CONTINUOUS EXTRACTION DEPLETION ZONE ISOTACHOPHORESIS (Edz-ITP)

Vasileios A. Papadimitriou, Loes I. Segerink, Albert van den Berg, and Jan C.T. Eijkel

BIOS-Lab on a Chip Group, MESA+ Institute of Nanotechnology, MIRA Institute for Biomedical Technology and Technical Medicine, Max Planck - University of Twente Center, The Netherlands

ABSTRACT

In this abstract we propose a method for continuous extraction of concentrated analytes out of their complex surrounding solution. An increase of the concentration by a factor of up to 10 is reported for the extracted analyte. In addition we were able to selective extract one of two concentrated fluorescent analytes.

KEYWORDS: Edz-ITP, Ion Concentration Polarization, Separation, Focusing

INTRODUCTION

Since the early days of microfluidics many separation techniques have been translated to Lab-on-chip systems. A special class of techniques thereby combines separation and focusing (e.g. isoelectric focusing, electric field gradient focusing and isotachophoresis (ITP)). Though these techniques offer very powerful analytical tools, the focused and separated analytes become "trapped" inside the chip in picoliter volumes. In this abstract we propose a method to continuously extract the separated and focused analytes from their complex surrounding solution. The method is akin to free-flow ITP [1], but also differs in several respects such as the use of concentration polarization.

THEORY

Depletion zone Isotachophoresis (dz-ITP) uses the depletion zone that is formed by ion concentration polarization as trailing electrolyte and the background electrolyte as leading electrolyte. At the border of both zones ITP occurs [2]. Our device uses the cation selectivity of Nafion to create the depletion zone. Nafion is introduced in the device and patterned via capillary valves as described in previous work [3]. In a novel design now two extraction channels are added, intercepting the sample channel perpendicularly (Figure 1, the channels with '-P': negative pressure). The analytes separate and create focused bands by dz-ITP, with the ions with the slowest electrophoretic mobilities closest to the depletion zone ('DZ' in figure 1). Since the analytes focus at the position where the bulk flow velocity and the opposing electrophoretic velocity cancel, we can control the focus position by tuning the reservoir potentials which tunes the bulk flow. In the new design, the analyte band can escape the sample channel when it is moved to the entrance of the extraction channel. The driving force for the extraction of the analyte is a low external negative pressure ('-P') applied at the extraction channel reservoirs. In addition, an excess pressure is induced by the electric field gradient, as the non-uniform EOF across the gradient creates a back pressure in the sample channel. The induced pressure opposes the external one, hence only when the external pressure overcomes the induced pressure the selective extraction of analytes will take place. The operating principle of the device is shown in Figure 1. The operation of the device resembles a free flow ITP device [1] in the sense that the electric field and ITP direction are perpendicular to the extraction flow direction.
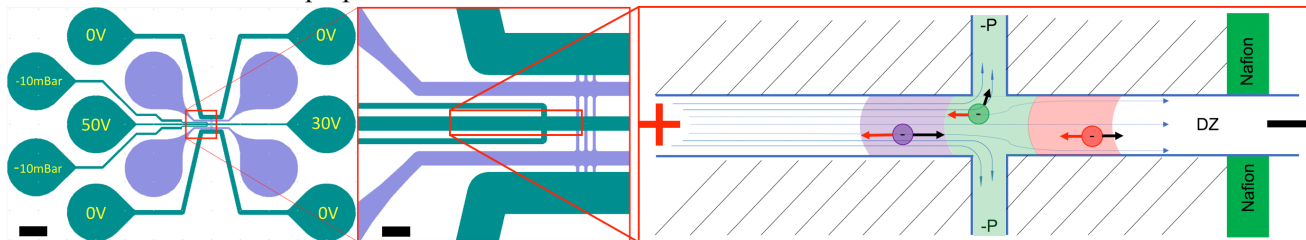

Figure 1: Left/middle: Layout of the chip and a typical operation scheme. Right: Operating principle of the device. During dz-ITP the anions are getting focused at the position where the bulk flow velocity (Black arrow) is cancelled by the electrophoretic velocity (Red arrow) resulting in zero net velocity. If the focused band overlaps with the extraction channel then the analyte will experience a bulk flow towards the extraction channel, breaking the zero net velocity and it will be extracted. The bulk flow is a combination of pressure-driven flow and electroosmotic flow shown by thin blue arrows. (Scale bars: Left – 1mm, middle – 100µm). DZ: depletion zone; -P: negative applied pressure.

RESULTS AND DISCUSSION

PDMS chips were prepared from SU8 masks, and after plasma treatment bonded to a standard microscope slide. 0.1x PBS was chosen as background electrolyte. First, the focusing and extraction was tested. 1.5μM of Bodipy disulfonate(BDP) was added to the background electrolyte. We were able to continuously extract the preconcentrated analyte in a stable manner for the entire duration of reservoir fluid depletion (5 min). The preconcentration factor depends on the applied potentials (Figure 2). To test the extraction of a single analyte from a composite sample, we added 1.5μM Alexa Fluor 647(AF647) as second fluorescent analyte. After focusing of both analytes we were able to selectively remove Bodipy by the extraction channel and retain only the band of Alexa Fluor 647 or the other way around (Figure 3).

CONCLUSION

We demonstrated a proof of concept for Edz-ITP with promising results for continuous purification, concentration and extraction of selected analytes.
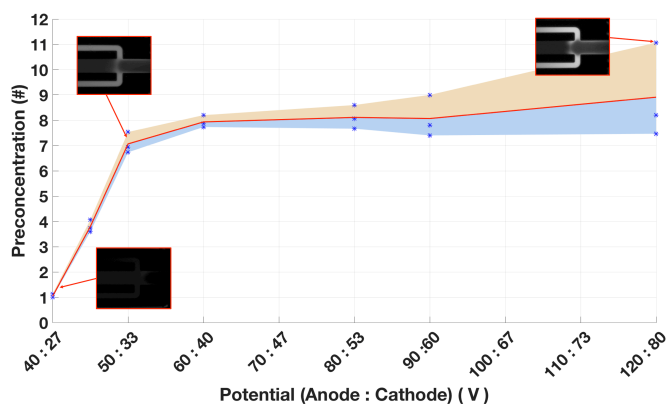

Figure 2. Preconcentration factor vs applied potential. A steady potential ratio between anode and cathode results in a steady focusing point location. By increasing the applied potential value with a steady ratio the extracted preconcentration rate increases. The saturation of preconcentration factor at higher potentials may have two causes. First, higher electric fields cause higher induced back pressure which will increase the flow through the extraction channel. Second, higher fields increase instabilities, vortices and electromixing which can also explain the lower reproducibility at higher potentials. 1.5μM BDP in 0.1xPBS. Data points indicated by *- represent separate experiments. The red line connects the average values of each set of experiments. Outer boundaries are indicated by the color bands.
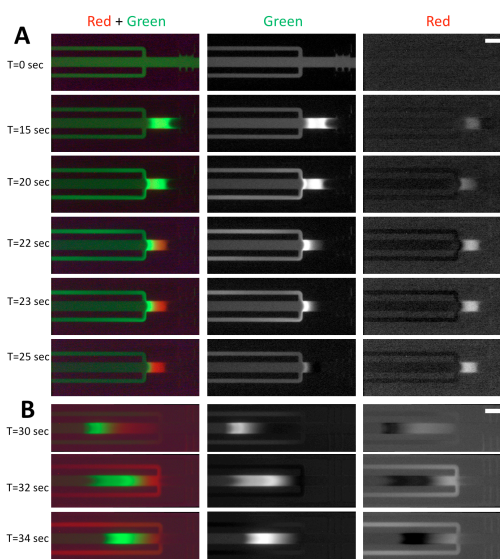

Figure 3. Separation and extraction of a single analyte from a mixture (BDP (green) and AF647(red) in 0.1xPBS). Red filter column indicates the AF647 concentration, Green filter column the BDP and Red+Green the composite image. We focus and create bands of both analytes for ±15sec and then release BDP (A) or AF657(B). The extraction channels remain dark in the red filter (A) and green filter (B) which indicates that only the targeted analyte is extracted. Note: A and B are two separate experiments and the time values are not related. Scale Bars - 100μm.

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CONTACT

V.A. Papadimitriou;phone: +31633621802; v.papadimitriou@utwente.nl