Clinical Chemistry 53:1
000–000 (2007)

Point-of-Care Testing

Microchip Capillary Electrophoresis for Point-of-Care Analysis of Lithium

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Background: **Microchip capillary electrophoresis (CE) is a promising method for chemical analysis of complex samples such as whole blood. We evaluated the method for point-of-care testing of lithium.**
Methods: **Chemical separation was performed on standard glass microchip CE devices with a conductivity detector as described in previous work. Here we demonstrate a new sample-to-chip interface. Initially, we took a glass capillary as a sample collector for whole blood from a finger stick. In addition, we designed a novel disposable sample collector and tested it against the clinical standard at the hospital (Medisch Spectrum Twente). Both types of collectors require <10 μL of test fluid. The collectors contain an integrated filter membrane, which prevents the transfer of blood cells into the microchip. The combination of such a sample collector with microchip CE allows point-of-care measurements without the need for off-chip sample treatment. This new on-chip protocol was verified against routine lithium testing of 5 patients in the hospital.**
Results: **Sodium, lithium, magnesium, and calcium were separated in <20 s. The detection limit for lithium was 0.15 mmol/L.**
Conclusions: **The new microchip CE system provides a convenient and rapid method for point-of-care testing of electrolytes in serum and whole blood.**

Lithium is one of the most important mood stabilizers for treatment of bipolar mood disorders *(1, 2)*. A disadvantage of lithium is the narrow therapeutic range (0.4–1.2 mmol/L) *(3)*, which necessitates therapeutic drug monitoring. Thus in some countries prescription of lithium is limited by lack of facilities, equipment, and/or trained personnel to measure lithium. A point-of-care test for lithium can solve these problems and allow improved treatment of patients.

Plasma or serum lithium concentrations are often determined with ion-selective electrodes (ISEs)[3] *(4)*. ISEs are also used in point-of-care testing to measure potassium and sodium in whole blood *(5)*. Recently a point-of-care test has been developed specifically for lithium *(6)*. The method measures the change in light absorbance by a porphyrin compound when it forms a complex with lithium. This test requires the sample to be applied onto a cell separator strip and subsequently to be transferred to a cuvette filled with the reagent. A sample volume of 50 μL is required for the test.

Compared to the above methods, capillary electrophoresis (CE) is advantageous because it is not limited to a single analyte. Developments in microchip CE show the potential for point-of-care applications *(7)*. In many instances the separations can be performed in the microchip in <1 min. For point-of-care applications microchip CE not only provides a short analysis time but also allows ease of sample handling, as well as user-friendly and robust instrumentation. Conventional CE has been used for a wide range of substances such as serum proteins *(8)*, β-agonists *(9)*, and amphetamines in whole blood *(10)*. Conventional CE has also been used to measure lithium in serum *(11)*. A review of additional applications is also available *(12)*. Miniaturization of conventional systems, in the form of microchip CE, offers the possibility of developing point-of-care tests for numerous compounds of interest. Microchip CE has also been demonstrated to be a potential tool for clinical diagnostics for analysis of renal markers in urine *(13)*, measurement of proteins *(14)* and valproate *(15)* in serum, and analysis of lithium in whole blood *(16)*. In addition to the speed advantage of microchip CE over conventional CE, the microchip manufactur-

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Received May 23, 2006; accepted October 26, 2006.
Previously published online at DOI: 10.1373/clinchem.2007.073726

[3] Nonstandard abbreviations: ISE, ion-selective electrodes; CE, capillary electrophoresis; BGE, background electrolyte solution.

ing process can provide methods for the integration of on-chip sample preparation that is required for point-of-care testing *(17, 18)*. A simple method to transfer patient samples to the microchip has not been developed, however. The ability to analyze finger-stick samples rather than venous whole blood would be a great improvement. We previously developed a new analytical method based on microchip CE for lithium detection directly from whole blood *(16)*. An elegant technique for on-chip sample pretreatment of whole blood was achieved by moving-boundary electrophoresis, but this method could not be fully validated for direct measurement from a patient finger-stick sample *(19)*. In addition, in our earlier configuration the channels of the microchip became contaminated with blood residue such as cells and large molecules, giving a high degree of uncertainty of the method. We thus discarded the chip after a single use. To facilitate a clinical trial that included repetitive direct sampling of capillary blood to the microchip, we had to modify the method used to introduce the sample onto the chip.

Materials and Methods

We performed routine clinical lithium testing (Medisch Spectrum Twente) on serum samples obtained by venipuncture and measured for lithium concentration by a robot with ISE detection. The introduction of sample collectors allowed us to measure whole blood samples, collected with standard procedures from healthy volunteers and from patients, on the same chip for proof of method. We then quantitatively compared the data obtained from serum by use of the routine clinical method with the data obtained by the chip method.

REAGENTS

CE separations were performed in a background electrolyte solution, pH 4.8, consisting of 30 mmol/L ammonium acetate (Sigma) and 30 mmol/L acetic acid (Merck) in deionized water (Millipore). For selected experiments the resolution between calcium and sodium was enhanced by adding 4 mmol/L tartaric acid (Baker) to the background electrolyte as a complexing agent. Calibration solutions were prepared by dissolving lithium chloride (Merck) into 140 mmol/L sodium chloride (Merck) in water, to create a concentration range of 0–2 mmol/L.

MICROFABRICATED ELECTROPHORESIS CHIPS

The Borofloat® glass CE microchips (Fig. 1A) were obtained from Micronit Microfluidics. We have previously described the principles of design and operation of this microchip *(16)*. In brief, the fluidic channels are etched to a depth of 8 μm and width of 56 μm at the top. The effective length of the separation channel, measured from the 200-μm long double-T injector to the detection electrodes, is 2 cm. The detection electrodes for measuring the conductivity consist of thin platinum films that are in direct contact with the electrolyte solution in the channel.

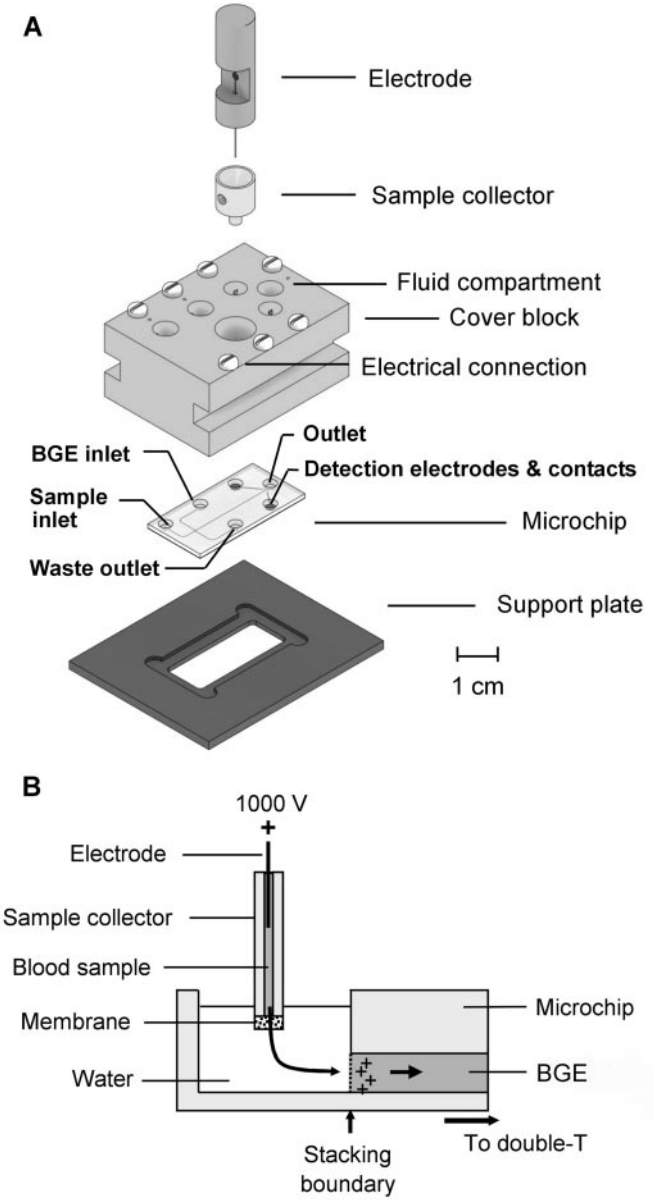

Fig. 1. (*A*) Components of the microchip CE system with the Plexiglas sample collector and (*B*) schematic representation of the sample-to-chip interface.
Fig. not to scale.

Using a procedure initially developed for fused silica capillaries, we coated the chips with polyacrylamide to reduce the electroosmotic flow and to prevent the adsorption of proteins onto the glass channel surface *(20)*. During this procedure the chip was filled with a solution made of 40 μL 3-(trimethoxysilyl)propyl methacrylate (Aldrich) in 10 mL water adjusted with acetic acid to pH 3.5. After 1 h the channels were washed with water and filled with a mixture of 30 g/L acrylamide (Aldrich), 1 mL/L N,N,N′,N′-tetramethylethylenediamine (Sigma) and 1 g/L potassium persulfate (Aldrich) in water. During the polymerization reaction the microchip compart-

ments were covered with a microscope cover slip to prevent inhibition by oxygen. After 30 min the solution was flushed out and the channels were washed with water. Because the microchip did not come into direct contact with the patient sample, a single chip was used repeatedly.

SAMPLE-TO-CHIP INTERFACE

A glass capillary was used as a sample collector. Glass disposable micropipettes, 0.5 mm inner diameter, 1.0 mm outer diameter (Drummond Scientific Company) were cut to a length of 1 cm. One end was sealed with a filter membrane (Millipore, mixed cellulose esters, 0.22 μm pore size) by heating the capillary in a gas flame and pressing it onto the membrane. The internal volume of the capillary, which defines the minimum amount of sample required, was 2.0 μL. We used a finger-stick sample from a healthy volunteer to test the sample collector for analysis of whole blood (Fig. 2). The capillary action instantaneously draws the sample into the center channel up to the filter membrane, which, together with the water plug in the chip compartment, creates the sampling interface. Microscopic observations of the chip during the CE analysis showed that the cells did not go through the filter. The chip therefore stayed clean and was used for multiple samples. For the clinical trials a special user-friendly sample collector was designed (Fig. 1A). These collectors were made from solid Plexiglas® blocks by computer numeric controlled milling. A 7.0-mm-long capillary with a diameter of 1.0 mm was drilled in the center, defining a volume of 5.5 μL. A conically shaped enlargement of the top opening of the capillary allowed for easier blood-droplet collection than a glass capillary. A filter membrane was heat bonded onto the bottom of the collector with a hotplate set at 120 °C. Each sample collector was sterile and used only once to prevent sample carryover.

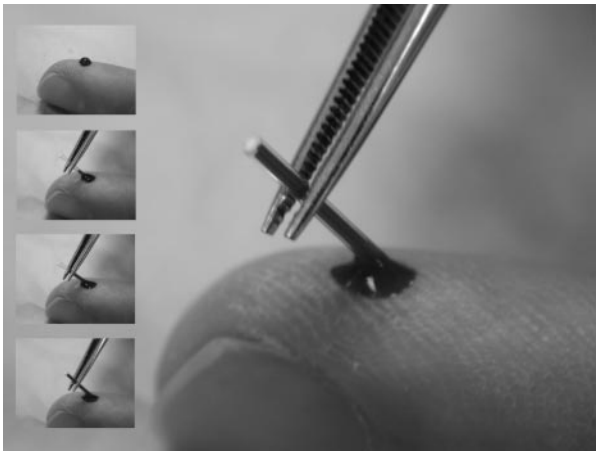

Fig. 2. Blood sample collection.

A finger stick sample is drawn into the glass sample collector by means of capillary action.

SAMPLES

Five serum samples, obtained from patients receiving lithium therapy, were received from the Medisch Spectrum Twente, Hospital Group, Enschede. The lithium concentrations were first measured in the hospital with an ISE (Cobas Integra 800). The procedures were done in accordance with the rules of the Institutional Ethical and Human Subject Research Committee of Medical Spectrum Twente, Hospital Group, Enschede, The Netherlands. The samples were subsequently analyzed on the same day with microchip CE. Whole blood, obtained from healthy volunteers not receiving lithium, was directly drawn into a sample collector after a finger stick (Hemolance disposable 21 gauge lancets with a puncture depth of 1.8 mm). A 2nd sample was collected in a sample vial by pipetting from the finger stick of the healthy volunteer. The second sample was anticoagulated by adding sodium citrate, and we subsequently added 2 mmol/L lithium. Finally, a heparinized plasma sample from a patient on lithium therapy was collected by routine venipuncture and kindly provided by the Medisch Spectrum Twente. The latter contained a lithium concentration of 0.62 mmol/L, as determined in the hospital laboratory.

INSTRUMENTATION

For the CE experiments we used a computer-controlled, high-voltage power supply, with 4 independently adjustable outputs (CU 411, IBIS Technologies). The power supply system also provided electrical current readout. The conductivity detector was custom made and commercially available (Sprenkels Consultancy). The detector signal was recorded with a data acquisition card (DAQCard 6036E, National Instruments) and an in-house written software package was used to control the power supply and acquire and process the data, which included signal filtering and peak area calculations.

MEASURING PROTOCOL

The chip was placed in a holder consisting of a Delrin® bottom-support plate and a Plexiglas cover block with fluid compartments (Fig. 1A). Platinum wires inserted into the compartments provide electrical connections to the high-voltage supply.

The analytical procedure started by filling the chip with the background electrolyte (BGE) solution. The channels were automatically filled by capillary action after 50 μL BGE was dispensed into the outlet compartment (Fig. 1A). Occasionally, we have seen air pockets trapped inside microchannels after they were filled. Therefore we insured that proper filling had taken place by microchip channel inspection with an inverted microscope (Leica DM/IRM). In cases in which clean and flawless microchips were employed, this inspection was considered unnecessary, and a simple electrical conductivity test to check for proper filling served as a substitute for the visual inspection. After inspection we filled the 3 remaining compartments with BGE and used BGE to perform a

blank separation to ensure that the system was working correctly. We started the CE experiment with a sample-loading step applying 1000 V to the sample compartment, 0 V to the waste compartment, and 1000 V and 800 V to the outlet and BGE inlet, respectively. This pinched injection procedure *(21)* electrokinetically transports the sample from the sample inlet compartment into the channels. A sample plug is defined in the double-T channel intersection by use of pinching fields from the sides to prevent diffusion of sample out of the double-T. After 60 s the separation was started by changing the voltages to 600 V on the sample and waste compartments, 1000 V on the BGE inlet, and 0 V on the outlet. The sample plug was injected into the separation channel, where it was separated. During the separation step, which lasts 60 s, the conductivity signal was recorded. If the measured electrical current through the 4 channels was within operating limits and the detector signal was free from interference, the chip was deemed ready for analyzing samples.

We first ran a calibration sample with 1 mmol/L lithium and 140 mmol/L sodium. Then BGE was removed from the sample compartment, which was cleaned and filled with 7 μL of water. A sample collector was filled with the calibration solution and subsequently inserted into the chip holder. The membrane on the bottom of the sample collector must make contact with the water in the compartment. A platinum wire electrode was inserted into the collector, and the analysis was started with the aforementioned voltage sequence. After the separation run was completed, 2 more separation runs were performed without changing anything in the system. The peak areas of the 3 electropherograms were used to obtain statistical information on the precision. To prepare the chip for a new blood sample, we first cleaned the compartment on the chip with water. The channels were flushed with BGE by pressurizing the BGE inlet compartment manually with a syringe for 10 s to insure that the starting conditions for each sample were always the same. To verify that the chip was clean, we performed a blank run with BGE. If there were no peaks in the electropherogram, the sample compartment was filled with 7 μL water, a sample collector filled with blood was inserted into the holder, and 3 subsequent separation runs were performed without refreshing the water plug. We then flushed the channel with BGE and refreshed the water plug. Placing the sample collector back into the inlet compartment, we obtained another set of 3 electropherograms, gaining a total of 6 repetitive measurements of the same sample (n = 6). With this protocol, including the disposable sample collector, multiple samples could be analyzed on a single CE chip with a highly reduced risk of chip contamination and sample carryover.

OPERATING PRINCIPLE

On a typical CE system the sample is placed directly in the microchip sample inlet. From the inlet reservoir the sample is driven into the channels for analysis by either electromigration or electroosmotic flow. We developed a sample collector that enabled us to measure samples from patients as well as whole blood from volunteers on the same microchip. The critical step is to create a defined sample volume in the double-T of the microchip with a chemical composition that is representative for the original sample being placed into the sample collector. The sample collector provided an additional interface between the sample and the microchip, which is suitable for clinical trial but requires validation as described here. In contrast to our previous work, with this technique the electrical contact between the collector and the separation electrolyte on the chip must be established through a small volume of water. This configuration allows sample stacking at the concentration boundary (see Fig. 1B). It should be noted that only a fraction of the ions in the sample enter the water plug in the sample compartment during this sample transfer step. The concentration of the ions in the water plug is therefore lower than in the sample itself. The water plug forms an integral part of the sample-loading procedure. By applying an electrical potential difference between the sample and the waste outlet compartment, the cations in the sample migrate through the filter membrane and enter the water plug. From there the ions continue to migrate into the channel filled with BGE, leading to the waste compartment. In this electrofiltration step the cells cannot pass the filter membrane, so they remain in the collector. The most important property of the system is that the water plug creates the conditions that enable field-amplified stacking *(22)*. The stacking efficiency is highly dependent on the conductivity difference of the water plug and the BGE. For example, no stacking occurs with the use of BGE instead of water. The sample matrix composition can also affect stacking. When an internal standard is used in the sample, however, it is possible to quantify the stacking efficiency in relation to other sample components. The use of an internal standard also corrects for any differences in the volume of the sample or water plug. Sodium has previously been used as an internal standard for reverse iontophoresis in vivo *(23)*. We also use the natural existing sodium concentration as an internal standard for quantification, taking small variations into account. It has even been suggested that the ratio between sodium and lithium is a more distinctive variable for monitoring lithium treatment *(24)*. For improved sample cleanup it is also possible to use sample collectors with a dialysis membrane instead of a filter membrane. In this way on-line electrodialysis can be performed. For lithium analysis, however, removal of only the blood cells is sufficient.

Results and Discussion

We used a calibration curve to determine the effect of sample stacking on the quantification of lithium. Because the peak areas are affected by the stacking efficiency, they are not necessarily proportional to the concentration in

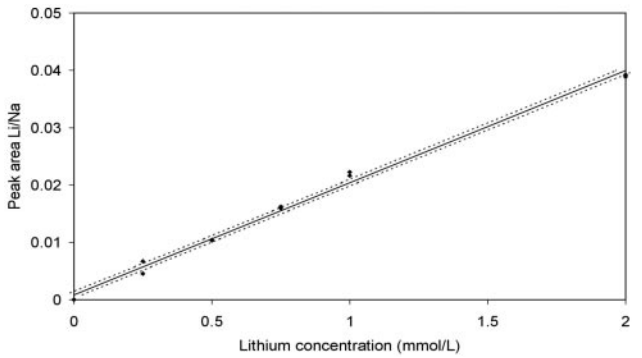

Fig. 3. Calibration curve with the 95% confidence interval for aqueous lithium standards in a matrix of 140 mmol/L sodium chloride.

BGE 30 mmol/L ammonium acetate/acetic acid BGE. Slope: 0.0196, *Y*-intercept: −0.000843, r^2: 0.994.

the sample. With sodium as an internal standard, we obtained a linear response for lithium for up to 2 mmol/L (Fig. 3), a result that confirmed the validity of the method for quantification of lithium in a high ionic strength sodium chloride matrix.

Because we wanted to measure samples directly from blood collected with a finger stick, we tested the microchip assembly with a glass capillary interface as blood collector for proof-of-method, using whole blood from a healthy volunteer. The sample was collected as demonstrated in Fig. 2, and the capillary placed into the holder. The lithium concentration of the patient plasma sample (the calibration sample in Fig. 4A) was 0.7 mmol/L (7% RSD, n = 3), which is in good agreement with the hospital value of 0.62 mmol/L. Fig. 4C shows the electropherogram containing sodium and magnesium in the expected ratio for whole blood. For quantitative measurements we used both the lithium-enriched blood sample and a heparinized patient plasma sample of known concentration. Both samples were pipetted into the sample collector for transfer to the chip. In the respective electropherograms (Fig. 4B and 4D), the lithium peaks can be clearly identified.

To compare the optimized Plexiglas sample collector against the hospital clinical method, we analyzed 5 blinded patient serum samples on the microchip. The chip was calibrated by running a clinical standard with 1.09 mmol/L lithium (determined with ISE) and 140 mmol/L sodium before running the patient samples. The ISE and microchip determinations correlated well with each other, but the imprecision was greater for the microchip analysis (Fig. 5). The relative mean SD was 10% for the microchip, compared with 2.3% for the ISE. Based on a calibration sample the detection limit, defined as a signal height of 3 times the noise, was 0.15 mmol/L. This value is below the therapeutic window. The limitation of the current system is the small size of the lithium peak, resulting in a relatively poor signal-to-noise ratio. Optimization of the sensitivity is possible by raising the BGE concentration, which increases the sample stacking efficiency. The size and shape of the conductivity detection electrodes in the microchip design have also not been fully optimized.

Potassium, magnesium, and calcium can also be detected with the microchip system. To determine potassium, 18-crown-6 can be added to the BGE to form a complex with potassium (data not shown), which decreases the conductivity of the potassium zone so that it can be distinguished from the background conductivity. Addition of other complexing agents to the BGE, such as tartaric acid, make it possible to separate sodium from calcium (Fig. 6). Tartaric acid decreases the effective electrophoretic mobility of calcium, and if a sufficient amount is added to the BGE, the calcium peak migrates behind that of lithium.

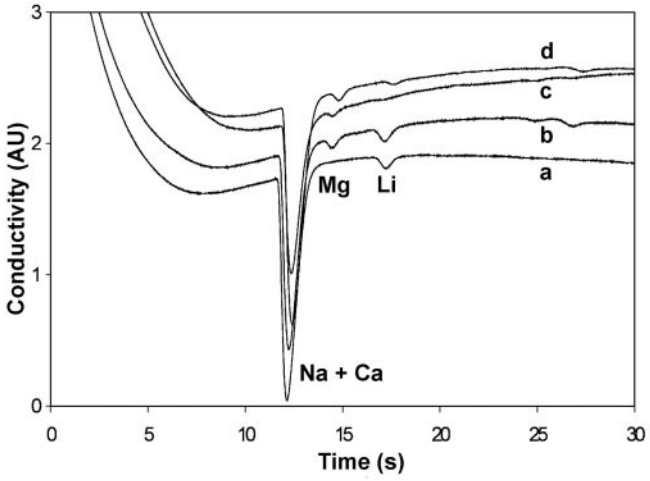

Fig. 4. Electropherograms of (*A*) an aqueous calibration mixture containing 140 mmol/L Na and 2 mmol/L lithium, (*B*) citrated whole blood enriched with 2 mmol/L lithium, (*C*) whole blood without anticoagulant and (*D*) heparinized plasma from a patient on lithium therapy containing 0.62 mmol/L Li.

Sampling by glass capillary collectors. BGE 30 mmol/L ammonium acetate/acetic acid.

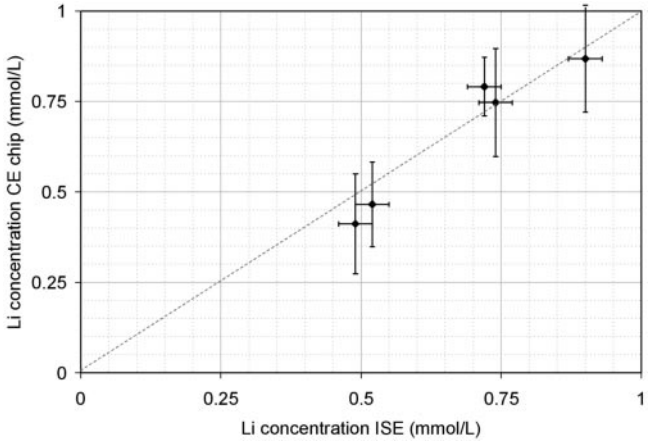

Fig. 5. Correlation plot of the lithium determined in 5 serum samples with the CE microchip and an ISE.

Each point is the mean of 6 measurements for the microchip system. The error bars represent 2 times the SD. Slope: 1.17, *Y*-intercept: −0.131, r^2: 0.937.

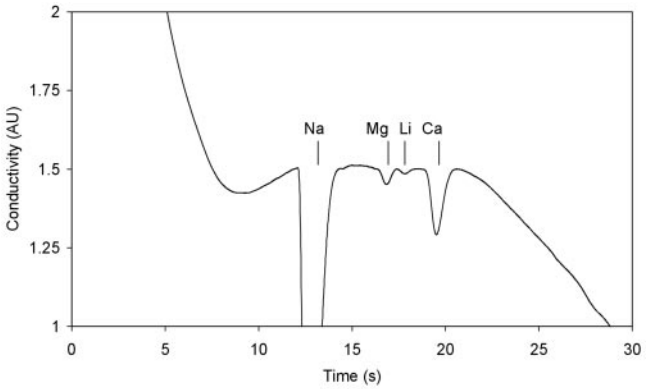

Fig. 6. Electropherogram of a serum sample with 144 mmol/L sodium, 0.74 mmol/L lithium, and 2.59 mmol/L calcium.

BGE 30 mmol/L ammonium acetate/acetic acid with 4 mmol/L tartaric acid. The baseline is corrected for drift.

The most important aspect for repeated use of the microchip is proper cleaning of the chip compartment in which the sample collector is placed. During the analysis the water is contaminated with ions from the sample, which can result in carryover effects. To turn the system into a viable product for point-of-care analysis, the chip will probably be disposable, thus avoiding these side effects without the need for cleaning. When analyzing whole blood, we were able to perform separations of sodium, magnesium, calcium, and lithium in <20 s without any off-system sample pretreatment. The disposable sample collectors presented here offer a convenient method for transferring a finger stick sample to the chip and also filter out potentially interfering blood cells. The chip itself does not come into direct contact with the sample and can be used repeatedly for method validation. The sample stacking using a water plug works reliably for 3 consecutive runs.

Microneedles that pierce the skin without inflicting pain have drawn a lot of attention *(25, 26)*. These microneedles have been used, for example, to sample interstitial fluid from the skin for glucose measurement *(27)*. In principle these needles can be integrated relatively easily with microfabricated devices such as CE microchips. In this case the microchip measurement protocol must be optimized for use with the nanoliter volumes typically delivered by microneedle arrays. The first results on the combination of microchip CE and microneedles are promising, but thus far only qualitative analysis of whole blood has been demonstrated *(28)*. The results gained from the small sample volumes, combined with a sample stacking procedure as demonstrated in this work, show very high potential for the integration of microneedle arrays as sample collectors with a microchip CE system.

The authors gratefully acknowledge Ir. Duwel and Mechatronics BV (Hoorn, The Netherlands) for designing and manufacturing the chip holder and the sample collectors. We also thank the laboratory staff members of the Medisch Spectrum Twente Hospital group (Enschede, The Netherlands) for the helpful discussions and preparation of patient samples, and specifically Dr. Kölling for her engagement as a psychiatrist in the project and promoting the benefits of point-of-care systems for lithium treatment to the medical community. This research was supported by the Technology Foundation STW, applied science division of the Netherlands Organization for Scientific Research (NWO), and the technology program of the Dutch Ministry of Economic Affairs.

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