FLUIDIC SCANNING OUTPUT FOR A FREE-FLOW ISOTACHOPHORESIS IN A GLASS CHIP

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ABSTRACT

Here we present a free-flow isotachophoresis (ITP) device with a selectable output by using flow control. ITP is a powerful mode of electrophoresis, which allows simultaneously preconcentration and separation of samples. Free-flow electrophoresis (FFE) is a method used for continuous separation. The combination of FFE and ITP allows us to have a continuous preconcentrated and separated sample which can be used for real time online detection. Device operation is demonstrated on fluorescein, with a concentration increase of over a factor of 30.

KEYWORDS: Isotachophoresis, Free-flow electrophoresis

INTRODUCTION

Isotachophoresis is a separation based on two buffers (leading and terminating) with ions of different mobility presence of electric field. The key advantages of isotachophoresis is that the sample can be concentrated and separated in the same time. Free-flow electrophoresis can be used for continuous separation. The combination of ITP and FFE was first suggested in 2006 by Dirk Janasek [1]. Since in ITP all the separated band is flowing adjacent to each other an optical detection is difficult. Therefore, acetylsalicylic acid (ASS) was used as a spacer which was placed in between of fluorescein and eosin G. One of the key advantages of FFE is that it is able to collect the desired sample continuously. However as already shown by Dirk Janasek, in free-flow ITP the separated band will be flow nearby. Therefore collecting the desired sample after ITP is difficult.

Here we represent a isotachophoresis glass device which is possible to select the separated sample to the output of desire by hydrodynamic control of the flow. Figure 1 shows the concept of the device, samples and buffers will be introduced to the five inlets by a pressure driven flow and perpendicular electric field is applied. By adjusting the flow rate from the outer two inlet the sample can be shifted to the desire outlet.

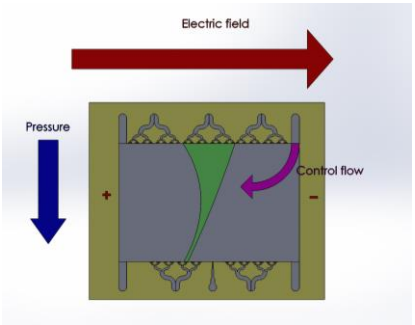

Fig 1. Schematic operation of the ITP device. By applying an electric field perpendicular to the flow direction, the sample will be focused. Positive pressure from the outlet will shift the stream to a desired outlet.

THEORY

Isotachophoresis (ITP) is one of the branch techniques of electrophoresis, where the ions are separated in an electric field according to their electrophoretic mobility. In ITP the sample is placed between two different electrolytes which is called as leading buffer and terminating buffer. The leading

buffer is composed of the highest mobility ions. Conversely, the terminating buffer has the lowest ion mobility of all three solutions. According to the Kohlrausch regulation all zones have a specific concentration. ITP does not broaden the band further, which is known as self-sharpening effect. If an ion diffuses out of its band into a higher or lower filed band, then it will forces to go back into it original band. These two unique features made Isotachophoresis attractive for microchip electrophoresis since its capable us to focusing and separating the sample in the same time [2].

Free-flow electrophoresis (FFE) is a method used for continuous separation. Its continuous sample separation makes it interesting for online detection application. The working principle of fee-flow electrophoresis is to apply perpendicular electric field to the buffer and sample flow direction. The first FFE device was designed in a relatively lager scale where the depth was in the range of millimeters [3]. Since 1994 several miniaturized device were developed and experimentally characterized, with several advantages . By reducing the size of the device the required amount of sample will be also reduce. This becomes especially interesting for clinical application, where the in the most case the sample amount is limited. The other key feature of miniaturizing the device is that the joule heating problem will be significantly reduced which obtains a higher resolution.

EXPERIMENTAL

Layout The layout of the device is shown in Figure 2 left. Arrays of pillars were used to avoid collapse of chamber during the thermal bonding process. The device consists of five inlets (I), a separation (main) chamber with the size of 23 × 15mm, two side chambers for connecting the electrodes and seven outlets (O). To achieve a high analytic concentration, the sample had to be injected from a wide inlet, therefore a binary tree structure was used in order to provide a equal distribution of samples to the chamber. The outer two inlets (I1 & I5) are used to guide the flow direction into the chamber. The outlets are designed in similar fashion as the inlets. However the outer two outlets (O1 & O7) are used for controlling the focused stream line and the middle outlet (O4) are used to collect the desired samples. The side chambers are separated from the main chamber by 25µm wide channels to prevent gas bubbles entering the main chamber. The side channels and the pillars as shown in Figure 1 right top. Reservoirs were used additionally for connecting the electrodes, and also to reduce the pH change inside the chamber. Figure 2 right bottom shows the side reservoir and the connectors which are used for connecting the syringes.

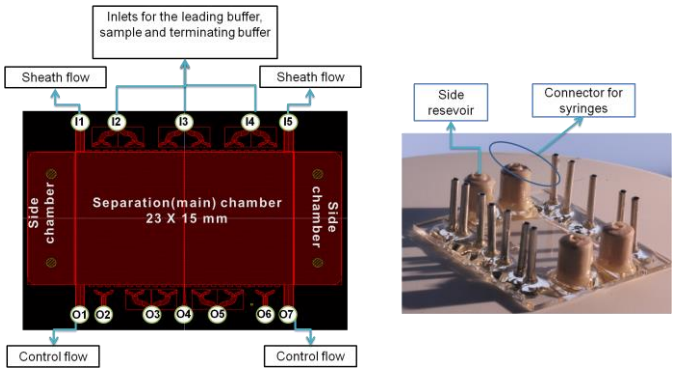

Fig 2 The right side shows the chip schematic. The side chambers are separated from the main chamber by 25µm wide channels and pillars were used in order to avoid collapse during thermal bonding. The left side shows the reservoir and the connectors for the tubing.

Chemicals All buffers were prepared as follows with resulting pH value of 9. The leading buffer was made by 50 mM NH_4OH mixed with 20 mM HCl, the terminating buffer by 50 mM NH_4OH mixed with 50 mM 2-(N-morpholino) ethanesulfonic acid. The sample was prepared by mixing 50 mM NH_4OH with 0.1 mM fluorescein.

Experimental Setup The leading buffer was introduced via the inlet 1&2 and the terminating buffer was injected from inlet 4&5. One of the problems by the filling procedure was the injection of bubble into the separation chamber which comes from the tubing. It was impossible to push the bubbles out by pressure driven force since inside the chamber the fluidic build up a network. Therefore a negative pressure was applied from the outlet to suck the bubbles out. After achieving a steady state flow of buffers the sample was introduced from inlet 3 with a flow rate of 5µl/min and a separation voltage of 200V was applied in order to focus the fluorescein stream.

RESULTS AND DISCUSSION

500 fold of concentration could be observed after applying the voltage. Since the samples was injected by a 3mm wide inlet and it was focused to a 60µm width stream line. Figure 3A shows the steady state situation before applying the voltage, and Figure 3B shows the fluorescein is focused after applying the separation voltage. Electroosmotic flow will drive the sample to the positive electrode, however by applying a positive pressure from the inlet (I1 & I5) it is possible to shift the focused stream to the desired outlet which is shown in Figure 3 C&D. Figure 3C shows that the stream is moving to the left side by applying 7µL/min flow from control inlet 1, vice versa Figure 3D shows the stream is moves to the right by applying 7µL/min flow from control inlet 5.

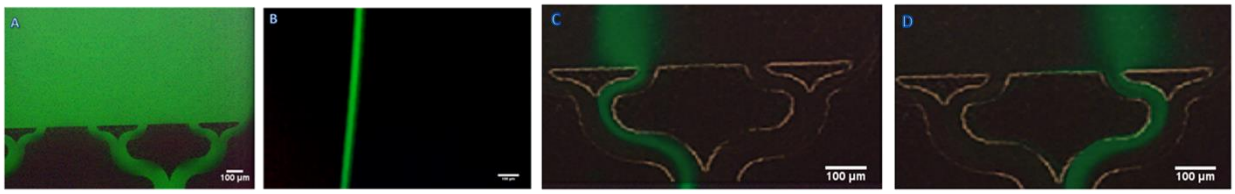

Fig 3 (A) Laminar flow before applying the separation voltage (B) Focused stream line of fluorescein when 200V separation voltage was applied. (C) The focused stream moves to the left side of the binary tree branch by applying 7µL/min flow rate from right side. (D) Conversely the stream shifts to the right side of the binary branch by applying the pressure from the left side.

CONCLUSION

A free-flow Isothachphoresis has been fabricated on a glass wafer with 5-µm depth channel. The device consists of five inlets, a separation chamber with the size of 23 × 15 mm, two side chambers for connecting the electrodes and seven outlets. The three middle inlets are split with a binary tree structure for equal distribution of samples and buffers. Two outer two inlets used to guide flow direction into the main chamber. The side chambers are separated from the main chamber by 15 µm wide channels to prevent gas bubbles entering the main chamber. The outlets are designed in similar fashion as the inlets. Steady flow of buffers and a sample was achieved by syringe pumps with flow rate of 5µl/min. Once the separation voltage with amplitude of 200V was applied the sample was focused. This focused stream was driven by positive pressure applied to two outer inlets. This configuration enables steering the focused stream to any output of our choice.

REFERENCES

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