THE DEVELOPMENT OF A POINT OF CARE CREATININE MEASUREMENT USING DISPOSABLE READY TO USE MICROCHIP CAPILLARY ELECTROPHORESIS

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ABSTRACT

We report on the determination of creatinine in human serum using a point-of-care device suitable for the use by untrained operators, the Medimate Multireader®. This device is based on electrophoretic separation and conductivity detection and its prefilled microfluidic chip has a single opening for sample introduction. The device was originally developed for the determination of lithium in blood [1]. Creatinine determination at a level of quantification of 300 µM was realized by optimizing the background electrolyte (BGE) pH and concentration, and the injection and separation potentials and time. This limit of quantification is in the relevant range for the detection of renal failure [2].

KEYWORDS: point-of-care device, Microchip-CE, creatinine.

INTRODUCTION

The contemporary biomedical research and clinical practice heavily depend on monitoring of many substances in biological materials, such as blood plasma, urine, cerebrospinal fluid, or tissue biopsy samples. The combination of these two facts has led to a revolution in clinical medicine and patient care with the development of point-of-care (POC) devices based on Lab-on-a-Chip technology.

Renal diseases such as acute renal failure, chronic kidney disease, and end-stage renal disease (ESRD) can be detected, in part, by an increase in serum creatinine. To give an example of the magnitude of the problem, ESRD is a disorder that affects the kidneys and their ability to remove waste products from the blood stream. ESRD affects more than 300.000 people annually in the USA with a 20% death rate in the first year after diagnosis [3].

Creatinine is an analyte of great interest, which serves as one of the most widely used markers of renal function. Unlike urea, the concentration of creatinine is not influence by the protein intake and therefore it is a more reliable indicator of renal function [4]. For this reason, the simplest and most widespread method of detecting kidney disease is through measurement of blood creatinine concentrations. Normal levels of creatinine in human blood are 50-110 µM in serum adult males, and 45-100 µM in serum adult females [2]. These levels can increase up to 1 mM in patients with renal failure [5].

We report on the determination of creatinine in human serum using a point-of-care device suitable for the use by untrained operators, the Medimate Multireader®. Creatinine determination at a level of quantification of 300 µM was realized by optimizing the background electrolyte (BGE) pH and concentration, and the injection and separation potentials and time. This limit of quantification is in the relevant range for the detection of renal failure [4].

THEORY

The measurement procedure employed a handheld analyzer (Multireader) in which a cartridge with a microfluidic chip is inserted. This device is based on electrophoretic separation and conductivity detection and its prefilled microfluidic chip has a single opening for sample introduction. Operator actions needed are solely the deposition of a sample drop at this opening and insertion of the chip into a handheld analyzer. The device was originally developed for the determination of lithium in blood [1]. Figure 1 shows a photograph and schematic of the microfluidic chip. The totally closed chips were filled with different buffer solutions. Vacuum filling was employed to fill the closed channel system through the single inlet opening and to leave an air bubble in a specifically designed reservoir.

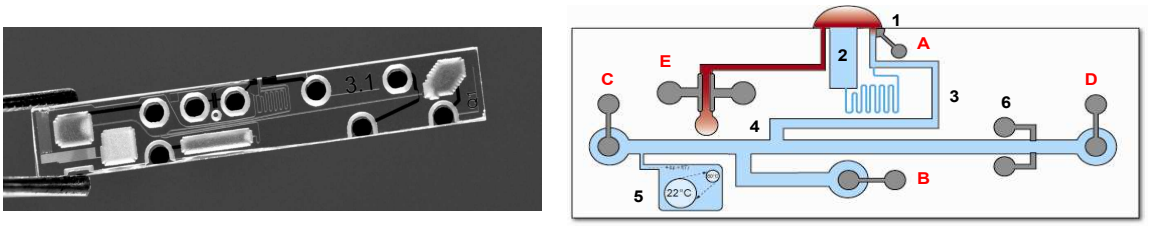

Figure 1 left: photograph of the microfluidic chip; right: Schematic indication of the different functional units

The exact locations slightly differ from the photograph for the sake of clarity. This way, the different functional units correspond to: 1) sample opening with applied sample droplet; 2) evaporation reservoir; 3) injection channel for injection of cations by moving boundary electrophoresis; 4) double-T injector; 5) reservoir with gas bubble for liquid expansion control; 6) conductivity detection electrodes; A, B) high-voltage injection anode and cathode; C,D) high-voltage separation anode and cathode; E) Electrodes for the conductimetric determination of the sample conductivity.

EXPERIMENTAL

The measurement procedure is based on the use of a disposable plastic cartridge containing the microfluidic chip prefilled with BGE. A drop of sample is deposited on the sampling opening and the chip is inserted into the handheld analyzer (Multireader).

After insertion of the cartridge into the analyzer, the measurement protocol was started. Different injection voltages and separation voltages were then applied for a specified time. The injection and separation potentials as well as injection and separation times were chosen according to an optimization process aimed at maximizing separation performance for creatinine.

RESULTS AND DISCUSSION

The POC device was converted from lithium to creatinine detection by altering and optimizing the BGE and the injection and separation potentials and times. Chip and electronics design were left unaltered and can still be optimized. Table 1 shows that under lithium protocol conditions (step 1) the Limit of Quantification (LOQ) was 2.295 mM. Optimization of the physical conditions (step 2, injection time and voltage and separation time and voltage) gave a 4-fold increase in sensitivity (slope b), but only a 2-fold decrease in LOQ due to the simultaneously increased standard deviation S. Additional optimization of the BGE (step 3) gave an almost 10-fold decrease of the LOQ to the physiologically relevant concentration of 289 µM.

Table 1. Figures of merit a: intercept, b: slope, S: standard deviation , R^2: regression coefficient, LOD: limit of detection (blank signal plus 3SD), LOQ (blank plus 10 SD).

Step	$a \pm S_a$	$b \pm S_b$	$S_{y/x}$	R^2	L.O.D (mM)	L.O.Q (mM)
1	-27 ± 99	433 ± 53	175	0.8712	0.688	2.295
2	696 ± 188	1840 ± 111	439	0.9022	0.307	1.023
3	45 ± 13	446 ± 7	30.1	0.9911	0.087	0.289

Five different BGEs were investigated, with pH values ranging from 4.7 to 3.5 and ionic strengths from 15-50 mM. The best results were found for the BGE of pH = 4.0 and ionic strength 15 mM. Figure 2 shows an electropherogram obtained under optimal conditions. A subsequent statistical analysis of 120 samples showed a normal distribution of the residuals, excluding systematic errors of the method.

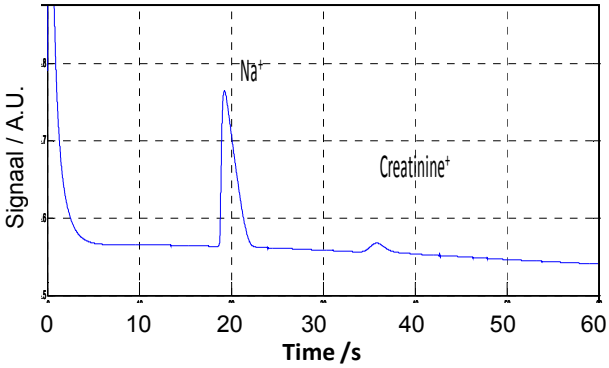

Figure 2: Electropherogram of a creatinine determination with optimized injection and separation protocols and background electrolyte. Sample: 140 mM sodium, 3 mM creatinine.

In order to demonstrate real-life applicability of the method, human serum samples spiked with three different concentrations of creatinine were analyzed. Every sample was measured three times. Table 2 shows the analysis results.

Table 2. Creatinine determinations of serum samples spiked with creatinine (3 determinations per sample).

Sample	Analyte	Conc. Added (mM)	Conc. Found (mM)	C.V. (%)
Serum 1	Creatinine	------	65 ± 38	85
Serum 2	Creatinine	100	221 ± 27	12
Serum 3	Creatinine	200	282 ± 71	25

Figure 3 shows an electropherogram of serum spiked with 100 µM creatinine.

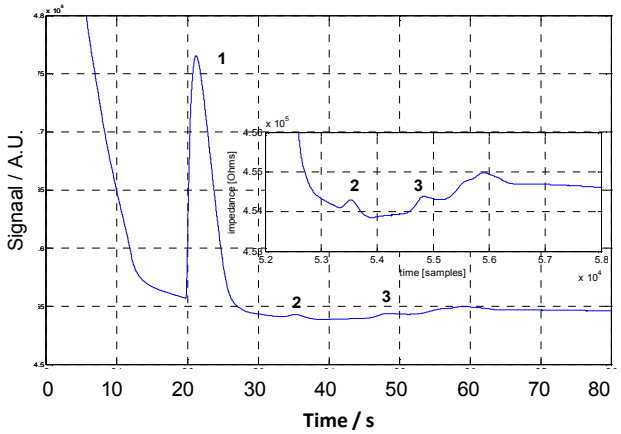

Figure 3. Electropherogram of human serum spiked with 100 µM creatinine; 1: sodium, 2: lithium, 3: creatinine

Finally, a more extensive study was carried out with the optimized protocol with the purpose of establishing the reliability of the measurement process. A total number of 120 measurements were executed, at 4 different sample concentration levels (0.250, 0.500, 1.0 and 2.0 mM), using 5 different Multireaders, and measuring at three different temperatures (16, 22 and 28 ºC). The statistical analysis of the residuals fitted a normal distribution implying there are not systematic errors in the measurement process.

CONCLUSION

We have shown that it is possible to use the Medimate Multireader for creatinine determination in serum by modification of the physical and chemical conditions. The limits of detection and quantification achieved are of the same order as the normal human levels in blood, ≈ 100 µM. The method can thus be used as screening method for possible kidney problems.

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