

Experimental verification of an improved method for conductivity detection in on-chip capillary electrophoresis systems

F. Laugere^{*}, A. Berthold^{**}, G.W. Lubking^{*}, J. Bastemeijer^{*}, R. M. Guijt^{***},
E. Baltussen^{***}, P.M. Sarro^{**}, M.J. Vellekoop^{*}

^{*}DIMES Electronic Instrumentation Lab., Mekelweg 4,
2628 CD Delft, The Netherlands, F.laugere@its.tudelft.nl

^{**}DIMES – ECTM, 2600 GB, Delft, The Netherlands

^{***}Kluyver Lab. for Biotechnology, 2628 BC, Delft, The Netherlands

SUMMARY

In this paper, we present the experimental results obtained with a miniaturised capillary electrophoresis device where detection is done by means of liquid conductivity measurements. The device is fabricated in glass. It includes four metal electrodes isolated from the solution with 30 nm of silicon carbide for contactless liquid conductivity detection. We show that the use of four electrodes instead of two is advantageous for liquid conductivity measurements. Reproducible separations of inorganic ions were successfully done and the detector showed a linear response for concentrations ranging from 1 mM down to 20 μ M (detection limit).

Keywords: conductivity detection, 4-electrode, capillary electrophoresis.

INTRODUCTION

Up till date, mainly optical measurement-techniques (absorption, fluorescence) are used for analyte detection in miniaturised capillary electrophoresis (CE) systems. Liquid conductivity detection has received renewed attention because it is potentially a sensitive method that can be integrated in the liquid channel and does not require any outside optical equipment [1,2]. A major problem when using bare metal electrodes in the liquid channel is the generation of gas bubbles under the presence of the electric field (up to 500 V/cm) applied for separation. An alternative method is capacitively coupled (contactless) conductivity detection. Electrochemical reactions between the metal electrodes and the liquid are prevented by covering the metal electrodes with an insulating material. A major drawback is that the insulation of the electrodes results in a very small usable frequency range. For every different buffer solution, the optimal frequency has to be found. In addition, the detector suffers from non-linearity, low accuracy, and low sensitivity. In a previous contribution [3], we presented an alternative solution: the use of a contactless 4-electrode measurement setup. It increases the frequency range considerably, which improves the linearity and sensitivity. Also, calibration for different buffer

solutions is not required. In this paper, we demonstrate the benefits of the improved conductivity detection method by means of measurements in a micro CE-device. Four isolated electrodes have been integrated in a microchannel fabricated in a glass chip. We compared the detector response obtained with a 2- and 4-electrode setup. The applicability of the 4-electrode conductivity detector has been demonstrated by monitoring separations of inorganic ions in the fabricated micro CE device.

DEVICE FABRICATION

The microdevice has been fabricated out of two glass wafers: one containing the channel and the liquid connections and the other containing the detector. In the top wafer, a 6 cm long channel is etched using wet etching (aqueous solution of 70 % H_3PO_4 and 5% HF at 70 °C). The channel has a depth of 20 μ m and a width of 70 μ m in the separation part, and it is widened to 170 μ m at the detector part. At the channel ends, access holes (vials) are etched through the wafer. Deposition of a 160 nm thick silicon nitride layer enables glass to glass anodic bonding [4].

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 by a 600 nm silicon nitride layer. The silicon

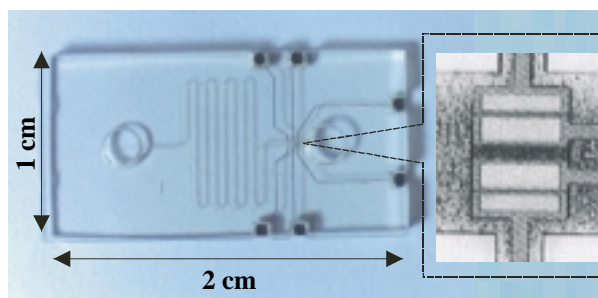


Fig. 1: Miniaturized capillary electrophoresis device containing an on-chip contactless 4-electrode liquid conductivity detector (insert).

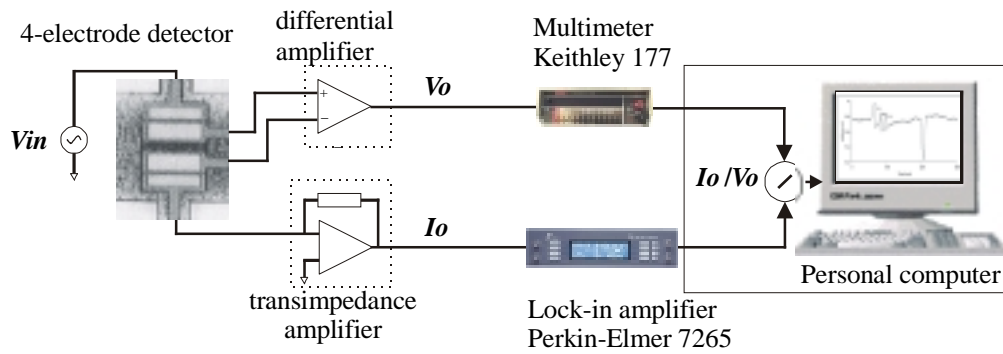


Fig. 2: Measurement setup for the capacitive 4-electrode liquid conductivity detection.

nitride is isotropically etched in an inductive 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. 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 anodically bonded at 400°C and 1000V for 1 hour. The bonding process leads to a sealed electrophoresis-channel including 4 isolated electrodes for capacitively coupled liquid conductivity detection (two outer-electrodes of $106 \times 25 \mu\text{m}^2$ and two inner-electrodes of $106 \times 35 \mu\text{m}^2$). The channel walls are entirely covered with nitride for a uniform electroosmotic flow. A picture of the device including a magnification of the detection area is shown in Fig. 1.

MEASUREMENTS

Measurement setup

The dispensing of the liquid is done using an autosampler (Gilson 221 XL) controlled by a computer using a dedicated injection procedure (IBIS Technologies B.V.).

The CE device is placed in the adapted dispensing rack of the autosampler. The voltage necessary for the electrophoretic separation is applied to the liquid by means of platinum wires placed in the vials.

The measurement setup for the liquid conductivity detection is shown in Fig. 2. In a classical 4-electrode setup, an open-loop current source imposes a fixed AC current value between the outer electrodes. The parasitic capacitances (from the connecting leads), which are of the same order as the electrode capacitance, induce a measurement error. Therefore, we have chosen to impose a voltage V_{in} and measure the corresponding current I_o with a transimpedance amplifier, although this implies real time division to get the conductivity value. This configuration is insensitive for parasitic capacitances in the frequency range we use (100 Hz - 1 MHz).

The two inner-electrodes are connected to a high input impedance ($100 \text{ M}\Omega$, 1 pF) differential-voltage amplifier (V_o). A voltmeter (Keithley 177 microvolt DMM) and a lock-in amplifier (Stanford Research SR830 DSP) read, respectively, the value of the differential voltage V_o (high level) and the current I_o (low level signal). Finally, the measured signals are sent to a computer through an acquisition card (KPCI 3108, Keithley), and the liquid conductivity is retrieved from the real time division I_o/V_o . Liquid conductivity detection by means of a 2-electrode measurement setup is possible using only the two outer electrodes. The conductivity value is retrieved from the ratio I_o/V_{in} .

Comparison between the 2- and 4-electrode setup

The two measurement setups are compared on the basis of their measured frequency response. The separation channel is filled with MES/His CE buffers (pH 6) of different concentrations: 0.2, 2 and 20 mM. The measured conductivity with the commercial conductivity meter Horiba ES14 is respectively 8, 38, and $317 \mu\text{S}/\text{cm}$ (@ 20.4°C). For each of the CE buffers, the response (measured impedance which is equal to the ratio V_o/I_o for the 2-electrode setup and to the ratio V_{in}/I_o for the 4-electrode setup) is measured in the frequency range 100 Hz - 1 MHz.

The frequency response obtained with a 2-electrode setup (Fig. 3A) presents characteristics which are similar for each buffer and which can be divided in three frequency bands. At low frequencies ($< 1 \text{ kHz}$), the insulating film which acts as a capacitor (C_{is}) in series with a double-layer capacitance (C_{dl}) dominates the measured impedance. The double-layer capacitance is the only parameter sensitive to conductivity changes. It results in a low sensitivity of the detector in that frequency range. At high frequencies ($> 100 \text{ kHz}$), stray capacitances C_{stray} (including the capacitive coupling through the liquid) shortcut the liquid impedance. The detector is not sensitive to conductivity changes in that frequency range. At medium frequencies, the measured impedance involves the liquid impedance and also

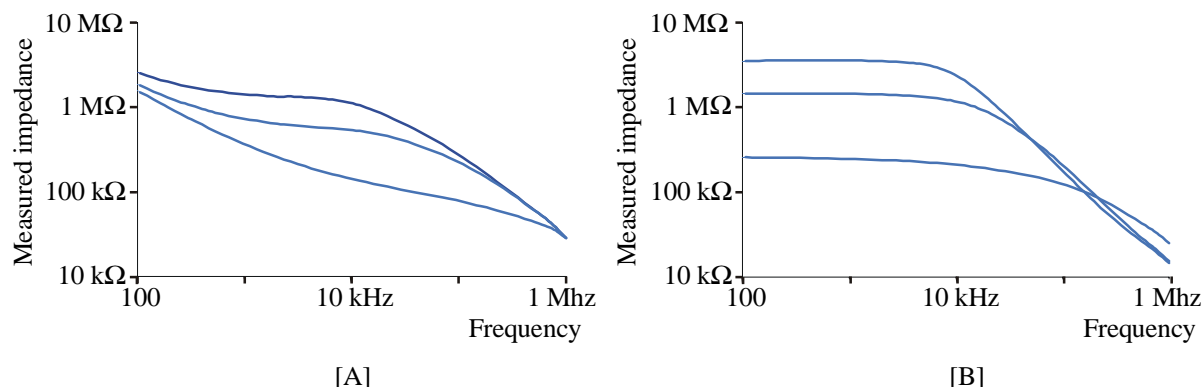


Fig. 3: Measured impedance with a contactless 2- and 4-electrode setup (A and B respectively). Liquids employed are standard CE buffers (MES/His @ pH 6) with concentration values equal to 0.2, 2, and 20 mM (respective conductivity of 8, 38, and 317 $\mu\text{S}/\text{cm}$). For both figures, the top curve corresponds to the 0.2 mM buffer, the middle curve to the 2 mM, and the bottom curve to the 20 mM.

partly the previously mentioned capacitances (C_{stray} , C_{is} , and C_{dl}). In that band of frequencies, the detector is sensitive to conductivity changes. However, the accuracy, linearity, sensitivity, and the dynamic range are determined by the value of C_{stray} , C_{is} , and C_{dl} .

As theoretically expected, there is a minor influence of C_{is} and C_{dl} on the frequency response of the 4-electrode detector (Fig. 3B). For frequencies up to 10 kHz, the detector response is linear with a constant and optimal sensitivity. Above 10 kHz, the stray capacitances affect the detector's response in the same way they affect the 2-electrode setup.

In a standard CE separation, the conductivity of the carrier electrolyte determines the base line of the output signal. Slight changes of this signal have to be detected when a plug with a different conductivity passes through the detection window. For that application, a capacitively coupled 2-electrode configuration is suitable. However, as mentioned before, the detector sensitivity is highly dependent on the measurement frequency. A proper adjustment of that frequency is necessary. In addition, changing the conductivity of the

carrier-electrolyte asks for re-adjustment of the measurement frequency in order to get optimal sensitivity, linearity, accuracy and dynamic range. This is not the case with a 4-electrode configuration, where detection performance is similar for each buffer when measuring below 10 kHz. Furthermore, separations achieved with highly conductive buffers tolerate a higher measurement frequency (about 50 kHz for a 20 mM MES/His buffer at pH 6).

Separation of inorganic ions

The separation is carried out in a 20 mM MES/His buffer (pH 6). The injected sample consists of potassium, lithium, and sodium with a 5 mM concentration (for each analyte) in purified water. The injection time is 3 seconds and the applied voltage for separation is 400 Volts. The results from the separation are shown in Fig. 4. The three peaks corresponding to the injected analytes are clearly seen. The negative peak (at 210 seconds) corresponds to the electroosmotic flow (EOF) which marks the migration of neutrals. Repetitive injections showed that the separation is reproducible.

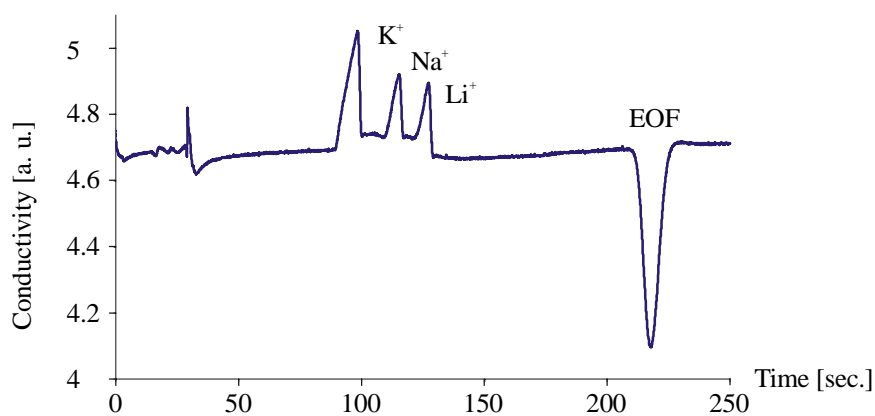


Fig. 4: Separation of 5 mM (each) potassium, sodium and lithium in a 20 mM MES/His buffer (pH 6). Liquid conductivity detection is achieved with the contactless 4-electrode setup. The injection time is 3 sec and the separation voltage is 400 Volts (83 V/cm).

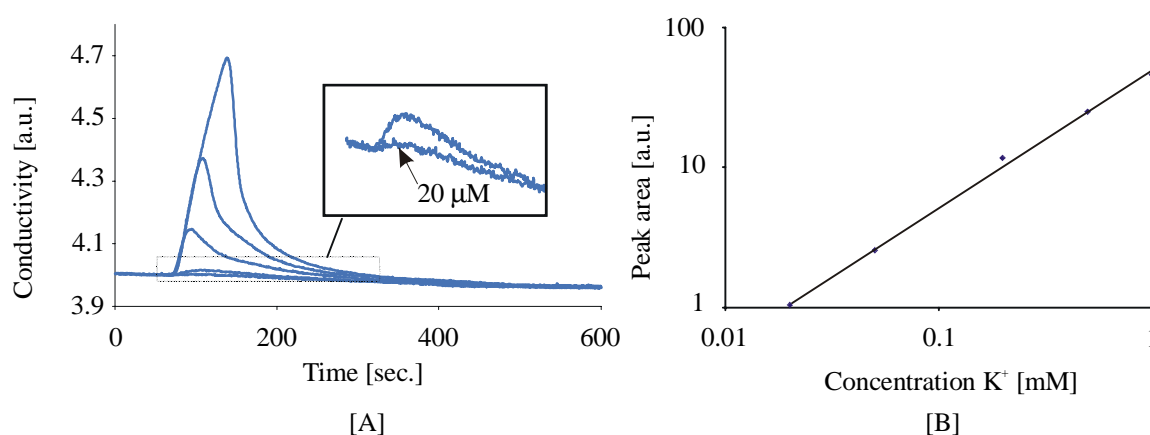


Fig. 6: (A) Measured potassium peak corresponding to injected sample with various concentration [20 μM - 1 mM]. The buffer is a 20 mM MES/His (pH 6). (B) Area of the measured peak. The conductivity is measured with the contactless 4-electrode setup.

The separation takes 3 to 4 minutes.

Similar measurements focussed on the separation of only potassium in the concentration range of 20 μM to 1 mM. Results are shown in Fig. 5A. Estimating the detection limit as three times the noise level, we deduce a corresponding value of 20 μM for potassium. For each concentration, a different peak height and width is observed. The concentration is retrieved from the area of the peak. The results are shown in Fig. 5B. The linearity of the curve indicates a proper separation and detection.

DISCUSSION

The measured peaks have a rather broad shape when compared to peaks obtained in conventional CE. That can be attributed to various factors. First, it has been shown that serpentine channels induce peak broadening due to a non-homogeneous distribution of the electric field in the corners. Second, it is known that in CE the separation efficiency is improved with high separation field strength, and values as high as 500 Volts/cm are applied in conventional systems. In our case, the potential applied for separation is limited to 400 Volts (field strength of 83 Volts/cm). Above that value, gas generation is observed at the silicon carbide electrodes. A thicker carbide layer (e.g. 100 nm) is necessary in order to allow a higher separation field strength. The use of a thicker insulating film over the metal electrodes will lead to a lower value of C_{is} and therefore make the 4-electrode setup even more advantageous over the 2-electrode setup (see the comparison between 2- and 4-electrode setup).

CONCLUSION

Liquid conductivity measurements have been done in miniaturized capillary electrophoresis device. We have shown that the 4-electrode setup is preferred over the classical 2-electrode setup. It allows a sensitive, linear

and accurate detection over a wide range of conductivity values. Calibration and adjustment of the measurement frequency is not required over a wide range of buffer concentrations.

The sensor shows a linear response in the range of 20 μM to 1 mM. The experimental results obtained on separation of inorganic ions demonstrate the applicability of the capacitively coupled 4-electrode liquid conductivity for detection in micro CE devices.

ACKNOWLEDGMENT

This research is supported by the Technology Foundation STW, applied science division of NWO and the technology programme of the Ministry of Economic Affairs (project DST.4351).

REFERENCES

- [1] F. Laugere, G.W. Lubking, A. Berthold, J. Bastemeijer, M.J. Vellekoop, "A novel high-resolution liquid-conductivity detector", Eurosensors XIII, The Hague, The Netherlands, 1999.
- [2] Graß B., Neyer A., Jöhnck M., Siepe D., Eisenbeiß F., Weber G., Hergenröder R., "A new PMMA-microchip device for isotachopheresis with integrated conductivity detector", Sens. Actuat. B 72, 249-258, 2001.
- [3] F. Laugere, G.W. Lubking, A. Berthold, J. Bastemeijer, M.J. Vellekoop, "Downscaling aspect of a conductivity detector for application in on-chip capillary electrophoresis", to be published in Sens. Actuat. A, 2001.
- [4] A. Berthold, L. Nicola, P.M. Sarro, M.J. Vellekoop, "Glass-to-glass anodic bonding with standard IC technology thin film as intermediate layers", Sens. Actuat. A 82, 224-228, 2000.