CHANNEL-TO-DROPLET EXTRACTIONS FOR ON-CHIP SAMPLE PREPARATION

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ABSTRACT

We report a new capability in on-chip sample preparation for digital microfluidic lab-on-chip systems: “channel-to-droplet” sample extraction by integrating proven capillary electrophoretic (CE) separation and electrowetting-on-dielectric (EWOD) droplet manipulation. This sample preparation approach consists of: a) separating particles within a channel using conventional in-channel CE methods and then b) extracting the concentrated particle bands from a channel opening in a mobile droplet form using EWOD actuations. The extraction enables the concentrated sample droplet to be further processed on chip or presented to ancillary devices for amplifications or sensing. The concept is successfully demonstrated by separating two types of bead particles by CE separations in a channel and extracting particles from a channel opening in droplet form with EWOD. Integration of proven CE technology with new droplet-based EWOD digital microfluidics will enable highly flexible sample separation and transport options while minimizing sample and reagent consumptions.

INTRODUCTION

While high performance liquid chromatography (HPLC) dominates capillary electrophoresis (CE) in analytical practice, CE is also popular to implement on microfluidic analysis and sensing chips due to simple fabrication methods and a low reliance on high pressure fluidics. Since early 1990’s [1], there have been numerous reports on microfabricated on-chip CE devices including demonstration and performance improvement. [2, 3] However, conventional CE separation methods typically require sample analysis to be conducted within or about the CE channel to minimize transport-driven diffusion/mixing of concentrated

sample/solute bands; this imposes certain limitations on the choice and interchangeability of sensing mechanisms. If the purified samples can be transferred to off-channel and user-selected analytical sites without undergoing diffusion or pressure-driven dispersion, highly flexible and multi-staged sample preparation and analysis processes can be devised, opening the door for many powerful and cost effective analytical or immuno-sensing devices.

To enable transport of channel-concentrated samples to off-channel analysis or extraction points with little or no reduction in sample concentration, we have developed novel “channel-to-droplet” sample extraction capabilities that combine well-proven on-chip CE separation methods [1] with electrowetting-on-dielectric (EWOD) based droplet transport [4]. In our approach (Fig. 1):

- unconcentrated samples can be pipetted or driven in a droplet form by EWOD transport into a channel,
- then separated and purified in the channel by CE (Fig. 1 (a)),
- and finally extracted out of a channel side opening in droplet form by EWOD (Fig. 1 (b)).

Figure 1. “Channel-to-droplet” sample extraction and purification is achieved by integrating channel based CE separation and EWOD droplet extraction.

- b. CE channel separates samples into concentrated particle bands
- c. Particle bands are extracted from channel opening in droplet form.

Figure 2. Conceptual lab-on-chip system combines proven CE separation methods with EWOD-based digital microfluidics to achieve channel-to-droplet sample extraction for: 1) routing of concentrated sample droplets to on-chip sensing sites (shown in the figure) or 2) off-chip transfer to ancillary analytical devices (not shown in the figure). Since the concentrated sample bands are isolated as discrete droplets, the concentration is not compromised by dispersion or diffusion during sample handling downstream.

The concentrated sample droplet (sub- μ l) can then be routed to other downstream processes (Fig. 2) or extracted for off-chip analysis. Figure 2 shows a conceptual EWOD digital microfluidic system with an integrated CE channel. Transportations of sample droplets and down stream processes are also illustrated.

EWOD based digital microfluidics enables the unique extraction and transport of sample droplets out of the CE channel, but also achieves: a) a reduction in sample and reagent consumption from more conventional continuous microfluidics, and b) a simplification of device design and fabrication since no pumps, channel structures or high pressure valve elements are needed.

To demonstrate the concept, CE separation of bead particles and extraction of sample with droplet by EWOD transport are designed and carried out. Two types of bead particles (i.e. $\sim 10\ \mu\text{m}$ red carboxyl modified atax (CML) beads and white amino-sulfate coated latex beads from Interfacial Dynamics Corp.) were selected in the tests to simulate biological particles.

DESIGN AND FABRICATION

A key for complete and repeatable EWOD droplet extractions

Figure 3. Chip design for channel-to-droplet extraction. (a) Refill droplet is introduced to mitigate channel capillary forces and enable full droplet extraction from channel opening. (b) Mask layouts, showing EWOD drive pads merge refill droplet to CE channel and extract sample droplet from it

from a channel side opening lies in the mitigation of the high pull-back forces within a channel (caused by hydrophilic channel interiors) that resist fluid extraction (Fig. 3(a)). This competition between inherent capillary forces and active EWOD wetting effects yields inconsistent, if not failed, droplet extraction. We found this pull-back can be mitigated by inserting an additional fluid volume (refill droplet) into one side of the extraction opening when a concentrated sample droplet is extracted out the opposite side. By merging a refill droplet into the channel just prior to or during droplet extraction, the resistive tension of the capillary forces was temporarily neutralized, enabling a successful and repeatable droplet extraction. This simultaneous insertion and extraction can be achieved with a column of EWOD drive pads positioned to pass through the channel opening (Fig. 3(b)).

Test chips (Fig. 4) consisting of top/bottom plates and $\sim 1.25'' \times 2.0''$ in dimensions were fabricated for concept demonstration. The bottom plate contained EWOD and CE electrodes patterned and passivated with SiO_2 . Additionally, a $100\ \mu\text{m}$ -wide and 60mm -long CE channel was constructed with photoreist (PR) walls. For sample extraction from the channel, the constructed channel has side openings or breaks to allow pass-through of the EWOD driving electrodes. The top plate is coated with ITO and SiO_2 and provides electrical referencing during EWOD operation. A Cytop® layer is spin-coated onto both plates to achieve hydrophobic surface conditions (inner channel surfaces were not exposed to Cytop® to enable capillary loading of buffer). The extraction site is shown magnified in the photo inset.

Figure 4. Testing chip. Top plate is removed for a better picture quality. The inset magnifies the extraction site (with top plate in place), showing the open section of the channel.

EXPERIMENT AND RESULT

To demonstrate the concept, we have tested CE separation and sample extraction by EWOD separately with the devices described above. The fabricated chips were evaluated on a testing stage, which provided the connections for programmed electrical signals from our control circuit for EWOD and microscope based imaging. For the separation tests, a DC power supply was connected to the test stage instead of EWOD control circuit connectors.

For CE separation, the microchannel was first filled with water or buffer solutions by capillary force to provide filling medium in the channel before loading sample solutions. Since inner surfaces of the channel are hydrophilic, an introduction of

liquid at any end of the channel fills the channel. However, it is important to ensure that water column is connected throughout the channel (including the channel break for extraction) between two CE electrodes. Once the channel was filled, a drop of solution containing mixtures of two bead particles (i.e. red CML and white Amino-Sulfate beads) was added at the sample input reservoir (Fig. 3 (b)) and a voltage (~ 75 V_{DC}) was applied across two CE electrodes located at the ends of the channel to initiate the separation.

Figure 5 illustrates the result of successful separation of bead particles. During the separation, the red CML beads move faster than white amino-sulfate beads due to mobility difference and become concentrated in band while moving to the extraction point (Fig. 5(a)). A concentrated band of white beads is also formed in the channel (Fig. 5(b)) as they became separated.

The two separated bands of particles then enter the extraction site, with the red beads entering first (Fig. 5(c)) and then followed by the white beads (Fig. 5(d)).

(a) Red beads are separated along the CE channel and are concentrated in a band while moving to the extraction site
(b) White beads are also concentrated in a band-like formation while moving through the CE channel to the extraction site

(c) Red beads arrive first at extraction site.
(d) White beads arrive later at extraction site

Figure 5. CE separation of two bead types and collection of the separated beads at the extraction site.

For the droplet extraction test, a water droplet (~ 2 μ l) was pipetted onto the bottom chip and pushed down with top cover plate during the assembly of the device. A solution with two types of beads particles was also preloaded in the channel. Successful sample extraction by EWOD is illustrated in Fig. 6.

A refill droplet was moved into the left side of the channel opening, just as droplet extraction was initiated to the right by EWOD actuation (~ 60 V_{rms} (@1kHz)). Refill droplet insertion and sample droplet extraction were performed simultaneously to neutralize the channel pull-back force. (Figs. 6(c)–6(f)) Based on visual inspection, more than 90% of particles were extracted from the channel break into a separated and mobile droplet. (Figs. 6(g)–6(h))

Figure 6. Photos show merging of refill droplet and extraction of sample droplet from channel break (opening). Channel and EWOD drive pad dimensions are annotated in extraction site close-up on Fig. 4.

CONCLUSIONS

The concept of “channel-to-droplet sample preparation” utilizing well-established capillary electrophoresis and EWOD digital microfluidics has been introduced and demonstrated with micromachined devices. We have successfully introduced and separated a mixture of red CML and white amino-sulfate bead particles in a CE channel. We also have successfully extracted samples from a channel in droplet form using EWOD actuations and established the proposed functionalities.

ACKNOWLEDGEMENT

This work was funded by Department of Homeland Security (HSARPA SBIR NBCHC050123). The authors would like to thank Professor Sungkwon Cho in University of Pittsburgh for valuable discussions on general EWOD principles.

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