LIQUID CHROMATOGRAPHYJ-MASS SPECTROMETRY INTERFACE FOR DETECTION OF EXTRATERRESTRIAL ORGANICS. A. E. Southard[1], Jerome Ferrance[2], Jamie E. Elsila[3], Ana Melina Espiritu[3], Carl Kotecki[3], Manuel Balvin[3], J.P. Dworkin[3], Daniel P. Glavin[3], Paul Mahaffy[3], Stephanie Getty[3], [1]University Space Research Association (Adrian.e.southard@nasa.gov), [2]J2F Engineering, [3]NASA Goddard Space Flight Center

Introduction: Planetary mass spectrometry has been a key enabling *in situ* analytical technique for investigating the composition of planetary atmospheres and surfaces. Similarly, much has been learned through exquisite terrestrial investigations of meteoritic materials, interplanetary dust, and returned cometary particles, including through the use of liquid chromatography coupled to mass spectrometry (LC-MS) [1]. Future high-priority planetary exploration will target destinations that are likely host to a broad diversity of inorganic and organic composition. Primitive bodies, such as comets and carbonaceous asteroids, are thought to have contributed an inventory of prebiotic chemistry to the early Earth, and cataloguing their present-day, cryo-trapped organic diversity can help to offer more detail about the breadth of organic astrochemistry that is representative of the Solar System's origins and evolution. Major classes of organics are likely to be present on these primitive, small bodies, including polycyclic aromatics, carboxylic acids, alcohols, aldehydes and ketones, amines, nucleobases, and amino acids. This diversity can be challenging to fully characterize by mass analysis alone, but liquid chromatography can offer additional resolution based on functional group chemistry and size effects to alleviate this ambiguity [2]–[4]. LC-MS has also been used to quantify enantiomeric ratios in extraterrestrial amino acids that have been found in well preserved meteorites delivered to Earth [4]. To exploit the advanced analysis offered by LC-MS, the goal of the instrument development effort described here is to implement LC-MS in a spaceflight-compatible package called OASIS (Organics Analyzer for Sampling Icy Surfaces) [5]. We estimate that an eventual flight-ready OASIS Instrument could weigh as little as 5 kg and consume only 3 W of power, even for use on the cold surface of an icy satellite or comet.

OASIS is designed to separate sample constituents present in a liquid phase based on their interactions with a solid stationary phase and subsequently analyze the eluent for definitive mass determination. The instrument includes an on-chip µHPLC analytical column, on-chip nozzle and compact time-of-flight mass spectrometer prototype [6]. This report will focus on the liquid-gas ion interface of the analytical column to the mass spectrometer. In the flight environment, it will be advantageous to operate at pressures lower than Earth ambient. To that end, we are exploring the use of heating of the spray to faciliate evaporation of sprayed droplets.

Fabrication: We fabricated the micro-Liquid Chromatography Thermo/Electrospray (µLCS) chip (see Figure 1) using semiconductor fabrication processes inside a Class 100 clean room. Fabrication of µLCS integrates two different functionalities onto the same chip: (1) the miniaturized High Performance Liquid Chromatography (HPLC) performed by a micro-Liquid Chromatography column (µLC) and (2) the ion spray interface to the Time of Flight Mass Spectrometer (ToF-MS).

The OASIS fabrication approach first defines the µLC channels and spray channels that terminate at the edge of the chip. All channels are designed to be round in cross-section, and this is accomplished by defining features in two separate wafers (on silicon and one Pyrex) that are bonded together to form the channel. The silicon channels are fabricated using the Bosch Process, also known as Deep Reactive Ion Etching (DRIE), followed by a sulfur hexafluoride plasma isotropic dry etch. On the mating Pyrex side, we employ wet chemical etching through a multi-layered chromium/gold etch mask to define the complementary channel feature. The wet etch solution is hydrofluoric acid with 15% (by volume) hydrochloric acid. The addition of the hydrochloric acid removes the passivating salts that are generated at the bottom of the etch surface, thereby creating a smooth surface. The round and smooth cross-section of the channels minimize turbulence inside the channels and enable stationary phase filling to complete the HPLC column.

An additional feature of the µLCS chip is an integrated heater capability defined on the back side of the silicon layer. Two discrete on-chip heaters are designed to independently control the temperature on the µLC and spray regions of the chip. The platinum resistive heaters are e-beam evaporated onto a titanium adhesion layer using a photolithography and liftoff process. The µLC heater is designed to maintain a steady temperature within the µLC column, even in icy environments. A heater dedicated to the spray nozzle enables the implementation of thermally assisted electrospray or thermospray to generate ions for mass analysis. When µLCS is in thermospray mode, required temperatures are expected to reach 300°C. This presents a problem for the µLC, where the aqueous solutions must remain a liquid. To enable cooling of the µLC, we deposited

and patterned heat-sinking gold around the µLC heaters. This gold serves a dual purpose, acting as a heat sink in thermospray mode and as an electrical connection to enable electrospray ionization. To further prevent heating of the µLC region, we added heat barriers between the spray and µLC component of the chips. This was done using DRIE to locally remove the thermally conductive silicon, thereby reducing the thermal cross-talk as well as creating a bridge between the µLC and ion spray regions of the chip.

The last steps in creating our µLCS chip are formation of the spray nozzle taper and anodic bonding. Prior to bonding, the pyrex wafer undergoes sand blast drilling of fluidic vias for macroscale connection to laboratory plumbing. The Pyrex and Silicon wafers are then cleaned in Piranha solution (3 parts H_2SO_4 : 1 part H_2O_2) and bonded anodically at 1000 V and 350 °C.

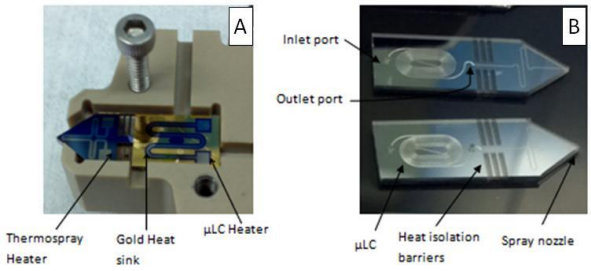

Figure 1 The bottom (A) of the OASIS µLCS microchip has separate heaters for heating the spray channels and µLC channels of the microchip and a heat sink. The top of the microchip (B) has inlet and outlet ports that allow packing of the µLC channels. The outlet port is blocked during analysis of samples but can be used to do post-column addition.

Ambient pressure testing: We successfully demonstrated the electrospray capabilities of the µLCS chip by interfacing it with commercial laboratory instrumentation. We used the OASIS test fixture in conjunction with a commercial mount to position the chip in the source region of a Waters LCT Premier time-of-flight mass spectrometer, orthogonal to the commercial skimmer. We used a Waters NanoAcquity nano-flow ultraperformance liquid chromatograph to provide a constant flow of a buffer containing 5 mM sodium formate with 10^{-4} M adenine and phenolphthalein. This flow was directed into the "outlet" port of the chip; the flow then split, with an uncalibrated amount flowing backwards through the µLC channel towards the "inlet" port and the remainder passing through the electrospray channel to the spray outlet. High voltage was applied to the chip through the test fixture. Waters MassLynx software was used to control flow rate and voltage and to acquire mass spectra. The position of the chip in the source region was controlled in the x, y, and z axes using a micrometer stage. The acquisition of a stable electrospray depended on the interaction of flow rate (0.1 to 1.5 µL /minute), voltage (2500 to 3500 V), and the position of the chip. Changes in these three variables affected the measured ion intensities and distributions. The best performance, or largest, stable signal, was obtained at a flow rate of 1.5 µL/min using a pressure of 760 psi and a voltage bias of 3500 V.The electrospray mass spectrum obtained under optimal conditions was compared with that obtained from a commercial electrospray tip (Waters Pre-cut TaperTip™, 20 µm inner diameter, 2.5" length) with the same buffer in the same NanoAcquity-LCT Premier instrumentation setup. The mass spectra obtained from the µLC chip and the commercial ESI tip were in reasonable agreement, although ion intensity distributions varied (see Figure 2). The total ion intensities were approximately one to two orders of magnitude lower with the µLCS chip than the commercial tip, although sensitivity was dependent on position, voltage, and flow rate.

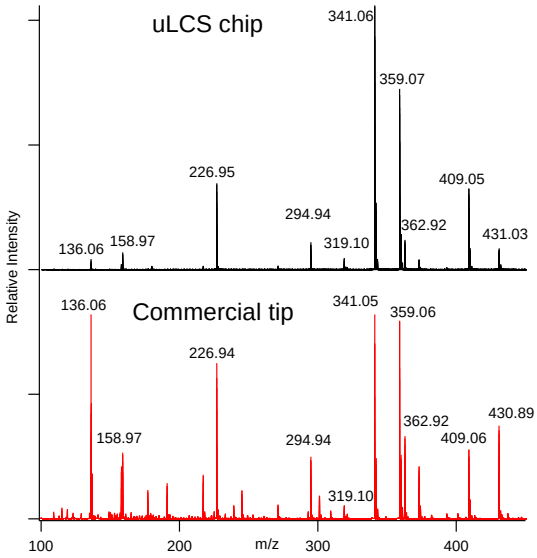

Figure 2 Mass spectra resulting from electrospray ionization using (top) the µLCS chip (x10) and (bottom) a commercial ESI tip of a 5 mM sodium formate with 10^{-4} M adenine and phenolphthalein buffer solution. The mass peak at 136.06 is attributed to adenine-H^+. The peak at 319.10 is attributed to phenolphthalein-H^+ while that at 341.05 is attributed to phenolphthalein-Na^+; the peak at 409.05 may represent addition of a sodium formate cluster to phenolphthalein-Na^+. The peaks at m/z=158.97, 226.9, 294.94, 362.92, and 430.89 are attributed to sodium formate clusters ($C_2H_2Na_3O_4$, $C_3H_3Na_4O_6$, $C_4H_4Na_5O_8$, $C_5H_5Na_6O_{10}$, and $C_6H_6Na_7O_{12}$, respectively). The identity of the peak at m/z=359.07 is unknown.

The mass spectrum shown here [7] is an excellent match to that expected for the chemical standards used in this component-level testing of the on-chip ion spray

nozzle. No significant contamination contributed to the laboratory test results based on the analysis of blanks run under the same conditions as the standards. During in situ OASIS operations, we will leverage the established approach of using blank analyses for assessing any contributions from terrestrial organic contamination.

Spraying under vacuum: A 260 mm diameter by 258 mm tall test chamber (Figure 3) was designed and built to allow testing of the ESI and thermo-spray chips in a moderate vacuum (5 to 10 torr). The chamber was sized to easily accommodate the x-y-z micrometer stage from a commercial Waters LCT Premier time-of-flight mass spectrometer. This enables us to optimize position and orientation of the chip with respect to the skimmer. The use of aperture and differential pumping stages allow reduction of pressure to small enough values to gauge sensitivity in with a commercial RGA (SRSRGA300).

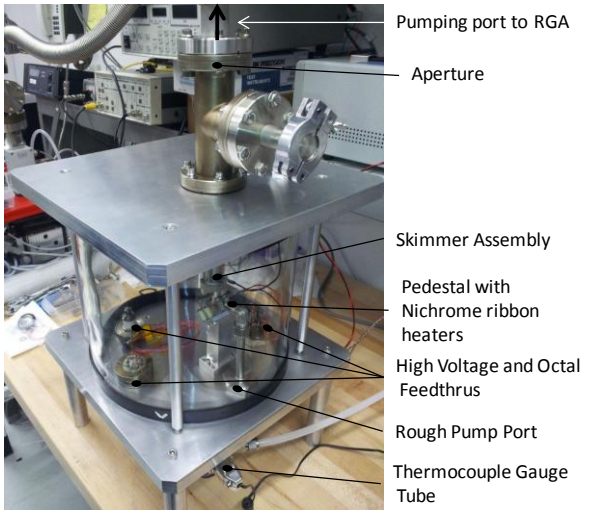

Figure 3 The OASIS test chamber allows testing of thermospray and electrospray under low pressure (~10 torr) conditions and contains the OASIS test fixture, pedestal with nichrome ribbon heaters, a skimmer, and various fluidic, electrical, and vacuum feed-thrus.

After determining a baseline sensitivity for detection of adenine and phenolphthalein, and optimizing this sensitivity, we will integrate the chip with a prototype time-of-flight mass spectrometer with a gridless multistage reflectron[5].

References: [1] Burton *et al. Chem. Soc. Rev.*, vol. 41, no. 16, pp. 5459–5472, Jul. 2012. [2] Glavin *et al. Meteorit. Planet. Sci.*, vol. 41, no. 6, pp. 889–902, 2006. [3] Elsila *et al. Meteorit. Planet. Sci.*, vol. 44, no. 9, pp. 1323–1330, 2009. [4] Callahan *et al. Proc. Natl. Acad. Sci.*, Aug. 2011. [5] Getty *et al.* (2013) *IEEE Aerospace Conference*, 2013, pp. 1–8. [6] King *et al.* (2008) *Proc. SPIE* **6959**, 6959E. [7] Southard *et al.* (2014) IEEE Aerospace Conference 2014, pp. 1-7.