A NOVEL DEVICE FOR HIGHLY EFFICIENT EXTRACTION OF NUCLEIC ACIDS FROM 100 MICROLITER WHOLE BLOOD SAMPLES

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ABSTRACT
We have designed a novel device to electrokinetically extract nucleic acids from 100 µL of lysed whole blood with high efficiency in less than 30 minutes. We performed this extraction using isotachophoresis (ITP), a technique that achieves selective, rapid pre-concentration and separation of molecules based on electrophoretic mobility. Previous efforts to extract nucleic acids from complex biological samples using ITP have typically operated in etched glass microchannels or capillary systems with separation capacity equivalent to 100 nL of blood or less. The device was designed for separation capacity of 100 µL. We have demonstrated a preliminary extraction efficiency of 25%.

KEYWORDS
Sample preparation, nucleic acids, isotachophoresis, scaling.

INTRODUCTION
Isotachophoresis (ITP) is an electrokinetic technique in which a two-buffer electrophoretic system creates shockwaves that both separate and concentrate ions based on electrophoretic mobility. We have demonstrated that ITP is a promising technique for separation of nucleic acids from complex biological samples such as blood and urine [1]. However, previous efforts to extract nucleic acids from using ITP have typically operated in standard etched glass microchannels or capillary systems. The small volumes of these chips have limited the throughput of the technique. A typical glass microchannel (for example, Caliper NS12A) has the capacity to separate order 100 nL of blood in a single batch process using ITP. We designed and fabricated a novel device with a separation capacity of 100 µL blood while maintaining a sample processing time of less than 30 min. We achieve this increase in throughput by using a high-aspect ratio geometry that rejects Joule heating and increases total channel volume 1000-fold. In addition, we have added a secondary buffering well for both the leading and trailing electrolytes. By separating the buffering reservoirs from the sample reservoirs, we have provided sufficient buffering capacity to prevent large pH changes due to electrolysis.

DEVICE
The device consists of stereolithographically-defined reservoirs (Figure 2) attached to 50 x 75 mm glass layers that define the length and width of the channel. The plastic reservoirs were fabricated by Fineline Prototyping (Rayleigh, NC) in Renshape 7820. They contain space for a 5 x 5 x 50 mm porous plastic separator that allows electrical connection between the buffering reservoir and the sample reservoir while reducing mixing between the two. The glass channel is constructed from two 50 x 75 mm glass microscope slides. These glass layers are spaced to form a 250 µm tall separation channel using polyisoprene spacers. This spacing produces a channel with total volume of with a volume of 900 µL.

Loading the wide, flat channel with buffer without introducing bubbles can be difficult. We fabricated phaseguides [2] on one of the glass surfaces using Rayzist SR3000 photoresist to make the loading process more consistent. These structures alter the hydrophobicity of the surface, causing the buffer to fill the channel one section at a time. However, Rayzist is somewhat water soluble. This causes the phaseguides to degrade after use. We are exploring other fabrication materials.

Figure 1. a) Image of device showing the wide central channel connecting two plastic reservoirs on each side. Blue strips are photoresist phaseguides to aid device loading. Porous spacers form a sample inlet, sample outlet, and two buffer/electrode reservoirs. b) CAD drawing showing details of the fabricated reservoir. The device uses a stereolithographically defined plastic chamber. A porous plastic spacer separates this into two reservoirs for electrode buffer and sample volumes. Stereolithography allows us to quickly and cheaply fabricate complex geometries for our devices.
EXPERIMENT

To prepare the device for operation, we loaded aqueous leading electrolyte (LE) buffer containing 10 mM 6-aminocaproic acid and 5 mM HCl into the separation channel. We added 100 mM 6-aminocaproic acid and 50 mM HCl into the leading buffering reservoir. We added 100 mM 6-aminocaproic acid and 50 mM acetic acid into the trailing buffering reservoir. We pipette in 100 µL of nucleic acid solution diluted in 10 mM 6-aminocaproic acid and 5 mM acetic acid into the sample reservoir and apply electric field to perform sample preparation. The nucleic acids are electrophoretically transferred through the channel toward the negative electrode, where they elute into the clean LE buffer. We then collected the nucleic acids, diluted in clean LE, and used this solution to perform quantitative PCR, as shown in figure 3. We show recovery of 25% of dispensed synthetic DNA based on qPCR threshold cycle.

We visualize operation of the device using Alexa Fluor 488 and fluorescein. We replaced 6-aminocaproic acid with bis-tris propane, and replaced acetic acid with hepes. This combination of buffers allows Alexa Fluor 488 to focus, while fluorescein remains behind in an unfocused zone. Both dyes were visualized by illuminating the device with a blue LED, and imaging the device under a stereoscope with 0.19x magnification. Images were captured using a Micromax CCD camera. Results are shown in figure 4a.

The device throughput is limited by heat dissipation. We designed the device geometry to minimize temperature rise during extraction, and analyzed the theoretical steady-state temperatures. We measured steady-state temperature as a function of operation al current using thermocouples inserted into the device. As shown in Figure 4b, the device can operate at up to 10 mA while maintaining a temperature within 10°C of room temperature, using the bis-tris/hepes buffer combination.

We hypothesize we can extract all DNA from 100 µl by balancing focusing dynamics, temperature, and buffering capacity.

Figure 3. Off-chip quantitative PCR curves showing the purity and abundance of extracted DNA from a 100 µL sample. Based on the threshold cycle of the extracted DNA sample, we calculate a preliminary yield of 25% from the sample. We hypothesize we can increase this to very near 100% extraction efficiency.

Figure 4. a) Fluorescence visualization of device operation. High-mobility Alexa Fluor 488 focuses into the ITP zone, while the lower-mobility fluorescein trails behind as an unfocused zone. The distance between the DNA simulant here (alexa fluor) increases from contaminants (fluorescein). The target sample ions (Alex Fluor) travel toward the anode, where they are collected, and the fluorescein discarded. b) Measured temperature in the extraction device (maximum temperature in channel) as a function of applied current. The device throughput is limited by Joule heating, but it can operate at currents as high as 10 mA with a temperature rise of approximately 10°C. We hypothesize we can extract all DNA from 100 µl by balancing focusing dynamics, temperature, and buffering capacity.
With this device, we demonstrate that isotachophoresis can be applied to a range of sample sizes. The extremely small volumes of typical microfluidic chips need not limit the volume of sample that can be processed. This is particularly important because samples containing rare sequences are subject to Poisson statistics. Small processed volumes may not contain important targets, rendering the extreme sensitivity of PCR amplification irrelevant. To our knowledge, our system is unique in addressing separation capacity, extraction efficiency, and throughput in a system for extracting nucleic acids from a biological sample using isotachophoresis.

REFERENCES

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