Analytical separations in microfluidic chips (K-111932)

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Further improvement of the microfluidic laboratory

The microfluidic laboratory at our university was established by our research group in 2010. The first instruments and tools (inverted microscope, peristaltic pump, spincoater, special UV lamp, programmable high voltage power supply) were used ones or purchased with the support of a previous OTKA grant and the Cetox Ltd, Hungary. Thanks to the current OTKA grant several other smaller instruments and tools could be bought (new syringe pump, used multi-channel peristaltic pump, special, intensive light source). Laser induced fluorescence detector applicable to CE was also purchased with an other (GINOP) grant, we hope that this detector can also be used with microchips. Recently, an other GINOP project made possible to purchase a modern Q-TOF-MS (Bruker), which was already started to apply with microchips (enzimatic reactor, chromatographic chips).

Designing and microfabrication of chromatographic microchips

The channel patterns were designated using the program Autocad, and then the chips were created by soft lithography [1]. The movement of the coloured or fluoresence components is monitored using an inverted microscope equipped with a color CCD camera which is enable to record movies and the images. The design of a microchannel system applied for multiple chromatographic separations was optimized. The pressure and velocity distribution in a complex microchip was simulated by COMSOL Multiphysics software in order to find the optimized geometry and pressure conditions [2]. The experimental results of the flow rates in the twelve parallel channels of the used microchip agreed well with the simulation results. COMSOL simulations were also applied to find adequate channel designs for the equalization of the flow rates in the parallel channels of a chip, which included many junctions of channels and bottlenecks. This approach can greatly expedite the time required for complex geometry based prototype fabrication.

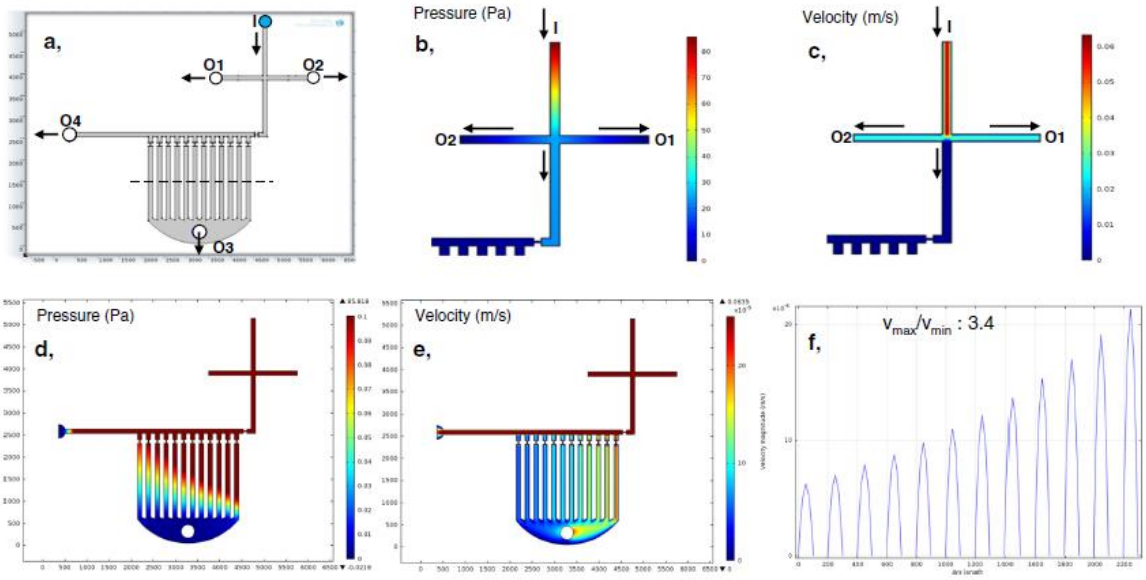

Fig. 1. COMSOL simulations of pressure distribution (b) and flow rates (c) in the sample introduction part (junction of fluid channels) and in the complete channel system (d, and e,) of the microchip. The flow rate applied at the inlet port I was 10 µL/min, and the pressure at the outlet ports O1–O4 were set to 0 Pa [2].

Electrophoretic separations in microchips

The ability to accurately inject small volumes of sample into microfluidic channels is of great importance in electrophoretic separations. While electrokinetic injection of nanoliter scale volumes is commonly utilized in microchip electrophoresis, mobility and matrix bias makes quantitation difficult. Recently, we described a new pressure injection method based on the simple patterning of the crossing of channels. Lab-made poly(dimethylsiloxane) microfluidic chips were matched to a capacitively coupled contactless conductivity detector (C4D) having external in-plane electrodes (eDAQ, Australia) [3]. The advantages of this type of C4D are the choice to reversibly place or remove the microchip onto/from the detector and to freely variate the position of the detection (separation length) on the microchip. The thickness of the bottom layer of the PDMS chip was optimized to achieve sensitive detection during the electrophoretic separation. PDMS chips with 100 µm bottom layer used with the C4D platform were tested by CZE of a mixture of seven anions and different types of real samples. Using split-flow pressure sample injection and effective length of 6.5 cm, the numbers of theoretical plates were in the range of 4 000-6 000 (63 000/m - 93 000/m) and the LODs amounted to 3.66 µmol/L-14.7 µmol/L (0.13-2.26 µg/mL) for the studied anions.

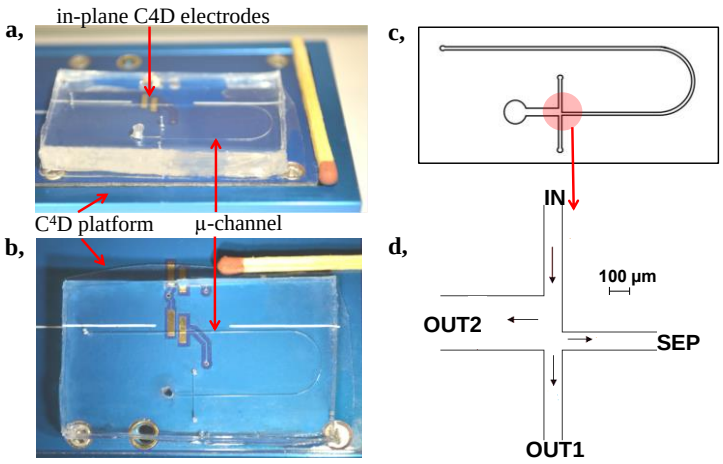

Fig. 2. A PDMSmicrochip with thin bottom layer (~100 mm) placed on the C4D platform to a position that the microfluidic channel crosses the two in-plane electrodes of the platform (top-side view (A) and top view (B)). The channel pattern includes a split-flow injection part for creating sample plug of subnanoliter volume in the separation channel (SEP) (C).

The electrophoresis was used to different pharmaceutical analytical studies. For instance we studied the role of equilibrium and kinetic properties in the dissociation of Gd[DTPAbis(methylamide)] (Omniscan) near to physiological condition [4] and the repeatibility of the determination of temozolomide by micellar electrokinetic capillary chromatography using internal standards [5].

Chromatographic separations in microchips

We continued our research started a few years on the development and investigation of multi-channels for 3-12 parallel chromatographic separations in order to gain high sample throughput analytical systems. Unfortunatelly, the application of high pressure could not be applied. The obtained chromatographic chips were used also for µSPE separations. The chromatographic micropackings in chips were used as support of high specific surface for enzym reactors.

We wrote and submitted two review papers in the topic of the OTKA project. One of those was invited by the editor (A.Kecskemeti, A.Gaspar: Particle based liquid chromatographic separations in microfluidic devices, Anal.Chim.Acta) [6].

Fig. 3. Cover page of Analytica Chimica Acta about our paper [6]

Microfluidic enzyme reactors (IMER) for efficient proteolysis for subsequant electrophoretic separation (with UV or MS detection)

We developed immobilized trypsin microreactors, capable of rapid and efficient protein digestion. The most appropriate immobilization techniques are based on adsorption onto the channel wall of a PDMS microfluidic chip [7] or covalent bond of trypsin on a packed bead microreactor using EDC/NHS method [8]. Both reactors were integrated into microfluidic chips, as these devices has high surface-to-volume ratio and require small sample volumes. These reactors are capable of rapid protein digestion, however trypsin adsorbed on PDMS lost its activity after 4 hours due to conformational deformation on the hydrophobic surface. Therefore, this reactor can only be used for protein digestion, if the PDMS surface is regenerated with trypsin directly before digestion. As a result, this immobilization technique (adsorption) was eliminated from further investigation, and another technique was applied to obtain durable reactors.

Using covalently immobilized reactor is advantageous, as it retains its activity for 1 month (if stored properly). In this period it can be used for digestion multiple times. For higher enzyme activity and for better reproducibility of peptide maps, the active site of trypsin was protected in the immobilization reaction with its competitive inhibitor, benzamidine. This way, reactors yielded reproducible peptide maps. Also, standard in-solution digestion was compared with the digestion on the packed bead microreactor. In both cases, most of the peptide products can be assigned (the majority of the products are the same). Reaction time in the described reactor is 10 seconds, while in-solution digestion requires 16 hours. The developed reactor can be used effectively for rapid protein digestion of realistic samples with high sample throughput (8 simultaneous digestion). We proved the applicability of the microfluidic chip IMER for rapid tear protein digestion. As the volume of a tear sample is limited, it is convenient to use the microfluidic chip IMER for protein digestion. The designed chip has a higher sample throughput, as it is possible to digest 8 protein samples at the same time.

The CZE peptide maps (peak pattern) obtained by microchip IMER digestion and in-solution digestion did not differ significantly, however, microchip IMER digestion takes < 10 s. Human serum was also digested efficiently with the IMER. The microchip IMER digested proteins were identified with LC-MS/MS, sequence coverage values were sufficient (29–50%). Trypsin autolysis peptides were not identified.

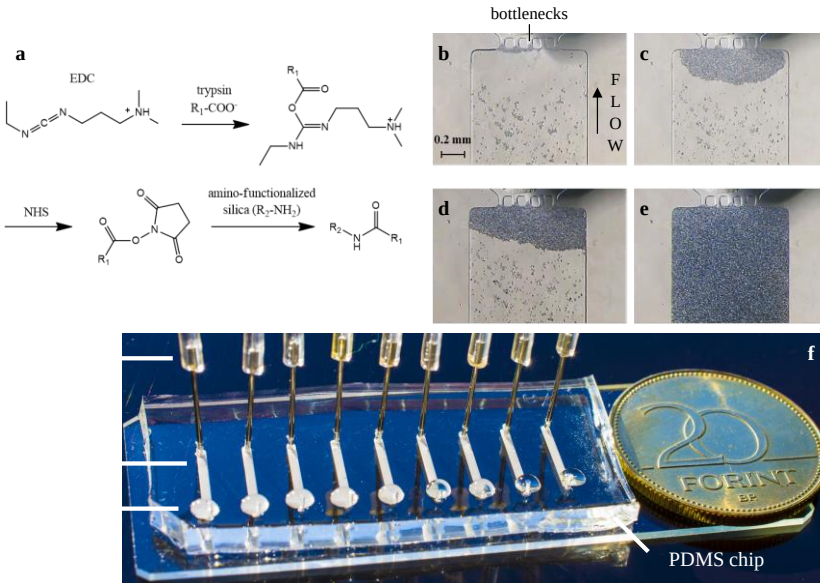

Fig. 4.(a) The covalent immobilization reactions with EDC/NHS between trypsin and amino-functionalized silica particles. (b–e) Optical micrographs of the packing formation process at the bottlenecks. The suspension of the silica particles in storage solution was pumped toward the bottlenecks. (f) Photograph of the microchip IMER with 9 simultaneous digestions. [8]

Development of a miniaturized flame atomic emission spectrometer („flame-on-a-chip")

Since atomic spectrometers typically need at least a few hundreds of microliters for analysis, only a very few number of works can be found in literature about the element-selective detectors hyphenated with microchips. Besides the ICP-AES or ICP-MS detectors, only one work was found using (flameless) atomic fluorescence spectrometry to microchip. The only one combination of chromatographic microfluidic chip with the traditional and often used flame atomic absorption spectrometer (FAAS) was shown by our group [9]. However, this was an off-line hyphenation. Now we developed a microchip including 3 PDMS layers: one layer makes possible to transport the sample liquid into the micro burner head, the other layer transport the fuel gas (propane-butane gained from a personal the gas lighter) and the third layer was about to support the fiber optic of the UV spectrometer [10]. On this chip a simple burner head was created to form a tiny flame. This flame was used to form a thermospray aerosol and to excite the alkali elements (in 2000 we described thermospray sample introduction for AAS [11]). Now we optimize this thermospray sample introduction conditions, which are the key parameters for the operation of this „flame-on-a-chip" device. According to our experiments even 1 microliter of a few ppm concentration of Na, Li, K, Cs can be measured through the detection of the light emission of these elements. Later we plan to integrate a chromatographic packing (before the FAES unit) into the chip. Such a lab-on-a-chip might be used in element speciation analysis, for which a complex instrumentation like HPLC-atomic spectrometer hyphenated technique is used.

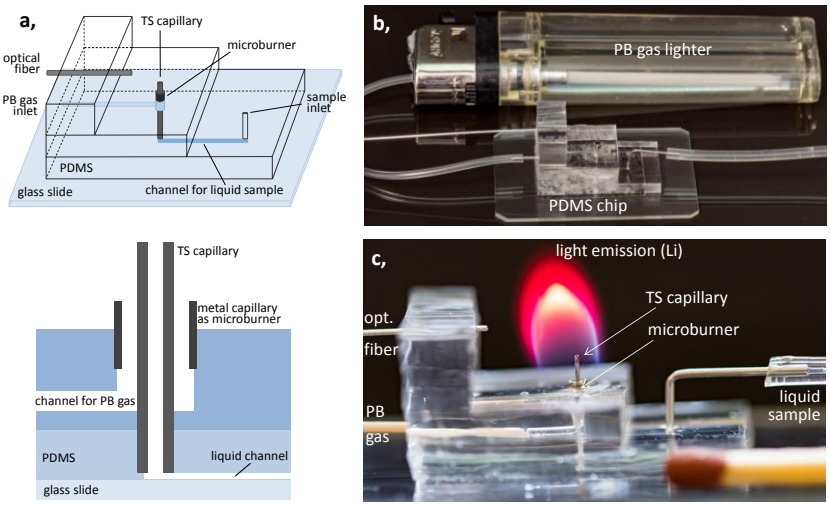

Fig. 5. Schematic construction of the miniaturized FAES system (left upper) and TS nebulizer/microburner (left lower) (a). Photographs of the miniaturized FAES system including microfluidic device and a propane/butane gas lighter (b) and the microfluidic device with flame colored by Li solution introduced via thermospray capillary (c) [10].

At first, the polydimethylsiloxane microfluidic chips were direct interfacing with laser-induced breakdown spectroscopy (LIBS) [12], which is a new atomic spectrometric detection. Our work focused on the solution of technical and analytical problems of coupling single-pulse LIBS detection with PDMS microfluidic chips in order to assess the feasibility and performance of the concept of creating a lab-on-a-chip device with LIBS detection (LOC-LIBS). Multiple optical and sample presentation schemes including in-channel and in-port detection were tested, but it was found that LOC-LIBS is only viable and practical with in-port detection outside the chip. It was shown that LOC-LIBS in this configuration is capable of the trace speciation analysis of chromium using as little as 0.5 µL solution volume. The achieved absolute limit of detection was 2 ng.

Study the application of isotachophoresis (ITP) using C4D and UV detection in capillary and chips

Firstly we tested the applicability of a commercial CE instrument (Agilent) for capillary ITP (CITP). The fused silica capillaries were flushed with polyvinylpyrrolidone (PVP) solution before each sample injection to suppress the EOF. As a dual-detection mode, commercial capacitively coupled

contactless conductivity detection and ultraviolet detectors were applied. The experiments showed that the detection gap of the capacitively coupled contactless conductivity detection limits the achievable LOD and the separation resolution when the analyte CITP zones are very narrow, therefore long (120 cm) CE capillary was used and it was largely filled with the sample solution. CITP analyses of several real samples (leather extract, red wine, juice, and fizzy drink) have been demonstrated. In peak mode of CITP when the zone of a chromophore analyte is positioned between nonchromophore zones, excellent sensitivity (in submicromolar concentration range) could be achieved by ultra-violet detection. The hazardous chromate in low concentration was determined in the aqueous extract of tanned leather. CITP can be used to selectively enrich analytes while eliminating unwanted impurities. This selective enrichment can be achieved by properly choosing leading electrolyte (LE) and terminating electrolyte (TE) ions whose mobilities bracket those of the target analytes. The UV and C4D in commercial CE instruments can be easily arranged and the application of the dual detection mode is preferred. Technical developments (such as shortening the detection gap/window or lengthening the analyte zone at the detector) can lead to improvements in apparent resolution and detection sensitivity.

The experiences and results obtained in the above isotachophoretic separation were utilized on the work of separation of cells in microfluidic chips. 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. 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. Not only ITP analyzes 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 greater channel distance.

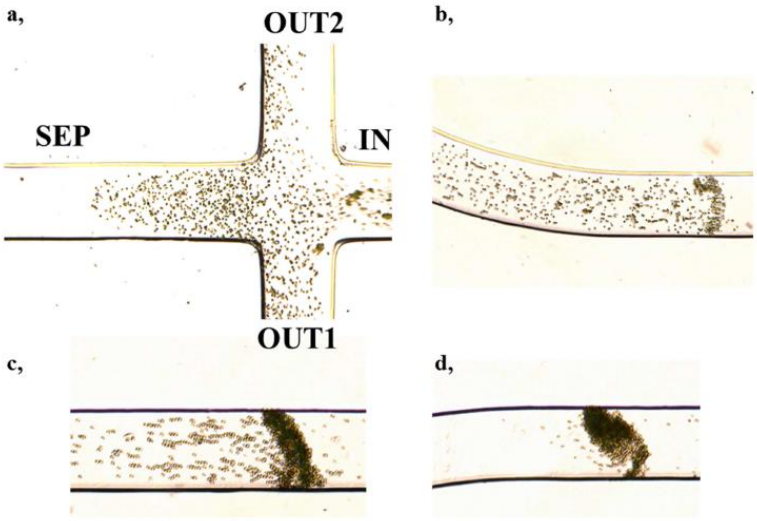

Fig. 6. Isotachophoretic separation/preconcentration of Microcystis aeruginosa bacterium cells in a microchip including a crossing of channels for injection of the cells (a,). Illustration of the zone sharpening effect due to ITP (b-d). These results are under publication.

Other achievements:

DSc thesis in the project topic was defended:

1. Attila Gaspar: Electrophoretic separation in capillary and microchip, 3rd November, 2015

PhD theses in the project topic were defended:

1. Andrea Nagy: Development of multichannel microfluidic systems for chromatographic applications, 2015
2. Peter Koczka: Application of pressure-driven injection for microchip electrophoresis, 2016
3. Adam Kecskemeti: Development of protein digesting enzymatic reactors in microfluidic chips, 2018

OTDK in the project topic:

1. Cynthia Nagy: Digestion of tear samples in trypsin based microfluidic enzimatic reactors, 2017, Miskolc. Third place.

References

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