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Direct measurement of lithium in whole blood using microchip capillary electrophoresis with integrated conductivity detection

The direct measurement of lithium in whole blood is described. Using microchip capillary electrophoresis (CE) with defined sample loading and applying the principles of column coupling, alkali metals were determined in a drop of whole blood. Blood collected from a finger stick was mixed with anticoagulant and transferred onto the chip without extraction or removal of components. The electrokinetic transport of red blood cells inside the channels was studied to find sample loading conditions suitable for the analysis of lithium without injecting cells into the separation channel. Both bare glass chips and chips coated with polyacrylamide were used showing the behavior of the cells under different electroosmotic flow conditions. In serum a detection limit for lithium of 0.4 mmol/L was reached. Proteins quickly contaminated untreated chip surfaces but devices with coating gave reproducible electropherograms. In addition, potassium and sodium were also detected in the same separation run. To our knowledge, this is the first device to directly measure ions in whole blood with the use of capillary zone electrophoresis on a microchip.

Keywords: Conductivity detection / Lithium monitoring / Microchip capillary electrophoresis / Microfluidics / Miniaturization / Whole blood DOI 10.1002/elps.200405885

1 Introduction

Direct analysis of whole blood without any sample pretreatment is still a largely unexplored field in separation science. Its complex matrix makes measuring even in serum or plasma a nontrivial analytical problem. At the present state of microfluidic chip technology, it is now possible to combine sample treatment steps with separation methods on a single chip. However, few devices have been developed that fully exploit combining multiple functionalities in so-called micrototal analysis systems (μTAS) [1, 2]. Instead of advanced multifunctions on chip, here we demonstrate that the measurement of alkali metals in a drop of whole blood can be performed on a capillary electrophoresis (CE) microchip with a standard double-T injector [3] applying the principles of column coupling.

In this paper, we will focus in particular on the analysis of lithium in blood. Under normal dietary conditions, lithium is not present in the human body in a significant amount. However, lithium salts are widely used in the treatment of manic-depressive illnesses where they have been shown to be very effective in stabilizing mood swings, either as a single drug or in combination with antidepressants. Because of differences in the excretion rates between patients, the daily lithium dosage can vary between 10 and 80 mmol, resulting in plasma concentrations of 0.5–1.2 mmol/L for effective treatment [4]. In addition, the therapeutic index, *i.e.*, the ratio between the toxic concentration (approximately 1.6 mmol/L) and the therapeutic concentration, is very low, and the two together make monitoring of the lithium concentration in blood throughout the treatment a critical issue.

In the clinical laboratory the first step of almost any blood test is to remove the cells to obtain either plasma or serum. Therefore, errors caused by continuing cell metabolism or cell lysis are avoided. Routine clinical methods for the determination of alkali metals in plasma or serum include flame emission spectroscopy, atomic absorption spectrometry and ion-selective electrodes (ISE). Though these techniques provide accurate results, there is currently no point-of-care test for lithium available. Both patients and physicians would therefore welcome a lithium analyzer that provides an almost instantaneous result without any sample preparation and reduces the necessary amount of sample to a single drop obtained by a finger stick. Commercially, there are a few point-of-care tests available that actually do measure directly in whole blood. Examples are the common glucose meter and the more sophisticated i-STAT clinical analyzer [5].

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Abbreviations: RBC, red blood cell; **ISE**, ion-selective electrode

The first instrument uses amperometry to measure the enzymatic conversion of glucose on a pair of electrodes. Measuring a large variety of components, the i-STAT system utilizes cartridges with different detection principles (*e.g.*, miniaturized ISE, conductivity and amperometry). Although it is possible to measure lithium with a conventional ISE there is ongoing research finding suitable ionophores with sufficiently high selectivity in respect to sodium [6]. Using a more generic method, for example, separation by CE, the analysis of ions in blood samples potentially suffers less from interference and has the additional advantage that a multitude of ions can be measured at once on the same device without the need for selective electrodes.

One of the problems of working with blood is the vast amount of cellular material in blood. Blood contains approximately five million red blood cells (RBCs) per microliter, accounting for approximately 40% of the total blood volume. Platelets take up another 6% of the volume or 250 000 cells per microliter while the white blood cell count is approximately 7000 per microliter. These solid blood components can interfere with the measurement method. Presently, there is research conducted towards the fabrication of micromachined devices that are capable of preparing plasma from whole blood on-chip. One way to generate plasma is to put the whole chip in a centrifuge after collecting a blood sample [7]. Alternatively, a blood sample can be pumped through a filtration structure to remove cellular material. Examples of these filters are composed of weirs or microposts that have been demonstrated to trap white blood cells [8], lateral percolation filters machined in silicon [9] or devices using dielectrophoretic principles to capture cells [10, 11]. A different approach is taken by the T-sensor or filter, which does not trap the cells but relies on diffusion of ions and molecules into an acceptor solution instead [12].

Once plasma or serum is available still the separation by CE is not trivial. The high sodium and chloride background concentrations of around 140 and 105 mmol/L, respectively, remain as a problem. Compared to most CE background electrolytes (BGEs), blood has a much higher ionic concentration and this mismatch between sample and BGE could result in extensive dispersion. The analysis of lithium with conventional CE was therefore performed on 20 or 50 times diluted serum [13]. Alternatively, Everaerts and Gebauer [14, 15] showed that there are conditions under which minor components can still be determined in an excess of matrix. This sample self-stacking relies on transient isotachophoretic conditions at the beginning of the separation changing to zone electrophoresis at the end. There, the matrix ion serves as either leading or terminating ion with the BGE co-ion as its complement. This allows sample components with a mobility between that of the matrix component and the BGE co-ion to be determined with high efficiency [16]. This principle has been applied, for example, to measure organic acids in undiluted serum [17]. In addition, matrix components like proteins can cause problems in CE due to wall adsorption shifting performance particularly over multiple runs. These adsorption effects have to be studied for method development but may not be a critical parameter for a single-use disposable device. Also many types of surface coatings can be found in literature to reduce the adsorption problem of proteins on silica [18].

The chip-based electrophoresis of alkali ions in aqueous samples has been shown before, including the separation of lithium [19–23]. The challenge is to take this a few steps further and use the specific advantages that chip technology offers to measure these ions in whole blood. In order to prevent interference from blood cells, it will be necessary to avoid injecting them into the separation channel. The largest concern of this type of measurements is that cells enter the separation channel and could lyse, releasing their content. In this study, we present for the first time the direct measurement of whole blood by microchip CE. The strategy is to use the fact that blood cells have a relatively low electrophoretic mobility compared to the alkaline metals, which allows cell-free sample plugs to be formed in the double-T of a typical microchip CE device.

2 Materials and methods

2.1 Reagents

Standards containing sodium, potassium and lithium were prepared by dissolving the chloride salts (Merck, Darmstadt, Germany) in deionized water (Millipore, Bedford, MA, USA). To model clinically relevant concentrations, aqueous solutions were prepared with 0.5 and 1 mmol/L lithium in the presence of 150 mmol/L sodium while the calibration curve extends to 5 mmol/L lithium. For the CE experiments, a BGE consisting of 50 mmol/L 2-(*N*-morpholino)ethanesulfonic acid (MES) (Sigma, Steinheim, Germany) and 50 mmol/L histidine (His) (Fluka, Buchs, Switzerland) with a pH of 6.1 was used. In certain experiments, 200 mmol/L glucose (Sigma) was added to adjust the osmotic strength of the BGE. Glucose does not increase the electrical conductivity of the BGE, and therefore we do not expect any interference with the separation or detection of alkali ions.

2.2 Blood samples

Blood was obtained performing the finger stick method on a volunteer using Haemolance (HaeMedic AB, Munka Ljungby, Sweden) disposable lancets. Approximately

30 μL of blood was collected with a pipette and transferred to a plastic tube. For experiments requiring whole blood, 10 μL of 0.0806 mol/L sodium citrate (Sigma) was added to stop coagulation whereas when serum was required the sample was allowed to clot for 10 min. Serum was collected after centrifuging the sample for 10 min at 11 500 × *g* (Biofuge Pico; Heraeus, Langenselbold, Germany). From each sample, an aliquot of 18 μL was spiked with 2 μL of 20 mmol/L lithium just before the start of an experiment in order to obtain a concentration of 2 mmol/L. To visualize the plug formation, a positively charged fluorescent dye of 1 mmol/L (rhodamine 123; Fluka) was added to the samples when required.

2.3 Microfabricated CE chips

Figure 1a shows a Borofloat glass chip with a double-T injector, which was purchased from Micronit Microfluidics BV (Enschede, The Netherlands). All channels were etched to a depth of 8 μm and a top width of 56 μm using hydrofluoric acid. The same figure also shows closeups of the conductivity detection electrodes (Fig. 1b) and the double-T injector (Fig. 1c). The length of the separation channel from the T-injector to the detection electrodes was 2 cm while the T-intersection had a length of 200 μm. The detection electrodes consisted of a layer of thin-film platinum, which is in direct contact with the electrolyte inside of the channel. The chips were placed in a holder made from Delrin™ consisting of a bottom support plate with an opening below the chip. A cover plate with platinum wires inserted into the fluidic compartments for electrical contacts to the high-voltage supply was placed on top of the chip. The holder containing the chip can be placed on an inverted microscope stage (Leica DM/IRM; Wetzlar, Germany) to follow the filling of the channels as well as tracking the cells.

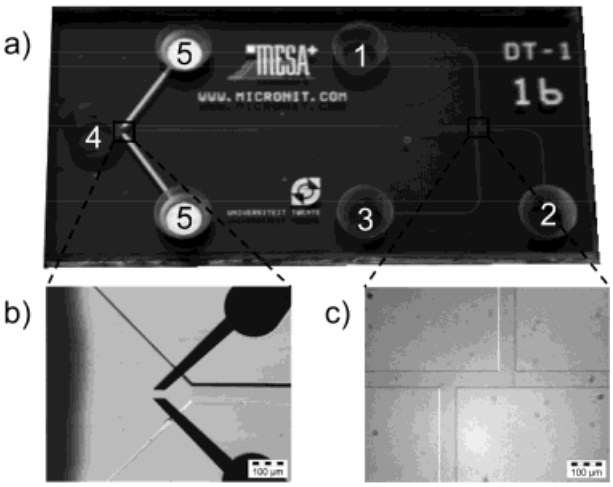

Figure 1. (a) Photograph of the microchip (dimensions, 3 cm × 1.5 cm). 1, sample compartment; 2, BGE compartment; 3, waste compartment; 4, outlet compartment; 5, detection electrodes. Close-ups of the end of the channel with (b) the conductivity detection electrodes and (c) double-T injector.

2.4 Surface coating

All experiments were performed on coated glass chips unless specified otherwise. Chips were coated with polyacrylamide according to the procedure of Hjertén [24] for fused-silica capillaries. In brief, the chip was filled with a solution of 40 μL of 3-(trimethoxysilyl)propyl methacrylate (Aldrich, Milwaukee, WI, USA) in 10 mL water adjusted to pH 3.5 with acetic acid (Merck) and allowed to react overnight. The channels were subsequently washed with water and filled with a mixture of 3% w/v acrylamide (Aldrich), 0.1% v/v *N,N,N',N'*-tetramethylethylenediamine (Sigma) and 0.1% w/v potassium persulfate (Aldrich) in water. During the polymerization reaction, the chip was covered with a microscope slide to keep out oxygen from the air. After 30 min the solution was removed and channels were washed with water after which the chip was ready for use.

2.5 CE on the microfabricated chip

For the CE experiments a computer-controlled high-voltage power supply (CU 411; IBIS Technologies BV, Hengelo, The Netherlands) with four independently controllable positive-voltage outputs and a custom-made AC conductivity detector (Sprenkels Consultancy, Lelystad, The Netherlands) were used. The detector signal was recorded with a data acquisition card (DAQCard 6036E; National Instruments, Austin, TX, USA). An in-house written software package combined the control of the power supply, acquisition of data from the detector and the subsequent data processing. A pinched sample loading procedure was used to fill the double-T with sample. The voltages were set at the sample compartment and at the outlet to 1000 V, at the waste to 0 V and at the BGE compartment to 800 V. These settings were maintained for 15 s on an uncoated chip and for 60 s on the coated microchip. To initiate the separation, the voltages were switched at the BGE compartment to 1000 V, at the sample and waste to 600 V, and at the outlet to 0 V. The separation was performed for 60 s on the uncoated chip and for 120 s on the coated chip. Throughout the experiment, the RBCs were tracked to determine the distance they traveled inside the channels and to verify whether they remain intact. The platelets and white blood cells were not seen under these conditions.

3 Results and discussion

3.1 Sample loading and electrokinetic transport of RBCs in uncoated channels

The first experiments were performed on a device without coating. Under physiological conditions, RBCs, white blood cells and platelets all have a negative net charge, which is mainly due to glycoproteins on the cell surface terminating in a sialic acid group. Because of this charge RBCs can be transported through capillaries [25] or since there is some heterogeneity, the electrophoretic mobility of individual cells can be determined [26]. On-chip manipulation of RBCs has been demonstrated too, showing the possibility to lyse cells by mixing in a stream with sodium dodecyl sulfate (SDS) detergent [27] or perform immunoelectrophoresis of RBCs [28]. However, in all these papers the authors used washed RBCs resuspended in buffer or at least diluted the blood sample in order to minimize interference from proteins and to obtain a suitable cell concentration.

In order to explain how the cells can be excluded from the cross or T-injector of the microchip (Fig. 1) without any additional features for sample preparation, a closer look to the sample loading is required. We consider the following loading configuration: the anode is positioned in the sample compartment while the cathode is in the waste compartment. In general, there is also a voltage applied to the remaining compartments to prevent leakage of sample into the separation channel, thus creating the conditions for full plug shaping [29]. Electrophoretic mobility values of RBCs between -1.6×10^{-4} cm^2/Vs and -3.0×10^{-5} cm^2/Vs have been reported [25, 26]. The mobility of the EOF in glass channels is around 4.2×10^{-4} cm^2/Vs (calculated from the EOF peak in our microchip CE experiments). Since the EOF is faster than the cells, they will enter the sample channel during the sample loading and consequently they will eventually fill also the double-T. However, for the measurement of relatively fast ions such as sodium and lithium, the sample loading does not have to be maintained that long, which gives the opportunity to start the separation before the cells reach the double-T.

For example, consider the situation depicted in Fig. 2a, showing the sample loading after a certain time. From the start of the loading, the bulk of the sample moves towards the injector because of the EOF. Cations that migrate out of the EOF-driven sample bulk form additional leading zones. Completely at the front, there is a zone containing only the fastest sample component, in this case potassium. This zone is followed by moving boundary zones in the order of decreasing mobility [30]. Due to this fact, a specific fraction of the sample can be selected by time-dependent transport to the T-injector and subsequently analyzed (Fig. 2b). When switching too late to the separation phase, also slow-moving components such as

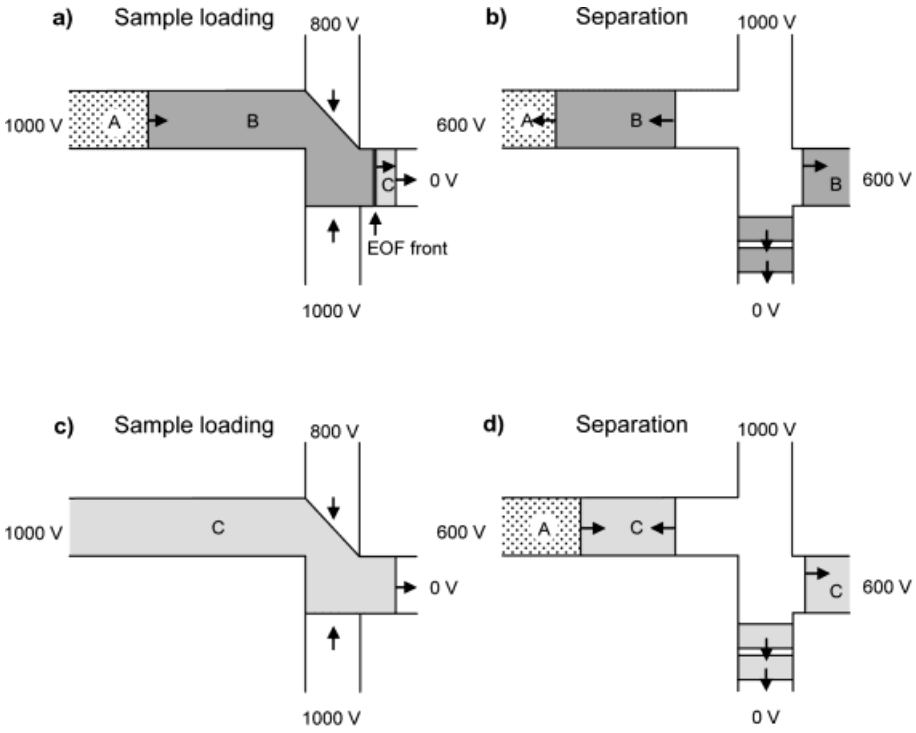

Figure 2. Schematic representation of the sample loading and separation procedure in (a), (b) uncoated and (c), (d) coated channels. Zone A, containing blood cells; B, EOF-driven zone free from cells: C, cations migrated out of the sample zone. Sample compartment is on the left of the picture, BGE on the top, waste on the right and outlet compartment is on the bottom.

the blood cells (zone A in Fig. 2) are injected into the separation channel. On the contrary, when the sample loading is too short the lithium has not reached the injector. Estimating the minimal loading time for lithium based on its electrophoretic mobility, 4.5 s would be sufficient to reach the injector at zero EOF conditions (normally during CE on glass chip the ions experience a positive EOF also, which would make their net flow even faster). By observing the migration of RBCs within a blood sample, the maximum allowable loading time can be determined.

On the uncoated chip, cells from a whole blood sample are indeed pumped from the sample compartment into the sample channel during the loading step (Fig. 3). Using the chip for the first time with whole blood, the cells almost reached the double-T in the loading time of 15 s as selected for this experiment. During the separation the cells ought to be pumped out of the sample channel again, however, a significant amount adhered to the surface. In addition, these cells were exposed to the low osmotic strength BGE during the separation mode, which caused them to lyse. Also a few cells were seen to travel into the opposite direction compared to the majority, which was probably caused by mobility differences between individual cells combined with local variations in the EOF due to protein adsorption. After the experiment, any cells that were still inside the channels were flushed out manually using a hydrodynamic flow of BGE. In a subsequent run, the cells travel only half the distance during the loading indicating that proteins were adsorbed during the first run and reduced the EOF. It was also observed that this protein layer reduced the interaction between cells and the channel wall, decreasing the risk for cell lysis during the pull-back of the separation run.

In all runs, the sodium peak was clearly identified, but lithium could not be resolved sufficiently (Fig. 4). For the calibration mixtures containing only 150 mmol/L sodium and 2 mmol/L lithium in water, the components were not resolved at all (curve I in Fig. 4). The difference in peak height between calibration and blood is proof for variations in the sample loading conditions. All these factors together prompted us to continue the experiments with microchips that had a defined coating on the surface of the channels in order to obtain more stable conditions.

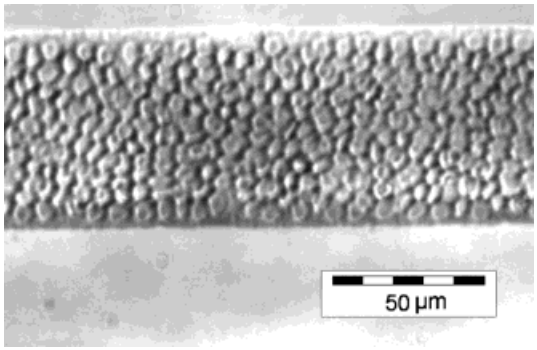

Figure 3. RBCs as observed in the experiments entering the sample channel of an uncoated device during sample loading. This photograph is taken from a device with slightly wider channels than used for all other experiments.

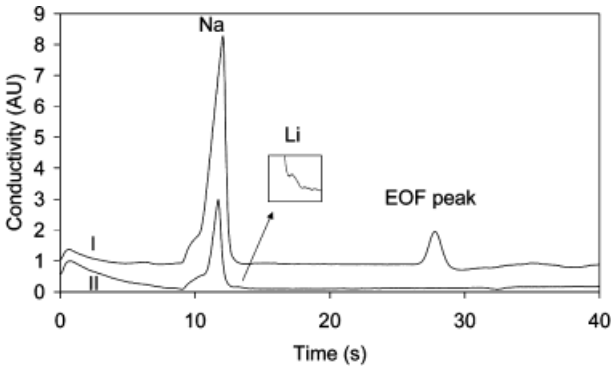

Figure 4. Separation on an uncoated device of (I) aqueous calibration mixture containing 150 mmol/L sodium, 5 mmol/L potassium and 2 mmol/L lithium; (II) whole blood spiked with 2 mmol/L lithium. BGE, 50 mmol/L MES/His.

3.2 Sample loading and electrokinetic transport of RBCs in coated channels

When applying EOF suppression, the net direction of the cells reverses as the mobility of the cells exceed that of the EOF. Under these conditions, the cells do not enter the channels during the sample loading and cells that are present there, *e.g.*, from a previous experimental run, will even migrate out (Fig. 2c). However, when a pull-back field is used during the separation, cells will now enter the sample channel (Fig. 2d) and depending on the duration of the separation as well as the electric field strength inside the channels, cells could eventually reach the double-T. Once reached the double-T, they would continue to move towards the BGE compartment, thus do not enter the separation channel and travel towards the detection region. Yet, this could interfere with subsequent experiments.

In general, if the EOF is faster than the mobility of the cells, the cells are always in a surrounding derived of the blood sample. In contrast, if the cells and bulk flow move in opposite directions, the cells can also migrate out of the blood matrix into the BGE. For example, when there is any residual EOF, the cell-depleted blood is pumped into the sample channel during the loading step while the cells themselves remain in the sample compartment. During the separation this blood matrix is again pumped out of the sample channel, but now the cells enter the sample

channel and at some point will cross the interface between the blood matrix and BGE. Consequently, when the osmotic strength of the BGE is not matched to blood, cells could lyse. To prevent this, 200 mmol/L glucose is added to the BGE during experiments with whole blood.

The loading experiments using whole blood samples on a coated device clearly showed that the EOF indeed has been suppressed substantially as cells did not enter the sample channel. Only during the separation when a small pull-back field is used cells slowly migrated down the channel. Cells traveled just around 2 mm within the 120 s of separation in this case. At the moment the next run was started, all cells quickly migrated out leaving none behind. Also for repeated injections there was no indication of changes in the behavior of the cells. It was observed that cells being exposed to the BGE during the separation step still showed some degree of swelling, however, there was no sign of cell lysis using the glucose-modified BGE. To summarize, the net transport of cells could be tuned by changing the surface conditions of the channels. The use of a polyacrylamide coating results in reproducible behavior of the RBCs for at least three consecutive runs.

3.3 Quantitation of lithium in serum and whole blood

Potassium, sodium and lithium peaks were identified in aqueous calibration mixtures (Fig. 5) and blood samples (Fig. 6) on a coated microchip. Electropherograms obtained from a coated chip show that the performance was superior compared to that of an uncoated one (Fig. 4). The higher efficiency might be attributed to the reduction of the EOF, which generally results in a better separation. Another important conclusion is that also under conditions of suppressed EOF described in this work, the electrophoretically pinched plug shaping works very well (Fig. 7).

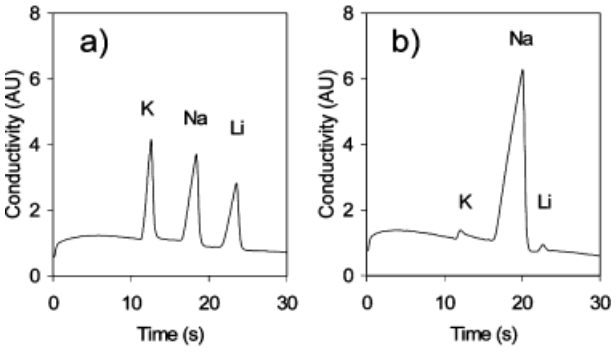

Figure 5. Separation of aqueous calibration mixtures on a coated chip. (a) 1 mmol/L of potassium, sodium and lithium; (b) 150 mmol/L sodium, 5 mmol/L potassium and 2 mmol/L lithium. BGE, 50 mmol/L MES/His.

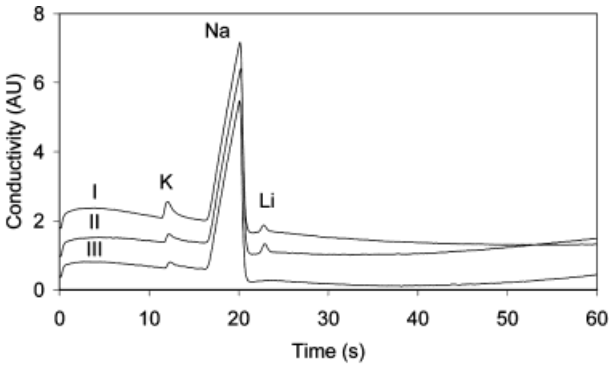

Figure 6. Separation of blood on a coated device; (I) Serum spiked with 2 mmol/L lithium; (II) whole blood spiked with 2 mmol/L lithium; (III) whole blood without spiking. BGE, 50 mmol/L MES/His with 200 mmol/L glucose.

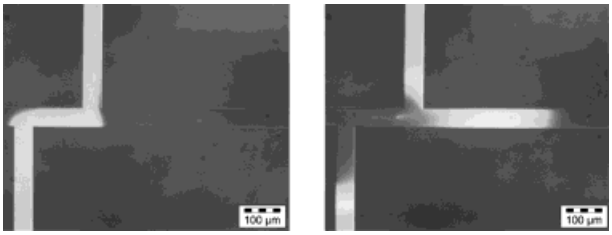

Figure 7. Sample plug formation in the double-T and subsequent injection into the separation channel. Whole blood sample with 1 mmol/L rhodamine 123.

For the quantitation of lithium only results obtained with the coated chip were used. At this point, it is important to realize that the sample loading and formation of the sample plug is solely based on the electromigration of ions instead of pumping the sample through the double-T by EOF. This has a fundamental consequence on the actual composition of the sample plug injected into the separation channel. Whereas with EOF loading the composition of the sample plug is similar to the bulk of the sample, now with only electromigration the concentration of sample components changes in a way described by Kohlrausch [31]. With the electromigration as transport mechanism, the cations from the sample displace the BGE coions. The initial BGE concentration and the sample matrix composition therefore determine the final sample concentration inside the channels, regardless of the concentration in the sample. In fact, what happens is that sample is either stacked or destacked during the loading. Since the blood sample has a higher ionic strength than the BGE the sample plug should consist of diluted sample. These effects were verified by examining the calibration curve of an aqueous mixture of sodium and lithium, which showed a nonlinear behavior (Fig. 8). For the concentration of lithium in the sample plug this means that it is more

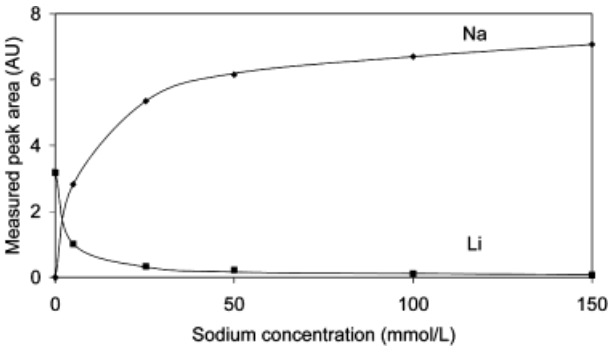

Figure 8. Calibration curve for aqueous sodium standards containing 2 mmol/L lithium.

and more diluted when the sample has a larger amount of sodium. For quantitation it is therefore necessary to correct for this matrix effect using an internal standard. The blood sodium concentration is fairly stable between 135 and 145 mmol/L and could be used as a readily present internal standard. In the calibration curve of lithium in a background of 150 mmol/L sodium (Fig. 9), sodium was actually used as internal standard by dividing the peak area of lithium by that of sodium to correct for changes in the loading conditions. This also presents us with the interesting opportunity to report sodium-lithium ratios instead of absolute lithium concentrations. It has been argued that sodium-lithium ratios reflect the patients' condition better [32]. This only requires knowledge about the relative detector sensitivity for sodium and lithium, skipping the calibration required to determine absolute values.

From the calibration experiments (Fig. 9), the relative lithium/sodium sensitivity was calculated, which was used to calculate the lithium concentration in the spiked blood samples. For the calibration standards a factor of 1.18 was determined. In order to calculate the lithium concentration, it was assumed that the plasma sodium concentration in prepared blood samples (see Section 2.2), which was used as internal standard, was 148 mmol/L. From the composition of blood as described in the introduction, the second assumption was that the volume of plasma is 55% of whole blood and that there is no exchange of components between cells and plasma. Spiking a blood sample of 18 μL with 2 μL of 20 mmol/L lithium chloride therefore theoretically results in a value of 3.36 mmol/L lithium. The experimental results show a lithium concentration of 2.7 mmol/L with an RSD of 2.1% (n = 3) calculated from the electropherograms. Hence, the recovery of lithium from whole blood is 81%. Since we could expect changes in the concentration profile of a whole blood sample, for example, due to the uptake of lithium by cells the experiment was repeated using serum as sample. This sample also was spiked to result a lithium concentration of 2 mmol/L. The concentration calculated from that experiment was 1.8 mmol/L with a detection limit of 0.4 mmol/L for lithium defined as three times the low-frequency noise level. Thus, in this case lithium was also not fully recovered. Using an average of 140 mmol/L sodium as the internal standard the error on the lithium concentration can be estimated to be up to 4%. The error in the measured peak area does also not sufficiently explain the error of approximately 10% on the recovery for the serum sample. Depending on the choice of buffer system, the concentration profile of ions in the sample plug may be influenced by the ions in the sample matrix as it was demonstrated for sodium (Fig. 8) and does not necessarily represent the original concentration ratios of the serum sample. To investigate the sources of error in detail, more experiments are necessary. It is furthermore interesting to note that the potassium peak in the serum sample is higher than in the whole blood sample (Fig. 6), indicating that not all the cells stayed intact during the described nonclinical sample treatment for serum. However, this measurement was performed on a single sample only.

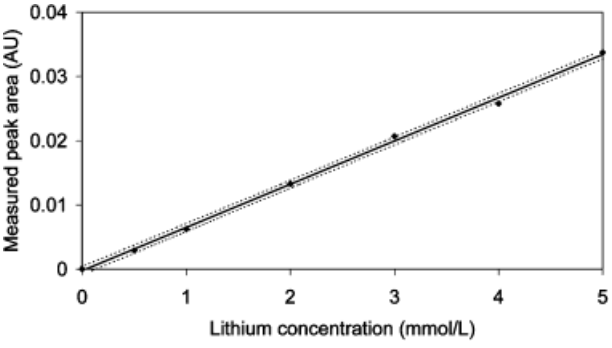

Figure 9. Calibration curve for lithium in a fixed matrix of 150 mmol/L sodium. The dotted lines denote the 95% confidence intervals.

4 Concluding remarks

The presented experiments demonstrate that it is possible to measure alkali ions in a sample as complex as whole blood with a microfluidic glass chip. Even a bare glass chip without a coating might be suitable for single use, although the current device did not have sufficient resolving power. The chip coated with polyacrylamide provided stable results over repeated injections with no adhesion of RBCs to the channel surface and increased resolution for the separation of sodium and lithium. The voltage sequence in respect to the exact timing of loading and separation to prevent injection of blood cells was found to be not critical.

Lithium could be determined in whole blood diluted only with anticoagulant and spiked with lithium. Because of the matrix effect shown in Fig. 8 it is absolutely necessary to use an internal standard for quantitation. In serum the detection limit for lithium is with 0.4 mmol/L on the high side because a quantitation limit of 0.2 mmol/L is desirable for clinical use. The main limitation for further optimization is the restriction of the dynamic range of the detector. Thus, increasing the sensitivity would only result in exceeding the working range of the detector which has been reached already for the sodium peak. Therefore, the aim will be to reduce baseline noise by improving the detector electronics and to increase sensitivity for lithium compared to that of sodium by the choice of an appropriate BGE.

Measurements on calibration samples in the clinically relevant range were presented. The next step towards the final application will be to measure in blood samples obtained from patients being on lithium therapy and compare the results obtained from the microchip with routine clinical methods. A larger supply of sample taking into account the variation from one individual to another will give us the opportunity to focus more on the quantitative aspects in order to improve the lithium recovery. Especially, the sample loading process under conditions of suppressed EOF needs to be studied in greater detail. Hence, our understanding of the process of stacking and destacking taking place in the intended application should increase. Preliminary results on these phenomena based on moving boundary zones have been published by us elsewhere [33].

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