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Nanoliter segment formation in micro fluid devices for chemical and biological micro serial flow processes in dependence on flow rate and viscosity

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Abstract

Micro segmentation of liquids inside micro channels and micro tubes opens an easy way for highly parallelized chemical, biochemical and microbiological processes. The production of liquid segments can be done as a fast serial process and leads to a linear arrangement of samples, which are always individually characterized by their position. The segment formation is a function of injector geometry, interface forces, viscosity of the carrier liquid and flow rate. The formation of droplets inside channels of about 500 μm diameter was investigated. Therefore, a micro module made by polymethyl-methacrylate (PMMA) was used for segmentation and integrated in a modular micro fluid arrangement. Microscopic imaging controlled fluidic processes. The inert aliphatic liquid tetradecane and a mineral oil were used as embedding liquids. Segmentation occurred in aqueous solutions of the cationic triphenylmethane dye Malachite Green. The influence of the flow rate on the segment volumes was found to be strongly dependent on the viscosity of the embedding liquid. Operations with series of segments in serial processes as well as possible applications are discussed.

Keywords: Segmented flow; Micro channels; Segment formation; Droplet; Digital chemistry

1. Introduction

Micro and nanofluidic devices are under investigation for integrated analytical systems as well as for miniaturized high throughput screening, combinatorial chemistry and highly parallelized biochemical, molecular biological and micro biological processes [1–6]. Micro channels play a deciding crucial role in these systems [7–10]. But, serial processes can hardly realize high throughput in micro channel systems in homogenous phase due to the high-dispersion effects. This problem is caused by the laminar flow. It can be overcome by the use of liquid/liquid two-phase systems where dispersion effects are completely suppressed. Such systems are proposed for miniaturized serial PCR [11], for the production of inorganic materials with narrow-size distribution of particles [12] and for other chemical and biological processes [13–15].

Serial processing in micro-segmented flows avoids serious problems of chemical operations in micro channels. Carrier liquids separate reaction compartments from each other. Micro motions inside single segments support mixing by interdiffusion if the segments are not too large [16]. The use of a liquid alkane as carrier and water as process solvent represents an easy way in the production of micro-segmented flows. This basic system is interesting for a lot of inorganic chemical processes but also for some organic reactions. Segmented aqueous solutions are of particular importance for nucleic acid and protein chemistry and for cell operations. But segmented flow can also be performed in inverse systems, in which water plays the role of the embedding liquid and an organic phase is segmented. The inverse system offers the possibility of handling organic solvents without wall

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contact. A lot of problems of material compatibility can be avoided by use of water as embedding liquid.

Recent investigations were mainly focussed on segment volumes in the submicroliter and the mediate nanoliter range. Segment diameters of a few 10th of 1 mm correspond to about some 10s to 100 nl. Segment volumes in the lower and mediate nanoliter range are particular suited for segmented flow generation and application. Generators, tubes and other components can be still prepared by traditional mechanics. Better reproducible results are obtained if micro-fabricated chip modules are used. The technique of micro-segmented flow should not be restricted to the submicroliter and nanoliter range. It is possible, that the picoliter range will also be addressed by the use of microlithographically prepared modules. Problems like evaporation and wetting, which frequently become very serious in open fluidic systems as micro and nanotiteroplates and droplet manipulation at surfaces, are not of importance for the miniaturization of segmented flow, because the whole segments are completely embedded in the carrier liquid. Optical control and measurement techniques should be easily applicable down to about 1 pl segments, which correspond to the characterization of objects in the range of about 10 μm diameter, which is far enough from diffraction limit.

Micro-segmented flows can be produced with high speed. Regular sizes and frequencies were observed up to generation rates of 30 segments in case of channel diameters of about 0.5 mm and tetradecane as embedding liquid. This means, that in principle more than 10^6 samples per day can be operated in one single channel [15]. This is of particular interest for quantitative operations. It becomes possible to carry out large experimental series with low but discrete stepwise change in concentration parameters or other conditions in closed systems. This enables the chemist and the biochemist to perform a “micro digital operation”. The digital principle relates to small, but well controllable steps in the composition of the huge number of droplets. Large multiparameter experiments could be performed in frame of combinatorial-segmented flow chemistry. Screening processes with biomolecules, cells or tissue fragments could be extended to detailed studies of concentration-dependent synergetic effects in multiparameter studies.

All these application fields for micro-segmented flow are strongly related to the precise control of process parameters. Therefore, the formation of segments is a crucial step in the whole segmented flow process. It determines the volume and the distance of segments. Both parameters being the central influencing factors in the control of quantitative operations and in the reproducibility of whole processes. Therefore, important influence parameters on segment formation were studied and are presented, here.

2. Experimental set-up

For segmentation, different types of devices were tested. All of them contain at least three channels—two for liquid input and one outlet. T-junction arrangements were prepared in all cases (Figs. 1 and 2). The straight channel was planned for the carrier (embedding liquid), whereas the third channel (rectangular oriented to the main channel) was thought for the supply of the embedded liquid.

In the simplest case, T-injectors, made mechanically were applied. Therefore, polymethyl-methacrylate (PMMA) was used as material. It can be processed by conventional mechanical techniques down to feature sizes of about 200 μm.

All experiments were carried out with alkanes as embedding liquid and water as embedded phase. For embedding tetradecane (VWR International, Darmstadt, Germany) and a mineral oil (Raveno, Werther, Germany) were used. Both liquids are aliphatic and chemically inert. The viscosity of tetradecane at 25 °C is 2.128 mPa s [17] and the ratio of the viscosities of Tetradecane and Mineral oil was determined to 1/5 at 27 °C. Malachitegreen-Oxalate (VWR International, Darmstadt, Germany) was chosen as dye for the labelling of aqueous phase due to their high-absorption coefficient. It was applied in concentrations of 0.1 M in order to yield absorbencies (extinctions) of about 120 that means high contrasts at

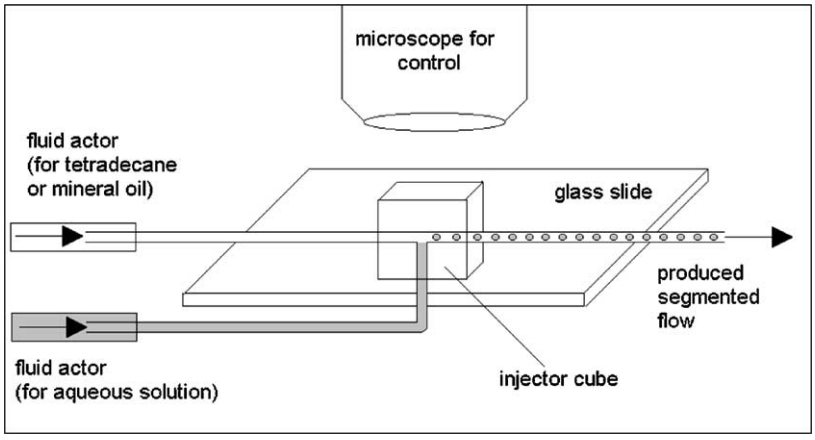

Fig. 1. Experimental set-up for the investigation of segment formation in PMMA segmentors using syringe pumps as fluid actors.