Electronic baseline-suppression for liquid conductivity detection in a capillary electrophoresis microchip

F. Laugere  
Electronic Instrumentation Laboratory  
DIMES, Department of microelectronics  
Delft University of technology  
Delft, the Netherlands  
F.laugere@its.tudelft.nl

J. Bastemeijer  
Electronic Instrumentation Laboratory  
DIMES, Department of microelectronics  
Delft University of technology  
Delft, the Netherlands  
J.bastemeijer@its.tudelft.nl

G. van der Steen  
Section Analytical Biotechnology,  
Kluiver Laboratory for Biotechnology  
Delft University of Technology  
Delft, the Netherlands  
G.vandersteen@tnw.tudelft.nl

M.J. Vellekoop  
Industrial Sensor Systems  
Institute of IEMW  
Vienna University of Technology  
Vienna, Austria  
Vellekoop@ienw.tuwien.ac.at

P.M. Sarro  
ECTM Laboratory  
DIMES, Department of microelectronics  
Delft University of technology  
Delft, the Netherlands  
Sarro@katana.dimes.tudelft.nl

A. Bossche  
Electronic Instrumentation Laboratory  
DIMES, Department of microelectronics  
Delft University of technology  
Delft, the Netherlands  
A.bossche@its.tudelft.nl

Abstract  
Liquid conductivity detection for application in chip-based capillary electrophoresis (CE) has received attention because the sensor electrodes can be integrated in the chip and there is no loss of sensitivity when downscaling the detector. A well known disadvantage of conductivity detection is the high level of the baseline (due to the conductivity of the carrier electrolyte) that has a high influence on the measured values and deteriorates the detection limit. By using a lock-in amplifier, making use of its two differential inputs and of its phase-locked sinewave output, we can generate a signal of controlled amplitude and phase, related to the level and phase of the baseline signal. By subtracting this signal from the AC output signal of the detector the baseline is significantly suppressed. Ideally, only the changes of the background signal are then measured. When applying the baseline-suppression method to real measurements, the level of the background signal was decreased 50 times. The detection limit (which is related to the signal-to-noise ratio) was found to be 10 times lower. Reproducible separations with concentrations from 1mM down to 10 μM of potassium, sodium, and lithium ions were obtained. For our separation and detection system, the concentration of 10 μM could not be detected without the baseline-suppression technique described here.

Keywords  
Capillary electrophoresis, microchip, baseline-suppression, conductivity detection.

INTRODUCTION  
Because of its high sensitivity and its ease of use, most often laser induced fluorescence is used for analyte detection in capillary electrophoresis (CE) systems. However, when miniaturizing the CE setup, the intensity of the fluorescent signal becomes smaller and the sensitivity decreases. Electrochemical detection techniques are receiving a lot of attention because of their compatibility with miniaturization (in terms of implementation and performance). Amperometry and potentiometry usually take advantage of miniaturization, and very low detection limits (pM) have been reported. Liquid conductivity detection has also been implemented in the microchip format [1]. However, The detection limit of conductivity measurements is relatively poor, compared to amperometry, because small changes of a high level background-signal (baseline) are measured. In standard CE application, The use of a chemical suppressor in combination with conductivity detection provides a low level of the measured background signal, thereby enhancing the detection limit. However, the fabrication of permeable membrane that is electrochemically reactive with the buffer can be an obstacle to miniaturization. Therefore, we have investigated the possibility of lowering the level of the background signal electronically. The corresponding setup is presented in this paper, as well as the first results obtained.

SUPPRESSED CONDUCTIVITY DETECTION  
Choice of the carrier electrolyte  
Conductivity detection has been extensively used in ion chromatography, but it is still not the case in CE. An explanation is that conductivity detection combined with CE suffers from conflicting requirements. The choice of the carrier electrolyte is at the source of this conflict. The carrier electrolyte is chosen in a way that zone dispersion is limited. Zone dispersion (or broadening) is partly a result of diffusion, convection, and Joule heating in the separation channel. Zone dispersion is also linked to the difference in velocity (therefore conductivity) between the analyte ion and the carrier electrolyte co-ion. Zone dispersion occurs when
the velocities differ. The zone dispersion is, most often, observed as tailing or fronting peaks [2],[3]. Various other peak-deformations or the formation of unwanted system peaks have also been noticed due to mismatches of velocities. On the other hand, the conductivity of the analyte ion must differ from the conductivity of the carrier electrolyte co-ion in order to get a signal out of the conductivity detector [4],[5]. Therefore, finding a suitable carrier electrolyte when using conductivity detection is a matter of compromise between separation efficiency and detection sensitivity. At the end, the difference in conductivity cannot be too high, and only small changes of a high-level background signal are observed when a zone passes the detector.

The high level of the background signal requires a large dynamic range of the read-out electronic, which has a negative consequence on the signal-to-noise-ratio (SNR). That is one reason why conductivity detection suffers from poor detection limit.

**Chemical suppressor**

Conductivity detection combined with chemical-suppression improves the detection limit in the presence of a highly conductive carrier electrolyte [6],[7]. A chemically active membrane is placed before the conductivity detector. This membrane chemically interacts with the carrier electrolyte exchanging ions with high conductivity for ions with low conductivity (e.g. Na\(^+\) ions for H\(^+\) ions). The resulting conductivity of the carrier electrolyte decreases. Then, it is possible to use a smaller dynamic range for the read-out electronic because a lower background signal is measured. Using a chemical suppressor, a detection limit of 10 nM was reported for inorganic ions [7].

**ELECTRONIC BASELINE SUPPRESSION**

The integration of the active membrane in the microchip format has never been reported till date, which is probably linked to technological limitations. In this section, we describe a method that allows to subtract electronically the value of the baseline before measurements and therefore to extend the usable dynamic range of the read-out electronic. A similar setup has been described for carrier suppression techniques in order to measure noise in oscillators [8].

**Hardware setup**

The baseline-suppression technique makes use of a lock-in amplifier (Figure 1). The signal proportional to the liquid conductivity is the differential AC voltage between the two inner electrodes. This signal is conditioned and amplified with the differential amplifier [9]. After, amplification, the signal amplitude \(V_o\) is measured with a lock-in amplifier (Perkin-Elmer 7265) and the value is send to the computer for on-line display of the electropherogram.

The differential input of the lock-in amplifier is used for the baseline-suppression. On the first input (A), the signal coming out from the detector is applied. On the other input (B), a signal of which amplitude and phase is related to the baseline signal is applied. This signal is self-generated by the lock-in amplifier (through the “sine out” output). When the proper phase and amplitude are set, only the changes of conductivity due to the peaks are measured. Then, the differential signal can be measured with a low input range and by taking more advantage of the dynamic range of the lock-in amplifier. It improves the signal-to-noise-ratio and thus it leads to a lower detection limit.

**Software setup**

The control of the lock-in amplifier is done with the software Labview, and the acquisition of the electropherogram is done according to the following sequence. (1) At the beginning of the recording, no baseline-suppression is applied. The input B of the lock-in amplifier is disconnected. The amplitude and the phase of the signal coming out of the detector are measured at the input A. (2) Shortly before the peaks come, the measured amplitude and phase are stored. They represent the amplitude and the phase of the baseline signal. (3) A signal with the same amplitude and phase is

![Figure 1. Measurement setup for four-electrode liquid conductivity measurements with electronic baseline suppression](image-url)
then generated at the output “sine wave” and the lock-in works in a differential mode (A-B). The baseline-suppression is now starting. (4) The input range of the lock-amplifier is adjusted to the right value.

MEASUREMENT RESULTS

The CE microchip
The CE microchip was fabricated out of two glass wafers, one containing the channel and the liquid connections, the other containing the detector. A picture of the device is given in Figure 2. In the top wafer, a 6 cm channel is etched using wet etching (aqueous solution of 70 % H$_3$PO$_4$ and 5 % HF at 70 °C). The channel has a depth of 20 µm and a width of 70 µm in the separation part, and it is widened to 170 µm at the detector. At the channel in- and outlet, access holes are etched through the wafer (wet etching). Deposition of a 160 nm thick silicon nitride layer enables glass-to-glass anodic bonding [10].

Contactless conductivity detection is done with four electrodes [11]. In the bottom wafer, a two step trench (600 nm each) is etched by reactive ion etching. In the lower trench the metal interconnects are buried. The upper trench is used to cover the metal and the bottom of the separation channel with a 600 nm silicon nitride layer. The silicon nitride is isotropically etched in an Inductively Coupled Plasma (ICP) etcher. This results in a planar surface that enables leakage free bonding. For the capacitively coupled conductivity detection, a very thin dielectric layer is required. Therefore, at the top of the electrodes the silicon nitride is removed and replaced by a 30 nm thick silicon carbide layer. After alignment, the wafers are bonded at 400°C and 1000V for 1 hour. The bonding process leads to a sealed electrophoresis-channel where the channel-walls are entirely covered with silicon nitride for a uniform electro-osmotic flow.

Separation of inorganic ions
For the experiments, all chemicals were of analytical reagent grade, and solutions prepared in MilliQ water (a Milli-pore SA system). Carrier electrolyte solutions were prepared daily. MES was obtained from Sigma Chemical Co. and histidine (His) from Fluka Chemie AG.

The chip was placed in the dispensing rack of an autosampler (Gilson 223). The voltage necessary for the electrophoretic separation was applied to the liquid by means of platinum wires placed in the reservoirs. Initially, the device and the reservoirs are filled with the carrier electrolyte. The injection procedure was typically as follows: empty the inlet and outlet reservoirs, fill the inlet with 70 µL sample, electrophoretic injection, empty the inlet reservoir via the drain port, fill subsequently the inlet and outlet reservoirs with 90 µL carrier electrolyte, and, finally, start the separation.

The first experiment consisted in injecting 1mM (each) of potassium, sodium, and lithium ions. 20 mM MES/His (pH 6) was chosen as the carrier electrolyte. The separation was performed at 600 Volts. No baseline-suppression was applied. The corresponding electropherogram is shown in Figure 3. The value of the baseline was in the range of 207.5 to 208.5 mV, and therefore the input range of the lock-in amplifier had to be set to 500 mV. The peak height is in the order of 4 mV, depending on the ion. The sodium peak (second peak) was taken into account to estimate the signal-to-noise-ratio (SNR). SNR was calculated as the ratio of the peak-height to the noise-amplitude (the peak-height is the difference in signal amplitude between the analyte zone and the carrier electrolyte). A corresponding value of 86.8 dB is found.

In the second experiment, the baseline-suppression technique was applied. It is visible in Figure 3 that peak shape remains identical to the previous measurements. Therefore, the baseline-suppression does not induce any distortion or broadening. After suppression, the baseline level decreased to a value in the range of 2 to 4 mV. That is a reduction of the background signal by a ratio of at least 50. Such a reduction of the baseline allowed setting the input range of the lock-in amplifier to 10 mV (instead of 500 mV). The SNR improvement is visible in Figure 3. The SNR is equal to 106.8 dB, which is 10 times higher than without base-

![Figure 2. Glass CE microchip with contactless four-electrode liquid-conductivity detector](image)

![Figure 3. Separation of 1 mM (each) of potassium, sodium, and lithium ions in 20 mM MES/His (pH 6) carrier electrolyte. The same separation was monitored with and without baseline suppression.](image)
In the third experiment, samples containing 1 mM, 100 µM, and 10 µM potassium, sodium, and lithium ions were injected. In Figure 4, the electropherogram corresponding to the injection of 10 µM sample is visible. Peaks at that concentration would not be visible without the suppression of the baseline. Taking the detection limit as three times the noise level, concentration down to 3 µM could be detected.

LIMITATIONS
A good suppression is only achieved when the two alternating signals have identical phase. In the setup described here, the acquisition of the phase and amplitude information of the baseline is done only once and shortly before the peaks pass the detection window. It is then expected that no phase changes occur afterwards. The recording of the phase information showed that when a zone passes, a variation of less than one degree is noticed. This is acceptable to achieve a good subtraction and as it can be seen in Figure 3, there is no distortion of the peak.

Baseline drift is another source of limitation for baseline suppression. For low sample concentrations, the baseline drifts are larger than the peak height. In that case, the sensitivity is fixed by the changes of the baseline which fixes the detection limit, as well. This is, for instance observed in Figure 4, when detecting concentrations of 10 µM.

CONCLUSION
A simple method for electronic baseline-suppression was presented. When applying the baseline-suppression technique, the level of the background signal was decreased 50 times. The detection limit (which is related to the signal-to-noise ratio) was found to be 10 times lowered. Reproducible separations with concentrations from 1 mM down to 10 µM of potassium, sodium, and lithium ions were obtained. The concentration of 10 µM could not be detected without the baseline-suppression technique described in this paper.

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REFERENCES