1,000-FOLD SAMPLE FOCUSING ON PAPER-BASED MICROFLUIDIC DEVICES
Tally Rosenfeld and Moran Bercovici

1Faculty of Mechanical Engineering, Technion – Israel Institute of Technology, ISRAEL
2Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, ISRAEL

ABSTRACT
We present a novel paper-based device and assay for isotachophoresis focusing, which is (i) self-contained on a simple piece of paper, (ii) does not require any specialized enclosures or cooling devices (iii) enables over 1,000-fold focusing in 6 minutes and (iv) allows processing of sample volumes as high as 30 μL. We believe this device can serve as the basis for low-cost, rapid and highly sensitive paper-based diagnostic platforms.

KEYWORDS: Isotachophoresis, Paper-based device, Paper microfluidics, Lab-on-a-chip

INTRODUCTION
Microfluidic paper-based analytical devices (µPADs) have gained significant attention in recent years due to their potential as a low-cost, durable, and simple to use diagnostic platform. These devices have now been applied for a variety of biochemical applications, however, many diagnostic needs cannot be met by the current sensitivity of such tests [1]. We here present a method for significantly increasing the sensitivity of such tests by coupling them with isotachophoresis (ITP).

ITP is an electrophoresis technique which allows for simultaneous separation and preconcentration of analytes based on their effective electrophoretic mobility. This technique is capable of focusing sample ions of interest at a sharp electric field gradient formed between a high electrophoretic mobility leading electrolyte (LE), and a low electrophoretic mobility trailing electrolyte (TE). We have recently demonstrated the use of microchannel ITP for improving the sensitivity of surface biosensors by two orders of magnitude [2]. Seeking to translate this technology to low-cost devices, we here present a novel paper-based device and assay for ITP focusing, and demonstrate over 1,000-fold focusing of 30 μL of sample in 10 min.

EXPERIMENTAL
Our fabrication technique, shown in Figure 1, is based on wax printing, in which wax is directly printed onto cellulose [3,4]. However, for compatibility with electrokinetic assays, we further developed
the technique to allow the fabrication of channels significantly shallower than the original thickness of the paper (~50 μm). Such shallow channels are critical in providing sufficient dissipation of joule heat and thus enable the use of high electric fields and short analysis time.

For the isotachophoresis assay, we used 100 mM HCl, 200 mM Bistris, and 1% 1.3 MDa poly(vinylpyrrolidone) (PVP) as the LE solution, and 10 mM Tricine, 20 mM Bistris, and 1% PVP as our TE solution. Our model analyte was DyLight650, which we mixed with the TE solution for an initial concentration of 10 nM in the reservoir.

Figure 2a-d presents the use of our fabricated µPAD for ITP focusing. We rely on capillary action for filling the channel with LE, and design a wax barrier to stop the flow and serve as a repeatable starting point for ITP. Upon introduction of TE and analyte to the sample reservoir, the electric circuit closes and ITP automatically initiates. We here image the ITP plug using a microscope (Figure 2f), however, for high initial concentrations (>1 μM) it can be seen by naked eye and imaged by consumer-grade camera (Figure 2e).

**RESULTS AND DISCUSSION**

![Figure 2: Demonstration of the use of our fabricated µPAD for ITP focusing.](image)

**Figure 2**: Demonstration of the use of our fabricated µPAD for ITP focusing. (a) We place electrodes in each of the reservoirs, and add the LE to the right reservoir (b) The channel is filled with the LE buffer by capillary action (c) After ~10 min, when the LE front stops at the barrier, we add a TE-sample mixture to the left reservoir (d) Contact is formed between the TE and LE buffers, and ITP automatically initiates. (e) Raw fluorescence image of ITP focusing of 1 μM fluorescein on a filter paper, imaged by a consumer-grade camera (f) Typical fluorescence image of ITP focusing imaged under a microscope.

![Figure 3: Experimental results showing continuous ITP focusing of a fluorescent dye on a filter paper channel.](image)

**Figure 3**: Experimental results showing continuous ITP focusing of a fluorescent dye on a filter paper channel. We injected 10 nM DyLight650 into the TE, and measured fluorescence intensities during ITP, at fixed distances from the TE reservoir. (a) Width averaged concentration profiles registered at each station. (b) Total accumulated sample at each station (calculated using values above 10% of the peak value threshold), showing continuous accumulation of sample at the interface. The solid line represents ITP theory for constant voltage (c) Raw intensity images corresponding to each station.
Figure 3 presents quantitative experimental results of ITP focusing in our shallow-channel μPADs. Figure 3c presents raw fluorescence images of the ITP front at the different stations along the paper channel (stations are printed as 1-8 in Figure 2). While the ITP front is significantly more dispersed compared to standard glass microchannels, focusing is nevertheless clearly evident and the ITP plug is well contained and steadily electromigrates along the paper channel. Figure 3a-b presents respectively the width averaged fluorescence signal and total accumulated sample as a function of time along the channel.

Figure 4 provides a quantitative assessment of peak and average concentration, showing that in less than 6 min, paper-based ITP provides an area averaged concentration enhancement of 200-fold, while the peak concentration is increased 1,000-fold.

**CONCLUSION**

We presented a novel paper-based analytical device for sample focusing using isotachophoresis, and showed over 1,000-fold focusing in several minutes on such devices. Obtaining high sample concentrations in paper has direct implications in accelerating reaction kinetics [2] and creating low-cost devices with much enhanced sensitivity. Another benefit of paper-based ITP is the ability to process large sample volumes, which can open the door to the use of ITP for detection of extremely dilute samples. Therefore, we believe that such devices can serve as the basis for low-cost, rapid and highly sensitive paper-based diagnostic platforms.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge support by the German-Israeli Foundation for Scientific Research and Development (GIF) no. 2287-2235.5/2011, and by Israel Ministry of Economy and Life Technologies, as part of the NOFAR program no. 50660.

**REFERENCES**


**CONTACT**

* Moran Bercovici; mberco@technion.ac.il