A MICROFLUIDICS-HPLC/DIFFERENTIAL MOBILITY SPECTROMETER MACROMOLECULAR DETECTION SYSTEM FOR HUMAN AND ROBOTIC MISSIONS. S. L. Coy[1], K. Killeen[2], J. Han[3], G. A. Eiceman[4], I. Kanik[1] and R. D. Kidd[1], [1]Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena CA, 91109, [2]Agilent Laboratories, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, [3]Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, [4]New Mexico State University, 1175 North Horseshoe Drive, Las Cruces, NM 88003.

Introduction: Our goal is to develop a unique, miniaturized, solute analyzer based on microfluidics technology. The analyzer consists of an integrated microfluidics High Performance Liquid Chromatographic chip / Differential Mobility Spectrometer (µHPLC-chip/DMS) detection system (Fig. 1).

The science objectives of this analyzer is to look for signs of: (1) extinct life by detecting fatty acids and lipids - the longevity and preservation of fatty acids and lipids [1,2] offer a chemical insight into potential primordial biological activity on Mars back when Mars was more Earth-like; (2) extant life by searching for fatty acids and lipids, peptides and proteins - macromolecules that strongly indicate a biotic origin; and, (3) carboxylic acids - thought to be stable under the highly oxidizing Martian surface.

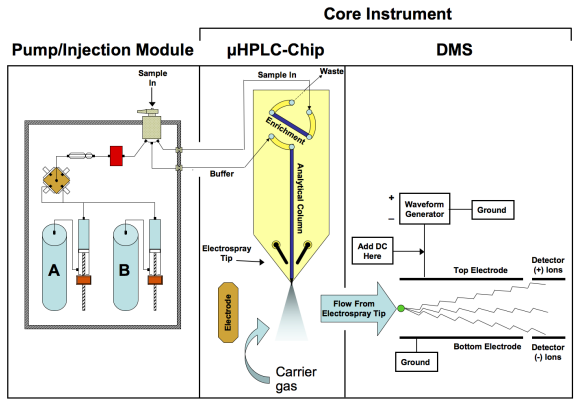

Fig 1. Schematic of the miniature breadboard instrument: Pump and Injector Module (PIM), µHPLC-Chip (which includes enrichment/desalting column, separation column and electrospray tip), and Differential Mobility Spectrometer (DMS).

Why µHPLC-chip? High Performance Liquid Chromatography (HPLC) is one of the most widely used tools in analytical chemistry due to its sensitivity, accuracy, and capability for identifying a wide range of organic compounds. It is well suited for analyzing complex samples of unknown composition. Reverse-Phase (RP) HPLC separation with C18 resin is a standard technique for separating compounds based on their hydrophobic character, and is capable of identifying molecular structure preferences (e.g. stereoisomers), fatty acid molecular weight distributions, non-homologous series, and ether-bound isoprenyl lipids (Archaea biomarkers). RP-HPLC is a proven method for identifying organic biomarkers in ancient sediments on Earth and has been used to separate terpane, sterane, and alkane biomarkers from crude petroleums and bitumens [1-3]. These data demonstrate the powerful capability of HPLC analysis to identify geochemically-stable biomarkers from a variety of depositional settings, and the potential of these compounds to preserve microbial source information and provide a record of evolutionary change.

Until recently, HPLC systems have been considered to be unsuitable for *in situ* planetary applications due to their large mass and operational complexity. However, in June 2005, Agilent introduced the first commercial microfluidic reverse-phase HPLC-chip instrument (µHPLC-chip), which is capable of separating a wide range of organic compounds (>50 species in less than 10 minutes) based on their varying elution times through the separation column [4]. The µHPLC-chip consists of a reusable microfluidic polymer chip with dimensions smaller than a credit card. The µHPLC-chip integrates the sample enrichment and separation columns of a conventional HPLC system, and incorporates the high voltage connections and spray tip used in electrospray ionization (for mass spectrometry) directly onto the polymer chip. The technology eliminates many of the traditional fittings and connections associated with HPLC systems, dramatically reducing the possibility of leaks and dead volumes and significantly improving ease of use, sensitivity, and reliability during analysis.

Why DMS? Differential Mobility Spectrometry (DMS) is a powerful technique for ion mobility analysis that was first demonstrated in the late 1990s by Gary Eiceman of New Mexico State University [5]. This DMS is capable of operating directly in Earth's ambient atmosphere (and on Mars) without a vacuum pump. The complete DMS instrument, including electronics, is contained in a volume of 10 cm × 4 cm × 6 cm with a mass of <500 g and power consumption of ~2 W - far smaller and simpler (no vacuum pumps, chambers, etc) than a mass spectrometer.

The DMS produces an oscillating asymmetric Rf electric field and a DC compensation electric field both of which are applied across two parallel plates producing a programmable high-field mobility filter. The Rf field is applied perpendicular to the motion of Ni^{63}-

ionized molecules, which causes the ions to move with a "zigzag" motion as the field is applied. Ions whose movement causes contact with either of the plates are neutralized and consequently pass by the electrometers at the end of the channel without being detected. Those ions whose net movement allows them to pass through the filter without touching the plates are subsequently detected.

Instrument Concepts: *HPLC-chip/DMS.* The principles of operation of the combined HPLC-chip/DMS instrument (Fig. 1) are as follows: (1) a liquid sample (obtained by melting planetary ice samples or performing an *in situ* extraction procedure) is injected into the µHPLC-chip. (2) The µHPLC-chip performs concentration, desalting, and separation of organic molecules via C18 reverse-phase chromatography. (3) The built-in electrospray tip on the µHPLC-chip ionizes the eluting compounds and injects them into the DMS. (4) The DMS detects the eluting compounds by determining their ion mobilities.

Ion concentration polarization (ICP). The high levels of salt on planetary bodies such as Mars, recently confirmed by probes including the Phoenix Mars lander [6], can interfere with liquid-phase *in situ* analytical instruments like the µHPLC-chip/DMS system that we are working on. Therefore, we have also proposed a new, compact, low power, chip-based desalinator as part of a robotic lander/rover sample preparation system where soil/ice samples need to be desalted prior to analysis.

The desalinator (Fig. 2) divides salty water into desalted and concentrated streams by ion concentration polarization (ICP), a phenomenon that occurs when an ion current is passed through ion-selective membranes [7]. During operation, both salts and larger charged particles are pushed away from the membrane (a nanochannel or nanoporous membrane), which significantly reduces the possibility of membrane fouling and salt accumulation, thus avoiding two problems that plague traditional membrane filtration methods.

Preliminary Results: The most complex aspect of this project is converting a liquid output from the µHPLC-chip to a gas for the DMS, an issue that mass spectrometers needed to overcome for HPLCs. As a first step, we have demonstrated DMS analysis of target compounds using traditional electrospray ionization (ESI) (Fig. 3). Next step is with the nanospray equipped µHPLC-chip and its lower flow rate of 150-300 nL/min, a distict advantage for ESI.

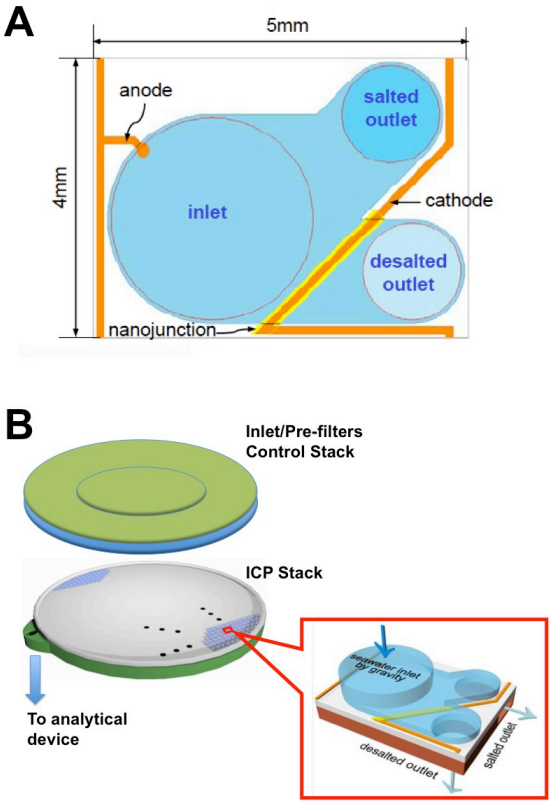

Fig 2. (**A**) Schematic diagram of an optimized unit design. (**B**) (Left) Gravity-fed ICP-stack desalination system. The ICP stack has many parallel microfluidic ICP-units for salt/particle removal. (Right) Perspective of a single unit in the stack.

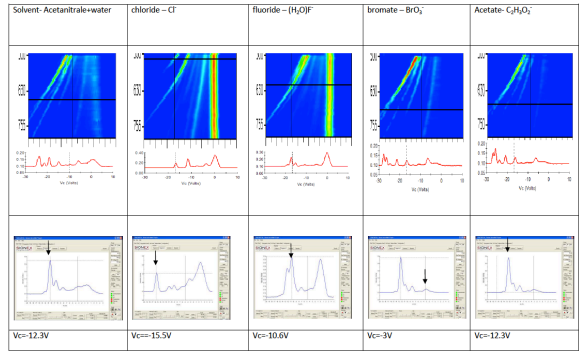

Fig 3. DMS dispersion plots, linear spectra for different Rf, and linear spectra for Rf = 500 V for five target ions.

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