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Chip modules for generation and manipulation of fluid segments for micro serial flow processes

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Abstract

Micro serial processing of continuous sample streams in microfluidic environments provides a new, powerful and scalable approach for micro scaled chemical reactions and analytical procedures. It is based on sample generation by embedding sample liquid in a continuous stream of an immiscible carrier fluid. Segmented streams are processed in capillaries with integrated microfluidic devices for chemical and analytical processing. Devices and methods were developed for generation of segmented flows and for dosing of liquid into compartments of a segmented sample stream. Segmentation is realized with predefined compartment volume and a high degree of uniformity in size and frequency. Injectors are optimized for interoperatibility with segmented flow handling in HPLC-capillary systems. For fabrication of microfluidic devices two half channels were prepared in glass by means of an isotropic etch process, followed by anodic bonding mediated by a nickel chromium metalization. Segmented streams are generated in the injector module and guided into a dosing module, where controlled infusion of liquid into the compartments of the segment stream is realized. The system was used for a neutralization reaction in compartments containing formic acid with sodium hydrogen phosphate.

Keywords: Chip; Fluid segments; Micro serial flow processes

1. Introduction

Serial processing of linearly organized samples represents the traditional approach for analysis of a plurality of analytical samples. This has been outperformed by modern micro titer plate, nano titer plate [1] or chip array based [2] highly parallelized approaches which represent the current state of high throughput screening. Sample throughput and efficiency of these approaches seemed not to be comparable with this classical approach of serial sample treatment. With respect to the development of novel concepts for micro serial processing of continuous sample streams in microfluidic environments this serial approach becomes a powerful alternative to array based methods [3,4]. It is based on the generation and manipulation of linear sample streams, containing a plurality of individual liquid sample segments, embedded in an immiscible separation medium in capillary systems. Digital reaction technology may be used for versatile generation and processing of a plurality of individual samples [5]. The segmented flow of analytical samples is thereby channeled through functional modules for sample processing and analytical readout of ingredients of the composition of the samples in compartments. These microfluidic chip modules are organized so, that all steps of the analytical procedure are performed as given by the protocol. A first implementation makes use of this concept for screening of micro organisms [6].

The time, required to process a single sample from generation to the analytical readout is given by the cycle time for the procedure. The throughput is given by the compartment rate. Chip modules and procedures presented in this work enable systems for generation and processing of sample segments with volumes ranging from 110 to 300 nl and sample rates between 1000 and 10,000 h^{-1}. By this way, up to 240,000 samples per day may be processed. In contrast to micro titer plate based screening procedures, this approach does not require subsystems for motion control and handling of sample carriers. This dramatically reduces the footprint of segmented flow based analytical systems and allows to use them as standard laboratory equipment as well as in mobile screening or analytical environments.

The number of available methods and devices for control, manipulation and analytical readout predetermines the scope of segmented flow systems.

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Segmented flow based applications depend on segment generation and controlled dosing of reagents into compartments. Additional operations are required in order to realize systems for particular applications [5]. Methods and devices for segmentation [3], thermal management [7] and extraction of individual compartments [6] from a segmented stream are generally available. However the customization of these modules for integration in systems for complex segmented flow based procedures remains to be solved. Therefore all subprocesses for generation, manipulation and analytical readout have to operate at identical flow rate. To realize maximum sample throughput with minimum requirement of reagents, it is necessary to generate and handle small compartments with a high segmentation rate.

In this paper we report on development of injector modules for controlled generation of segmented flows and for dosing of liquid into compartments of a segmented sample stream. Segmentation is realized with predefined compartment volume and a high degree of uniformity in size and frequency. Injectors are optimized for interoperatibility with segmented flow handling in HPLC-capillary systems. In order to realize symmetrical micro channels without edges for reliable segment manipulation, two half channels were prepared in glass by means of an isotropic etch process, followed by anodic bonding mediated by a nickel chromium metalization. Segmented streams generated in the injector module may be guided into a dosing module, where controlled infusion of liquid into the compartments of the segmented stream is realized. These two processes were investigated. Finally the system was used for the neutralization of compartments containing formic acid with sodium hydrogen phosphate.

2. Demands on translation of segmented fluid streams in capillaries

Reliable transport of segmented flows between functional modules without carry over of compartment ingredients, joining or fragmentation of segments is the most fundamental requirement for segmented flow based systems. Therefore, plug flow provides a maximum of reliability. This ensures, that all compartments are moved with an identical feed rate and effects of gravity and friction of the compartments on the capillary walls are minimized. Compartments, which do not completely plug the capillary are moved with a slower feed rate. In this case, separation fluid bypasses the compartment and friction and wetting of the compartment at the capillary wall results in a slower motion with respect to the other compartments. This will result in fusion of the compartment with the following one.

With respect to this it its helpful to define a parameter, that represents the minimum volume of a compartment, which is able to plug a given channel geometry completely. In order to eliminate the influence of wetting properties the volume is defined for nonwettability and a contact angle of 180°:

> The “Ideal Minimum Compartment Volume” IMCV of a microchannel describes the volume of the minimum compartment, which is able to plug the channel completely under nonwetting conditions with a contact angle between the liquid interface and the channel wall of about 180 degrees.

For channels with circular cross-section the IMCV will be a sphere with the diameter of the channel. In particular cases of special contact angles a volume smaller than the IMCV may be able to plug the channel completely. In this case, it depends on the special conditions, whether it may form a thermodynamic stable plug or a metastable one which may collapse to a droplet that is settling on the capillary wall.

The IMCV of PTFE–HPLC capillaries with an inner diameter of about 0.5 mm is 65.4 nl. The shape of this IMCV is a sphere with the diameter of the capillary. These capillaries are reported for storage and transport of segmented flows between chip modules [6].

The control of wetting in micro channel systems for manipulation of segmented flows is essential for reliable segmented flow management. Wetting properties are influencing the shape of compartments as well as the friction of the compartments at the channel wall. With increasing wettability, the risk of droplet settling at discontinuities in micro channels and interconnectors increases. Droplets, remaining at discontinuities cause fluid exchange with compartments passing this region. This results in cross contamination.

In our experiments, we use tetradecane as separation medium and aqueous solutions for segment generation and embedding. The contact angle between water and a PTFE surface was determined to 147.8°. In order to realize nonwetting conditions for water inside the glass chip modules functionalization of its surface is necessary. This is realized by treatment with alkyltrichlorosilanes. The wetting properties for water on functionalized glass surfaces in the ternary system water, tetradecane and surface are given in Table 1. Minimum wettability is found after treatment with octadecyl-trichlorosilane. This functionalization results in nearly ideal nonwetting behavior of the channel surface and was used in all experiments.

For generation of segmented flows the volume streams of the separation medium and the injection medium are joined in an orthogonal T-shaped junction. The droplet size (and

Table 1
Wetting of water to functionalized glass surfaces and PTFE bulk material in the ternary system water, surface and tetradecane, measured using the sessile drop method

Surface	Contact angle (°)
n-Octadecyl-trichlorosilane	>170
n-Decyl-trichlorosilane	>170
Perfluorooctyl-trichlorosilane	145.93 ± 4.1
Heptafluoro-isopropoxy-propyltrichlorosilan	141.2 ± 2.8
Chlorodimethylethylsilan	98.07 ± 2.5
PTFE bulk material	147.8 ± 1.3

thereby the volume) in these simple injector system is controlled by the flow and decreases with increasing flow rates [3]. The compartment tears off, if the energy deposited into the growing droplet by the passing separation fluid exceeds the required surface energy. Generation of small segments with volumes down to the IMCV using an ordinary T-shaped junction requires either high flow rates or high viscosity of the separation medium.

For segmented flow applications the compartment stream is guided through capillaries over long distances. The maximum length of the capillary system depends on the maximum system pressure. This is limited by fluid interconnectors and syringe pump capabilities. With increasing viscosity of the segmentation medium the maximum length for the capillary system decreases. Therefore a separation medium with low viscosity is preferred for segmented flow based applications. For sensitive analytical readout of, e.g., fluorescence or luminescence a residence time of the sample in the detection window of at least some milliseconds is required. This is compatible with low flow rates, which also enable long incubation times for enzymatic procedures in short length capillary tubes. So a residence time of 11 min per meter capillary loop (i.d. = 0.5 mm) could be reached at a flow rate of 2 ml h^{-1}, typical for systems reported in this work.

In order to realize injector modules, that are capable of creation of small segments for low flow rates and for low viscosity media a variety of injector modules were designed, prepared and tested with respect to their segmentation behavior. Best results were obtained with a T-shaped junction, equipped with a nozzle. This module was adapted for interoperatibility with 0.5 mm HPLC capillaries and successfully tested for the generation of segmented flow, continuous dosing of liquid to segmented streams and application in volumetric analysis.

3. Microfabrication of injector modules

For preparation of microchannels and injectors, as shown in Fig. 1, two half channels were fabricated by isotropic chemical wet etching. The two half channels were anodically bonded via a nickel chromium support layer.

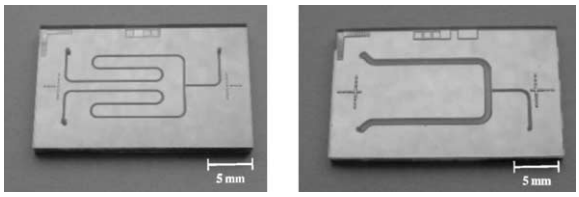

Fig. 1. Injector modules (16 mm × 25 mm) with a T-shaped junction and transparent channels for segmented flow generation and dosing of liquid to embedded fluid segments. Left: Injector with an IMCV of 7.1 nl used for evaluation of different injector geometries, Right: Injector equipped with a T-shaped junction with an integrated nozzle, optimized for interoperatibility with HPLC capillaries and connectors, IMCV 109 nl.

Both sides of the glass substrates (BOROFLOAT33, Schott Jena, thickness 0.7 mm) were covered with a 150 nm Ni/Cr mask. Micro channels, nozzles and support structures were transferred into the metal layer using photolithography. Micro channels were etched using a HF etchant. Holes for fluidic interconnections with a diameter of 0.5 mm were prepared by ultrasonic drilling. For anodic bonding, the Ni/Cr metalization of the ion conductive substrate was removed using a Ni/Cr etchant. The substrates were aligned using a mask aligner and anodically bonded at 450 °C/800 V. Finally, the residual Ni/Cr metalization was removed in the channels using Ni/Cr etchant. With respect to the transparency of the material and the coplanar channel surfaces at the top and bottom of the channel, this geometry allows for optical readout in transmission using sensors or camera systems.

4. Results

4.1. Evaluation of injector systems for segmented flow generation

For investigation of injector geometries for segment generation at low flow rates using low viscous separation medium different injector geometries were designed and fabricated. These geometries are shown in Fig. 2. Microchannels have a channel width of 250 μm, a depth of 200 μm and were prepared as described using a mask width of 50 μm and a etch depth of 100 μm. This results in an IMCV of about 7.1 nl.

Experiments were performed using the separation medium tetradecane and bromophenol blue stained phosphate buffer as injection medium. The ratio of the flow rates was fixed to a ratio of 5:1 between separation medium and injection medium.

For comparison of the injector modules the volume of the generated compartments was measured for flow rates of the injection medium of 0.2 and 1.0 ml h^{-1}. The range

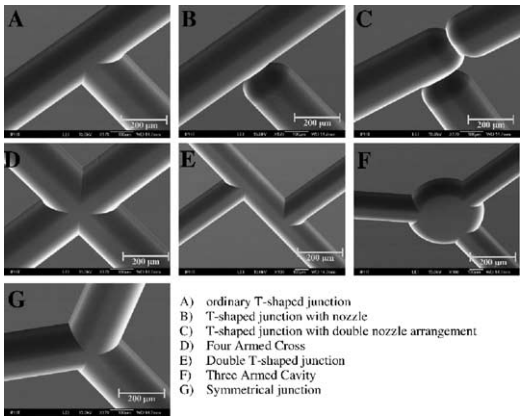

Fig. 2. (A–G) SEM of injector geometries prepared by isotropic wet etching of glass, developed and integrated in injector modules evaluated for segmented flow generation.

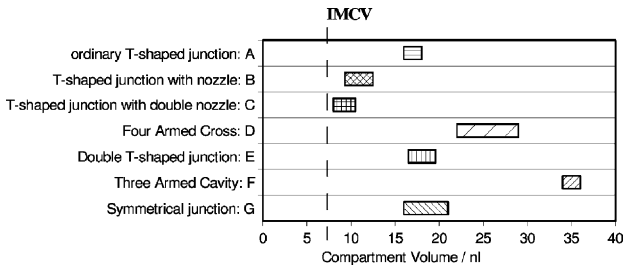

Fig. 3. Range of compartment volumes, which may be generated at flow rates of the injection medium between 0.2 and 1.0 ml h^{-1} for individual injector geometries shown in Fig. 2. Experiments were performed using a fixed ratio between separation medium tetradecane and bromopheol blue stained aqueous injection medium of 5–1.

of compartment volumes, which may be generated using the individual modules is shown in Fig. 3. Small segment volumes down to the IMCV are available using T-Injectors, equipped with nozzle systems.

Fig. 4 shows the shape of the fluidic interface inside the nozzle region for these injectors at the time immediately before tear off occurs. In order to demonstrate the segmentation capabilities these images have been registered at a very low flow rate of 0.1 ml h^{-1} for the injection medium.

Both nozzle T-injectors (B) and (C) (Fig. 2) allow for segment generation with low scattering in compartment volume. A detailed analysis of the segmentation behavior with respect to reliability, scattering of segment volumes and error frequency was performed for these modules. The compartmentalization was analyzed at flow rates of the injection medium between 0.05 and 1.2 ml. The results are shown in Fig. 5 as notched box and whisker plots [8] as generated with the statistical package R [9]. The whiskers show the minimum and maximum points that are not outliers. The boxes show the position of the first and third quartiles. Notches mark the ±95% confidence interval for the medians. Outliers are represented by circles.

For the double nozzle arrangement (C) (Fig. 2), the compartment volume falls below the IMCV for flow rates above 0.9 ml h (see Fig. 5). Furthermore this arrangement causes a higher frequency of outliers with higher volume.

Using the single nozzle injector, as shown in Fig. 2B, the compartment volume generally exceeds the IMCV. At higher flow rates it seems to converge towards the IMCV (see Fig. 5). This demonstrates that the T-shaped junction equipped with a single nozzle as shown for injector (B)

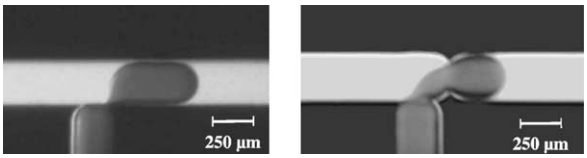

Fig. 4. Shape of the fluid interface inside the nozzle region immediately before tear off of a compartment. Left: T-shaped junction equipped with a single nozzle (B). Right: T-shaped junction with a double nozzle arrangement (C).

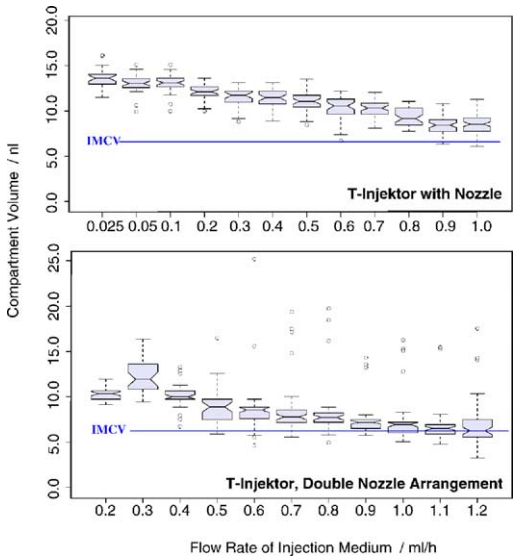

Fig. 5. Comparative analysis of segmentation using T-shaped injectors for flow rates between 0.025 and 1.2 ml h^{-1} for the aqueous injection medium and a fixed ratio to separation medium tetradecane of about 1–5. Segment volume distribution is shown as notched box and whisker plot which shows outliers, minimum, maximum, first and third quartiles, and median with confidence intervals. At least 10 images were recorded and processed for each flow rate. Top: Injector with a singel nozzle as shown in Fig. 2A, Bottom: Double nozzle arrangement as shown in Fig. 2C.

(Fig. 2) provides optimum segmentation capabilities and enables generation of segment volumes near the IMCV for low flow rates and low viscous separation media. In general, the segmentation occurs as follows: the injection solution grows spherical outside the nozzle without wetting the channel surface. Tear off occurs immediately after the growing droplet has plugged the channel completely. During tear off the interface at the nozzle and an equal area at the droplet has to be newly generated. Therefore, the area of the interface created during tear off is given as double of nozzle cross-section. With respect to this the energy required for tear off may be calculated from nozzle cross-section and interface tension as given in Eq. (1).

$$E_{\text{tear off}} = 2 \times A_{\text{nozzle}} \times \sigma_{\text{sepl inject}} \quad (1)$$

Thus, the energy required for tear off directly correlates with the nozzle cross-section and the interface tension between separation medium and injection medium. With respect to this we can conclude, that the IMCV represents a parameter for single nozzle T-Injectors, which defines the segment volume obtainable for a given injector geometry. Therefore it is possible to predict the segmentation behavior for this type of injector. This is a fundamental advantage for scaling and development of such injectors for particular segmented flow systems.

4.2. Dosing of liquid to compartments

Dosing of liquid to embedded micro compartments is fundamental for chemical, analytical or biological processing

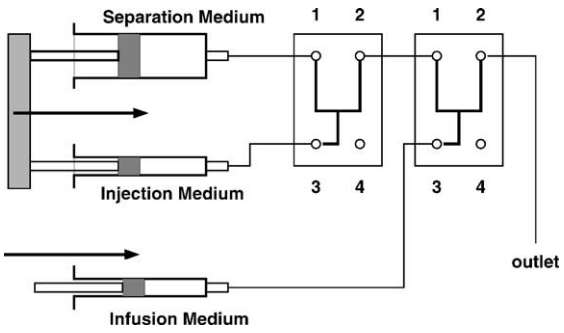

Fig. 6. Experimental setup for dosing experiments.

of the ingredients of a micro compartment. Thus, development of segmented flow based systems for screening, analysis and chemistry strongly depends on these methods. With respect to this, single nozzle T-injectors were tested for segment generation and continuous dosing of liquid to a continuous stream of segments. For transport of segmented flows between functional modules, PTFE capillaries with an inner diameter of 0.5 mm have been used successfully in development of segmented flow based applications [6]. For fluidic interconnection, a system based on U-profiles and HPLC fittings [10] was adapted to the requirements of segmented flow based applications [6]. Single nozzle T-Injectors as shown in Fig. 2B were developed and realized for interoperatibility with this system. These modules have a nozzle cross-section of 0.02 mm^2, a channel width of 760 μm, a depth of 280 μm and were prepared using a mask width of 480 μm and an etch depth of 140 μm. This results in an IMCV of 108 nl.

The experimental setup is given in Fig. 6. Segmented flow is generated in Chip 1, using the separation medium tetradecane at a flow rate of 1.5 ml h^{-1} and the injection medium bromophenol blue stained phosphate buffer at a flow rate of 0.3 ml h^{-1}. This results in a total flow rate of the segmented flow of 1.8 ml h^{-1} and a compartment volume of about 150 nl. The segmented flow is guided through the main channel of a second injector chip, where the compartments are fed at the injector structure with continuous flow of injection medium with adjustable flow rate. Compartment volume was measured before and after dosing using a CCD camera, image processing and data analysis.

For each flow rate at least 15 images were registered and used for analysis. As expected a linear correlation between infusion rate and the resulting compartment volume is observed. Compartments are generated with a rate of $1940 \pm 60\ h^{-1}$ and transferred into the main channel of chip 2 with a velocity of 2.5 mm s^{-1}. They have a settling time of 4 s inside the channel segment for monitoring with the CCD camera.

The compartment volume before infusion is 150 ± 5 nl. Infusion increases the relative error up to 6%. The relative error of the infusion process itself ranges from 5 to 8% for infused volumes up to 350 nl.

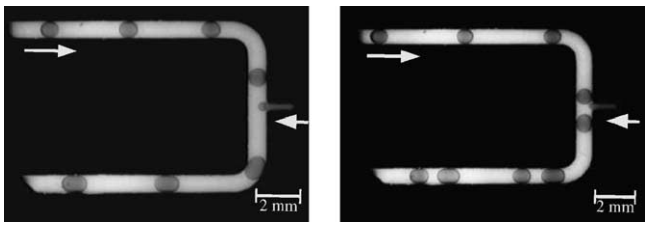

Fig. 7. Channel view of the infusion process. Arrows indicate the flow direction of the segmented flow and the infusion medium. Left: Compartments, passing the infusion chip at a infusion rate of 0.15 ml h^{-1}. Right: Compartments, passing the chip at a infusion rate of 0.45 ml h^{-1} resulting in superposition of infusion and segmentation. A small compartment of the infusion medium tears off before the compartment has reached the nozzle and is infused with residual infusion medium. By this way, two nested compartment classes with different ingredients and volume are stacked.

These results demonstrate the suitability of the developed injector geometry for precise continuous dosing of liquid to segmented flow systems. In contrast to standard junctions no settling and fusion of the passing compartments is found especially for small compartment volumes (not shown).

The standard infusion process is shown in Fig. 7 (left). At higher flow rates, tear off from an infusion medium segment may occur before the next compartment reaches the nozzle (Fig. 7, right). In this case, a droplet of infusion medium is inserted into the segment stream. At these conditions a superposition of infusion and segmentation results in generation of repetitive sequences of segments. This behavior may be particular useful for creation of complex sequences of compartments.

4.3. Neutralization of formic acid in microcompartments

Based on these results the system was used for titration experiments of formic acid with disodium hydrogen phosphate solution. 10 mM Formic acid in water, stained with 0.3 mM bromophenol blue as indicator dye was used for segment generation at a flow rate of 1.5 ml for tetradecane and 0.3 ml h^{-1} for formic acid. Segments were guided as described through the main channel of chip 2 (Fig. 7). At the injector sodium hydrogen phosphate solution was added with variable flow rates.

Fig. 8 shows the steps of neutralization of formic acid using sodium hydrogen phosphate with a flow rate of 0.1 ml h^{-1}. Fusion of the growing interface of the phosphate solution with the formic acid compartment results in a high alkaline concentration at the top of the acid compartment. This is visible as a color change of the indicator bromophenol blue in Fig. 8D.

A complete color change of the indicator dye inside the monitoring window of the chip was only observed at titrations with a flow rate ratio of formic acid/sodium-hydrogen phosphate of 0.3–0.2 ml h^{-1}. At lower ratio only a partial switching was monitored inside the chip, whereas the switch

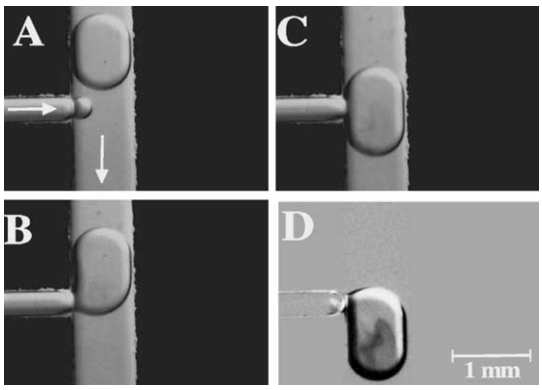

Fig. 8. Dosing of sodium hydrogen phosphate to formic acid compartments, stained with bromophenol blue. (A) Immediately before a acid compartment reaches the nozzle with the elongated interface of the phosphate solution, (B) fusion of the interfaces, (C) dosing of buffer and (D) background reduced, enhanced image of the compartment immediately before tear off, the dark color in the frontal part indicates indicator switch.

was completed in the capillary between outlet of chip 2 and the waste. This demonstrates, that mixing of reactants inside the compartments is limited. It was shown, that a circular flow inside the compartment may be induced at curvatures of the channel. This gives rise for future development of injection modules, equipped with curved channel segments for efficient mixing of reactants.

5. Conclusion

We have reported on design and preparation of devices for generation and manipulation of segmented flows with high uniformity in size and frequency. The modules, fabricated with respect to interoperatibility with HPLC capillaries allow for preparation of segmented flows with segment rates between 1000 and 10,000 h^{-1}. Segment volumes range from 110 to 170 nl with an relative error of about 3%. These modules were interconnected using HPLC capillaries, integrated into systems for segmented flow processing and used for dosing of liquid reagents into compartments of a segmented stream. By that way, a segmented flow was generated and transferred to an infusion chip for addition of up to 250 nl to compartments of 150 nl. At a rate of 2000 compartments per hour relative errors below 6% were reached. Integration of functional systems into single chips avoids the problem of segmented flow handling and transfer in HPLC-capillaries, and enables segment generation with volumes between 8 and 15 nl at segment rates of about 100,000 samples h^{-1}. By that way, particular lab on a chip systems may be realized with ultra high throughput. Chip devices, developed for segmented flow processing allow for optical readout in transparency mode. Thus they are qualified for integration of optical sensors for process monitoring and analysis of ingredients of the compartments.

6. Experimental procedures

6.1. Functionalization of inner channel surfaces using alkyl- and perfluoroalkyl-chlorosilanes

Micro channel surfaces were pretreated with a mixture of H_2O_2/H_2SO_4 (ratio 1/4 for 5 min), followed by rinsing with water. After drying, the channels were incubated for 4 h in 4 mM alkyl-trichlorosilane (4 mmol l^{-1}) dissolved in *n*-heptane (dried over molecular sieves), followed by washing steps with *n*-heptane, toluene, isopropanol and water. Contact angles were measured on a contact angle system OCA 30 (DataPhysics Instruments GmbH) using the sessile drop method. Results for chlorosilanes are listed in Table 1.

6.2. Monitoring of segment generation for different nozzle types

Chip devices with a channel width of 250 μm and a channel depth of 200 μm were used in this experiment. Chips were prepared using an etch mask width of 50 μm and an etch depth of 100 μm. The IMCV for these channels is about 7.1 nl. Monitoring of segment generation was performed using the separation liquid tetradecane and a 0.3 mM solution of bromophenol blue in a 10 mM phosphate buffer. Fluid control was performed using a 1 ml glass syringe for aqueous solution and a 5 ml glass syringe for tetradecane, both mounted on a KDS 210 infusion/withdrawal syringe pump. Syringes were interconnected with the injector chip using PTFE–HPLC capillaries, HPLC fittings and ferrules (Jasco). Monitoring and image data acquisition was done in transmission mode using a CCD camera equipped with a zoom objective and an interference filter 575–625 nm. Illumination was realized using a KL1500 light source in conjunction with a milk-glass-plate. With respect to the 100 Hz intensity modulation of the light source the exposition time of the CCD device was adjusted to 10 ms. By that way, exactly one period is used for exposure and images are registered with identical illumination. At least 10 images per flow rate were processed for estimation of the segment volumes using a software module for image processing and image–object recognition and analysis [11], followed by calculation of the segment volume based of width, height, position and shape of recognized image objects prior to a statistical analysis of these data [9].

6.3. Monitoring of dosing process

Chip devices with a channel width of 760 μm, and a channel depth of 280 μm were used in this experiment. Chips were prepared using an etch mask width of 480 μm and an etch depth of 140 μm. The IMCV for these channels is about 108 nl.

Monitoring of continuous segment feeding was done using the separation liquid tetradecane and a 0.3 mM solution

of bromophenol blue in 10 mM phosphate buffer at a constant flow rate of 0.3 ml h^{-1} for the aqueous solution and 1.5 ml h^{-1} for tetradecane.

Segment generation was performed as described. Segments were guided through the main channel of a second injector module as shown in Fig. 7. A 1 ml glass syringe was mounted on a second syringe pump SP210 and connected with the injector inlet of the second chip. Dosing was performed at flow rates up to 0.4 ml h^{-1}.

Data acquisition and analysis for chip 2 was done as in the experiment previously described. For estimation of the volume before and after dosing two regions of interests were defined, which cover the channel segments before and after dosing. These regions were analyzed separately for the whole set of images.

6.4. Neutralization of formic acid compartments using sodium hydrogen phosphate

The experimental environment was used as described for monitoring of the dosing process. Segments were created using tetradecane and a 10 mM solution of formic acid in 0.3 mM bromophenol blue in water at constant flow rates of 0.3 ml h^{-1} for the formic acid solution and 1.5 ml h^{-1} for tetradecane. Compartments generated in chip 1 were guided through the main channel of chip 2. Sodium hydrogen phosphate 30 mM was diluted into the injector channel of chip 2 with flow rates between 0.03 and 0.3 ml h^{-1}.

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