OPTIMIZATION OF FLOW CONTROL AND CHANNEL PATTERNS DESIGNED FOR USE IN SEQUENTIALLY COUPLED MICROFLUDIC CHIPS AND NANOPARTICLE CHARACTERIZATION BY SINGLE PARTICLE ICP-MS

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Abstract
In this study, we developed polydimethylsiloxane (PDMS) - glass microfluidic chips (MCs) with the ability to function in a sequentially coupled way. We optimized their channel pattern and demonstrated that this feature makes them a more effective tool for carrying out sample preparation steps in single particle inductively coupled plasma mass spectrometry (spICP-MS) such as dilution, counting, and characterization of nanoparticles (NPs).

Introduction
Single particle inductively coupled plasma mass spectrometry is a novel and effective measurement technique, which is used for the characterization of the dispersions of nano- or sub-micron-sized particles. By individually detecting thousands of particles per minute and evaluating the recorded signal profiles, the technique can provide information about the size distribution, number concentration, elemental and isotope composition of the nanoparticles in the sample with great statistical relevance [1, 2]. Another outstanding advantage of spICP-MS is that the particle number concentration that has to be set for the measurement is low (10^3-10^5 mL^{-1}), thus the sample requirement is very small [2]. In case of solid samples, setting the required NP concentration is no problem, however, the concentration of real-life samples is typically not known, and its adjustment requires a lengthy procedure. As we have shown it in our earlier publications, this task can be automated by utilizing a microfluidic chip as an on-line sample preparation device interfaced with an ICP-MS instrument [3]. MCs can also be potentially used to automate more complicated sample preparation steps, such as extraction, enrichment, etc. MCs are cost-efficient, easy to produce, compact and versatile devices with the ability to handle low-volume fluid samples. MCs are usually made of polydimethylsiloxane (PDMS) and glass [4]. Despite the MC's great potential in ICP-MS sample preparation, they are not yet widely used. In spICP-MS, one of the obstacles in the way of their use is that the high-level dilution (several orders of magnitude) needed can not be achieved by using a single flow mixer-splitter pattern. For this reason, in this study we developed new MCs that can be used in a sequentially coupled manner, to extend the on-line dilution range of the system.

Experimental
An Agilent 7700X inductively coupled plasma mass spectrometer (ICP-MS) was used in all experiments. Sample introduction was performed through our microfluidic devices in which the flow was generated by utilizing three Gilson Minipuls 3 peristaltic pumps (Gilson Inc., USA). While the outflow was pumped into a MicroMist type nebulizer equipped with a Peltier-cooled Scott spray chamber. The sample uptake rate was 600 μL/min. The integration time was

set to 500 ms for the measurement of solution samples and 6 ms for nanodispersions, whereas the acquisition time was set to 60 s.

The microfluidic chip molds were fabricated utilizing a Form 3 professional 3D printer using "High Temp" resin (Formlabs, USA). Prior to the molding process, a thin (tridecafluoro-1,1,2,2-tetrahydrooctil)trichlorosilane layer (Gelest Inc., USA) was applied to the mold to facilitate the removal of the cured PDMS from it. The chips were cast using Sylgard 184 silicone elastomer and curing agent (Dow Corning, USA) and sealing the PDMS to a microscope slide with the help of cold oxygen plasma treatment (PDC-002, HarrickPlasma, USA).

Cobalt sample solutions were prepared from 1000 mg/L CertiPUR monoelemental standards (Merck, Germany). In spICP-MS measurements, ultra uniform polyethylene-glycol-capped 47.8 (1.8) nm gold nanospheres were used (Nano-Composix, USA). Trace-quality de-ionized water from MilliPore Elix 10 device equipped with Synergy polishing unit (Merck, Germany) was used for the preparation of all solutions. Ismatec S3 E-LFL Tygon tubings (IDEX, Germany) with 0.27 or 0.48 mm inner diameter were used for the aspiration of liquid samples. To drive the liquid samples to and from the MCs, stainless steel capillaries with 1.2 mm outer diameter, fabricated from medical needles, were placed in the inlet and outlet ports. For the connection of peristaltic tubing, the inlet and outlet needles and the ICP-MS nebulizer, a PFA tubing with 0.3 inner diameter (Agilent Technologies, USA) and patches prepared from silicone tubing with 1.0 mm inner diameter (Deutsch & Neumann, Germany) were applied. For the inspection of the flow conditions inside the MCs, an Optika Ti600-FL type microscope equipped with a digital camera (Optika, Italy) was used, as well as a Kiralux monochromatic CMOS scientific camera (Thorlabs Inc., USA).

All data processing was performed within the Agilent MassHunter (Agilent Technologies, USA), Origin (OriginLab Corp., USA), and MS Office Excel (Microsoft Corp., USA) software.

Hydrodynamic simulations on flow conditions were performed utilizing the COMSOL Multiphysics software package (COMSOL Inc., USA), in which the models were created utilizing the AutoCAD (Autodesk Inc., CA, USA) and Solid Edge (Siemens PLM Software, , USA) engineering design software.

Results and discussion

For the sequential coupling of the chips, the sample and the diluent flows had to be united and properly mixed, finally the hopefully homogenous flow had to be split into two distinct channels using the same flow rate which was used when the two flows were combined. In an earlier publication of ours we found that the "W" junction is the most effective pattern for combining the flows [3], thus we kept on utilizing this pattern. At the same time, we had to study and optimize the mixing process – the mixing is not cruical if only a single chip is used, because then the ICP-MS spray chamber provides adequate final mixing after nebulization, but in the case of the use of sequentially coupled chips, proper transfer of liquid from chip to chip is only possible if the mixing is good.

For the purposes of mixing pattern optimization, we reproduced four patterns which are commonly used in the literature, each with different operating principles [5, 6, 7]. The mixing in the "serpentine" pattern is strictly based on diffusion, while the "micropillars", "fishbone" and "tesla valve" patterns are designed to cause disturbances in the laminar flow with their protrusions, thus splitting and recombining the flow over and over. To make the mixer designs comparable, we designed them with roughly the same surface area; also, because compactness is a crucial feature in microfluidics. Before the production of the chips, several hydrodynamic simulations were carried out on each design in a search for potential flaws, and to assess their approximate efficiency. Some of the results are shown in Figure 1. and Table 1.

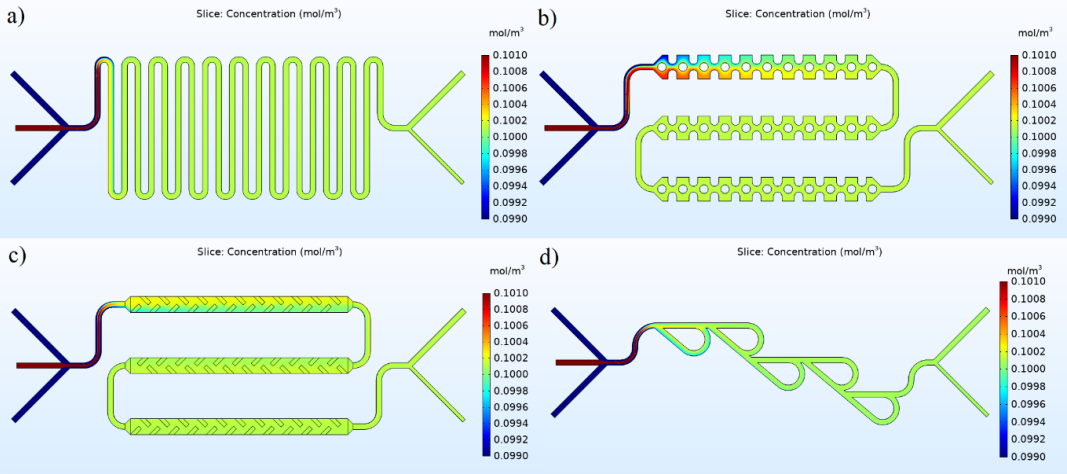

Figure 1. Lateral concentration profiles, plotted for the mid-height plane of the channels (a.) serpentine, b.) micropillar, c.) fishbone, d) Tesla valve)

Mixer design	Serpentine	Micropillars	Fishbones	Tesla Valve
Number of inlets, their dimensions	3, 700 × 350 µm			
Cross section of outlets	700 × 350, 438 ×350 µm			
Pressure drop [Pa]	2347	742	2451	303
Internal volume [µL]	105.5	75.0	47.2	35.3
Concentration difference between the two outflows [mol/m^3]	$2.11 \cdot 10^{-9}$	$5.99 \cdot 10^{-8}$	$2.18 \cdot 10^{-6}$	$4.74 \cdot 10^{-6}$

Table 1. Geometric, flow and mixing properties of chip designs

Based on the simulation results, all four designs can complete the mixing process within their length. However, some properties differ for the designs like their flow resistance (closely related to the pressure drop, which, if too high, could burst the microchannels open), and their internal volume (which should be kept as low as possible due to processing speed considerations). In additon to hydrodynamic simulations, we also performed microscope-based imaging experiments using fluorescent dyes. These revealed that the mixing is actually less satisfactory than shown in the simulations, especially in case of the Tesla valve pattern. All in all, the serpentine seemed to be the best mixing geometry, since it provided the highest level of homogeneity at the outlets. Also, its simple geometry (without any constrictions or protrusions), minimizes potential problems associated with the poor wetting properties of PDMS (e.g., bubbles and blockages). These problems were frequently observed with every other chip design, as shown in Figure 2.

In order to optimize the flow splitting process, several experiments were done. We found that if the splitting is done in a passive way, only controlled by the different flow resistances of waste and sample outlet channels with calculated channel cross section ratios, the system shows high sensitivity to random disturbances, thus it was decided to use forced flow (pumping) to be applied. We tested the effect of pumping either the waste or sample outlet ports by gravimetric experiments. Based on five repetitions, an average of 0.67% deviation from the desired flow rate with a 0.81 RSD% was found with the pumping of the waste branch, whereas in the case

of pumping the sample branch, these numbers were much improved, namely 0.01% and 0.4%. Therefore, pumping the sample outlet is more beneficial, which was kind of expected, since the error of the pumping rate are proportional to the flow rate, and the sample outlet flow is always smaller.

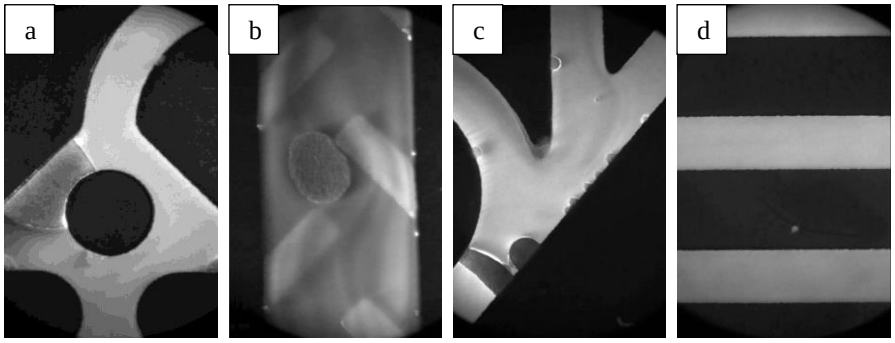

Figure 2. Flow imperfections inside a.) micropillar, b.) fishbone, c.) Tesla valve and d.) serpentine channel patterns.

The mixing efficiency was finally determined by diluting a standard 10 ppb Co solution with water. With a tenfold dilution applied, we found only 2.57% deviation from the expected concentration with a relative standard deviation of 2.62% (N= 6). This reflects a very efficient mixing.

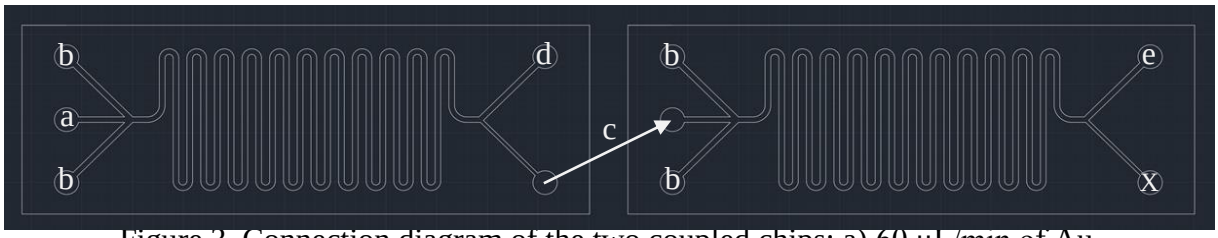

Figure 3. Connection diagram of the two coupled chips: a) 60 µL/min of Au nanoparticle dispersion flow introduced with $4 \cdot 10^5$ mL^{-1} particle number concentration, b) 270 µL/min of deionized water flow introduced, c) 60 µL/min of diluted Au nanodispersion flow transferred into the second chips sample inlet, d) 540 µL/min of waste flow generated, e) 600 µL/min of highly diluted nanodispersion flow, which gets introduced to the ICP-MS, X) blocked outlet.

Finally, an experiment was performed on two sequentially coupled serpentine chips, using the arrangement shown in Figure 3. As the sample, a 47.8 nm gold nanodispersion with the number concentration of $4 \cdot 10^5$ mL^{-1} was introduced, and diluted in the 1 to 225 range, shown on Figure 4. Overall, the number of detection events showed a good linear proportionality with the number concentration of the sample nanodispersion at every dilution rate used. Except in the case of the undiluted sample, when the number of detected particles showed a significant fall-back from the expected value. This difference is caused by the too high particle number concentration, which led to co-detections of particles. Actually, this is the same problem we could face if the measurement of an undiluted nanosuspension would be attempted.

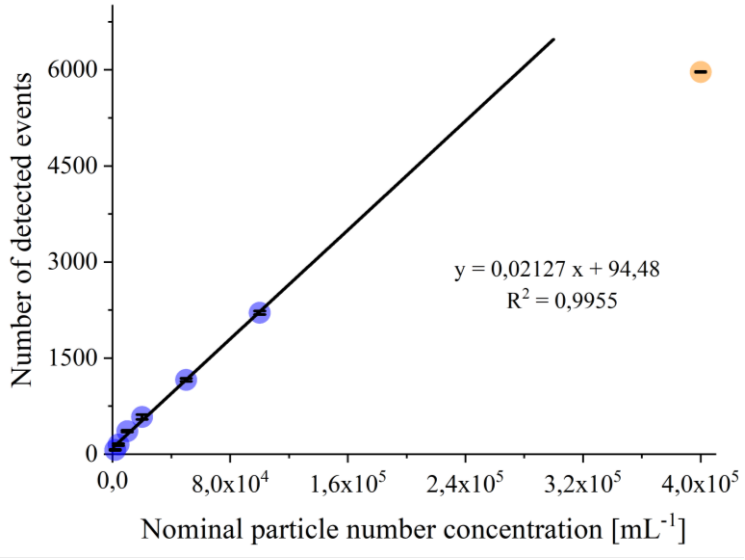

Figure 4. On-line 1 to 225-fold dilution of an Au nanodispersion with 47,8 nm particle diameter and initial particle number concentration of $4 \cdot 10^5$ mL^{-1}.

All in all, the chip coupling proved to be a useful and well applicable technique, which extends our capabilities in terms of MC-based on-line nanodispersion preparation. In principle, any number of chips can be sequentially coupled, thereby increasing the maximum achieavable by more than ten times. With 2-4 unit, a 1000-10000-fold dilution can be easily reached, which is fully adequate for most spICP-MS experiments. At the downside, each chip adds to the total channel (dead) volume, resulting in a longer stabilization time and a higher sample volume requirement, thus the pros and cons have to be weighed.

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