Ned Tijdschr Klin Chem Labgeneesk 2004; 29: 295-296

Direct measurement of lithium in whole blood using a capillary electrophoresis microchip

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Introduction

Receiving clinically relevant blood parameters even for off-chip cleaned up sample is a nontrivial analytical problem. Few devices have been developed that fully exploit combining multiple functions in so-called micro total analysis systems (1, 2). Instead of advanced component integration on chip, here we demonstrate that the measurement of alkali metals can be performed in a drop of whole blood using an established type of capillary electrophoresis microchip (3-7) by applying the principles of moving boundary electrophoresis. The emphasis will be on the determination of lithium, which is used in the treatment of manic-depressive mood disorders. The low therapeutic index of lithium in blood is critical throughout the treatment and is therefore an ideal candidate for the demonstration of a microchip for self-testing.

Materials and methods

Microfabricated CE chips

The inset of Figure 1 shows the CE chip made of glass. Electrodes were integrated in the device for conductivity detection. To reduce both the electroosmotic flow (EOF) and the adsorption of proteins to the chip surface the channels were coated with polyacrylamide according to the procedure of Hjertén for fused silica capillaries (8). During the experiments the chip was placed in a custom-made holder, which was placed on an inverted microscope to follow the filling of the channels with the buffer as well as for tracking the cells. For the CE experiments a background electrolyte (BGE) consisting of 50 mmol/l 2-(N-morpholino)-ethanesulfonic acid (Sigma, Germany) and 50 mmol/l histidine (Fluka, Switzerland) with a pH of 6.1 was used. When whole blood was used as sample 200 mmol/l glucose (Sigma) was added to the BGE to adjust the osmotic strength. For the separations a computer controlled high voltage power supply with four independently controllable positive voltage outputs was used together with a custom-made conductivity detector. An optimized voltage scheme was applied to form a sample plug in the double-T (9). The separation was performed using 1000 V.

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Blood samples

Blood was obtained performing the finger stick method on a volunteer using Haemolance (HaeMedic AB, 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 minutes. Serum was collected after centrifuging the sample for 10 minutes at 11,500 g. 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.

Results

Performing capillary electrophoresis on the microchip the sample was diluted on-chip in the BGE during the sample loading step. Due to electrophoretic mobility difference between the cells and the analytes of interest a cell-free sample plug was formed in the double-T. Because of the EOF suppression, electromigration dominates and therefore the blood cells, which carry a negative charge, migrate in the opposite direction of the cations. We observed that the formed sample plug matches the conductivity of the BGE, which can be explained by moving boundary electrophoresis during sample loading. In figure 1, the control run using (a) whole blood without lithium is compared with (b) the same blood sample spiked with lithium. Separation of the blood sample plug resulted in three peaks originating from potassium, sodium and lithium. For determining the lithium concentration, the sodium peak was used as internal standard assuming a fixed concentration of 140 mmol/l. By doing so an error of less than 4% will be introduced in the calculated lithium concentration due to variations within the normal limits for sodium. For quantitation of whole blood samples prepared by manual spiking with lithium there is an uncertainty in the exact concentration of lithium in the plasma since it is not known how fast the cells take up the lithium. The actual concentration of the spiked sample can therefore be in the range of 3.4 mmol/l (no lithium uptake) to 2.0 mmol/l (equal distribution between plasma and cells). Based on calibration runs a concentration of 2.7 mmol/l (2.1% RSD, n=3) was calculated from the electropherograms. This was compared to experiments performed on serum in which the lithium concentration was exactly 2.0 mmol/l (Fig. 1c). In that case the concentration was determined at 1.8 mmol/l

with a detection limit of 0.4 mmol/l. The measurement error in the size of the peak area does 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 matrix composition of sample and does not necessarily represent the original concentration. To investigate the sources of error in detail more experiments are necessary.

Conclusion

The experiments demonstrated that lithium was separated from a drop of whole blood with capillary electrophoresis on a microchip within two minutes. Currently further investigations are conducted studying the process of concentration adjustment during the sample loading in order to improve on the accuracy for quantitation. In addition, potassium was detected by this method and more study is currently dedicated to separate calcium and magnesium in order to utilize the full potential of these microchips for 'point of care'-testing.

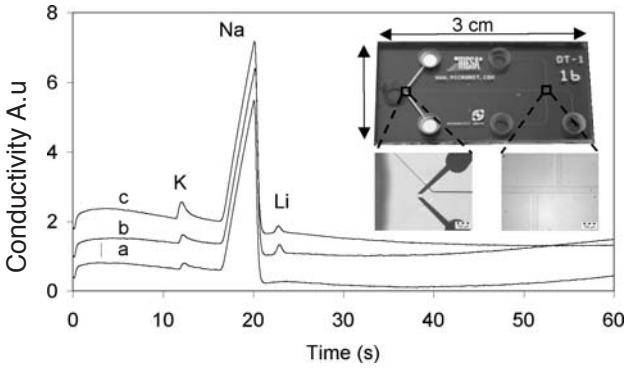

Figure 1. Results of a separation of a) whole blood without lithium, b) whole blood spiked with 2 mmol/l lithium and c) blood serum spiked with 2 mmol/l lithium. The inset shows a photograph of the CE chip with a capillary length of 2 cm (Micronit Microfluidics, The Netherlands) with a blow-up of the end-column conductivity detection electrodes and the double-T injection region defining the size of the sample plug dispensed into the separation channel.

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Ned Tijdschr Klin Chem Labgeneesk 2004; 29: 296-297

Apoptosis induced kinetic changes in autofluorescence of HL60 cells – application for single cell analysis on chip

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Introduction

Natural cellular autofluorescence (AF) can be a useful tool to unravel intracellular pathophysiological processes and distinguish normal from diseased tissue. Many cellular metabolites exhibit autofluorescence, e.g. NAD(P)H and flavins, which colocalizes strongly within the mitochondria and in some extent to the lysosomes (1-6). Both components are actively involved in a number of metabolic processes within the cell and play an important role in the energy household of the cell. This paper presents a new method using AF to study apoptosis. Apoptosis or programmed cell death plays an important role in maintaining a homeostatic equilibrium between cell proliferation and cell death. Induction of apoptosis results in shrinkage of the cell and fragmentation into apoptotic bodies (7). AF intensity is first measured conventionally at the flow cytometer (FCM) and finally the results will be translated on to a microfluidic chip to perform single-cell analysis.

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Autofluorescence measurements

Human promyelocytic leukemic HL60 cells were incubated with camptothecin (CPT), tumour necrosis factor (TNF)-a in combination with cycloheximide (CHX), or irradiated with 6 or 10 Gy, during varying