MULTI-LAYER MICROFLUIDIC DEVICES FOR AMINO ACID ANALYSIS: THE MARS ORGANIC ANALYZER

Alison M. Skelley, James R. Scherer, Jeffrey L. Bada[1], Pascale Ehrenfreund[2], Frank J. Grunthaner[3] and Richard A. Mathies

Department of Chemistry, University of California, Berkeley, CA, 94720, USA; [1]Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA, 92093, USA; [2]Leiden Institute of Chemistry, Einsteinweg 55, 2300 Ra Leiden, The Netherlands; [3]Jet Propulsion Laboratory, 4800 Oak Grove Drive, Pasadena, CA, 91109, USA

Abstract

Sensitive amino acid composition and chirality analysis is demonstrated using the Mars Organic Analyzer (MOA), a portable microfabricated capillary electrophoresis (CE) instrument. The MOA integrates all high voltage power supplies, pneumatic controls, and fluorescence detection optics. The microfabricated device is a novel multi-layer structure combining glass separation channels [1] and microfabricated pneumatic membrane valves and pumps [2]. The Mars Organic Analyzer has been successfully field tested demonstrating part-per-trillion sensitivity when analyzing jarosite, a key sulfate-rich mineral recently detected on Mars that is associated with liquid water [3].

Keywords: microfabricated valves and pumps, capillary electrophoresis, astrobiology

1. Introduction

The detection of life on Mars requires identification of a suitable biomarker and development of sensitive yet compact instrumentation capable of performing in situ analyses. Our approach to this problem has focused on amino acid analysis because they are more resistant to decomposition than other biomolecules, and because amino acid chirality is a well-defined biomarker. Microfluidic devices provide an ideal platform for such analyses, allowing facile integration of dense microfluidic structures for sample preparation and capillary electrophoresis separation channels for sensitive and efficient analysis. Previously, we developed a prototype microfabricated capillary electrophoresis (CE) chip and analysis method where the amino acids were labeled with fluorescein and electrophoretically analyzed in under 3 minutes [4]. Our microchip analyzer has now been improved by using fluorescamine, a fluorogenic dye with much faster labeling kinetics [5]. Fluorescamine-labeled amino acids are separated using analogous conditions to those described earlier, resulting in similar separation times and identical elution orders. The present work is focused on developing the Mars Organic Analyzer (MOA), a portable analysis system that integrates the previously developed amino acid sample extraction capabilities of the Mars Organic Detector (MOD, [6]) with the composition and chiral analysis capabilities of microchip CE [1].

2. Results and Discussion

The microfabricated device for performing amino acid analysis is shown in Figure 1. The glass wafers are patterned and developed using standard photolithographic techniques [5]. The device includes microfabricated glass channels and as well as valves and pumps [2] for fluidic handling. The glass-enclosed separation channel is formed by thermally bonding the bottom channel layer with the middle manifold layer. The manifold wafer is blank on the bottom and patterned with the pneumatic manifold features (for vacuum/pressure lines and displacement chambers) on top. The fluidic wafer contains discontinuous channels patterned on its bottom surface to form the valve and fluidic routing structures. These membrane valves and pumps are integrated with the glass-enclosed separation channel by using a novel multilayer design in which sample enters the top fluidic layer for routing and is directed through drilled via holes to the bottom layer for analysis.

The microfabricated device is operated by the portable CE instrument shown in Figure 2 which contains pressure and vacuum pumps and solenoids for controlling fluidic valves, electronics for performing electrophoresis, a thermoelectric cooler and temperature sensor, a 15 mW 400 nm diode laser, confocal detection optics and filters, and a fiber-optic coupled photomultiplier for fluorescence detection. The device has a mass of ~ 11 kg and a peak power consumption of ~15 W.

The MOA was characterized by determining the limit of detection using different injection schemes. The "regular injection" consisted of a cross injection from sample to waste, presenting an unbiased population in the plug, followed by analysis. Alternatively the regular injection can be enhanced by injecting different lengths of plug directly toward the cathode in a 2-step process. A 2 second direct injection resulted in a 10 x increase in signal over the cross injection alone; a 10 second direct injection resulted in a 100 x increase although some amino acid resolution was lost.

The limit of detection for each injection technique was found through serial dilutions of the standard. The limit of detection of valine was 13 nM for the 10 second cross injection, 1.3 nM for the 2 second direct injection and 133 pM for the 10 s direct injection (Figure 3) which translates to part-per-trillion sensitivities in soil samples. Comparing the performance of the portable system to our previous bench-top system [5], identical concentrations run on the same microdevice resulted in equal separation efficiency and resolution but the portable system had 4-fold superior sensitivity.

The portable CE instrument, in combination with MOD, was recently successfully field tested using soil samples rich in jarosite from Panoche Valley, CA (Figure 4). Jarosite has recently been detected on Mars and is a key mineral indicating that liquid water was once present on the planet's surface. [3] Jarosite samples from soil were sublimed by MOD, and the microfabricated pumps were used to direct buffer through the MOA sipper to dissolve the sample. The sample was redirected to the separation channel for analysis. The jarosite sample was found to contain low levels of methyl and ethylamine (5 ppb), alanine/serine (0.5 ppb), glycine (0.2 ppb), glutamic (0.05 ppb) and aspartic (0.1 ppb) acid as well as a high concentration (~100 ppb) of valine.

3. Conclusions

The complete end-to-end field testing of the Mars Organic Analyzer has demonstrated the utility of this instrument for performing *in situ* amino acid analysis. The sensitivity and portability of the CE instrument and novel multi-layer microfabricated device demonstrates the utility of this approach for developing a wide range of versatile portable chemical and biochemical analysis microdevices. The Mars Organic Analyzer is currently in competition for the Mars Express (ESA) 2009 Mission, as well as the Mars Science Lander (NASA) 2009 Mission. For more details and pictures go to ***http://astrobiology.berkeley.edu.***

4. Acknowledgements

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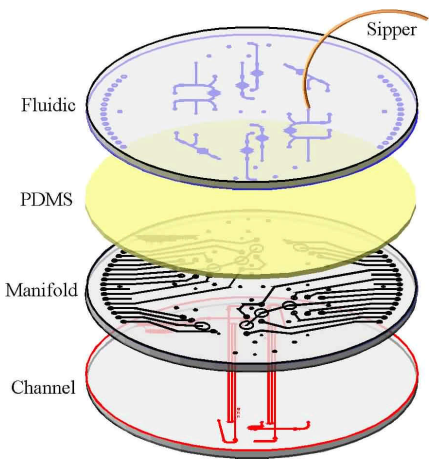

Figure 1. Microfabricated wafer for sample preparation and amino acid analysis. The 100-mm diameter microfabricated wafer stack is composed of a 4-layer sandwich of glass and PDMS to create channels and pumping structures.

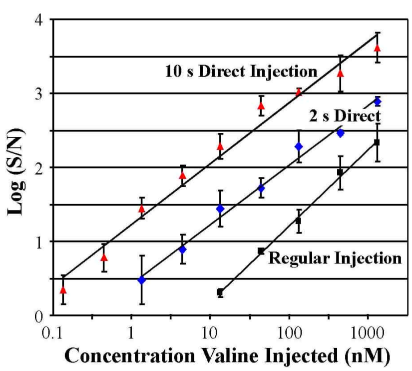

Figure 3. Limits of detection of the MOA system. The cross injection can detect down to 13 nM valine, the 2 second direct injection limits are 1.3 nM, and the 10 second direct injection limits are 130 pM.

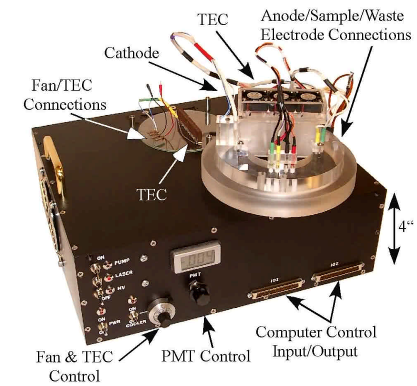

Figure 2. The Mars Organic Analyzer (MOA). The portable CE instrument, measuring 4" x 10" x 12", integrates all necessary pneumatic actuation, high voltage power supplies and confocal optics for laser excitation and fluorescence detection.

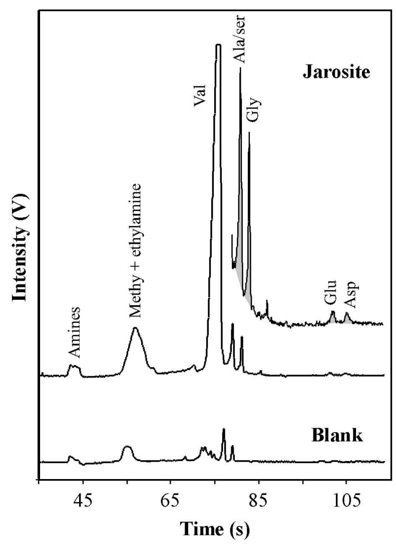

Figure 4. MOA field analysis in Panoche Valley, CA of a jarosite sample prepared by MOD. The peak identities were confirmed by spiking with standard samples.