FREE-FLOW DEPLETION ZONE ISOTACHOPHORESIS (FFdz-ITP)

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ABSTRACT
In this abstract free-flow depletion zone isotachophoresis (FFdz-ITP) is proposed. The device combines the high sample throughput and convenient analyte extraction of free flow separation techniques with the simple and efficient separation and focusing of depletion zone isotachophoresis.

KEYWORDS: Ion concentration polarization, electrokinetic focusing, free-flow, blood plasma, dz-ITP, ICPF

INTRODUCTION
Lab-on-chip systems provide an appealing platform for electrophoretic separations due to the high controllability of fluid flow and electric fields on the micrometer scale. The inherent disadvantage of miniaturized separation techniques, their low sample throughput, is addressed by two special classes of techniques, ones that combine *separation* and *focusing* and ones that use the free-flow format. In this abstract we report Free-Flow depletion zone Isotachophoresis (FFdz-ITP), a free-flow separation and focusing technique that does not require any sample or special electrolyte preparation; and allows high throughput extraction of separated and concentrated analytes directly out of blood plasma.

THEORY
In "Free-Flow" separation systems, the separation direction is perpendicular to the flow direction allowing continuous high-throughput separations [1]. Particularly powerful are the free-flow forms of focusing techniques, such as isotachophoresis (ITP)[2]. ITP needs a leading and trailing electrolyte, and a simpler form is *dz*-ITP, which uses the depletion zone that is formed by ion concentration polarization as trailing electrolyte and the background electrolyte as leading electrolyte [3]. Here we demonstrate FFdz-ITP. Figure 1 shows a schematic of the device and its operating principle. The device uses the cation selectivity of Nafion to create a depletion zone by ion concentration polarization. The sample flows (via syringe pump) through the separation chamber while an electric field (i.e. the separation driving force) is applied perpendicular to the flow. The electric field plays a double role, forming the depletion zone and bringing analytes towards the depletion zone via a combination of electroosmotic flow (EOF) and electrophoresis. The analytes separate and create concentrated streams, with the analyte with the slowest electrophoretic mobility closest to the depletion zone.

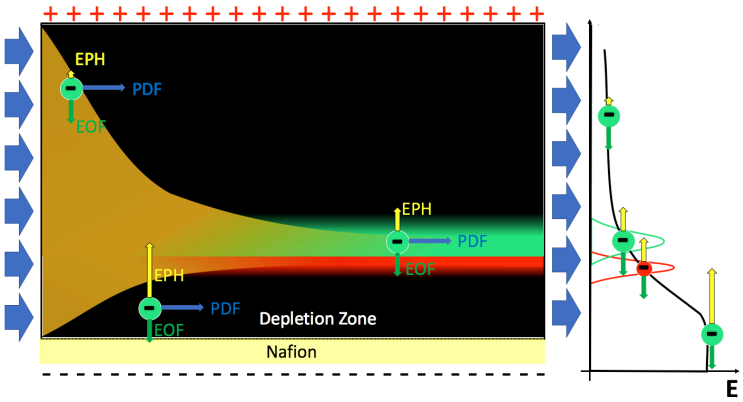

Figure 1- Schematic of operation principle of FFdz-ITP. The sample enters and flows across the separation chamber by pressure driven flow (PDF) and a E-field is applied perpedicular to the flow (in contrast to simple dz-ITP where the E-field and flow have the same direction). The analytes focus at the position where the EOF and electrophoretic flow (EPH) are equal and opposite. A different analyte with a different electrophoretic mobility requires a different electric field to acquire the same EPH hence it will focus at a different position in the E-field gradient (present between depletion zone and bulk solution).

RESULTS AND DISCUSSION

PDMS chips were prepared from a SU8 mask (Fig.2), and after plasma treatment bonded to a standard microscope slide. Nafion is introduced and patterned via capillary valves as described in previous work[4]. A demonstration of FFdz-ITP is shown in figure 3 where 0.1xPBS was used as a background electrolyte spiked with Bodipy(BDP) and Alexa Fluor 647(AF647). For the characterization of the device (figure 4) human blood plasma was used as electrolyte and spiked with 1.5µM of BDP and 1.5µM AF647. Since the analytes focus at the position where the contribution of bulk flow velocity and the opposing electrophoretic velocity cancel, we can control the focus/extraction position by tuning the reservoir potentials and the flow rate (Fig.4c). The concentration factor depends on the amount of analytes reaching the depletion zone during passage time. If the flow rate is reduced there is more time for the analyte to reach the depletion zone. Similarly, if the electric field is increased, the EOF will increase hence the concentration factor will increase (Fig.4d). The limiting factor for maximizing throughput and preconcentration is the Joule heating, which is exacerbated by the high conductivity of blood plasma. The thin channel adjacent to the anode functions as temperature fuse, avoiding heat damage to our biosample in the separation chamber, as current density is highest there. We report a maximum flow rate of 20µl/min at 150V for blood plasma and 200µl/min at 500V for 10x dilute blood plasma while still having separations and focusing.

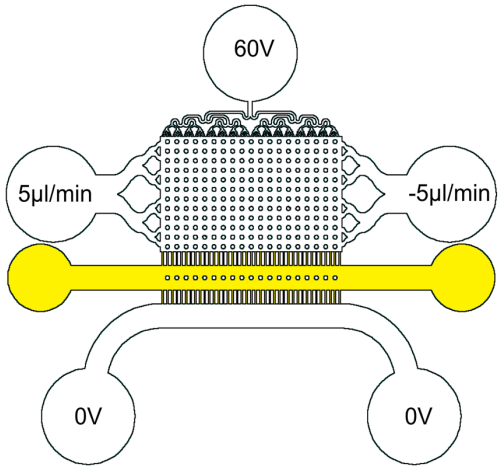

Figure 2 - Schematic of the device along with typically applied potentials and flow rates. The yellow area is filled and patterned with Nafion via capillary forces.

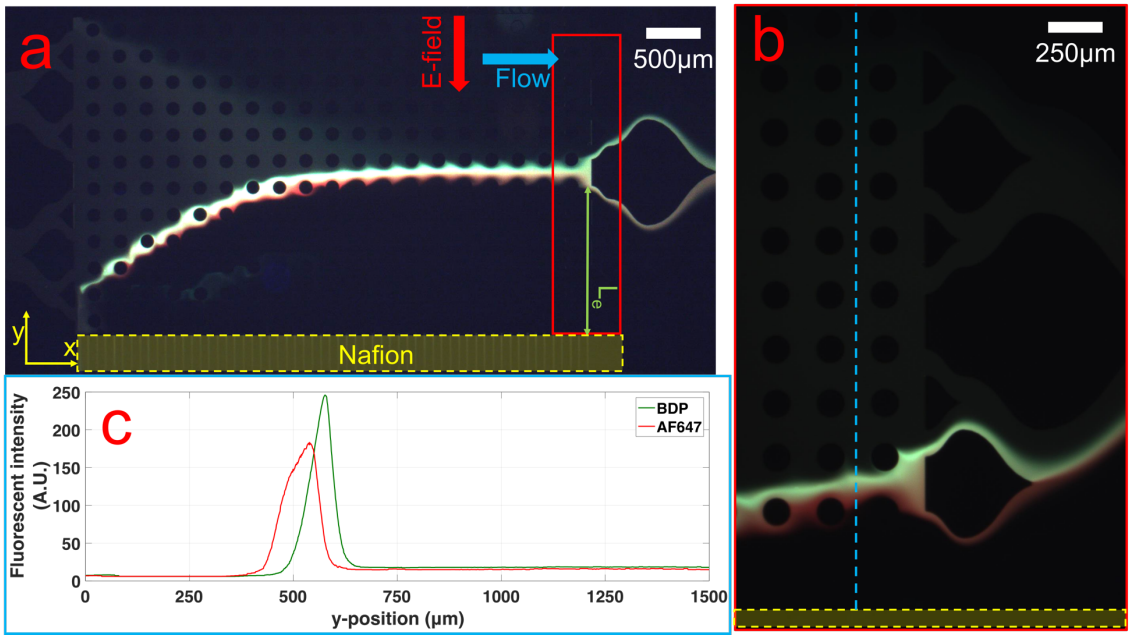

Figure 3– a) Fluorescent microscopy image of FFdz-ITP of 0.1xPBS. Focused streams of BDP and AF647 can be seen (5µl/min at 500V) .b)Close up fluorescent microscopy image of the extracted streams (10µl/min at 250V) c) Fluorescence intensity profile of BDP and AF647(along the blue line of image b), the maximum intensity is approximately ten times higher than the bulk intensity. Note: Images a and b are part of different experiments.

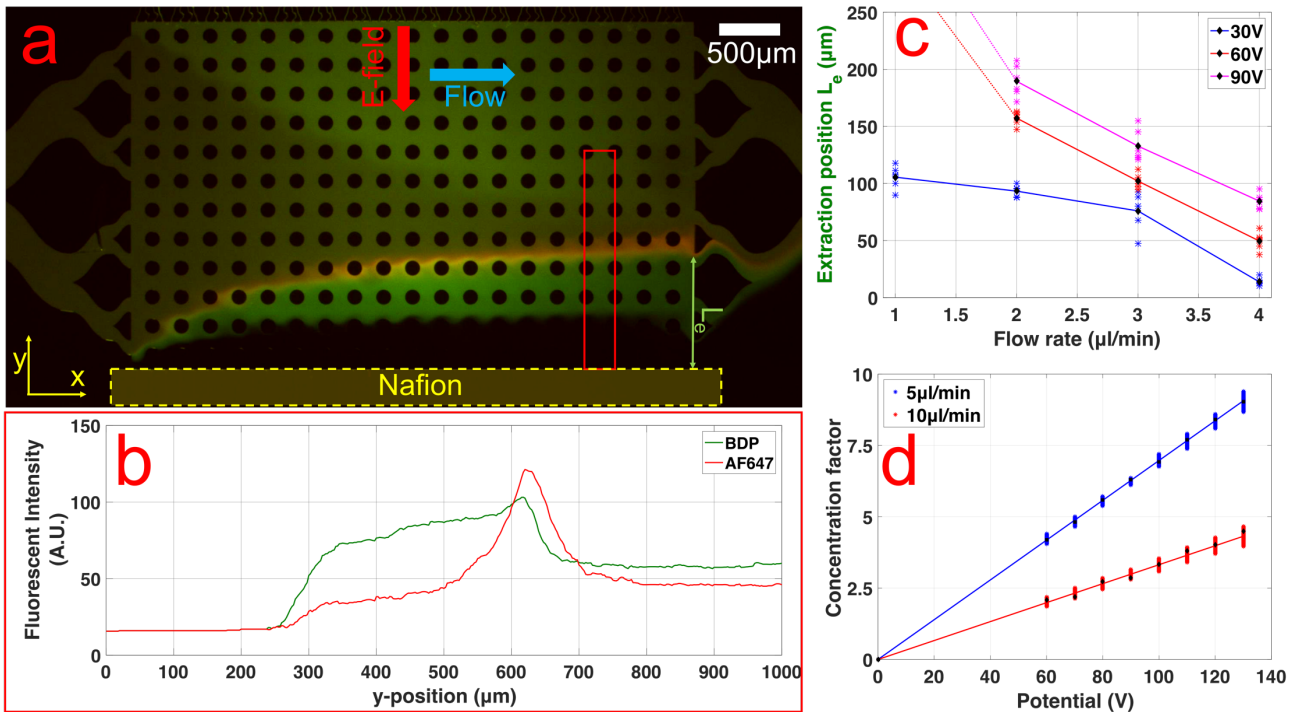

Figure 4– a) Fluorescent microscopy image of FFdz-ITP of human blood plasma. Focused streams of BDP and AF647 can be seen. b) Fluorescence intensity profile of BDP and AF647, the maximum intensity is approximately two times higher than the bulk intensity. c) Extraction position versus flow rate for different applied potentials. As extraction position the location of the maximum intensity of BDP was chosen. Each point (*) corresponds to a separate experiment with the same device and the lines connect the average values. For a flow rate of 1µl/min the extraction position of 60V and 90V was outside the observation window. d) Concentration factor versus potential for two flow rates. Each point (*) corresponds to a different measurement of the same experiment at a different time over a duration of 100 seconds to demonstrate the stability of the system. The lines show a linear fit with R^2 of 0.9999 and 0.9934 and a slope of 0.0697 and 0.0337 V^{-1} for 5 and 10 µl/min, respectively.

CONCLUSIONS

We have shown the use of dzITP in a free flow format. We demonstrated separation of model anionic analytes while concentrating up to 10 times in continuous flow. Finally, the applicability of FFdz-ITP for biomedical samples was shown with the use of human blood plasma as a background electrolyte.

ACKNOWLEDGEMENTS: This work was supported and funded by Horizon 2020 Framework Programme of the European Union under the project H2020-PHC-634013 (PHOCNOSIS).

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