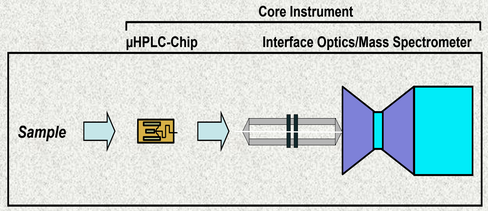
Miniature Liquid Chromatographic Systems for Human and Robotic Missions

Richard D. Kidd[1], Nicholas Scianmarello[2], Josh Wiley[1,2], Stojan Madzunkov[1], Y.-C. Tai[2]

[1]Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena CA, 91109, [2]California Institute of Technology, Pasadena, CA 91125

OVERVIEW

In designing *in situ*, liquid-based analytical instruments, three areas need to be addressed: *Sample Acquisition*, *Sample Preparation* and *Separation/Detection*. At JPL, we are pursuing research into all three areas focusing, thus far, on developing a unique, miniaturized solute analyzer based on microfluidics technology. This macromolecular analyzer consists of a microfluidics High Performance Liquid Chromatographic chip integrated to a Paul Ion Trap Mass Spectrometer (μHPLC-chip/MS).



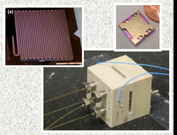
INTRODUCTION

- SMD (Science Mission Directorate): Our instruments are meant to address two fundamental questions in Astrobiology, namely, "How does life begin and evolve?" and "Does life exist elsewhere in the Universe?" For these reasons, the Science Objectives for the analyzer are to look for:
  - ✓ Signs of extinct life by detecting Carboxylic Acids and Lipids - the longevity and preservation of carboxylic acids and lipids offer a chemical insight into potential primordial biological activity.
  - ✓ Extant life by searching for Peptides and Proteins - macromolecules that strongly indicate a biotic origin.
- Provide organic molecular detection and life detection capabilities for future landed missions to Mars, Europa, Titan, Enceladus, and other planetary bodies.
- Astrobiology field research on Earth.
- HEOMD (Human Exploration & Operations Mission Directorate): Experiments on ISS, astronaut health monitoring, environmental monitoring.
- Of the three main chromatographic technologies (GC, CE, LC), liquid chromatography is the least advanced regarding miniaturization, portability, etc.

JPL/CIT MEMS CHROMATOGRAPHY

Gas Chromatography (GC)

- Can be coupled to a MS
- Very fast
- Can separate volatile small molecules
- Need to derivatize amino acids, fatty acids, etc.



Madzunkov, S.M., MacAskill, J.A., Simcic, J., Kidd, R.D. & Darrach, M. (2013). Recent developments in gas chromatographs and mass spectrometers for crewed and robotic space missions. *J. Am. Inst. Aeronautics & Astronautics*. Electronic 2012-3453.

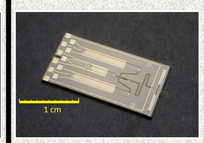
Capillary Electrophoresis (CE)

- Coupled to laser-based fluorescence detector
- Very sensitive/ specific
- Can separate small molecules to macromolecules

Willis, P. A., Stockton, A. M., Microchip Capillary Electrophoresis for In Situ Planetary Exploration. In *Capillary Electrophoresis and Microchip Capillary Electrophoresis*, John Wiley & Sons, Inc.: **2013**; pp 277-291.

High Performance Liquid Chromatography (HPLC)

- Can be coupled to a MS
- Well suited for non-volatiles
- Can separate small molecules to macromolecules
- No derivatization required

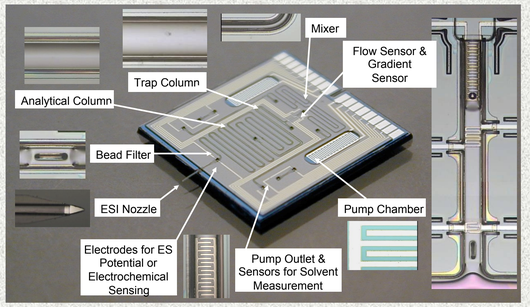


Xie, J., Miao, Y., Shih, J., Tai, Y.-C. & Lee, T.D. (2005). Microfluidic platform for liquid chromatography-tandem mass spectrometry analyses of complex peptide mixtures. *Anal. Chem.* **77**; 6947-6953.

HPLC-CHIP

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 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].

Until recently, HPLC systems have been considered to be unsuitable for *in situ* planetary applications due to their large mass and operational complexity. However, starting in 2004 the Caltech Micromachining Laboratory demonstrated the first *complete* microfluidic reverse-phase HPLC-chip instrument (pumps, injector, mixer, column), which is capable of separating a wide range of organic compounds based on their varying elution times through the separation column [4]. The HPLC-chip is based on Micro-Electro-Mechanical Systems (MEMS) technology and consists of a reusable microfluidic polymer chip with dimensions smaller than a quarter. The HPLC-chip integrates three electrolysis based electrochemical pumps, one for loading the sample and the other two for delivering the solvent gradient; a static mixer; a column packed with silica-based reversed-phase support; and an electrospray nozzle directly on the polymer chip. The technology eliminates many of the traditional fittings and connections associated with traditional HPLC systems, dramatically reducing the possibility of leaks and dead volumes and significantly improving ease of use, sensitivity, and reliability during analysis.



REFERENCES

[1] Simoneit B.R. et al. (1998) *Orig. Life Evol. Biosph.* **28**, 475–483.
[2] Brocks J.J. et al. (1999) *Science* **285**, 1033–1036.
[3] Kawamura K. & Kaplan I.R. (1987) *Geochim. Cosmochim. Acta* **51**, 3201–3207.
[4] Xie J. et al. (2005) *Anal. Chem.* **77**, 6947–6953.
[5] Fenn J.B. et al. (1989) *Science* **246**, 64–71.
[6] Page J.S. et al. (2008) *Anal. Chem.* **80**,1800–1805.
[7] Madzunkov S.M .et al. (2013) *J. Am. Inst. Aeronautics & Astronautics*. Electronic 2012–3453.
[8] Boynton W.V. et al (2009) *Science* **325**, 61–64
[9] Hecht M.H. et al. (2009) *Science* **325**, 64–67.
[10] Skelley A.M. et al. (2005). *PNAS USA.* **102**, 1041–1046.
[11] Aubrey A.D. et al. (2008) *Astrobiology* **8**, 1–13.
[12] Willis P. A. (2013) In *Capillary Electrophoresis and Microchip Capillary Electrophoresis*, John Wiley & Sons, pp 277–291.
[13] Kidd R.D. et al. (2015). *ICES Conf. Proc.* Electronic ICES-2015-141.

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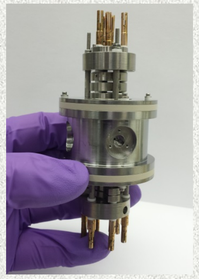
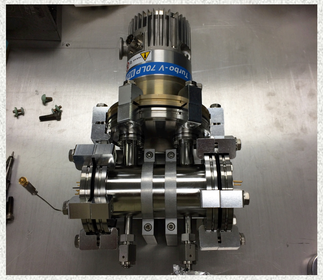
ION TRAP MASS SPECTROMETER

Why MS? There are a variety of compact detectors commonly used for HPLC: UV/VIS, refractive index, fluorescence, electrochemical, conductivity, evaporative light scattering to name a few. However, none of these types of detectors can give a definitive identification of molecules nor can they identify, and in some cases, even detect unknown compounds. The coupling of LC to MS is recognized as the premier technique for any application, which requires high sensitivity, selectivity, and complete unambiguous identification of an unknown collection of chemical species. These are exactly the requirements and conditions found in a planetary robotic exploration. The coupling of LC to MS via electrospray ionization (ESI) is the terrestrial standard and is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized through other methods (e.g. electron impact, laser ionization). The development of electrospray ionization for the analysis of biological macromolecules [5] was rewarded with the attribution of the Nobel Prize in Chemistry to John Bennett Fenn in 2002. A further advantage for LC/MS is that the need for chemical labeling (for optical detectors) and/or derivatization (for gas chromatography) are eliminated. The clogging and reliability of prior ESI efforts is being addressed in the present work through the inclusion of a MEMS desalting column before the MEMS-HPLC column.

The Planetary Surface Instruments Group at JPL is drawing upon its experience in successfully developing and constructing MS flight instruments for the MEMS-HPLC/MS system. The JPL PSI Group has been responsible for the original research and flight development of several different miniature mass spectrometers including a Paul quadrupole ion trap (QIT) MS that was used in the Vehicle Cabin Atmosphere Monitor (VCAM) and will be used in the Spacecraft Atmosphere Monitor (S.A.M.) and other instruments.

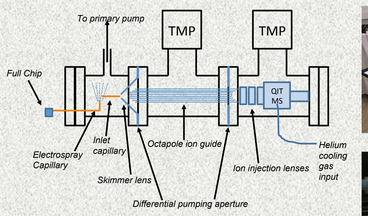


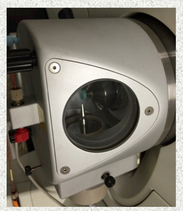


Photographs of our MS system. (Left) Our 10 mm Paul ion trap mass spectrometer [7]. ***(Right)*** The MS in a custom chamber, 4.5CF.

ELECTROSPRAY IONIZATION

In our project the nanospray source, operating at Mars-like pressure (~0.64 kPa), will be interfaced to the ion-trap MS through a conventional arrangement of a hexapole ion guide and DC lenses. Recent studies have shown that nanospray sources can be efficiently operated at near-Mars ambient pressures [6]. Even at these low pressures there was adequate droplet desolvation while avoiding electrical discharge.

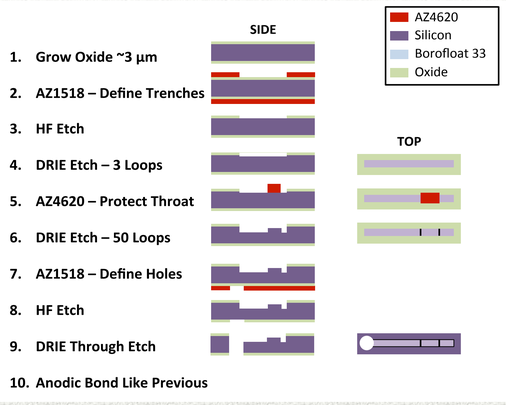




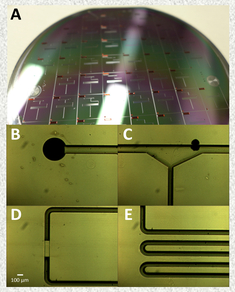
Pursuing a Thermo architecture:

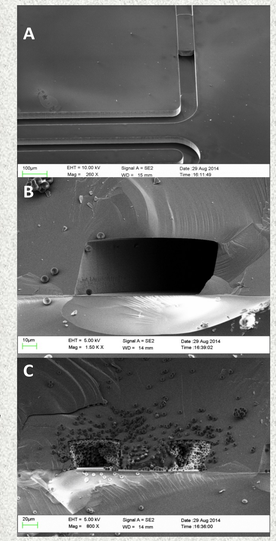
- Capillary electrospray.
- Traditional electrospray in vacuum (Thermo system), minimal pumping needed.
- Minimizes vacuum sealing issues on chip side. Safest route.

FABRICATION



Fabrication process for the chip. Silicone oxide is grown and patterned on silicon as an etching mask for the channel (steps 1-3). Step 4 shows the DRIE etch of the trench down to the height of 3 μm for the frit for the chromatography beads. Steps 5 & 6 is the application of a mask protecting the bead filter and 50 μm etching using DRIE to define the channel. Steps 7-9 show the through-hole etching. The channels are sealed by anodically bonding silicon to borosilicate glass.

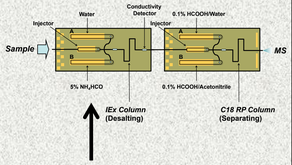




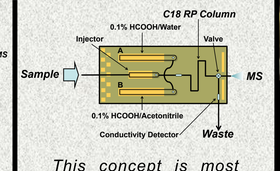
(Top) Photograph of the chip. (A) Post hard bake of 2nd mask. (B) Images of a through-hole chip interconnect, (C) on-chip evaporative tee union, (D) bead filter, (E) column channel. ***(Right) Cross-section of a packed channel.*** Electron micrographs of (A) chromatography media filter (3 μm x 80 μm), (B) unpacked microchannel cross- section, and (C) PRP-C18 5 μm packed microchannel cross-section.

DESALTING

The high levels of salt on planetary bodies including Mars, recently confirmed by probes such as the Phoenix Mars lander [8,9], can interfere with liquid-phase, *in situ* analytical instruments like capillary electrophoresis systems [10-12] that have been proposed for future landed missions. Therefore, we have proposed several desalting-chip concepts.



This front-end chip has lead to an ion chromatograph (IC)-chip [13].



This concept is most analogous to commercial HPLC/MS systems

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