

Summary of PhD thesis

Development of multichannel microfluidic systems for chromatographic applications

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Debrecen, 2015.

I. Introduction and objectives

An explosion in the development of microfluidic research has been started in the recent years. The development study and analytical applications of lab-on-a-chip systems are still very intensively. Nowadays one of the new trends in analytical chemistry is the miniaturization of chromatographic separation techniques, the preparation of chromatographic packings in microfluidic channels, due to the increasing demand for the analysis of the larger number of samples in a fast and relatively cheap way.

The analytical applications of microfluidic chips have many advantages. Only a few nL sample is required for the fast analysis, the used volume of the reagents, solvents is smaller than in the conventional analytical methods. The preparation and operating costs of the microchips are significantly less than in the case of conventional analytical instruments. Their use is economic and environmental friendly. A large number of biological and environmental analytical methods were developed and described in microchips.

Relatively few chip-based chromatographic separation systems have been developed, because of the difficulties in the preparation of chromatographic packing in microchannels. Several systems are known where the chromatographic particles were retained in the channels by frits (frit-like barriers). However the use of frits can cause various technical problems (for example, the preparation of the frits in the channels is a difficult process or these frits can catalyze the formation of the bubbles in the channels).

The main goal of our work was the miniaturization of separation techniques, the preparation and application of chromatographic packing in microfluidic chips. We would like to develop a fast and reproducible packing process, where the chromatographic particles were retained without the preparation of any special frit.

Another goal was the designing and the preparation of multichannel systems, where it is possible to prepare different chromatographic packings in the parallel channels for simultaneous separations. One of the drawbacks of these systems is the lack of sensitive detection methods. We were examined the applicability of atomic spectroscopy detection methods for our microfluidic systems.

II. Applied methods and equipments

The preparation of microchips

AutoCAD software was used for designing and drawing of microfluidic systems. The channel pattern was printed as a high resolution (3600 dpi) photomask. The negative type photoresist (SU-8 2025, Microchem, Newton, MA, USA) was spin-coated onto a 3" (7.6 cm) diameter silicon wafer (3000 rpm, 30 s). This photosensitive layer was lightened for 10 minutes with UV light (365 nm, Spectroline FC-100/F lamp, Spectronics Corporation, Westbury, New York, USA) through the photolithographic mask. The channel patterns were developed with 1-methoxy-2-propanol-acetate (mr-Dev 600, Micro Resist Technology, Berlin, Germany), on the wafer only 40 μm high channel patterns remained. The 10:1 mixture of the polydimethylsiloxane (PDMS) and curing agent (Sylgard 184, Dow Corning, Midland, MI, USA) was poured onto the mold and it was cross-linked in an oven at a high temperature (65 °C degrees, 1 hour). After polymerization the plastic was peeled from the mold, the chips were cut to size, the ports were formed by hand punch (the diameter of the ports was 0.7 mm). The PDMS microchip was sealed onto a glass slide after oxygen plasma treatment (Harrick PDC-32G, Harrick Plasma, Ithaca, New York, USA).



Fig. 1. A mold for the preparation microfluidic chip containing three identical channel system (left), a microchip sealed onto a glass slide and the channels filled with different color dyes (right).

The investigation of microchips, detection methods

The liquid was pumped to the channels by a low-rate peristaltic pump (Ismatec IPC, Ismatec SA, Glattburgg, Switzerland), the processes in the microchannels were observed by an inverted microscope (Axio Observer A1, Zeiss, Germany) equipped with a high speed CCD camera (AxioCam ICC3, Zeiss) and image/video recorder software (AxioVision 4.6.3, Zeiss).

The determination of the separated and preconcentrated chromium was carried out with a 240FS atomic absorption spectrometer (Agilent Technologies, Santa Clara, CA, USA) and a laser induced breakdown spectrometer (LIBScan 25+, Applied Photonics Ltd, Skipton, New Yorkshire, UK).

The simulations of the liquid flows in the channels were performed using COMSOL Multiphysics (COMSOL, Inc., Palo Alto, CA, USA) software.

III. New scientific results

1. Chromatographic packings were prepared in polydimethylsiloxane microchip by a fritless process using bottlenecks in the channels.

1.1. We demonstrated that the chromatographic particles can be retained in the channels with the use of bottlenecks which was created by the reduction of the channel height.

Decreasing the channel height to 15-20 μm was enough for the retainment of the 5 μm diameter chromatographic particles. It was achievable by the tapering of the channel pattern at the proper location.

1.2. During the designing of the channel patterns the bottlenecks were created by the appropriate reduction of the channel width. These bottlenecks were able to retain the chromatographic particles.

The microchips' channel width was reduced from 100 μm to 15-20 μm , which allowed the reproducible preparation of chromatographic packings from the 5 μm diameter particles. The length of the packings was freely variable from 200 μm to more centimeters. Using of this process it was possible to prepare chromatographic packings in multichannel systems.

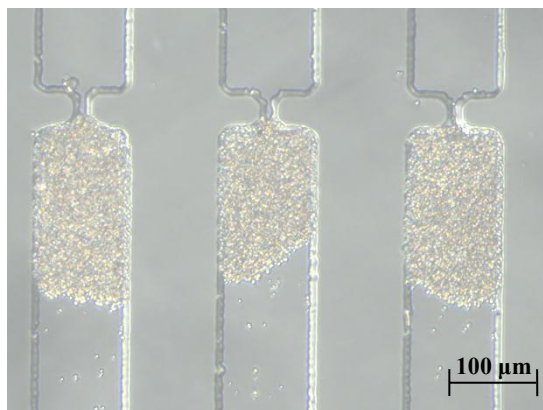


Fig. 2. The preparation of chromatographic packings in the parallel channels using bottlenecks created by the reduction of the channel width.

1.3. We demonstrated the importance of the keystone-effect during the chromatographic packing creating procedures which were detailed in the 1.1. and 1.2. sections.

The stability of the prepared chromatographic packings was investigated by the application of different bottleneck diameters. Stable chromatographic packings were prepared with the use of a maximum three times bigger bottleneck than the retainable particles, due to the keystone-effect. (For

example, a bottleneck with 15 μm diameter was able to retain the 5 μm diameter chromatographic particles, but when the diameter of the bottleneck was increased to 25 μm the packing became instable.)

The length of the bottleneck has not got effect for the stability of the packings when the length was minimum two times longer than the diameter of the chromatographic particles. (In our systems a 10 μm long bottleneck would be enough but we designed our channel systems with 50 μm long bottlenecks.)

We found that a suitable bottleneck can be created even if the contour of the bottleneck (a 10 μm wide and 50 μm long channel) was drawn directly onto the photolithographic mask and proper lithographic conditions were applied. The diameter of these bottlenecks was impressible with the thickness of the photosensitive layer and the period of the exposure time. We observed that the use of a thinner photosensitive layer or a longer exposure time resulted wider channels and bottlenecks.

2. We prepared multichannel microfluidic systems which are suitable for the parallel chromatographic separations at the same time.

2.1. In microfluidic chips 3-12 parallel chromatographic packings were prepared by the process detailed in the 1.2. section.

We demonstrated that the size of the 12 parallel chromatographic packing is really small compared to the size of the microfluidic chip. The number of these parallel chromatographic packings is ultimately limited by the number and the position of the outlet and injection ports. If the system contains only one injection part the number of the chromatographic packings can be increased even to 60. These multichannel systems can contain independent or merged channels. In the independent channel systems different parallel chromatographic packings can be created, while in the merged channels the packings can be formed at the same time through a common outlet port. In both type of microchips we have the possibility to measure one or more sample at the same time.

2.2. We proved that for the sample injection a multiple cross section was necessary, the injected sample distributed between the channels and only a small part of the liquid flowed to the direction of the packings.

For example, in the case of a 1 mm length C18 chromatographic packing (5 μm) from the injected 1 μL sample volume only 1 nL sample flowed to the packing, due to the high hydrodynamic resistance. We observed that the volume of the sample was independent from the applied flow velocity, it depended on the original sample volume.

In those systems where the parallel channels were packed the injected sample can be split to several equal parts before the packings, because the hydrodynamic resistance of the parallel packings was equal.

3. The chromatographic properties of the various types of packings were studied.

3.1. We separated two component food dye mixture on chromatographic packings prepared from 5 μm diameter C18 commercial chromatographic particles.

The two components could be separated within 30 s. The smallest theoretical plate height was 0.75 μm , the obtained highest plate number value was 2500 (1 330 000/m). The overall capacity of a packing was calculated to be $7.5 \cdot 10^{-12}$ mol/ μm for the Brilliant Blue FCF (E133) dye.

3.2. Different modified aerogel chromatographic packings were prepared in the channels of our microfluidic systems.

Before the packing procedure the aerogel bulk was grinded, a methanolic suspension was made from the particles and after sedimentation the diameters of the particles were around 1 μm . The aerogel packings were tested with food dyes, the enrichment factor was around 50, the separation of the two component food dye mixture took only 6-8 seconds (0.5 mm length C16 modified ground silica aerogel packing, isocratic conditions (35% methanol-water mixture), 1 nL food dye mixture, 3 bar).

3.3. Chromatographic separations were performed in microfluidic systems containing three different chromatographic packings.

Three different types of chromatographic packings (from 5 μm diameter RP-1, C8 and C18 particles) were made in microfluidic systems. Parallel chromatographic separations of food dye mixtures were made in these systems (the mobile phase was 25% methanol-water mixture). The injected sample volume reduced, due to the hydrodynamic resistance. It splitted to several equal parts between the parallel channels after the cross section. These systems were suitable for the fast comparison of chromatographic packings or the selection of the appropriate chromatographic packing for the analytical problem, thus the development of the new methods can be faster.

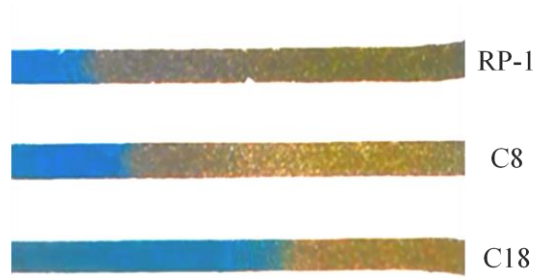


Fig. 3. Separation of food dyes on three different chromatographic packing at the same time.

3.4. We demonstrated the applicability of simulation softwares for the modeling of the flows in our multichannel systems, the optimizing the geometry of the channel patterns.

During our experiments we observed that the flow rates in the parallel channels were different (0.014-0.024 mm/s), reduced towards the outlet ports (the ratio of the maximum and minimum velocity was 2.2). For equalizing of the flow rates in the system we designed a new channel pattern, where this flow rate ratio was reduced for 1.05. The results of the simulations agreed well with the experimental results.

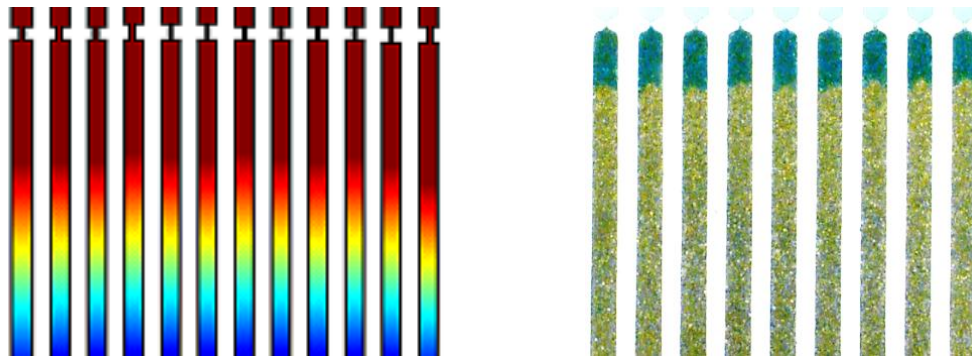


Fig. 4. The equalizing of the flow rates in the parallel channels by simulations (left) and the experimentally measured flow velocities in the modified channel pattern (right).

4. Atomic spectrometric detection methods were implemented to chromatographic packing containing microfluidic systems.

4.1. Interfacing the flame atomic absorption spectrometer (FAAS) with our microfluidic systems containing chromatographic packings were demonstrated. The sample volume was increased with the modification of the microfluidic channels/packings dimensions.

The minimal sample volume was 30 μL , this was enough for the reproducible and sensitive FAAS detection. In the modified channel systems the 5 μm C18 packings were 20 mm long, 1 mm wide and 0.1 mm thick. With the developed method preconcentration and separation of the chromium(VI) were demonstrated. The limit of the detection value for the Cr(VI) was 0.0031 $\mu\text{g/ml}$ with the injection of 80 μL sample into the microchip.

4.2. The absorbed Cr(VI) on the packings were detected with laser induced breakdown spectroscopy (LIBS).

After the elution of the Cr(VI) to the outlet port of the microchip, the dried sample was determined with the laser beam of the spectrometer. The obtained limit of detection value was 28,9 ng/mm^2 for chromium. The detection of the Cr(VI) can be directly performed on the chromatographic packings, too.

IV. The expected practical applications of our results

Nowadays the microfluidics is the most intensively examined field of the analytical chemistry. The expected practical applications of the developed analytical methods and microchips are widespread in several industrial, medical and environmental analytical areas.

However the research related to microfluidic chips is mostly basic research, these systems have many useful advantages. In the recent years a lot of analytical system containing microfluidic chips were developed by the big instrument developer companies. The industrial applications require fast and cheap separations. The microfluidic chips which were developed by us, were allowed for the preparation of parallel chromatographic packings in multichannels (3, 12 or more parallel channels). In these packings we have the possibility to perform several parallel chromatographic separations and the analysis time and costs are greatly reducible.

These systems were suitable for the fast comparison of chromatographic packings or the selection of the appropriate chromatographic packing for the analytical problem, thus the development of the new methods can be faster. These PDMS microchips packed with chromatographic particles can be prepared in a fast and reproducible way, the costs of the preparation and operation are inexpensive. With the economic development there will be a high demand for the performance of more and cheaper analytical determinations, the widespread distribution of the microfluidic systems for analytical analysis can be expected.



Registry number:
Subject:

DEENK/88/2015.PL
Ph.D. List of Publications

Candidate: Andrea Nagy

Neptun ID: GC83MV

Doctoral School: Doctoral School of Chemistry

MTMT ID: 10048170

List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (3)

1. **Nagy, A.**, Baranyai, E., Gáspár, A.: Interfacing microfluidic chip-based chromatography with flame atomic absorption spectrometry for the determination of chromium(VI).
Microchem J. 114, 216-222, 2014. ISSN: 0026-265X.
DOI: <http://dx.doi.org/10.1016/j.microc.2014.01.008>
IF:3.583 (2013)
2. **Nagy, A.**, Gáspár, A.: Packed multi-channels for parallel chromatographic separations in microchips.
J. Chromatogr. A. 1304, 251-256, 2013. ISSN: 0021-9673.
DOI: <http://dx.doi.org/10.1016/j.chroma.2013.06.065>
IF:4.258
3. Gáspár, A., **Nagy, A.**, Lázár, I.: Integration of ground aerogel particles as chromatographic stationary phase into microchip.
J. Chromatogr. A. 1218 (7), 1011-1015, 2011. ISSN: 0021-9673.
DOI: <http://dx.doi.org/10.1016/j.chroma.2010.12.091>
IF:4.531

Total IF of journals (all publications): 12,372

Total IF of journals (publications related to the dissertation): 12,372

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

22 April, 2015