DEPLETION ZONE ISOTACHOPHORESIS (dzITP): BEATING THE SIMPLICITY OF ELECTROPHORESIS

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ABSTRACT

A radically novel approach towards isotachophoresis is presented in which a nanochannel-induced ionic depletion zone replaces a trailing electrolyte. This makes depletion zone isotachophoresis (dzITP) a single-electrolyte focusing and separation technique. Moreover, focusing strength and positions of analyte zones can be precisely controlled by a three-point voltage actuation scheme. Thus, dzITP provides unprecedented versatility and simplicity.

KEYWORDS: Isotachophoresis, ion exclusion, nanofluidics

INTRODUCTION

Isotachophoresis (ITP) is arguably today's most powerful technique for the concentration, separation, purification and quantification of ionic analytes, especially when performed on chip [1]. In practice, however, it lags in popularity compared to the much simpler technique of zone electrophoresis. A reason is that its discontinuous buffer system renders method development and sample introduction rather complicated. As a further complication, analyte zone positions are difficult to control in conventional ITP.

Here we present how the mentioned limitations can be overcome by a radically different approach towards ITP employing nanofluidic phenomena. The technique, which we named depletion zone isotachophoresis (dzITP) is achieved in a common H-shaped nano/microchannel setup [2, 3] (figure 1). After an electric field is applied, anion/cation asymmetry in the nanochannel [4] leads to depletion zone formation [5]. Meanwhile, electro-osmotic flow (EOF) in the microchannel opposes depletion zone growth and supplies analytes that focus at the depletion border. These analytes separate into zones of pure compound, which are arranged in order of their electrophoretic mobility. For dzITP, the depletion zone acts as a trailing electrolyte (TE) while the background electrolyte acts as leading electrolyte.

dzITP requires a single electrolyte only, which is a significant simplification over conventional ITP. Moreover, utilizing the advantage that dzITP is a quasi-static method, precise control over focusing strength and deliberate positioning of analyte zones is enabled by a unique three-point voltage actuation scheme.

EXPERIMENTAL

Micro- and nanochannel structures were consecutively fabricated in Pyrex wafers by photolithography followed by a deep reactive ion etching step. Etched depths were 1.7 µm and 60 nm for the micro- and nanochannels respectively. The etched wafer was thermally bonded with another Pyrex wafer in which access holes were drilled using a diamond drill. The channel system was filled with analyte dissolved in 2.0 mmol/L lithium carbonate as a background electrolyte. The chip was connected with electrodes through electrolyte-filled reservoirs. Our setup employed 3-point voltage actuation using a downstream voltage A, upstream voltage B and a third voltage C, which was typically grounded (see figure 1a).

RESULTS AND DISCUSSION

Figure 1b shows an example of an dzITP separations. Two fluorescent analytes have been focused at the border of the depletion zone and have been ordered in adjacent zones. Thus, a ITP separation has been achieved with a nanochannel-induced depletion zone as a terminating electrolyte.

Injections are simply performed by placing sample in the upstream well of the separation channel and filling the channel hydrodynamically or electrokinetically. For continuous injections, the sample was kept in the reservoir to provide a

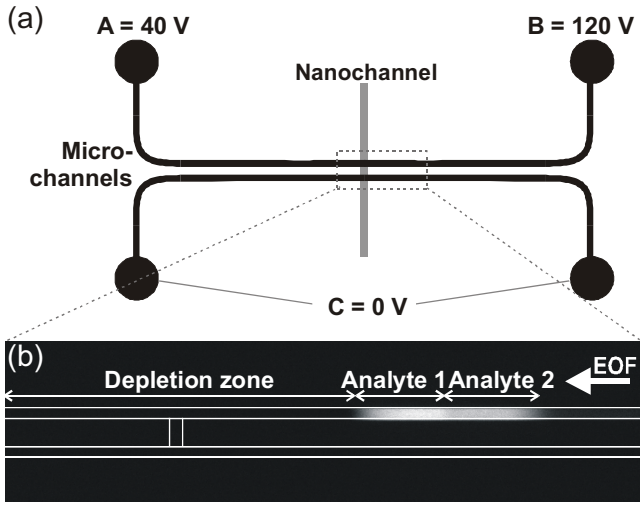

Figure 1. a) Schematic representation of the chip layout, showing two microchannels connected by a nanochannel, and an example of voltages during three-point voltage-actuation. b) Example of dzITP separated zones at the border of a depletion zone. Micro- and nanochannels have been indicated by lines that were drawn upon the photomicrograph

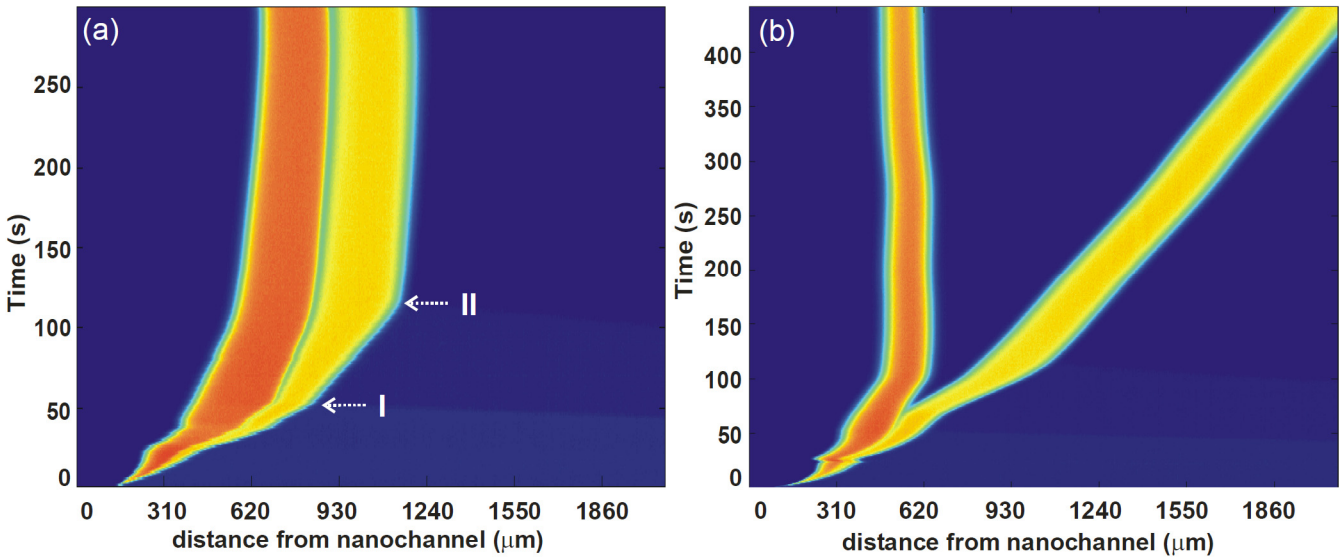

Figure 2: Spatiotemporal plots of dzITP separations. a) Discrete injection of fluorescein and 6-carboxyfluorescein. Arrows I and II indicate exhaustion of fluorescein and 6-carboxyfluorescein respectively. d) Discrete injection of fluorescein and 6-carboxyfluorescein combined with a continuous injection of acetate.

practically inexhaustible supply of analytes; for discrete injections, the sample was replaced with electrolyte such that only the separation channel contained analytes. This was demonstrated in experiments using fluorescein and 6-carboxyfluorescein as analytes; voltages were 120 V (upstream) and 40 V (downstream). A discrete injection is shown in figure 2a; analyte concentrations were 50 μmol/L in this experiment. An EOF transports the analytes towards the depletion zone border, where they are focused and separated isotachophoretically. This process continues until all analytes are focused, after which the zone-widths become constant. The time point at which focusing of an analyte is complete depends on the ionic mobility of the analyte: the higher the mobility, the faster it moves opposite of the EOF and the longer it takes to arrive at the depletion zone border. In the figure, this time points at which the analytes are exhausted are indicated by arrows.

Figure 2b shows a combination of a discrete injection of fluorescent analytes (30 μmol/L of both fluorescein and 6-carboxyfluorescein) and a continuous injection of a non-fluorescent spacer compound (100 μmol/L sodium acetate). Acetate travels from the sample reservoir through the separation channel and arrives after approximately 70 seconds. As acetate has intermediate mobility between fluorescein and 6-carboxyfluorescein, it spaces between the zones of these analytes. The continuous supply of acetate pushes 6-carboxyfluorescein in upstream direction, while fluorescein remains positioned at a near-stable position at the border of the depletion zone. Both zones remain focused during the process. This demonstrates that dzITP spacer addition is a powerful method for purification and sample transport.

Variation of voltages by the three-point actuation scheme indicated in figure 1a allows both zone sharpening (figure 3a and b) and positioning (figure 3c and d). Sharper zones and better separations are achieved by increasing the voltage magnitude. Zone positions remain approximately constant when the ratio between the upstream and downstream voltage is also kept constant, although at higher voltage magnitudes shifts occur.

By varying the voltage ratio, zone positioning is performed. The zones can be shifted over a range of 1.4 mm when the voltage ratio is changed from 0.25 to 0.67 (downstream voltage : upstream voltage). This is accompanied with some defocusing, but separation is maintained.

CONCLUSION

dzITP will prove particularly useful for our metabolomics research. Low abundant metabolites from complex biological samples can be separated and strongly enriched, while removing low-mobility proteins and high-mobility salts.

As a single-electrolyte method, dzITP possesses great simplicity. Injection is very straightforward and even simpler than in on-chip zone electrophoresis. Meanwhile, dzITP has great functionality and versatility. Complete control over analyte zone position and sharpness is a crucial and unique advantage of dzITP over conventional ITP methods.

The increased simplicity and functionality of our approach thus paves the way for claiming ITP's rightful position as best electrokinetic separation technique, not only in theory, but also in practice.

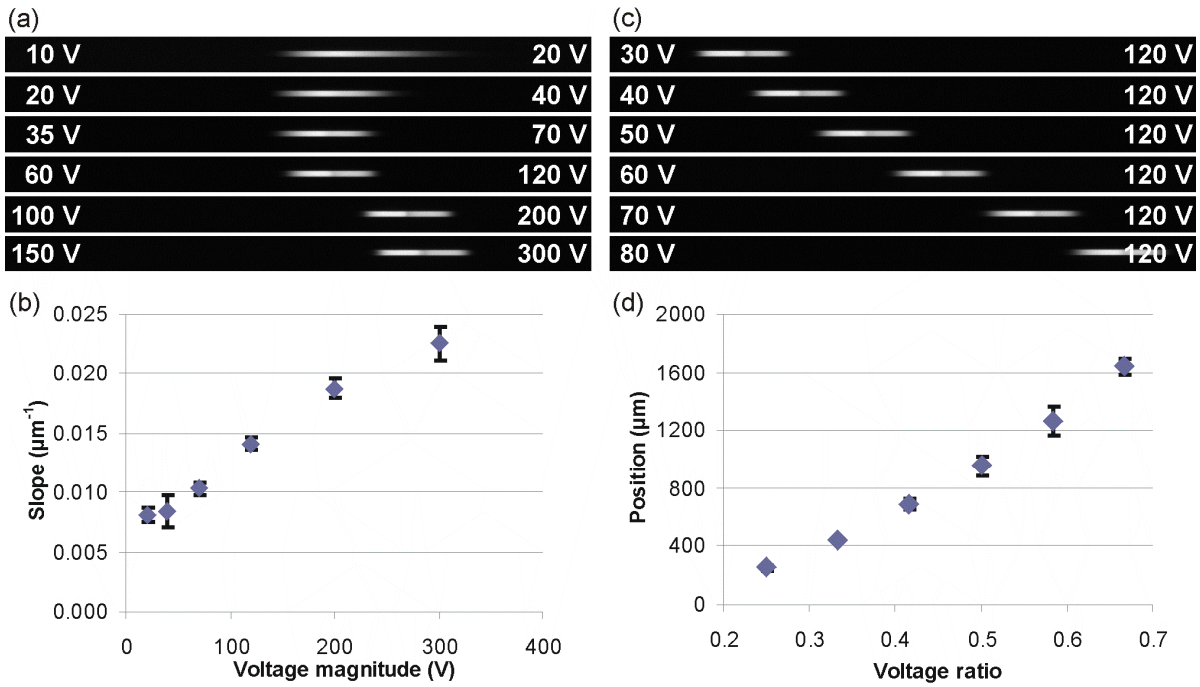

Figure 3: Three-point voltage actuation. a) Focusing and separation of fluorescein and 6-carboxyfluorescein at several voltage magnitudes. b) Dependence of focusing strength on the voltage magnitude. Focusing strengths are represented by the steepness of the slopes between the fluorescein plateau and the depletion zone; voltage magnitudes are represented by the upstream voltage. c) Fluorescein and 6-carboxyfluorescein zones at different ratio's between upstream and down-stream voltages. d) Distances of the edge between the depletion zone and the fluorescein zone from the nanochannel.

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