

## Summary of PhD thesis

### Application of pressure-driven injection for microchip electrophoresis

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## **I. Introduction and objectives**

In recent decades, reducing the size of the measurement systems, miniaturization techniques have been developed greatly. First, the newest science achievements appeared in computer technology and electronics science. By developing integrated circuits with size of few  $\text{cm}^2$  scale, unprecedented technological development began, which continues unabated today. Later, other disciplines have also adopted the miniaturization trends. In the early 90s the idea for creating miniaturized total analysis system (micro total analysis system, lab-on-a-chip) which contains a compact, complete chemical laboratory, was formulated. In addition to reducing the size of the separation unit, more objectives were to develop miniaturized sample dispensers enabling accurate sample introduction and sensitive detection devices. To achieve this the experts from different fields work was needed. So, now microfluidics is a dynamic interdisciplinary research field.

The use of microchips for analytical chemistry purpose has many advantages. In the miniaturized system very small volume of sample (often subnanoliter) and solvent (a few microliters) are required for the analyses. In addition, it enables fast, consecutive or parallel, low-cost analysis. Owing to its small size lab-on-a-chip systems are portable, inexpensive and economical production of its promoted widespread usage. Despite in several sub-areas of microfluidics breakthrough was achieved, further improvements will be needed in the area of sample introduction and detection methods. That is why creation, study and application of the lab-on-a-chip continue intensively.

In this work basic research studies were planned and our main goal was to develop pressure-driven sample introduction methods using simple tools which provide the injection of small sample plug (a few tenths nanoliter) in fast and reproducible way without sample bias. Using the developed injection methods creation of portable separation systems based on electrophoresis was intended. The proposed systems were tested by analysis of model and real samples.

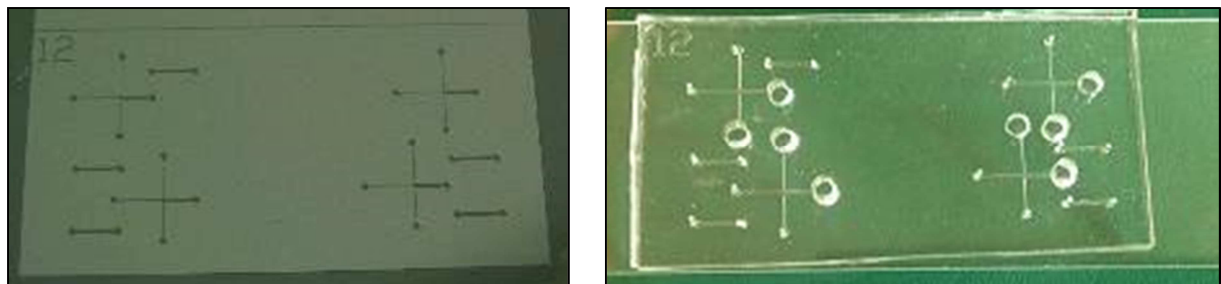
In order to achieve more sensitive detection electrophoretic sample preconcentration process (e.g. isotachopheresis) and different commercially available detectors applied for microchips were studied.

## II. Applied methods and equipments

### Preparation of microchips

Microchips made from polydimethylsiloxane (PDMS) by soft lithography technique were used for experiments. The channel pattern was designed by using AutoCAD (v.2013, Autodesk, San Rafael, CA, USA) software and printed to the transparent foil with high-resolution (3 600 dpi) printer to prepare the photolithography mask. Approx. 0.5 g negative-type photosensitive polymer resin (SU-8 2025, Microchem, Newton, MA, USA) was poured onto a thin sheet of silicon (Silicon Quest, Santa Clara, CA). To obtain ideal thickness of resin (35  $\mu\text{m}$ ) spincoater was used (3200 rpm for 30 s). Silicon wafer containing thin film of resin was placed into the oven at 95°C for 15 min. After the solidification of resin photolithographic mask was put onto the Si wafer and irradiated with UV light ( $\lambda = 365$  nm, Spectroline FC-100/F lamp, Spectronics Corporation, Westbury, NY, USA) for 10 min. Si wafer was immersed into developer (mr-Dev 600, Micro Resist Technology, Berlin, Germany) to remove residues of the photoresist not irradiated by UV light.

A molding method was used to create the PDMS microchip containing the channel pattern by pouring a 10:1 mixture of PDMS oligomer and crosslinking agent (Sylgard 184, Dow Corning, Midland, MI, USA) onto the mold and was solidified at 65°C for 1 hour. After reticulation the replicas were peeled off from the mold, cut to size and the connection ports were created by a hand puncher. In order to close the channels the PDMS layer was irreversibly bonded to a glass slide or to a PDMS bottom layer after air plasma treatment (Harrick PDC-32G, Harrick Plasma, Ithaca, New York, USA).



**Fig. 1.**

A mold used for the preparation microfluidic chip (left) and a complete microchip bonded to a glass slide (right).

### **Other applied devices and detectors**

Samples and buffer electrolytes were pumped to the channels by a low-rate peristaltic pump (IPC, Ismatec) or a syringe pump (Bee Hive MD-1020, Bioanalytical System Inc., equipped with syringe of 2.5 mL). Injection and electrophoretic processes occurring in the microchannel were observed by an inverted microscope (Axio Observer A1, Zeiss, Germany) assembled with a high speed CCD camera (AxioCam ICC3, Zeiss). For evaluation of data image/video recorder software (AxioVision 4.6.3, Zeiss) was used.

After the injection, high voltage power supply (Microply-01, 0-2 kV, max. 0.25 mA and Microply-31, 0-2.5 kV, max. 0.6 mA, Cetox Kft. or Spellman CZE 1000R, 0-30 kV, max. 0.3 mA, Ithaca, New York, USA) was applied.

The UV detection was performed by a miniaturized fiber optic spectrometer (Avaspec-2048-2, Avantes, Netherlands). Obtained values were recorded by using Avasoft software (Avantes, Eerbeek, Netherlands).

Capacitively coupled contactless conductivity detection on the microchip was accomplished on a platform (ET121, eDAQ, Australia) by using a C<sup>4</sup>D Amplifier (eDAQ, Australia) connected to a data acquisition system (PowerChrom 280, eDAQ, Australia). Contactless conductivity detector with headstage (TraceDec, Innovative Sensor Technologies, Strasshof, Austria) was employed for detecting separations in the capillary. Data were processed and evaluated by Chart v.5.5.13 software.

CE and CIEP analyses were conducted in HP 3DCE and 7100 CE instruments (Agilent, Waldbronn, Germany) by using polyimide coated fused silica capillary (Polymicro Technology, Phoenix, AZ, USA). The obtained electropherograms were evaluated by ChemStation 7.01 software (Agilent).

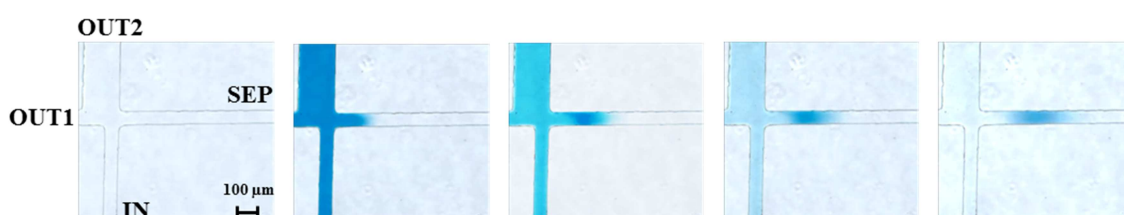
Flow conditions occurring during split-flow injection was simulated by COMSOL Multiphysics (COMSOL, Inc., Palo Lato, CA, USA).

### III. New scientific results

1. Sample introduction method has been developed, called **split-flow**, for injecting subnanoliter volume of sample into the separation channel of microchip in reproducible way.

1.1. Microchip with channel pattern was designed in which crossing of channels consisted of a separation channel and a wider channel with ratio of their diameter 1:4, that allowed the injection of subnanoliter sample volume.

Split-flow injection was based on Hagen-Poiseuille law in which the volume of a homogeneous fluid passing through a capillary tube into the separation channel is directly proportional to the fourth power of internal diameter of channel and inversely proportional to length of channel, but independent from the flow rate. If 1  $\mu\text{L}$  volume of sample was pumped into the chip, only a fraction of initial volume (less than 1/2500 part) was flown toward the separation channel that provided small sample volume ( $\sim 0.4$  nL) for zone electrophoretic separation in microchip. In this injection method only pressure was applied, therefore the known quantitation errors (biases) of electrokinetic injection did not occur, because the voltage does not influence the moving of charged analytes (and thus the composition of the sample was not changed). Reproducibility of the developed injection method was tested by consecutive injection of blue dye (N=6) and the achieved precisions were 4.8 RSD% and 3.7 RSD% for the peak heights and peak areas, respectively. These values were similar to those obtained with other injection technique used for microchips.



**Fig. 2.**

Split-flow injection of blue dye ( $V_0=1$   $\mu\text{L}$ ,  $V_{inj.}\sim 0,4$  nL).

**1.2. To investigate flow conditions occurring at the crossing channels in the proposed microchip designed for split-flow injection, COMSOL simulation software was used. From the velocity distribution diagram obtained at different channel width ratios (1:4, 1:6 and 1:10 split ratios) we found that 0.102% (at the ratio of 1:4), 0.053% (at the ratio of 1:4) or 0.037% (at the ratio of 1:10) of initial volume of the injected sample ( $V_0=1 \mu\text{L}$ ) was introduced into the separation channel, respectively.**

Thus, depending on the ratios of channel widths volumes of sample in the range of 0.37 to 1.02 nL (1:10  $\rightarrow$  1:4 split ratios) were injected. Injected volumes of samples at different ratios of channel widths calculated from the Hagen-Poiseuille law and obtained from the simulation were compared resulting values in similar magnitude, but despite the same initial volumes of sample, five-fold differences were observed in the injected volumes.

**2. We proved that after the split-flow injection, the formed small sample plug was suitable for zone electrophoretic separation in PDMS microchip.**

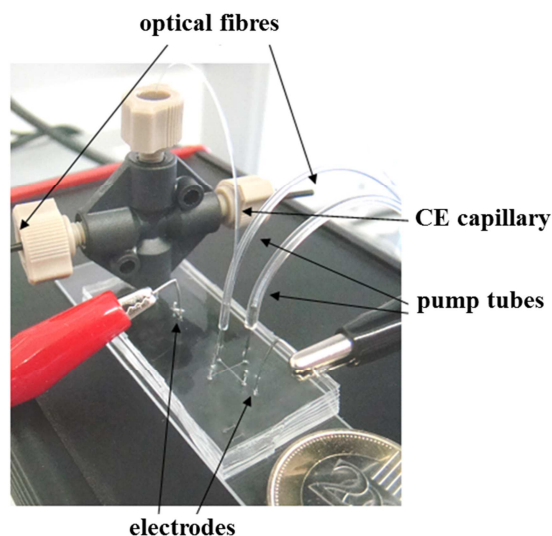
**2.1. Since in the originally hydrophobic PDMS channel system, significant EOF flowing toward the cathode was present, the use of methyl cellulose as surface modifier was proposed. After surface modification the applicability of rapid (less than 15 s) CZE separation in microchip with split-flow injection was demonstrated.**

For the reproducible CZE separations, different surface modifying agents (anionic, cationic and neutral) were used before the injection in order to create stable, homogeneous channel wall. After the channel wall was rinsed with neutral (methyl cellulose of 0.1%) modifier for 5 min, baseline separation of the two negatively charged components was achieved less than 15 s, at 1 cm effective length.

**2.2. A microchip-based miniaturized capillary electrophoresis system ( $\mu\text{CE}$ ) has been developed, wherein the sample introduction was executed in a PDMS chip using split-flow injection method and the separation of compounds was performed in a fused silica capillary attached to the ports of the PDMS chip.**

In the assembled system, PDMS microchip had dual function, it did not only include channel pattern for the split-flow injection, but also held the 20 cm length of CE separation capillary. The flexible PDMS used as material for microchip preparation allowed the formation of leakage-free connection with a relatively low dead volume. The optical fibres of the photometer were used for

detection, like in the commercial CE apparatus, directly detected through the capillary.



**Fig. 3.**  
Miniaturized CE system.

**2.3. Analytical applicability of the developed  $\mu$ CE system was confirmed by CZE separation of six cephalosporin antibiotics. The obtained results were compared with separations achieved in commercial CE instrument.**

The baseline separation of six cephalosporins, having relatively similar chemical structure, was completed in less than 8.5 minutes, at 18 cm separation length. In the  $\mu$ CE system the achieved separation efficiency (number of theoretical plates ranged from 5 000 to 15 000) was in similar magnitude to be measured by traditional CE apparatus. The reproducibility of the  $\mu$ CE system was tested by consecutive injection and repeated separation of mixture of two cephalosporins. Peak heights and peak areas were ranged from 1.89 to 1.96% and 3.34 to 3.58%, respectively. Owing to its reduced size, battery operated high-voltage power supply and detector device,  $\mu$ CE system can be considered portable.

**3. The use of contactless conductivity ( $C^4D$ ) platform detection with PDMS microchip having a 100  $\mu$ m thin bottom layer was presented. The proposed PDMS chip- $C^4D$  system was applied to separate anions in model and real samples.**

To accomplish the contactless conductivity ( $C^4D$ ) platform detection, a very thin layer has to be used for making the bottom of the PDMS microchip acting as an insulating layer between the sensing electrodes and the liquid inside the microfluidic channel. We examined how different thickness of PDMS and glass

layers affect the response signals. ~100  $\mu\text{m}$  thick PDMS layer was found to be optimal for sensitive detection. One advantage of the proposed system is disposability, because the PDMS chip can be removed from the platform in case of clogging and due to its flexibility a new microchip can be smoothed simply onto the sensing electrodes. The other advantage of the use of  $\text{C}^4\text{D}$  with external electrodes is the possibility to freely vary the separation length in the microchip.

PDMS microchip- $\text{C}^4\text{D}$  system was tested by separation a seven-component containing mixture as a model solution, including inorganic and organic anions. Using 0.5  $\mu\text{L}$  sample as initial volume after the split-flow injection small sample plug was got into the separation channel. The length of 6.5 cm was long enough for baseline separation of the seven components less than 3.5 min obtaining the numbers of theoretical plates in the range of 4 000-6 000 (63 000/m-93 000/m).

The proposed microchip- $\text{C}^4\text{D}$  system was successfully applied to analyze different types of real samples (tap and mineral water, toothpaste, saliva). Arranging the two types of  $\text{C}^4\text{D}$  detectors in line, a dual detection could be performed to directly compare the performances of the two  $\text{C}^4\text{D}$  detection systems using the same electrophoretic run.

#### **4. Capillary isotachopheresis (CITP) sample preconcentration and separation were performed in the conventional CE instrument.**

**4.1. We have studied the possibility of reducing electroosmotic flow (EOF) induced by applying voltage in the fused silica capillary. Thus, before the injection the capillary wall was rinsed with solution of polymers having high molecular weight. Rinsing with PVP of 2% for 10 min was enough to minimize the EOF, a stable, dynamic layer was formed on the capillary wall.**

The alteration of EOF was studied by following the changes of migration time of neutral marker (benzyl alcohol). For the further CITP experiments the use of PVP-coated silica capillary was concluded to be optimal and therefore flushing the capillary with PVP solution before each sample injection was applied, but the buffer solutions did not contain PVP.

**4.2. Detailed studies were conducted about the applicability of the  $\text{C}^4\text{D}$  headstage and UV detection for CITP analysis carried out in commercial CE instrument. It was found that the use of  $\text{C}^4\text{D}$  headstage for detecting narrow sample zones obtained during isotachopheresis was very limited.**

The main reason was the relatively large distance between electrodes of the detector (1-2 mm). To improve the resolution and reach wider zones 75% of the 120 cm length of capillary was filled with the sample (chromate ion) prior to the CITP analysis. Calibration diagram for chromate was shown good

linearity in the concentration range of 0.1  $\mu\text{M}$ -5 mM for UV and 0.4 mM-5 mM for  $\text{C}^4\text{D}$ . The LODs values for chromate were 0.02  $\mu\text{M}$  and 0.2 mM obtained with UV and  $\text{C}^4\text{D}$  detection, respectively. Repeatability of CITP analysis was tested, the obtained RSD values were 1.49% for migration times and 1.98% for peak areas (N=5). We found that CITP with spectrophotometric detection in case of UV active component (if adjacent (neighbouring) zones were non UV-absorbing components) can be advantageously applied for determining trace chromophore analytes (<1  $\mu\text{M}$ ). With CITP, various real samples were successfully analyzed (e.g. chromate in aqueous extract of tanned leather, organic acids in wine samples and preservatives in juice).

**4.3. Among the first, we performed capillary isotachopheresis preconcentration in PDMS microchip and demonstrated that using CITP components can be moved at long distance in microchip without dispersion.**

Split-flow injection was successfully used to inject larger sample volume (a few nL) required for microchip ITP by using smaller ratio of channel diameter or higher initial volume ( $V_0$ ). ITP analyses of one and multi-component mixtures of dyes were performed in PDMS microchip. The sharp, narrow zones of the sample were monitored and recorded using a camera in real time. The formation of ITP zones was examined and the experimental results were consistent with the simulations obtained by Simul 5 Complex software.

**4.4. We confirmed that bacterial cells can be concentrated largely in PDMS chip using isotachopheresis.**

Not only ITP analyses of liquid samples were carried out, but also extended investigations were conducted with shaped components (bacterial cells). During the split-flow injection moving of cells was significantly different from the previously described flow profile of liquids. Therefore, channel pattern with 1:1 ratio of diameter was used for injection of the cells. Using ITP conditions, cells were concentrated into the compressed zone (narrower or wider depending on the injected volume) and this zone can be transported without band dispersion even at longer channel distance.

#### **IV. Possible utilization of the results**

Owing to continuous development of analytical microfluidics, newer and newer lab-on-a-chip devices have been appeared. Consideration of the emerging market demands and the applicability of the developed device in specific areas are important aspects during the development of microfluidic devices. A lab-on-a-chip system will be commercially available, if it has concrete usability in industrial, medical diagnostics or environmental areas. The microfluidic systems can be competitive with other conventional (instrumental) analytical methods due to low cost of its operation, production and the small sample request. Lab-on-a-chip devices are portable owing to small sizes and since their use is rapid, consecutive or (many) parallel analyses can be performed.

Although we primarily carried out basic research studies, we tried to implement methods based on a relatively simple principle. Sample introduction method has been developed, called split-flow, for injecting small volume of sample in reproducible way that may be a good alternative in the future for microchips used electrokinetic injection with quantitation errors (biases). The developed injection method does not require any special equipment. Thus, consequently it can be easily transposed to other microfluidic systems.

In the developed miniaturized systems different real samples were analyzed successfully during short time. The obtained analytical performance values were in similar magnitude to be measured by commercially available instruments. In the future, further expansion of the use of microfluidic chips can be predicted in analytical chemistry, for which more sensitive detection methods will be needed. This can be achieved by improving the existing detectors as well as introducing new types of miniaturized detectors and applying effective sample preconcentration techniques (e.g. isotachopheresis).



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Subject: Ph.D. List of Publications

### List of publications related to the dissertation

#### Foreign language scientific articles in international journals (4)

1. **Koczka, P. I.**, Bodoki, E., Gáspár, A.: Application of capacitively coupled contactless conductivity as an external detector for zone electrophoresis in poly(dimethylsiloxane) chips.  
*Electrophoresis*. 37 (3), 398-405, 2016. ISSN: 0173-0835.  
DOI: <http://dx.doi.org/10.1002/elps.201500335>  
IF: 3.028 (2014)
2. **Koczka, P. I.**, Bodor, R., Masár, M., Gáspár, A.: Application of isotachophoresis in commercial capillary electrophoresis instrument using C4D and UV detection.  
*Electrophoresis*. [Epub ahead of Print], 2016. ISSN: 0173-0835.  
DOI: <http://dx.doi.org/10.1002/elps.201600194>  
IF: 3.028 (2014)
3. **Koczka, P. I.**, Gáspár, A.: Application of a capillary-assembled microfluidic system for separation of cephalosporins.  
*Electrophoresis*. 35 (17), 2534-2537, 2014. ISSN: 0173-0835.  
DOI: <http://dx.doi.org/10.1002/elps.201400118>  
IF: 3.028
4. Gáspár, A., **Koczka, P. I.**, Carmona, H. A., Gomez, F.: Split injection: A simple introduction of subnanoliter sample volumes for chip electrophoresis.  
*Microchem J*. 99 (2), 180-185, 2011. ISSN: 0026-265X.  
DOI: <http://dx.doi.org/10.1016/j.microc.2011.05.001>  
IF: 3.048

The Candidate's publication data submitted to the iDEa Tudóster have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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