A PDMS Sample Pre-treatment Device for the Optimization of Electrokinetic Manipulations of Serum

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Abstract

Raw sample pretreatment is a critical step for rapid diagnostic tools that involve the detection of biomarkers from complex biological samples. In order for detection to occur, target markers are usually isolated from their sample of origin by means of various sample preparation techniques. However, some sample properties are not optimized for μ–processes, such as electrophoresis and dielectrophoresis. To address these concerns, a pretreatment PDMS, microfluidic chip has been fabricated that demonstrates selective tuning of key parameters of blood serum in order to enable effective sample processing and Electrokinetic manipulations. In this work, the optimized solution parameters include viscosity, pH, and conductivity. Using previous literature related to capillary electrophoresis[1,2,3], a bench-scale pretreatment protocol was developed to tune these parameters to an optimal range. A PDMS device based on a previous AC micromixer design[4] was fabricated and used to combine raw sample with specific buffer solutions. Off-chip electrodes were used to induce Electrokinetic micromixing in the mixing chamber. Homogeneous analyte mixing was achieved in 1.5 seconds using an 800 V DC pulse.

Motivation / Background

Most point-of-care diagnostic instruments employ the following modular paradigm:

From this common paradigm, it is abundantly clear that without proper sample preconditioning, desired processing and detection are not possible; hence, the focus of this project is the selective tuning of pH and conductivity to optimize blood serum samples to enable downstream Electrokinetic manipulations. Most of the current preconditioning protocols are performed off-chip following sample collection. Therefore, we propose that we can accomplish desired sample preconditioning directly on chip by introduction of raw sample and a sample-specific buffer solution. In this work, we demonstrate this hypothesis using bovine calf serum.

Methods

Pretreatment: Buffer Selection

Since the primary downstream processing steps will likely involve bulk separations of blood serum into its five main protein fractions, our operational buffers were based on data from capillary electrophoresis of serum protein fractioning[1,2,3].

The two buffer solutions used were 0.1M MGA-0.1M EACA* and 0.1M MGA - 0.1M GABA** which have been found to yield high resolutions in electropherograms of serum protein fractions[1]. After testing different concentrations and amounts of each of the three buffers, we determined that a 5:1 (v/v) ratio of 0.1M MGA to 0.1M of EACA or GABA were suitable to pre-condition a sample of bovine calf serum to an optimal pH range. Conductivity was linearly dependent on the volume ratio of serum added to the buffers (Figure 1).

*[0.1M N-methyl-D-glucamine / 0.1M ε-amino-caproic-acid]

** [0.1M N-methyl-D-glucamine / 0.1M γ-amino-butyric acid]

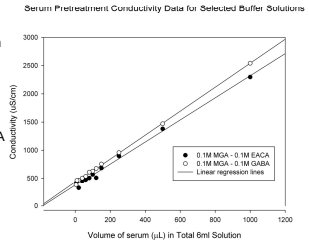


Figure 1. Serum conductivity during pretreatment process.

Chip Design

A PDMS chip (Figures 2,3) was fabricated (Stanford Microfluidics Foundry) and plasma bonded to a glass substrate. While the chip design was based off the Electrokinetic instability micromixer of Oddy and Santiago[4], our design also incorporated a passive mixing element, namely repeating mixing gates in the exit channel. COMSOL™ analysis revealed that the inclusion of the gates resulted in more efficient mixing; however, experimental results showed accumulation of analyte/biofouling concerns.

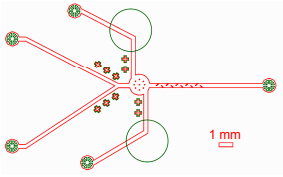
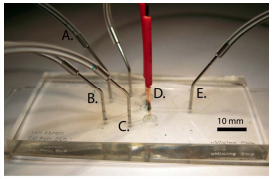


Figure 2. AutoCAD™ mask drawing of chip.



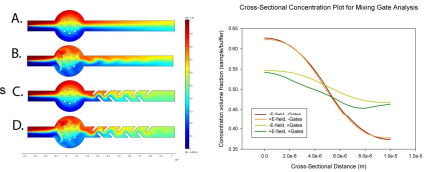
A. Sample inlet port
B. Buffer inlet port
C. Electrolyte reservoir filling port
D. Off-chip electrodes
E. Outlet port

Figure 3. Labeled PDMS chip with connections.

Results

Modeling Results

Computer simulations performed using COMSOL™ were useful in validating chip design and developing experimental operating conditions. Before chip fabrication, a simulation was run to validate the presence of the mixing gates (Figures 4,5). Four different operational configurations (electric field on/off, with/out gates) were compared based on the exit concentration profiles. Our metric for demonstrating desired mixing was an even distribution of analyte, 50% ± 5%, through the cross-section of the exit channel. Based on this analysis, it was shown that the presence of the gates greatly enhanced the mixing efficiency of the chip.



Figures 4,5.
COMSOL™ results:
A. No E-field, no gates
B. E-field, no gates
C. No E-field, gates
D. E-field, gates

Micro-mixing Results

Local Electrokinetic mixing was observed in the mixing chamber using a solution of 0.2μm yellow-green fluorescent microspheres as the sample and DI water as the buffer after pulsing a 50% duty cycle DC voltage of at least 200 Volts (LabSmith HVS). The distance that the dye propagated across the exit channel was measured in ImageJ[5] and compared for different applied voltages (Figure 7). We determined that an applied amplitude of 800 Volts was sufficient to induce complete mixing within 1.5 seconds.

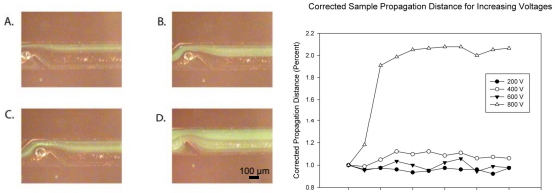


Figure 6. Sample propagation at 1.5 seconds after E-field excitation for A) 200V, B) 400V, C) 600V, D) 800V.

Figure 7. Corrected dye propagation distances for 200V, 400V, 600V, 800V

Conclusions

We have shown that sample pretreatment is possible on-chip by combining specific buffers with a raw sample in a pulsed wave DC-micromixer. Our next steps will include the observation of a serum sample spiked with a pH-indicator dye (thymolphthalein) undergoing treatment by our prepared buffers. Future work includes the adaptation of on-chip microfluidic valving to regulate volume ratios of serum to buffer, which will enable more precise control of the resultant sample conductivity. Eventually, this chip will be combined with various separation devices in order to demonstrate its intended use in a point of care diagnostic system.

Works Cited

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