LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY INTERFACE FOR DETECTION OF EXTRATERRESTRIAL ORGANICS

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Abstract*—The OASIS (Organics Analyzer for Sampling Icy surfaces) microchip enables electrospray or thermospray of analyte for subsequent analysis by the OASIS time-of-flight mass spectrometer. Electrospray of buffer solution containing the nucleobase adenine was performed using the microchip and detected by a commercial time-of-flight mass spectrometer. Future testing of thermospray and electrospray capability will be performed using a test fixture and vacuum chamber developed especially for optimization of ion spray at atmosphere and in low pressure environments.

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1. 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, using 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). 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.

2. INSTRUMENT DESIGN

OASIS is designed to separate sample constituents present as solutes in a liquid phase based on their interactions with a solid stationary phase and their mass. The instrument includes an on-chip μHPLC analytical column, on-chip nozzle and custom built time-of-flight mass spectrometer[5].

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This report will focus on the liquid-gas ion interface of the 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 thermally assisted electrospray ionization (ESI) or thermospray to promote the compatibility of ion spray techniques with low pressure.

3. ESI MICROCHIP DESIGN

We conducted fabrication of the micro-Liquid Chromatography Thermo/Electrospray (µLCT/ES) chip (see Figure 1) by utilizing semiconductor fabrication processes inside a Class 100 clean room, using silicon and Pyrex wafers as the substrates. Fabrication of µLCT/ES integrates two different functionalities onto the same chip. On one end, the miniaturized High Performance Liquid Chromatography (HPLC) is performed by the micro-Liquid Chromatography column (µLC); this serves to separate analytes of interest, such as chiral amino acids. At the other end of the chip is the interface to the Time of Flight Mass Spectrometer (ToF-MS), which is in the form of thermospray or electrospray.

Our fabrication commences by development of the µLC channel and a spray channel, which serves both for electrospray or thermospray. We fabricate the channels by using the Bosch Process, also known as Deep Reactive Ion Etching (DRIE), on the silicon wafer. We use DRIE to create the channels by cycling sulfur hexafluoride and octafluorocyclobutane plasmas on and off. When we achieve 2/3rds of the desired depth, we turn off the octafluorocyclobutane plasma and solely use the sulfur hexafluoride plasma as an isotropic dry etch. This technique allows for fabrication of round channels on silicon. On the mating Pyrex side, we employ wet chemical etching instead of DRIE. Initially we deposit a multi-layered chrome-gold mask as the etch mask. The wet etch solution is hydrofluoric acid with 15% hydrochloric acid, by volume. 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 enable microbead filling, which allows the µLC to function as an HPLC column.

In addition to creating the channels, we fabricated on-chip heaters on both the µLC and spray components. The heaters are deposited over patterned photoresist using e-beam evaporation of platinum with a titanium adhesion layer. We subsequently dissolve the resist in acetone and lift-off the unwanted platinum and titanium thereby creating the platinum heaters. The µLC heater helps maintain a steady temperature during chromatography on icy bodies and the spray heater enables thermospray into the ToF-MS. When µLCT/ES is in the thermospray mode, temperatures can exceed 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 the µLC from heating, we added heat barriers between the spray and µLC component of the chips. This was performed by using DRIE to remove the thermally conductive silicon, thus reducing the thermal cross-talk as well as creating a bridge between the µLC and thermo/electrospray components.

The last steps in creating our µLCT/ES chip are formation of the spray nozzle and anodic bonding. The spray nozzles are created using DRIE on the silicon and by sand blast drilling on the Pyrex. Prior to bonding, the Pyrex wafer undergoes sand blast drilling of vias to fabricate the fluid inlets and outlets for macroscale connection to the OASIS testing fixture. The vias on the Pyrex are tapered at 22° in order to allow leak-free pressure connection to the macroscale. Upon completion of the DRIE and sand blast drilling for the tapered vias, the Pyrex and silicon wafers are cleaned in Piranha solution (3 parts H_2SO_4 : 1 part H_2O_2) then bonded anodically at 1000 V and 350 °C.

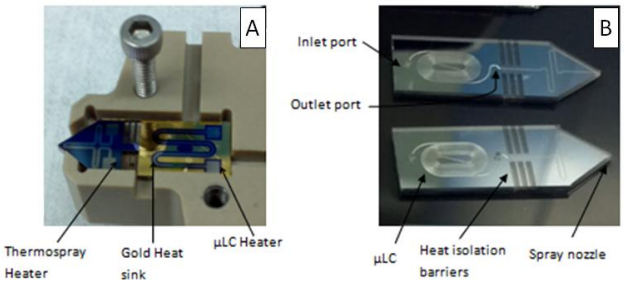

Figure 1 – The bottom (A) of the OASIS µLCT/ES microchip has two heaters, for heating the spray channel and µLC channel, and a heat sink. The top of the microchip (B) has inlet and outlet ports that allow packing of the µLC channels. The outlet port is blocked during analysis of samples but can be used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

4. TEST FIXTURE

The OASIS test fixture (Figure 2) is fabricated from PEEK (PolyEtherEtherKetone) 450G thermoplastic for stiffness, high temperature performance, electrical isolation, low outgassing, and machinability. Electrical connections to the spray and µLC channels, heat sink electrode, and temperature sensors are provided through surface mount, spring loaded pins located on the underside of the fixture and soldered to a micro-D sub connector. A separate socket adjacent to the micro-D sub connector is used for providing high voltage to the chip and is wired directly to one of the pins. Fluidic connections are provided through Upchurch Scientific NanoFerrules installed on modified NanoMixer clamp assemblies. The fixture's asymmetric front profile provides clearance to the gas supply line and baffle of the

commercial TOF used for laboratory testing, and enables positioning to the skimmer to within 1.2 mm. Rotational adjustability to the skimmer is accommodated to a range of ±30° through 43.3° chamfers at the rear of the fixture.

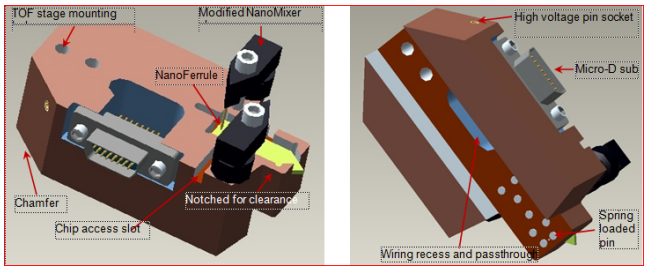

Figure 2 – The OASIS fixture holds the µLCT/ES microchip. Spring loaded pins support the chip as nanoferrules are pushed into the inlet and outlet ports. The fixture provides an interface of the chip's heaters and electrical connections to a Micro-D sub connector.

5. ESI TESTING

We successfully demonstrated the electrospray capabilities of the µLCT/ES 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 and 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 µLCT/ES chip and the commercial ESI tip were in reasonable agreement, although ion intensity distributions varied (see Figure 3). The total ion intensities were approximately one to two orders of magnitude lower with the µLCT/ES chip than the commercial tip, although sensitivity was dependent on position, voltage, and flow rate.

The mass spectrum shown here 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.

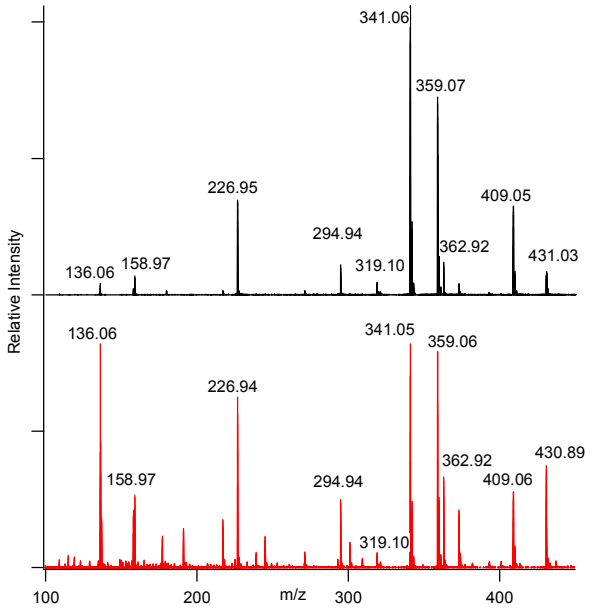

Figure 3 – Mass spectra resulting from ESI using (top) the OASIS chip (x10) and (bottom) a commercial tip to spray a 5 mM sodium formate with 10^{-4} M adenine and phenolphthalein buffer solution. The mass peak at 136.06 is attributed to adenine-H^+. The peak at 319.10 is attributed to phenolphthalein-H^+ 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.

6. OASIS TEST CHAMBER

A 260 mm diameter by 258 mm tall test chamber (Figure 4) was designed and built to allow testing of the ESI and

thermo-spray chips in a moderate vacuum. 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 first test the chips in a lab ambient environment optimizing the location of the chip tip with respect to the location of the skimmer inlet in a commercial TOF, than transfer our custom test fixture with the chip still mounted, into our test chamber. We are using a spare skimmer assembly from the same TOF-MS in our chamber to allow direct comparison of the results by locating the chip tip at the same relative location.

The chamber bell jar is Pyrex and the base and top plates are both 305mm square, 25.4mm thick aluminum plates. We attached the mounting tube for the skimmer, which is also the inlet tube for the residual gas analyzer or RGA (SRS RGA300), to the inside of the chamber lid as well as a BNC feed-thru to enable applying a bias to the electrically isolated skimmer. There are also four external guide rods to accurately locate the lid with respect to the baseplate. All other components and feed-thrus are mounted to and through the baseplate. This was done to minimize the number of connections to make and break every time the chamber is opened to install or remove a sample chip. The skimmer is attached to the lid to eliminate the possibility of a gravity assist to ions entering the skimmer. Baseplate penetrations include two standard 25.4mm octal feed-thrus for heater and thermocouple connections, a capillary tubing feed-thru for the sample fluid, a 3/8"NPT hole for the thermocouple pressure gauge tube, a 25.4mm high voltage feed-thru for the ESI high voltage and a standard 25.4mm diameter feed-thru with a 9.5mm diam. stainless steel tube used for pumping the chamber. An elbow was attached to the end of the tube inside the chamber so that the fluid sprays directly into the inlet of the roughing pump. A pedestal was designed to hold nichrome ribbon heaters that maintain the temperature of the vapor in thermospray mode[6] and act as a repeller in either thermospray or electrospray mode. A Ted Pella dry scroll vacuum pump was chosen so that the test fluid solvents do not degrade the performance of the pump as they would with an oil based pump. In addition, there is no possibility of pump fluid back-streaming into the chamber and contaminating our test results.

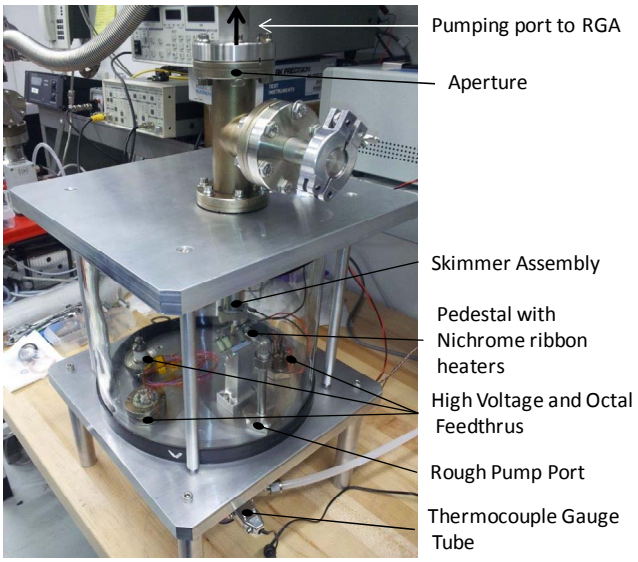

Figure 4 – The OASIS test chamber allows testing of thermospray and electrospray under low pressure (~7 torr) conditions and contains the OASIS test fixture, pedestal with nichrome ribbon heaters, a skimmer, and various fluidic, electrical, and vacuum feed-thrus.

7. THERMOSPRAY TEST PROTOCOL

Once construction of the vacuum test chamber is completed, thermospray testing from the μLCT/ES microchip will be initiated. Efficiency of the thermospray is not based on the spray itself, but has to be evaluated based on the number of charged ions reaching the RGA connected to the vacuum chamber system. This will be influenced by a number of variables in the system, all of which will be evaluated during this testing protocol. Initially, the system will be set up with a flow rate of 0.1 μL/min, a microchip spray temperature of 250˚C, a ribbon heater temperature of 250˚C, and a pinhole size of 100 μm. This will set the initial ion count, which can then be used to evaluate the effect of changes to the system parameters. Adjustments of the microchip outlet relative to the skimmer inlet will be performed to maximize ions collected under these initial conditions; it is not expected that changes to other system parameters will affect this chip positioning, so this testing will set the ultimate position of the microchip.

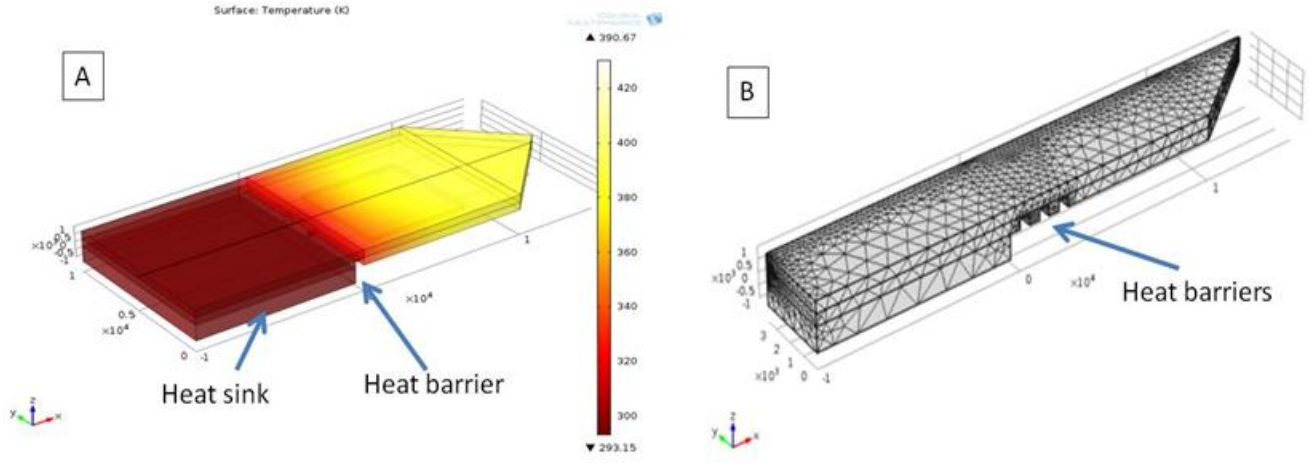

Figure 5 – COMSOL model was used to determine the temperature within the microchip given a geometry with one heat barrier (A) and three heat barriers (B).

The pinhole between the vacuum spray chamber and the RGA restricts flow between the higher pressure, ~7 torr, and lower pressure, 0.1 to 1 mtorr, regions in the chamber. A smaller pinhole creates a greater pressure differential, potentially drawing more ions into the low pressure region. A smaller pinhole also limits the area through which the ions can enter, however, and an optimal compromise between area and pressure difference will be obtained by changing the size of the pinhole in the system. With an optimal pinhole selected, subsequent experiments will evaluate the temperature and position of the nichrome ribbon heaters relative to the skimmer inlet, and the temperature of the microchip outlet generating the thermospray. The outlet microchip temperature will be somewhat dependent upon the flow rate through the chip; thus, additional experiments to determine a relationship between flow rate and spray temperature will also be performed.

8. THERMOSPRAY MODELING

A finite element simulation (COMSOL) was performed to determine the power necessary to heat liquid water flowing through the capillary to a temperature above its boiling point of 100 °C. Here, the platinum heater geometry was approximated by a block heated with a fixed heating power. The length and width of the block were chosen to heat an area of the chip comparable to that area heated by the platinum heater wire. A flow of 1 uL/min through the capillary was assumed and the LC side of the chip was heat sunk to a block at ambient temperature, 290 K. All surfaces were allowed to convect to their static, ambient temperature surroundings with heat transfer coefficient h=5 W/(m^{2}K). The energy input to induce a phase change was not accounted for in this simulation thus it is only accurate up to the boiling point. Initially, a model with one heat barrier was investigated and a heating power of between 7 and 7.5 Watts was necessary to achieve temperatures of 373.15 K on the chip (**Error! Reference source not found.**a). However, with the introduction of three heat barriers, as described in the fabrication section and shown in **Error! Reference source not found.**b (mesh view of half the geometry), a heating power of only 2 Watts was necessary. Using this heating power, a temperature of 373.15 K was reached at a distance of 7 mm from the heat sink. The minimum heating power necessary to heat the sprayer orifice of the microchip to 373.15 K was the same whether or not a capillary was included in the model. In other words, the heat flux into the liquid flowing through the capillary was negligible compared to the flow via conduction to the cooled side of the chip and convection from the chip's surface. Additional modeling will be performed in the future to account for a phase change in the capillary, slight changes in the geometry of the chip, and the use of a mixture of methanol and water as the mobile phase.

9. CONCLUSIONS

Design and fabrication of a microchip with channels for liquid chromatography and ion spray was completed using MEMS fabrication techniques. A test fixture for interfacing the microchip to a high voltage source enabled testing of electrospray from the microchip into a commercial mass spectrometer. Successful detection of the nucleobase adenine in the presence of a sodium formate buffer solution was demonstrated, although sensitivity of the mass peak was smaller than that achieved from a commercial ESI tip. The microchip also incorporated heaters for both the LC and ion spray channels that will enable thermospray testing (as well as electrospray testing) in a custom built vacuum chamber. Modeling of heat flow on the microchip indicates that temperatures of the chip should exceed 100 °C using only a few watts of power. In the future, a more mechanically robust chip will be fabricated and thermospray testing will be performed.

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BIOGRAPHY

Adrian Southard *received his bachelor of arts in physics from New College of Florida in 2000 and his Ph.D. in chemical physics from University of Maryland in 2009. His Ph.D. research focused on transport in organic semiconductors and novel device fabrication methods. He joined the VAPoR team at NASA Goddard in 2009 to work on development of a Time-of-Flight Mass Spectrometer. He is currently serving as a product development lead on the ExoMars MOMA-MS team and supporting simulation, design, and testing associated with OASIS. Dr. Southard is a member of the American Society for Mass Spectrometry and American Physical Society.*

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