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Polyelectrolyte-modified short microchannel for cation separation

Three alkali cations, potassium, sodium, and lithium, have been separated within 15 s in a 1 cm long polymer microchip. The separation microchannel is modified by a polycation, poly(allylammonium chloride), which makes the channel surfaces positively charged leading to a reversed electroosmotic flow (EOF) when compared to bare channels. Due to the decreased apparent mobility of the cations, the separation resolution is improved allowing the use of shorter channels.

Keywords: Capillary electrophoresis / Cation separation / Microchannel / Miniaturization / Polyelectrolyte / Surface modification
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1 Introduction

Micrototal analysis systems (μTAS) allow fast and efficient chemical separation within small volumes. This is the main reason why they have attracted so much interest in recent years [1]. For pumping, electroosmotic flow (EOF) is usually the method of choice as it is rather easy to implement experimentally compared to pressure-driven flow [2–5]. However, the main weakness of EOF is the stability of the flow rate, and perhaps this is why capillary electrophoresis (CE) as a separation technique is not always as reproducible as required. To stabilize the EOF, it has been proposed to control the pH of the eluent, or to fix the zeta potential of the capillary either by chemical modification or by adsorption. In the latter case, adsorption of polyelectrolytes is rather an easy route to achieve this goal [6–10]. Except for modulating the EOF, these adsorption methods, both covalent and noncovalent, have also been used to prevent nonspecific adsorption, or as a means to attach active molecules (such as antibodies) to the surface. In the present paper, we demonstrate that polyelectrolyte adsorption can be used both to obtain a stable EOF as well as to improve the separation efficiency.

The separation of small ions is routinely carried out by traditional CE in silica capillaries [11–19], the ions being typically separated in minutes when the capillary length ranges from 10 to 80 cm. Analysis of small inorganic/organic charged species has also been performed on microchip platforms by capillary zone electrophoresis. For example, separation of potassium, lithium, and sodium [20] was observed on a 8.4 cm long glass microchip within 1 min. Calcium and magnesium have also been separated within 15 s in a 2.5 cm long quartz microchip [21]. The separation of ammonium, methylammonium, and sodium has been obtained within 40 s in a 4 cm long polymer chip by using a movable contactless-conductivity detector [22].

Fast anion separation has been obtained using the co-electroosmotic separation mode on adding a cationic surfactant to the electrolyte that reverses the EOF, so that the anionic solutes electrophoretically migrate in the same direction as EOF [23–25]. Here, the same methodology is applied to generate a counter-electroosmotic mode of cation separation in order to decrease the length of the capillary. The length, as one of the size parameters, is an important aspect of microchip design where patterning density is a major issue. The present work describes a capillary electrophoresis analysis of Li^+, Na^+, and K^+ on a polymer chip using a polyelectrolyte coating of the micro channel surface with the objectives of performing a separation in 15 s within a 1 cm long separation channel. The long-term objective of this work is to design a lithium sensor for EOF reversed electrophoresis of extracted subcutaneous fluid where sample volume is a major issue.

2 Materials and methods

2.1 Chemicals

Polyethylene terephthalate (PET) sheets (100 μm thick Melinex) were purchased from Dupont (Geneva, Switzerland). 2-(*N*-Morpholino (ethanesulfonic acid) (MES), L-histidine (His), poly(allylamine hydrochloride) (PAA-HCl) and hydroquinone (HQ) were obtained from Sigma (St. Louis, MO, USA). The standard ionic solutions with a concentra-

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Abbreviations: His, L-histidine; **HQ**, hydroquinone; **PAA-HCl**, poly(allylamine hydrochloride) **PE**, polyethylene; **PET**, polyethylene terephthalate; **SHE**, standard hydrogen electrode

tion of 0.1 M were from Fluka (Buchs, Switzerland). Milli-Q water (Millipore, Bedford, MA, USA) was used to prepare all solutions. Freshly prepared solutions were used for the experiments.

2.2 Microchip fabrication

The fabrication of microchip has been previously described [26]. Briefly, the PET sheet is photoablated by a UV excimer laser (Argon Fluor Excimer Laser at 193 nm; Lambda Physik LPX 2051, Göttingen, Germany). The typical channel obtained has a trapezoidal cross-section shape. Carbon ink is pasted into a photoablated microchannel (with a depth of 20 μm and a width of 100 μm) and dried at 80°C for 30 min to form a conductive track. A photoresist solution (Shipley Europe, Herald Way, Coventry, UK) is used to seal and protect the carbon track, by spin-coating over the carbon track and thermally treating at 90°C for 1 h. In a second step, the main separation channel (corresponding to the horizontal channel shown in Fig. 1a) is photoablated perpendicularly to the carbon track so as to expose two face-to-face microelectrodes on the opposite vertical walls of the separation channel (Fig. 1b). The separation channel is usually about 40 μm deep and 50 μm wide at the top. Two side channels (corresponding to the two vertical side channels shown in Fig. 1a) are photoablated for injection purposes, which feature a so-called double-T design with a typical center-to-center distance of 100 μm. The microchip obtained is thermally laminated by a polyethylene/polyethylene terephthalate (PE/PET) layer (35 μm thick; Morane, Oxon, UK) at 135°C and 2 bar. The obtained microchip is similar

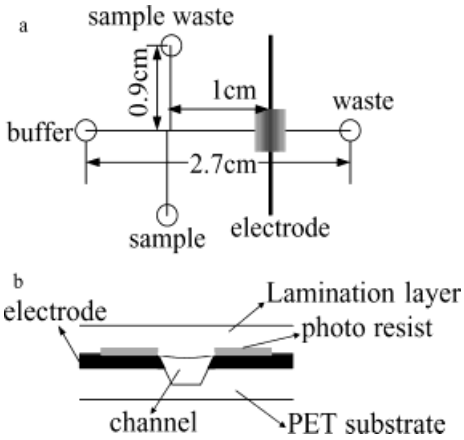

Figure 1. Scheme (a) of the top view of microchip and (b) the cross-section at the detector position. Two 0.9 cm long side channels (vertical) feature a double-T for injection purpose, two face-to-face microelectrodes are perpendicularly located at the end of the main separation channel.

to that shown in Fig. 1a (the top view) and Fig. 1b (the cross-section) except the effective separation length (from the double-T to the detection electrode) which may be different depending on the chip surface chemistry. The chip shown in Fig. 1a is indeed a polycation-coated microchip that has a shorter effective length of 1 cm.

2.3 CE on microchip

The microelectrodes are connected to a Metrohm conductivity meter (732 conductivity detector; Metrohm, Herisau, Switzerland) controlled by a PC through an interface (762 interface, Metrohm) [27]. A floating battery with 5 kV output and a locally made switching box are controlled by a PC through a Labview VI program (National Instruments, Austin, TX, USA). An electrokinetic floating injection is used to inject the sample: a positive potential of 500 V is applied for 20 s between the sample and sample waste reservoirs to fill the sample segment of the channel between these two reservoirs while the buffer and waste reservoirs are kept floating (no pinch). A positive potential of 800 V or 2 kV is applied between the buffer reservoir and the waste reservoir during a typical separation time of 50 s whilst the sample reservoirs are floating. Special attention has been given to the volume of the solutions in all the reservoirs. It is observed that when the solution volume in the buffer reservoir is smaller than that of other reservoirs, the sample plug in the double-T section migrates to the buffer reservoir during injection due to the effect of the hydrodynamic pressure, and a sample leakage from the sample channel to the main channel is observed after injection. A solution of 20 mM MES with 20 mM His (pH at 6) is used as background electrolyte (BGE) to separate cations [18]. HQ is added to the BGE to avoid the bubble generation due to an electrical cross-talk between the AC detection field (between the two face-to-face carbon electrodes) and the main DC high voltage. According to the redox potential of HQ (0.699 V *vs.* standard hydrogen electrode (SHE)), it can be easily oxidized into benzoquinone before water (1.23 V *vs.* SHE) to prevent the electrolysis of water that leads the formation of hydrogen (at cathode) and oxygen (at anode). The EOF measurement follows Huang's current monitoring method [28]. The principle of the measurement is to follow the conductivity change in a channel by monitoring the current at a constant voltage. When a solution of slightly higher conductivity is pumped (using a high voltage) inside a microchannel containing the same solution with lower conductivity, an increase and finally a stabilization of the current is observed, which indicates the time required by the solution to run through the channel. Measurement is performed with 4 mM and 20 mM MES/His

solutions to obtain an obvious current change, and a typical electrical field of 330 V/cm (by Spellman CZE 1000R Power Supply; Hauppauge, NY, USA) is applied to the microchannel.

2.4 Surface modification of microchannel

The surface modification of the PET channel by PAA-HCl is similar to that in a previously published work [29]. It was reported that the PAA-HCl absorbs on PET at low pH but reacts by amidation at high pH as a free base to form a covalently attached PAA-HCl layer. Therefore, PET-NH_2 samples prepared at high pH (such as 11.5) contain physisorbed PAA-HCl as well as chemisorbed PAA-HCl. Briefly, after washing with distilled water, the microchannel is filled with a PAA-HCl solution of 0.02 M repeat unit (corresponding to the concentration of the monomer) at pH 11.5 (adjusted by addition of 0.1 M NaOH) for 1 h. The channel is washed again with distilled water before filling with HCl (pH 2) for 30 min. The channel finally needs to be cleaned by distilled water and kept in air when it is not in use. The formation of the PAA-HCl layer is confirmed by EOF measurements. The electroosmotic mobility of a bare PET channel was measured to be 3.15×10^{-4} $cm^2V^{-1}s^{-1}$ and the electroosmotic mobility of a PAA-HCl coated PET channel was -3.52×10^{-4} $cm^2V^{-1}s^{-1}$. It shows that the magnitude of the electroosmotic mobility does not change much after surface coating but the flow direction is reversed. This layer is quite stable when the chip is kept in air, similar results can be reproduced after several days.

3 Results and discussion

A baseline separation of three cations, potassium, sodium, and lithium, is obtained in a bare PET microchip with an effective length of 4.5 cm (electric field of 308 V/cm), as shown in Fig. 2. The resolutions between potassium and sodium, sodium and lithium are 2.14 and 1.63, respectively. The theoretical plate number is calculated as 35 600/m for potassium ion.

The resolution of the separation is given by

$$R_s = \frac{t_2 - t_1}{\frac{1}{2}(w_1 + w_2)} \tag{1}$$

where t is the migration time and w is the baseline bandwidth of the signal, proportional to the difference of the migration time, Δt. This difference can be expressed in terms of the apparent mobility u

$$\Delta t = t_2 - t_1 = \frac{L}{E}\left(\frac{u_1 - u_2}{u_1 u_2}\right) \tag{2}$$

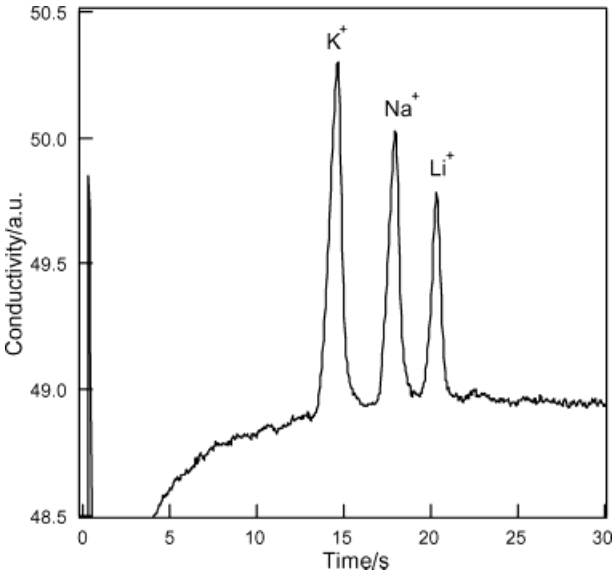

Figure 2. Electropherogram of ions. Channel dimension, 50 μm wide, 40 μm deep, and with 4.5 cm effective separation length; sample, 1 mM K^+, Li^+, and Na^+, injected at 500 V for 25 s and separated at an electrical field of 308 V/cm; BGE, 20 mM MES/His, pH at 6.

where L is the effective length of the separation channel and E is the electrical field strength. The resolution is also related to the width of the peak, which can be represented by the variance σ and therefore to the apparent mobility,

$$w = 4\sigma = 4\sqrt{2Dt} = 4\sqrt{\frac{2DL}{E}\frac{1}{u}} \tag{3}$$

where D is the diffusion coefficient. Then, the separation resolution can be written as

$$R_s = \frac{u_1 - u_2}{u_2\sqrt{u_1} + u_1\sqrt{u_2}}\sqrt{\frac{L}{8DE}} \tag{4}$$

The apparent mobility is the sum of the electroosmotic mobility and the electrophoretic mobility. The sign of the former depends on the sign of the zeta potential. For a bare channel, the charges on the channel are negative, *e.g.,* carboxy groups, the zeta potential is therefore negative and the electroosmotic mobility is positive, *i.e.*, the EOF goes in the same direction as the electric field. The sign of the electrophoretic mobility depends on the sign of the ions, and is positive for the cations. By reversing the charges on the microchannel walls by adsorption of the polycation, the electroosmotic mobility becomes negative. The difference $u_1 - u_2$ in Eq. (2) is not altered by reversing the flow direction, but the apparent mobility u of the cations becomes smaller when the flow is reversed. Indeed, for a bare channel the electroosmotic and the electrophoretic mobilities add for cations, whereas in the modified channel the electroosmotic mobility is sub-

tracted from the electrophoretic one. As a consequence, the ratio $\frac{1}{(u_2\sqrt{u_1}+u_1\sqrt{u_2})^{-1}}$ is increased upon the modification of the channel. In other words, to obtain a similar separation with a fixed resolution, either the length of the channel could be decreased or the electrical field strength could be increased.

The following experiment confirms this approach. A microchip with a short effective separation length, 1 cm (as shown in Fig. 1a), is used to separate the same cations as mentioned before. The electrophoregrams obtained in this microchip before and after PAA-HCl coating are compared in Fig. 3. As it can be seen from Fig. 3a, although

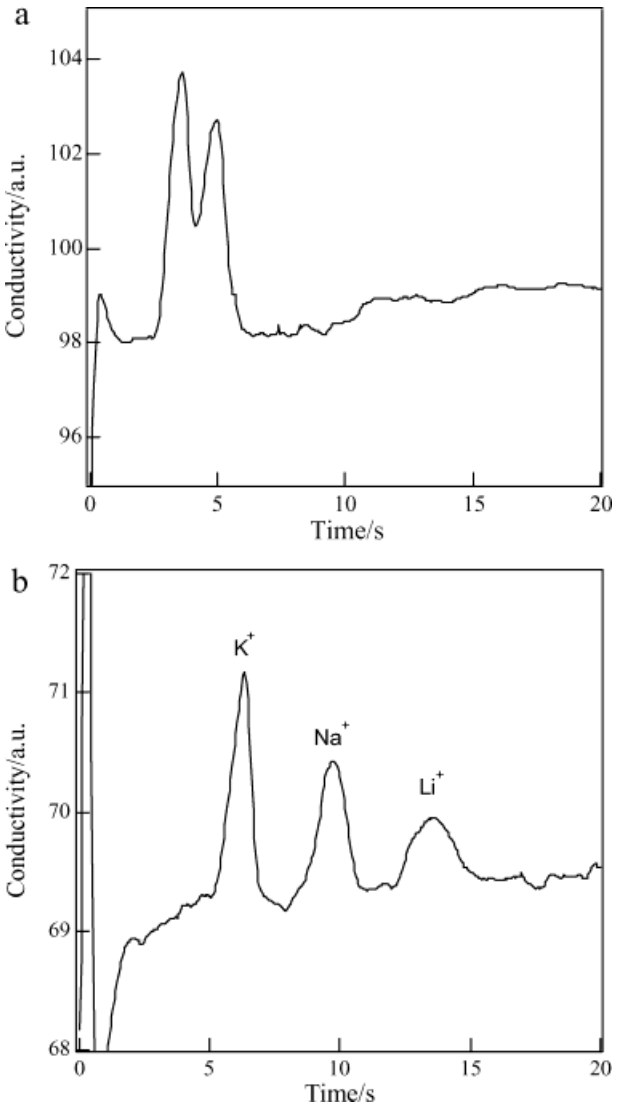

Figure 3. Comparison of the electropherograms obtained on a microchip (a) before and (b) after a PAA-HCl coated layer on the channel surface. Effective length, 1 cm; sample injection at 500 V for 20 s, separation at an electrical field of (a) 296 or (b) 741 V/cm; BGE, 20 mM MES/His+4 mM HQ, pH at 6. Other conditions as in Fig. 2.

each ion has not been identified respectively, the three ions cannot be separated in the bare PET microchannel when the electrical field is 296 V/cm, *i.e.*, about the same electric field strength as in Fig. 2. It has to be pointed out that the other broadening effects, such as the detector region width and the injection plug width, may improve the detected signal. For instance, the separation can be accelerated by injecting a very small plug, and a confocal fluorescence detection focusing in the center of the channel can also improve the resolution of the separation [30]. However, those are out of the scope of the present work and therefore have not been evaluated here. According to Eq. (4), when the effective separation length is decreased by a certain factor, the electrical field should be decreased by the same factor in order to obtain a separation with the same resolution. For this microchannel with an effective length of 1 cm, the electrical field should be 68 V/cm to obtain a separation similar to that shown in Fig. 2. When the channel is coated with a PAA-HCl layer, a baseline separation is obtained even at an electrical field of 740.7 V/cm, (Fig. 3b). With this flow reversal method, a high electrical field, instead of a much lower electrical field as in the usual CE, can be applied when the effective length is highly decreased. The resolution between potassium and sodium is calculated to be 1.68 and 1.23 between sodium and lithium. The relatively small value of the resolution between sodium and lithium is mainly due to the broadening of the lithium signal. The theoretical plate number for potassium is 29 000/m. Compared to the separation shown in Fig. 2, both the resolution and the theoretical plate number are only slightly decreased. As shown in Eqs. (2) and (4), the migration time can be further decreased by increasing the applied electrical field, at the cost of reduced resolution (linear dependence *vs.* square root dependence). Those data show that the short channel coated with a PAA-HCl layer yields a similar electrophoresis efficiency for the small cations separation compared to the longer bare channel.

The presented experiments show a separation of cations for whose electrophoretic mobility is higher than the electroosmotic one. This method can also be applied to cations that have relatively small electrophoretic mobility. In this case, the electric field direction needs to be reversed for the cations to pass the detector and the apparent mobility is then negative.

In conclusion, we developed a surface modification method for polymer microchip to decrease the effective separation length of the chip whilst maintaining the resolution. This is important to microdevice design strategies as microfabrication techniques impose restriction on the

size of the devices. The present method therefore allows higher packing densities when fabricating CE, *e.g.*, by plasma etching methods [31].

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