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ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 2011

Impact Factor: 5.64 · DOI: 10.1021/ac202095k · Source: PubMed

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Toward Total Automation of Microfluidics for Extraterrestrial In Situ Analysis

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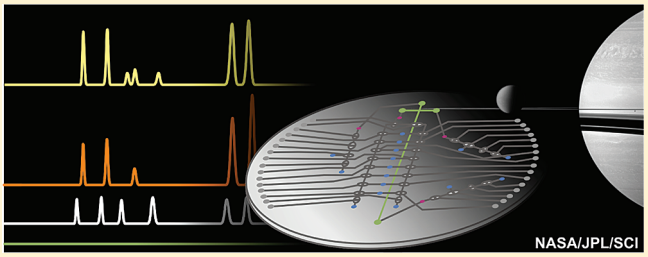
Supporting Information

ABSTRACT: Despite multiple orbiter and landed missions to extraterrestrial bodies in the solar system, including Mars and Titan, we still know relatively little about the detailed chemical composition and quantity of organics and biomolecules in those bodies. For chemical analysis on astrobiologically relevant targets such as Mars, Europa, Titan, and Enceladus, instrumentation should be extremely sensitive and capable of analyzing a broad range of organic molecules. Microchip capillary electrophoresis (μCE) with laser-induced fluorescence (LIF) detection provides this required sensitivity and targets a wide range of relevant markers but, to date, has lacked the necessary degree of automation for spaceflight applications. Here we describe a fully integrated microfluidic device capable of performing automated end-to-end analyses of amino acids by μCE with LIF detection. The device integrates an array of pneumatically actuated valves and pumps for autonomous fluidic routing with an electrophoretic channel. Operation of the device, including manipulation of liquids for sample pretreatment and electrophoretic analysis, was performed exclusively via computer control. The device was validated by mixing of laboratory standards and labeling of amino acids with Pacific Blue succinimidyl ester followed by electrophoretic analysis. To our knowledge, this is the first demonstration of completely automated end-to-end μCE analyses on a single, fully integrated microfluidic device.



One of the primary goals of planetary exploration is to determine the potential for past, present, or future life on extraterrestrial bodies. Despite multiple orbiter and landed missions to extraterrestrial bodies, such as Mars and Titan, we still know relatively little about their detailed chemical composition. Quantitative chemical compositional analysis provides vital chemical information on the processes that shape these environments, including details on abiotic or potentially biotic sources of organic chemistry. For example, abiotic processes lead to a racemic statistical distribution of organic molecules, while biotic processes enrich the few organic molecules essential for life resulting in homochirality.[1,2] In order to learn as much as possible about these environments, which may contain only trace quantities of organic material, fully automated instrumentation capable of sensitive analysis of a broad range of organic molecules is needed.

Capillary electrophoresis (CE) is a powerful liquid-based analytical technique that has been widely used for the analysis of a large range of biomolecules,[3−8] including amino acids,[9−12] and thus is a well-suited technique for in situ extraterrestrial chemical analyses. CE provides highly efficient separations with minimal sample consumption and short analysis times. There have been significant developments in the field of CE in the past decade in miniaturization to create portable separation and detection units.[13−16] In the field of planetary exploration, miniaturized CE systems have been incorporated into instrument prototypes for detection of life on other planetary bodies, including Mars.[17−20] CE has proven to be easily miniaturized to lab-on-a-chip (LOC) systems offering great versatility, custom design, high-throughput, and even lower volumes and times of analysis.[13] These microchip CE (μCE) devices have low mass, volume, and power requirements, making them well-suited for the scientific payloads of planetary probes.

Although several materials can be used to fabricate microchips, glass is highly suitable for in situ planetary exploration due to its compatibility with planetary protection and contamination control procedures used to ultrasterilize spacecraft probes and instruments. Additionally, the surface chemistry of glass is well-understood, which allows precise control of electro-osmotic flow, yielding highly efficient and reproducible electrophoretic separations. Finally, glass microchips have high mechanical strength, high chemical resistance and low conductivity and are compatible with optical detection techniques.[21]

A μCE analysis requires fluidic manipulation, which involves the incorporation of valves or switches into the design. A variety of microfabricated valves and pumps have been reported for on-chip fluidic manipulation.[22−31] Multiple devices for in situ planetary analyses have been published based on these designs, including

Received: August 9, 2011
Accepted: October 5, 2011
Published: October 05, 2011

simple fluidic routing structures, based on pneumatically actuated monolithic membrane microvalves.[17,19] These devices rely on a polymer elastomer, which can be PDMS or a fluorinated polymer such as Teflon or perfluoropolyether.[29,30] Typically, PDMS is used in the laboratory because it allows easy and rapid prototyping. While work to partially automate sample processing steps including fluorescent derivatization, serial dilutions, and mixing with a standard have been reported, to date these methods have inadequately integrated sample processing steps with sample analysis into a single device. Although the Mars Organic Analyzer (MOA) incorporated fluidic routing features into microfluidic systems, these methods required operator involvement and the use of PEEK tubing interfaces between two separate microdevices. To date, these devices have not incorporated all processing steps into a single microfluidic device.

Here, we present a fully integrated four-layer microchip electrophoresis device for end-to-end μCE analysis of amino acids. The device consists of bonded layers of Borofloat glass wafers and a flexible PDMS membrane. We demonstrate labeling, dilution, and separation of amino acids with this device with minimal operator intervention. The solutions were placed in the appropriate reservoirs at the beginning of the experiment, and all subsequent fluidic manipulations were performed via a microvalve circuit designed for autonomous investigations. The objective of this work was to develop and demonstrate a complete system that could thus be used for future in situ extraterrestrial exploration. All that would be required of the spacecraft probe would be to deliver an aqueous sample to a system having the capabilities described here. To the best of our knowledge, this is the first report of a LOC automated end-to-end μCE analysis of amino acids where ***all*** of the sample preparation and chemical analysis steps are performed on a single, fully integrated device.

EXPERIMENTAL SECTION

Reagents and Solutions. All chemicals were analytical reagent grade and used as received. Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Isopropyl alcohol was purchased from Sigma-Aldrich (St. Louis, MO). All aqueous solutions were prepared using 18 M$\Omega \cdot$cm water. The pH was adjusted using either 1 M NaOH or 1 M HCl (Sigma-Aldrich, St. Louis, MO) and measured using a glass electrode and a digital pH meter (Orion 290A, Thermo, Waltham, MA). L-Valine, L-alanine, L-serine, glycine, and L-citrulline were purchased from Sigma-Aldrich (Saint Louis, MO). Pacific Blue succinimidyl ester (PB) was purchased from Invitrogen (Carlsbad, CA). Stock solutions of amino acids (10 mM in water) and Pacific Blue (20 mM in dimethylformamide (DMF)) were prepared and kept frozen (−20 °C) when not in use. The labeling reaction was performed on and off-chip by mixing amino acids and PB (200 μM) and allowing the reaction to proceed for at least 1 h. The reaction was performed in 25 mM tetraborate buffer, pH 9.2. Other amino acid solutions were prepared by diluting the corresponding amount of stock in buffer.

Microfabrication. Multilayer microdevices were prepared as previously described with minimal modifications.[17,27] For the μCE/pneumatic layers, 100 mm diameter 1.1 mm thick Borofloat wafers were coated with >2000 Å aSi using LPCVD. The wafers were spin-coated with SPR 220-7 photoresist and patterned with the desired features. The aSi hard mask was patterned using SiF_6 plasma, and the Borofloat was wet-etched using 49% HF. The electrophoretic layer was patterned first, while the pneumatic layer face was protected with blue tape. The pneumatic layer was patterned second, while the electrophoretic layer was protected with blue tape, except during backside alignment steps. Fluidic and electrophoretic access holes were drilled in the wafer with a diamond-tipped drill bit, then the aSi hard mask was stripped. After dipping the wafer and a 700 μm backing wafer in AZ developer, the two wafers were manually pressed together to create a low-temperature temporary bond. This bond was solidified by bonding between two Macor blocks under weights at 668 °C for 12 h. A fluidic wafer was patterned and etched in a similar manner, and both the bonded two-wafer stack and the fluidic wafer were ashed with O_2 plasma prior to being bonded in a four-layer stack utilizing a 250 μm PDMS gasket sandwiched between the glass layers.

The microfabrication process produced separation channels that were 8.5 cm long, 50 μm wide, 20 μm deep, with a 1.0 cm long cross injection channel located at 0.5 cm from the anode. Pneumatic features were etched to 300 μm depth, while the fluidic features were etched to 150 μm depth. Holes were punched immediately post-assembly in the PDMS membrane to enable fluidic access between the fluidic wafer and the μCE reservoirs. The assembly of the microdevice is depicted in Figure 1A. Additional fluidic reservoirs were made in 3 mm thick PDMS using a 4 mm diameter circular punch and bonded to the uppermost glass surface of the fluidic wafer. Finally, pipettor tips were placed in these PDMS fluidic reservoirs to hold larger volumes (<1 mL) when needed.

Microdevice Operation. Devices were mounted inside a polycarbonate fixture designed in our laboratory (see Figure SI-1 in Supporting Information). The assembly was placed on the microscope stage for fluorescence detection. The top fixture was used to make individual pneumatic connections between each of the 32 pneumatic access holes and a manifold containing 32 solenoid valves. Depending upon the state of the solenoid valve, microvalves are either open (i.e., when solenoid is connected to vacuum) or closed (when solenoid is connected to N_2). A GAST vacuum pressure pump, Xantrex XHR 300-2 dc power supply, National Instruments cDAQ-9172 CompactDAQ chassis, and National Instruments 9472 8-channel output module were used for this purpose. Figure 1B shows a schematic drawing of the 32 valve device used for all of the experiments described here. A National Instruments LabView 8.5 virtual instrument interface was employed to control opening and closing of the valves.

Microchip Capillary Electrophoresis. A LabSmith HVS448 high voltage sequencer (Livermore, CA) was used to control voltages applied to electrophoresis wells during injection (25 s) and separation (200 s). Laser-induced fluorescence detection was performed with a commercial Nikon Eclipse TE2000-U inverted microscope system. A 405 nm Melles Griot diode laser (CVI Melles Griot, Carlsbad, CA) was used for excitation, and emission was detected by a CCD camera (Cascade 650, Photometrics). Tetraborate buffer (25 mM, pH 9.2) was used for labeling reactions and all CE separations. The separation channel was conditioned before use with 0.1 M NaOH for 10 min, followed by water and buffer for 5 min each. Initial experiments to optimize labeling conditions were performed on a commercial microchip (Micralyne Inc., Edmonton, Canada). For the four-layer device, prior to each experiment, buffer was pumped to each reservoir and a baseline electropherogram was acquired. Then, the sample reservoir was emptied, the sample was pumped into the reservoir, and the CE separation was performed. After each

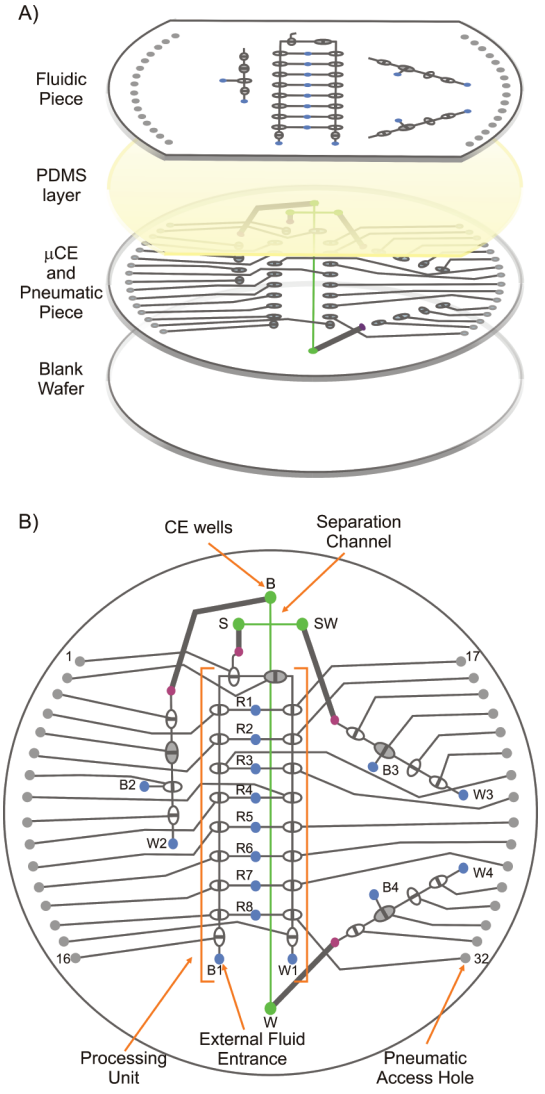


Figure 1. Microfluidic circuit for end-to-end μCE analysis. Schematic representation of (A) assembly of the four-layer device and the features of each layer, and (B) microfluidic circuit layout including separation channel and μCE reservoirs: sample (S), sample waste (SW), buffer (B), and waste (W), as well as fluid reservoirs. Reservoirs containing buffer for delivery to μCE wells are R1, B2, B3, and B4. Buffer reservoir B1 is used to rinse the processing unit to W1 (waste) between runs. The wells S, SW, B, and W can be emptied to the respective waste reservoirs W1, W3, W2, and W4. The processing unit contains reservoirs R1 to R8 for sample, standards, and reagents handling.

run, the sample was pumped in and out of the CE well for 10 cycles in order to ensure complete mixing, and then the next separation was performed. During injection, 700, 1000, 0, and 900 V were applied to buffer, sample, sample waste, and waste, respectively. After 25 s, the potentials were switched to 3000, 1700, 1700, and −2000 V. Data were processed using PeakFit (Systat Software Inc., San Jose CA). Electropherograms were baseline-corrected and filtered using a 0.2% Loess function. Peak migration times were corrected using Origin 8.1 (OriginLab Corporation, Northampton, MA) to account for run-to-run variations.

Off-Chip Fluorescence Measurements. A Fluorolog-3 fluorescence spectrometer (Horiba Jobin-Yvon) was used to determine the fluorescence of solutions of Pacific Blue. Solutions were placed in quartz cuvettes (1 cm path length), and the emission spectra (excitation at 405 nm, emission from 410 to 550 nm) were obtained in triplicate. All spectra were obtained as a ratio of corrected signal to corrected reference (S_c/R_c) to eliminate the effect of varying background radiation in the sample chamber; emission intensities are in units of counts per second per microampere (cps/μA). Spectra were processed with PeakFit (Systat Software Inc., San Jose, CA) to obtain the maximum signal.

RESULTS AND DISCUSSION

Pacific Blue was selected as the fluorescent label for this study based on its high extinction coefficient (46 000 cm^{-1} M^{-1}) that enables low limits of detection of amino acids in extraterrestrial sample extracts.[20,32] A set of five amino acids that are well-resolved by microchip capillary electrophoresis was used as a standard mixture to demonstrate the capabilities of the device proposed here. Among the amino acids that are expected in extraterrestrial samples,[33−35] we selected for our standard mixture two major amino acid components of meteorites[33] that have also been produced by some abiotic model syntheses[36] (alanine and glycine), two minor components of meteorites (serine and valine), and we also included a terrestrial contamination marker from the urea cycle (citrulline[37]).

In order to optimize the sensitivity of LIF to trace quantities of amino acids, we studied the response of a mixture of amino acids (Cit, Val, Ser, and Gly) labeled with Pacific Blue over a range of different experimental conditions (see SI Figure 1 and SI Figure 2 in Supporting Information). The highest labeling efficiency was obtained at pH ∼9. It was also observed that a high ratio of PB to amino acid improves the labeling efficiency; we thus selected a ratio of 20 to ensure the proper labeling of low concentrations of amino acids on the sample.

Device Characterization. The design of the microfluidic device used for all of the experiments described here is shown in Figure 1B. There are 32 valves arranged in three sets of four valves used to pump fluid in and out of the μCE wells (B, SW, and W) and 20 valves located in the processing unit. This unit contains eight fluidic reservoirs (R1−R8) that can be used for storage of samples, reagents, and standards as well as for performing all of the sample pretreatment steps before electrophoretic separation. This unit is connected to the sample μCE well (S), so any solution placed on the fluidic reservoirs (R1−R8) can be analyzed by the CE system.

Each microvalve consists of an etched displacement chamber on the top surface of the pneumatic manifold wafer, an elastomeric membrane layer, and a discontinuous channel structure on the bottom of the fluidic wafer. Applying vacuum to the displacement chamber pulls the membrane down, opening the valve and allowing connection of the fluidic channels across the discontinuity and movement of fluids. The valve is closed by releasing the vacuum or applying a slight pressure. As previously described,[27] by actuating three serially connected valves in a cycle, a self-priming diaphragm pump is created. These valves were fully characterized by Grover et al.[27] The flow rates obtained with this type of pump depend on the dimensions of the fluidic channel, the displacement chamber volume, and the valve actuation times. The actuation times also determine the total time required for each pumping step, so optimization is required in order to pump the highest volume of liquid in the least possible time.

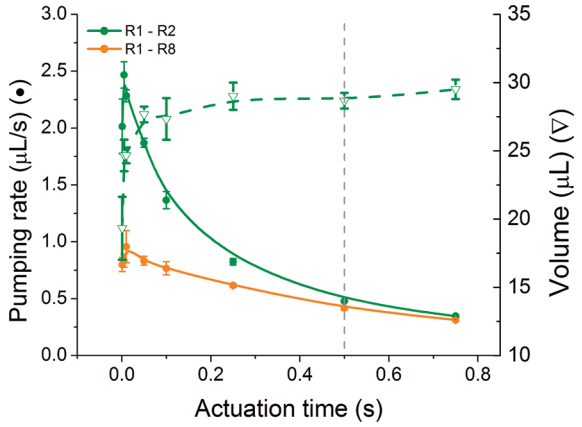


Figure 2. Pumping rate (●) and volume (∇) for 20 cycles pumping from R1 to R2 (green line) and R1 to R8 (orange line). Volumes were measured in triplicate, and error bars were calculated as the standard deviation of the three measurements.

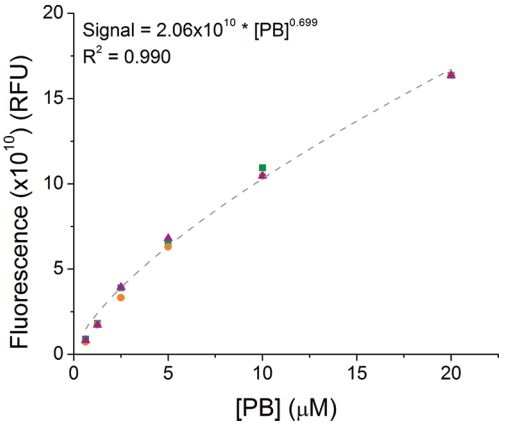


Figure 3. Fluorescence signal obtained for solutions of PB diluted sequentially from a 20 μM solution, (green ■) off-chip, (orange ●) on-chip repetition 1, (violet ▲) on-chip repetition 2. Dashed line represents the power fit for the standard dilution performed off-chip.

Several elastomeric polymers have been integrated with glass to form monolithic membrane microvalve devices. While fluorinated membranes have been developed in our lab and proven suitable for the extreme conditions associated with extraterrestrial analyses, we selected polydimethylsiloxane (PDMS) membranes based on their ease of fabrication for rapid prototyping.[22,27]

In order to determine the optimal actuation time for our fluidic design, the volume of water transferred in 20 cycles from R1 to its closest and farthest neighbor reservoirs, R2 and R8, respectively, was measured for actuation times ranging from 10 to 750 ms. Figure 2 shows the pumping rate as a function of actuation time for R1 to R2 and R1 to R8 and also the total volume displaced from R1 to R2. For the minimum-distance transfer (R1−R2), the pumping rate decreases, but the volume increases with longer actuation times. At fast actuation times, the valves do not fully open or close in each cycle, so less volume than the maximum is transferred in each cycle. For actuation times ≥250 ms, the valves have enough time to fully open and close, and consequently, the volume pumped per cycle no longer increases. It can also be observed in Figure 2 that the pumping rates for R1 to R8 are smaller than those for R1 to R2 for actuation times less than 500 ms. Because the fluidic resistance is higher for reservoirs and valves farther apart in the layout, longer actuation times are required to transfer the same volume of fluid. Even though a 500 ms actuation time increases the total time required for each cycle, it was selected as the optimum value for the rest of the experiments because it permits a uniform pumping rate throughout the processing unit, simplifying the operation of the system. To evaluate chip-to-chip reproducibility, the volume pumped from R1 to each of the other reservoirs with an actuation time of 500 ms was measured for two microfluidic devices. The average volumes obtained for each device were 12 ± 1 and 11.2 ± 0.8 μL, indicating that although the alignment may be slightly different for different devices due to deviations in human hand-to-eye coordination, the pumping rates are consistent. All valves remained functional after the device was stored for a period of eight months. Additionally, we did not observe any microdevice failure (by PDMS irreversible sealing to the glass) after approximately 100 000 actuations over a period of 10 days of continuous use.

To validate the proposed microfluidic circuit for quantitative manipulation of fluids, serial dilutions of a 20 μM PB solution were performed both manually off-chip and on-chip in duplicate. The fluorescence of the solutions was then measured and correlated to the expected concentration. Figure 3 shows the signal as a function of the concentration of PB for the three sets of sequential dilutions (20 to 0.625 μM). As can be observed in the figure, the dilutions performed using the automated pumping system correlate well with the dilutions performed manually off-chip, thus validating the system for quantitative handling of solutions prior to analysis by μCE.

End-to-End Analysis. Once the operation of the processing unit was optimized and validated, an automated μCE end-to-end analysis of amino acids involving labeling, dilution, and spiking was performed. For the end-to-end analysis, R1 was filled with buffer, R2 with 50 μL of PB (500 μM), R3 with 250 μL of the three unlabeled amino acid (simulated unknown sample) mixture (10 μM each), and R8 with 250 μL of the PB-labeled amino acid standard (100 nM each). The rest of the reservoirs were used for dilution of the labeled sample (R5 and R6) and for mixing with the standard solution (R7). The sequences of mixing and dilutions performed are described briefly here; more detail is provided in the Supporting Information. The experiments were performed in the following sequence. First, sample and dye were mixed; the solution was then left to react for at least 1 h and then diluted to a final concentration of 160 nM. The processing unit was then rinsed to remove residual sample (labeled or unlabeled amino acids) to avoid contamination or false positives. Then, buffer was pumped to each CE well (50 μL), and a blank electropherogram was obtained (Figure 4A). The sample reservoir was emptied and filled with the amino acid standard solution, and three electrophoretic separations were acquired (Figure 4B). After analyzing the standard, the processing unit was rinsed with buffer, S was emptied and filled with buffer, and a blank run was acquired again to ensure that the system was clean. Next, S was emptied and then filled with the on-chip-labeled sample, and electropherograms were obtained (Figure 4C). As can be observed in the figure, the sample was successfully labeled on-chip. The system was then rinsed, and the blank run was acquired again. Finally, the on-chip-labeled sample and the standard were mixed and pumped to S for analysis (Figure 4D). The presence of five peaks in Figure 4D indicates that the sample and the standard were well mixed on-chip. A dilution effect (smaller peaks) is observed for Ser and Gly, which were only present in the standard,

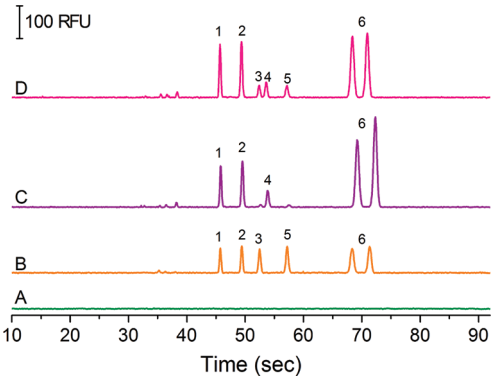


Figure 4. Results from a completely automated on-chip analysis of amino acid mixtures. Labeling and analysis of amino acid mixtures on-chip. (A) blank run (buffer only); (B) standard solution containing 100 nM of PB-labeled (1) Cit, (2) Val, (3) Ser, and (5) Gly; (C) ~160 nM sample of (1) Cit, (2) Val, and (4) Ala diluted from 10 μM sample labeled on-chip; (D) mixture of (B) and (C). Conditions: 25 mM tetraborate buffer, pH 9.2, V_{SEP} = 5 kV.

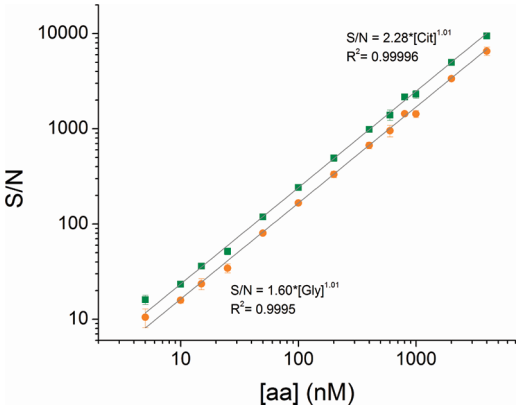


Figure 5. Calibration curves for (green ■) Cit and (orange ●) Gly. Conditions: 25 mM tetraborate buffer, pH 9.2, V_{SEP} = 6 kV. Each concentration was measured in triplicate, and error bars were calculated as the standard deviation of the three measurements.

and for Ala, which was only present on the simulated sample. Although each solution was measured in triplicate, Figure 4 only shows one electropherogram for clarity of data presentation.

The sensitivity of our system was tested with the standard mixture of amino acids described earlier. Figure 5 shows the calibration curves obtained manually for solutions of Cit and Gly diluted from a stock labeled with PB. The LOD values were calculated as the concentration yielding a signal-to-noise ratio (S/N) of 3. LOD values for Cit, Val, Ser, Ala, and Gly were 0.94 ± 0.01, 0.70 ± 0.01, 1.17 ± 0.02, 1.05 ± 0.01, and 1.33 ± 0.02 nM, respectively.

Due to the significant increase in capability represented by the novel microdevice demonstrated here and the challenge of obtaining relevant samples of astrobiological interest, the automated analysis of real samples remains outside the scope of this work. We are currently pursuing collaborations to obtain relevant samples; their autonomous analyses will be published separately.

CONCLUSIONS

This paper describes a novel four-layer fully integrated microfluidic device that successfully executes automated end-to-end microchip capillary electrophoresis analyses. The performance of the device was demonstrated by electrophoretic analysis of a sample of amino acids labeled, diluted, and spiked on-chip. The entire process was exclusively controlled via computer, and the only intervention of the operator was to place the solutions in reservoirs prior to beginning the experiment. To our knowledge, this is the first demonstration of a completely automated end-to-end μCE analysis of amino acids on a single, fully integrated microfluidic device. This effort is critical in advancing the technology readiness level of μCE systems for future in situ spaceflight investigations to targets such as Mars, Europa, and Titan, etc., where complete automation is required. In addition, the fully autonomous fluidic processing for microchip capillary electrophoretic analyses reported here also has a number of terrestrial applications, ranging from point-of-care analyses to environmental monitoring.

ASSOCIATED CONTENT

Supporting Information. Additional information as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

The first two authors contributed equally to this work. The research described in this paper was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Financial support for this project was provided by NASA's Astrobiology Science and Technology Instrument Development (ASTID) Program (Project #104320), the NASA Harriett G. Jenkins Predoctoral Fellowship Project (JPFP) Mini Research Award, and the NASA Postdoctoral Program (NPP) at the Jet Propulsion Laboratory, administered by Oak Ridge Associated Universities through a contract with NASA. We thank Dr. Morgan L. Cable for her help with the fluorescence measurements, and Dr. Adrian Ponce's lab for the use of the Fluorolog-3 fluorescence spectrometer.

REFERENCES

(1) McKay, C. *Space Sci. Rev.* **2008**, *135* (1), 49–54.
(2) McKay, C. P. *PLoS Biol.* **2004**, *2* (9), e302.
(3) Mora, M. F.; García, C. D. *Electrophoresis* **2007**, *28* (8), 1197–1203.
(4) Smith, J. T.; el Rassi, Z. *Electrophoresis* **1993**, *14* (5−6), 396–406.
(5) Arvidsson, B.; Johannesson, N.; Citterio, A.; Righetti, P. G.; Bergquist, J. *J. Chromatogr. A* **2007**, *1159* (1−2), 154–158.
(6) Guzman, N. A.; Blanc, T.; Phillips, T. M. *Electrophoresis* **2008**, *29* (16), 3259–3278.
(7) Blanes, L.; Mora, M. F.; Ayon, A.; Do Lago, C. L.; Garcia, C. D. *Electroanalysis* **2007**, *19*, 2451–2456.
(8) Simionato, A. V. C.; Carrilho, E.; Tavares, M. F. M. *Electrophoresis* **2010**, *31*, 1214–1226.

(9) Zhou, S. Y.; Zuo, H.; Stobaugh, J. F.; Lunte, C. E.; Lunte, S. M. *Anal. Chem.* **1995**, *67* (3), 594–599.
(10) Schultz, C. L.; Moini, M. *Anal. Chem.* **2003**, *75* (6), 1508–1513.
(11) Garcia, C. D.; Henry, C. S. *Anal. Chem.* **2003**, *75* (18), 4778–4783.
(12) Simionato, A. V. C.; Moraes, E. P.; Carrilho, E.; Tavares, M. F. M.; Kenndler, E. *Electrophoresis* **2008**, *29*, 2051–2058.
(13) Dittrich, P. S.; Tachikawa, K.; Manz, A. *Anal. Chem.* **2006**, *78* (12), 3887–3908.
(14) Berg, C.; Valdez, D. C.; Bergeron, P.; Mora, M. F.; Garcia, C. D.; Ayon, A. *Electrophoresis* **2008**, *29* (24), 4914–4921.
(15) Jackson, D. J.; Naber, J. F.; Roussel, T. J., Jr.; Crain, M. M.; Walsh, K. M.; Keynton, R. S.; Baldwin, R. P. *Anal. Chem.* **2003**, *75*, 3311–3317.
(16) Felhofer, J. L.; Blanes, L.; Garcia, C. D. *Electrophoresis* **2010**, *31* (15), 2469–2486.
(17) Skelley, A. M.; Scherer, J. R.; Aubrey, A. D.; Grover, W. H.; Ivester, R. H. C.; Ehrenfreund, P.; Grunthaner, F. J.; Bada, J. L.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (4), 1041–1046.
(18) Hutt, L. D.; Glavin, D. P.; Bada, J. L.; Mathies, R. A. *Anal. Chem.* **1999**, *71* (18), 4000–4006.
(19) Benhabib, M.; Chiesl, T. N.; Stockton, A. M.; Scherer, J. R.; Mathies, R. A. *Anal. Chem.* **2010**, *82* (6), 2372–2379.
(20) Chiesl, T. N.; Chu, W. K.; Stockton, A. M.; Amashukeli, X.; Grunthaner, F.; Mathies, R. A. *Anal. Chem.* **2009**, *81* (7), 2537–2544.
(21) Harrison, D. J.; Manz, A.; Fan, Z.; Luedi, H.; Widmer, H. M. *Anal. Chem.* **1992**, *64* (17), 1926–1932.
(22) Hosokawa, K.; Maeda, R. *J. Micromech. Microeng.* **2000**, *10* (3), 415–420.
(23) Chen, C.-F.; Liu, J.; Chang, C.-C.; DeVoe, D. L. *Lab Chip* **2009**, *9* (24), 3511–3516.
(24) Weibel, D. B.; Kruithof, M.; Potenta, S.; Sia, S. K.; Lee, A.; Whitesides, G. M. *Anal. Chem.* **2005**, *77* (15), 4726–4733.
(25) Unger, M. A.; Chou, H.-P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288* (5463), 113–116.
(26) Grover, W. H.; Muhlen, M. G. v.; Manalis, S. R. *Lab Chip* **2008**, *8* (6), 913–918.
(27) Grover, W. H.; Skelley, A. M.; Liu, C. N.; Lagally, E. T.; Mathies, R. A. *Sens. Actuators, A* **2003**, *89* (3), 315–323.
(28) Hisamoto, H.; Funano, S.-i.; Terabe, S. *Anal. Chem.* **2005**, *77* (7), 2266–2271.
(29) Willis, P. A.; Greer, F.; Lee, M. C.; Smith, J. A.; White, V. E.; Grunthaner, F. J.; Sprague, J. J.; Rolland, J. P. *Lab Chip* **2008**, *8* (7), 1024–1026.
(30) Willis, P. A.; Hunt, B. D.; White, V. E.; Lee, M. C.; Ikeda, M.; Bae, S.; Pelletier, M. J.; Grunthaner, F. J. *Lab Chip* **2007**, *7* (11), 1469–1474.
(31) Guillo, C.; Karlinsey, J. M.; Landers, J. P. *Lab Chip* **2007**, *7* (1), 112–118.
(32) Stockton, A. M.; Chiesl, T. N.; Lowenstein, T. K.; Amashukeli, X.; Grunthaner, F.; Mathies, R. *Astrobiology* **2009**, *9* (9), 823–831.
(33) Botta, O.; Bada, J. L. *Surv. Geophys.* **2002**, *23* (5), 411–467.
(34) Ehrenfreund, P.; Glavin, D. P.; Botta, O.; Cooper, G.; Bada, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (5), 2138–2141.
(35) Glavin, D. P.; Dworkin, J. P.; Sandford, S. A. *Meteorit. Planet. Sci.* **2008**, *43* (1−2), 399–413.
(36) Parker, E. T.; Cleaves, H. J.; Dworkin, J. P.; Glavin, D. P.; Callahan, M.; Aubrey, A.; Lazcano, A.; Bada, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (14), 5526–5531.
(37) Curis, E.; Nicolis, I.; Moinard, C.; Osowska, S.; Zerrouk, N.; Bénazeth, S.; Cynober, L. *Amino Acids* **2005**, *29* (3), 177–205.