Hydrogel plug for independent sample and buffer handling in continuous microchip capillary electrophoresis

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ABSTRACT

In microchip capillary electrophoresis most frequently electrokinetic sample injection is utilized, which does not allow pressure driven sample handling and is sensitive for pressure drops due to different reservoir levels. For efficient field tests a multitude of samples have to be processed with the least amount of external equipment. We present the use of a hydrogel plug to separate the sample from clean buffer to enable independent sample change and buffer refreshment. In-situ polymerization of the gel does away with complex membrane fabrication techniques. The sample is electrokinetically injected through the gel and subsequently separated by a voltage between the second gel inlet and the buffer outlet. By blocking of disturbing flows by the gel barrier a well-defined ion plug is obtained. After each experiment, the sample and the separation channel can be flushed independently, allowing for a continuous operation mode in order to process multiple samples.

Keywords: Microchip capillary electrophoresis, polyethylene glycol diacrylate, hydrogel, microfluidic device, capacitively coupled contactless conductivity measurement

INTRODUCTION

Since the 1990s, Lab-on Chip has experienced a tremendous expansion in the world of technology[1]. Miniaturized laboratories yield important advantages compared to bulky standard lab equipment. Capillary electrophoresis on microchip (μCE) allows shortening measurement times to less than a minute, smaller sample volumes from mL to pL, and easier quantitation compared to standard capillary electrophoresis (CE)[2,3]. Difficulties to overcome in microfluidic systems include the "real world" connection and sample introduction in the microchannel. The increasing number of injection methods reported in literature[4, 5] confirms a shift from basic research towards field-applicable microsystems. The injection of a sample in a CE chip is delicate due to the pL volume in microchannel junctions. Variations in the injection process prevent reliable measurement results. Usually, the sample is introduced by hydrodynamic or electrokinetic forces into the capillary[5]. For the hydrodynamic injection, the sample is driven into the channel by applying a vacuum at the end of the separation channel or by an overpressure at the injection side[6]. Another possibility is to introduce the sample by gravity injection (the sample vial is lifted above the electrolyte level)[5-7]. The advantage of using pressure driven injection is that a representative aliquot of sample is introduced into the separation channel during the injection. On the other hand, additional instrumentation is required to create the pressure difference in the channel to mobilize the sample. A key issue in order to achieve efficient field tests is to process a multitude of samples with the least amount of external equipment.

For electrokinetic injection, the sample is introduced into the channel by electrophoretic migration. This is realized by applying a voltage over the electrodes immersed in the sample solution at each end of the channel[5, 7]. However, the quantity of injected ions depends on the electrophoretic mobility. Ions that have a high mobility are introduced in larger concentrations into the capillary than ions with a low mobility, which yields an overestimation of these analytes[2, 7]. Another challenge that has to be overcome for electrokinetic injections is the ion migration into the microchannel during separation, which influences the reproducibility of quantitative analysis.

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Usually, samples of interest are only present in small volumes and have a low analyte concentration. In standard analytical systems such low analyte concentration might be under the detection threshold. To overcome this problem, sample pre-concentration has proven to be very successful, evidenced by the number of reported methods in literature. Intrinsic pre-concentration in CE can be accomplished by sample stacking, which is based on differences of the electrophoretic mobility of analytes[2]. Another promising method is field amplified sample injection[8]. However, the implementation of these techniques in microchips is challenging as the location of the sample plug during pre-concentration is difficult to control[2]. This problem can be overcome by a clever channel design, specific gate injections, and a porous membrane between the sample reservoir and micro channel. The integration of membranes in microfluidic chips has seen an increasing interest due to their versatile sample handling abilities[9]. However, the fabrication of on-chip membranes may involve complex technologies which are not easily accessible. Polydimethylsiloxane (PDMS) soft lithography has become the main technology for microfluidic fabrication and membrane integration[9-11]. Unfortunately, the hydrophobic surface and the unstable electroosmotic flow make PDMS not very suitable for microchip electrophoresis applications[12].

Herein, we describe the design and realization of a sample introduction method based on a porous hydrogel plug. Photopatterned hydrogel membranes have been utilized in microchip electrophoresis to preconcentrate biomolecules at the edge of the gel prior to the separation process[13]. We utilize a hydrogel as a barrier between the sample and buffer allowing for independent and continuous sample change and buffer refreshment. The sample is electrokinetically injected through the hydrogel, separated by the applied voltage and finally detected by capacitively coupled contactless conductivity method (C4M) [14].

MATERIALS AND METHODS

The micro capillary electrophoresis chips are fabricated on a microscope slide format (76 mm x 26 mm). Platinum electrodes for contactless conductivity measurements have been structured by sputtering 200 nm onto the glass substrate in a standard lift-off process. The electrodes are passivated by hot roll laminating an acrylic permanent dry film resist (*Ordyl SY300*, *Elga Europe*) onto the substrate. After exposure the film is hard-baked at 120 °C. The thickness of that insulation layer has been measured to 13 µm. The microchannels are fabricated in another layer of 100 µm dry film resist, structured by photolithography.

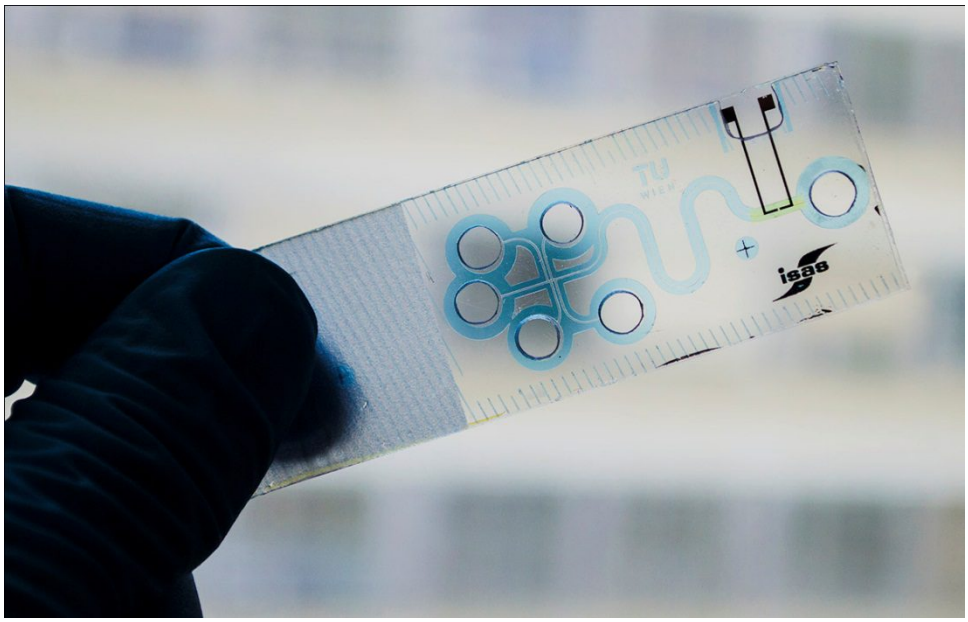

Figure 1. Micro capillary electrophoresis device fabricated in a thick photoresist laminate on a glass substrate. The device comprises passivated electrodes for capacitively coupled contactless conductivity measurements (C4M) and on-chip reservoirs for sample and separation buffer, respectively.

Reservoirs for sample and clean separation buffer (each reservoir can hold 25 µl liquid) are powder-blasted into the top glass layer, which is directly bonded to the dry film resist by hot roll lamination. After the assembly of the chip it is hard-baked at 120 °C for 2 hours. A fabricated, assembled device, consisting of a microfluidic channel with a quad-T injector, is shown in Fig. 1. UV curing polyethylene glycol diacrylate (PEG-DA), mixed with buffer is used to produce the fluidic barrier within the chip in a single step. A porogen (PEG) adjusts the pore size of the hydrogel to the size of analyte ions. The hydrogel precursor is prepared by adding 1.5 % of the photoinitiator 2-hydroxy-2-methyl-1-phenyl-1-propanone to polyethylene glycol diacrylate MW 700. (*Sigma Aldrich*). The precursor solution is mixed with a porogen solution (20 % w/w polyethylenglycol MW 35000 in 20 mM Mes/Histidine) in a ratio of 3:7. After injection of the gel into the chip via one inlet and a negative pressure at the second inlet, it is cured by UV exposure at 365 nm. Alternatively the gel can be cured by exposure through a mask with subsequent removal of uncured prepolymer solution. As seen from Fig. 2 the inner double-T is occupied by the hydrogel, dividing the device into two microfluidic channels for sample and buffer liquid, respectively. Prior to the experiments the separation channel is filled with buffer solution (20 mM Mes/Histidine, 0.1 % methyl cellulose, 4000 cP) and the chip is placed into a holder where it is electrically connected by spring contacts.

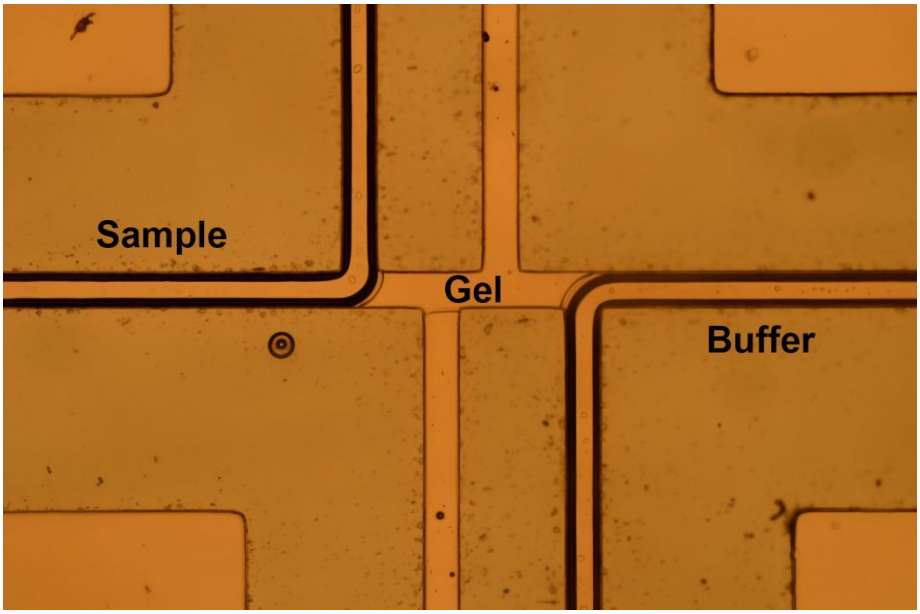

Figure 2. Photocurable hydrogel is introduced in the inner double-T injector and cured by UV exposure. The porous hydrogel separates the sample in the left channel from the separation channel at the right for independent handling of samples and refreshment of buffer liquid.

RESULTS & DISCUSSION

The sample is electrokinetically injected into the inner double-T through the gel as illustrated in Fig. 3a by a potential difference between one of the sample reservoirs A and the buffer waste reservoir B. In a second step the ion separation is started by a voltage between the gel inlet C and the end of the separation channel D (Fig. 3b). By blocking disturbing flows by the gel barrier a well-defined ion plug is obtained. If a longer sample plug is desired, also the remaining gel inlet can be used in the separation step, otherwise it remains unused. In addition to the shape-based injection also time-based injection is a possible method by applying an injection voltage between A and D for a short time (e.g. 0.5 s) and the separation voltage between C and D. The operation in different modes can be of interest to shift the limit of detection for different analytes in complex samples. Feeding different samples continuously into the chip via the separated channel has shown to allow quick sample change and, therefore, enables high throughput.

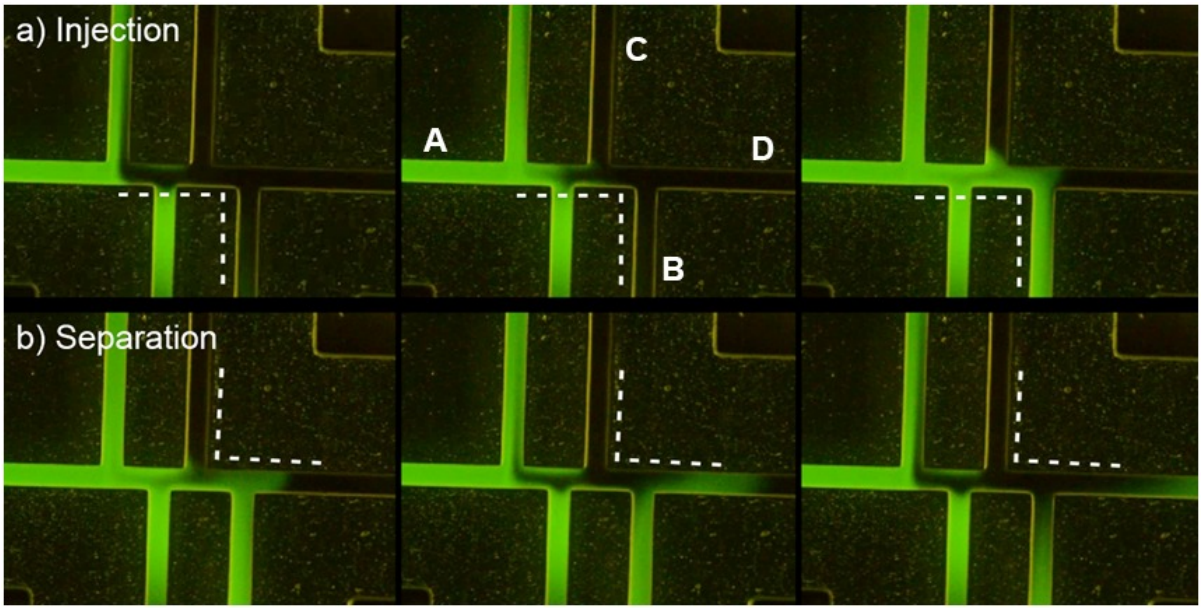

Figure 3. Injection and Separation facilitated by the flow-blocking hydrogel plug. a) The sample is injected through the gel by a voltage between the sample reservoir A and buffer waste B. b) Sample plug is wandering through the separation channel by a potential difference between gel inlet C and channel inlet D.

Samples in environmental monitoring often contain microorganisms, such as algae, bacteria or fungi which to filter (usually with 0.22 µm filters) would mean an additional time consuming sample preparation step. As microorganisms exhibit a negative surface charge, they will move in the electric field and interfere with the detection of anions. The exclusion of microorganisms by the hydrogel has been tested by contaminating a sample with a high density of *S. cerevisiae* cells. As seen in Fig. 4 the cells accumulate at the hydrogel barrier, while the channel on the right side remains clear, proving that the channel is tightly sealed by the gel and the pore size is small enough to exclude microorganisms from interfering with capacitively coupled contactless conductivity measurement (C4M).

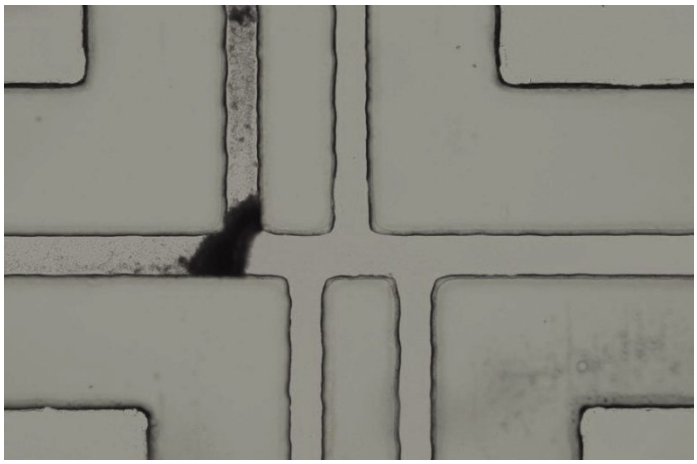

Figure 4. Microorgansim filtering of the gel with a sample contaminated with a high density of *S. cerevisiae*. Due to the negative surface charge of cells, they accumulate at the gel, showing the tight channel sealing of the plug and a sufficient gel strength to prevent them from migrating through the gel.

In order to test the chip together with the C4M method, separation experiments with nitrate (NO_3^-) and chloride (Cl^-) have been conducted (Fig. 5). Nitrate is a main source of nitrogen for plants and, therefore, and important measure for soil fertility, whereas chloride plays an important role in plant disease suppression[15]. Experiments in Fig. 5a show the detection of 250 µM chloride and nitrate with good reproducibility of the ion peaks. After introducing another sample with 500 µM concentration of both ions, the measurements have been repeated (Fig. 5b) with immediately similar reproducible results and accordingly higher peaks.

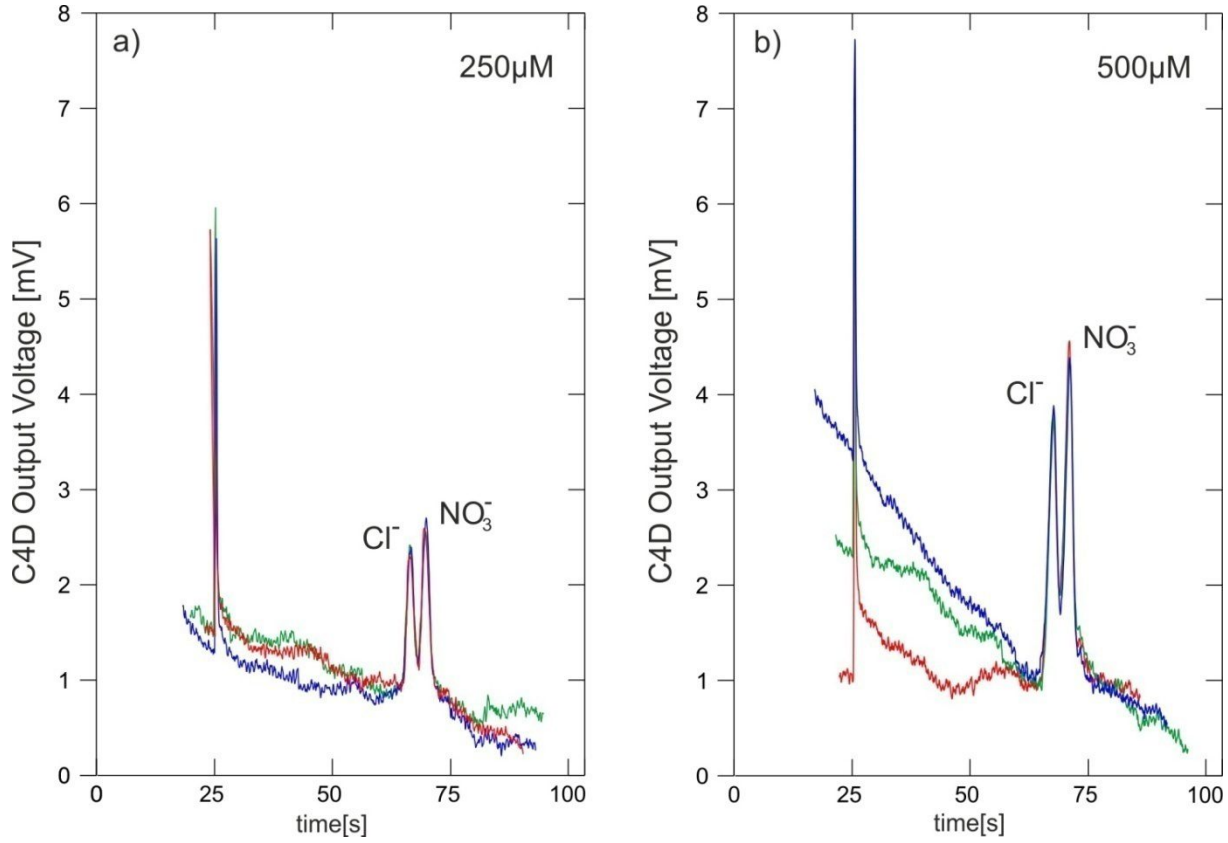

Figure 5. Separation and capacitively coupled contactless conductivity measurement experiments with nitrate and chloride samples at a) 250 µM and b) 500 µM concentrations. The reproducible results suggest a reliable injection method.

CONCLUSION

For environmental monitoring systems ease of operation and handling is a key factor. We have presented a microchip electrophoresis method for robust injection and convenient sample handling. In-situ polymerized hydrogel stabilizes the operation by preventing disturbing fluid flow and sample leakage. Furthermore it acts as a filter to exclude microorganisms, which are ubiquitously present in samples for environmental monitoring. The on-chip reservoirs enable quick operation without external fluid handling equipment, while the independent sample channel configuration facilitates sample supply for a high throughput.

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