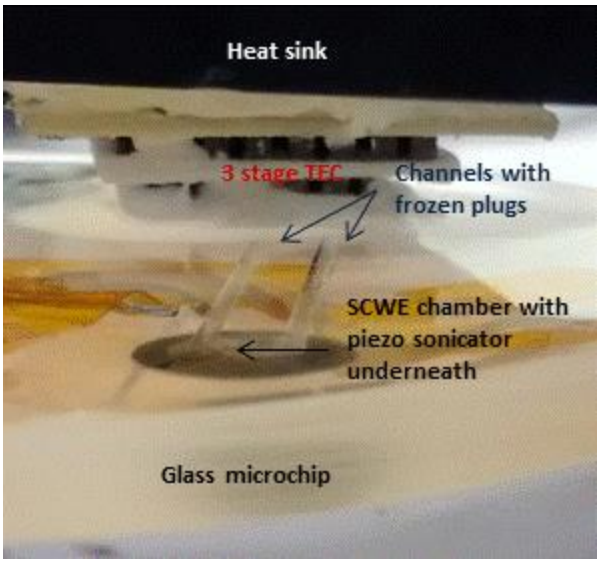

Figure 2 Image of SCWE extraction microchip

Frozen plugs can be maintained and the chamber heated to ≥ 150°C with both pure water and slurry solutions. Preliminary tests of SCWE extractions are ongoing.

3. Concentration using an H-Cell: Prototype microfluidic devices for testing diffusion transport of amino acids in H-Cells have been built. Preliminary data demonstrating the development of the LFDI under laminar flow conditions has been demonstrated and tests of the desired flow conditions to allow optimal diffusion have begun.

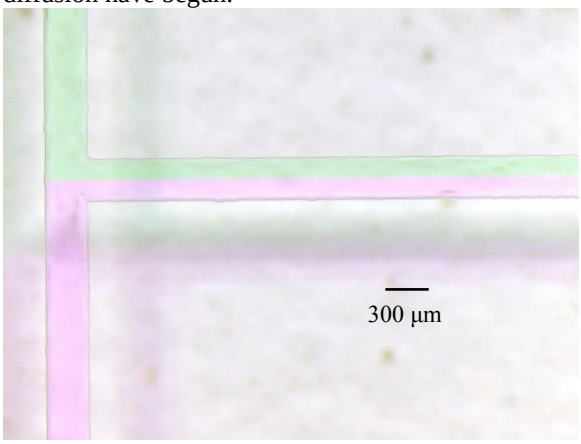

Figure 3 Red and Green food coloring demonstrating the lack of mixing in the H-Cell devices

Optical evidence of the LFDI is clearly seen using two different colored dyes. The next version of the H-Cell devices will include a loop on one half of the H-Cell to allow recirculation of the concentrating flow as it continuously strips the analyte of interest from the sample flow.

Conclusions: A first prototype of all three portions of the ABN have been designed and tested. Critically, the challenges of performing SCWE on chip because of the temperature and pressures accessed has been met through the use of local heating via sonication and sealing of the SCWE chamber via frozen plugs in the entrance and exit microchannels. Continued testing and integration of all three sections will lead to a small instrument for extraction and concentration of organics from solid powder samples.

References:

[1] Mora, M.F., et al. (2011) *Anal. Chem.*, *83*, 8636-8641.
[2] Skelley, A.M., et al. (2005) *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 1041-1046.
[3] Grunthaner, F.J., et al. (2012) *Concepts and Approaches for Mars Exploration 4303*. Houston, TX: Lunar and Planetary Institute.
[4] Hasenoehrl, J., et al. (2013) *Advance in Microfluidics and Nanofluidics P11*. University of Notre Dame, IN.
[5] Amashukeli, X., et al. (2007) *JGR: Biogeo*, *112*, G04S16.
[6] Stockton, A.M., et al. (2008) *Anal. Chem.*, *81*, 790-796.
[7] Kamholz, A.E., et al. (2001) *Biophys. J.*, *80*, 1967-1972.
[8] Kamholz, A.E. and P. Yager. (2001) *Biophys. J.*, *80*, 155-160.