A continuous-flow droplet-based concentrator using ion concentration polarization†

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We propose a method to continuously generate droplets with programmable concentration using the ion concentration polarization (ICP) phenomenon. The concentration of a sample in continuously formed droplets can be tuned with a combination of flow rate and applied voltage. The nanoporous junction needed for ICP was fabricated by embedding a Naion membrane inside a PDMS mixture and then self-sealed by curing it. Compared to other methods previously reported in the literature, our fabrication method has the advantages of simplicity, reliability, repeatability and low-cost. Sample droplets with up to 100-fold concentration were generated continuously with our device.

Introduction

Ion concentration polarization (ICP) is a fundamental transport phenomenon that occurs near ion-selective membranes and is often called an ion-depletion and ion-enrichment process.1 Recently, ICP has attracted a great deal of attention from the lab-on-a-chip research community because of its potential applications in biochemical analysis, such as concentration,2–5 desalination,6,7 separation and mixing.6,9 Further applications of ICP have been reported recently. Jeon et al.10 presented a novel separation method, which can continuously separate the particles based on the ICP phenomenon and the electrophoresis mobilities of micro- and nano-sized particles. MacDonald et al.7 reported a method of out-of-plane ion concentration polarization for scalable water desalination, which has the advantages of increasing the throughput and the ability of integrating many devices.

A concentrator, that increases the number low-abundant molecules in a given sample volume, is an important tool for biochemical analysis and drug testing. Chen et al.11 introduced an integrated microfluidic device that consists of a biomolecule concentrator and a micro-droplet generator. Concentrating the sample before forming droplets could enhance the limited sensitivity of these droplet-based enzyme essays. However, this device could only generate droplets in an on-demand mode. First, the enzyme molecules were accumulated by a concentrator into a plug, and then, was released by turning off the voltage and transported by pressure-driven flow. Kwak et al.5 presented a continuous-flow nanofluidic biomolecule/cell concentrator, which could accumulate biomolecules and cells into a concentrated plug before the ICP boundary and guided into the narrow, concentrated channel by hydrodynamic force. More recently, Yu et al.12 reported an on-demand nanofluidic concentrator based on ICP. The device injects concentrated sample in the form of a droplet into an oil stream. The concentration and droplet size depend on the voltage, accumulation time and the period of the injection pulse. A continuous-flow microfluidic device that can both accumulate the low-abundance sample and generate the concentrated droplets at the same time with simple and reliable fabrication process is a critical need for biological applications of microfluidics. In this paper, we first introduce a novel and simple fabrication method to fabricate microfluidic devices with nanoporous materials suitable for establishing ICP. Our method integrates a Naion membrane into a PDMS layer to form the ion-selective membrane required for ICP. Next, we demonstrate the proof-of-concept of a working concentrator device by continuously generating mono-disperse micro-droplets with up to 100-fold concentration of the inlet sample.

Material and methods

Device concept and fabrication

Our device is a combination of a continuous-flow concentrator2 and a droplet generator11 based on the ion concentration polarization. Fig. 1 shows the device design and its operation concept. The sample is introduced into the main channel with a width of 500 μm. At the preconcentrated zone where the sample is concentrated, the inlet channel is bifurcated into a small 50 μm wide concentrating channel and a 450 μm wide filtering channel. The concentrating channel is slanted 45° to allow the stream of concentrated sample to be introduced into the droplet formation part. To establish the preconcentrated zone with ICP, a 50 μm thick Naion membrane was patterned along the
slanted concentrating channel. A buffer channel with 500 μm width and two 8 mm reservoirs form the electric ground for the ICP process. Platinum wires are connected to both sample inlets, and one buffer hole to conduct the electrical current passing through the ion-selective membrane. The filtered, diluted outlet is floated electrically. The gap between buffer channel and main channel is 50 μm. In the droplet formation part, the oil inlet channels have a width of 50 μm. Droplets of the concentrated sample are formed with the flow-focusing configuration. Droplets sample are collected at the 8 mm large outlet reservoirs. The whole system is controlled by the inlet flow rates of the sample inlet and the oil, the applied voltage at the inlet and the outlet pressure. In our later experiments, a voltage ranging between 0 and 150 V was applied between the inlet of the main channel and the buffer channel across the Nafion junction.

Sample flow rates ranging from 10 to 100 μL h⁻¹ were set by a syringe pump. The outlet pressure is adjusted by the outlet height with a precision lever. On application of an electric field (V⁺) across the nanoporous membrane, the ICP zone was developed based on force balance between the depletion force and hydrodynamic force. In Fig. 1, the arrows indicate the directions of hydrodynamic force and depletion force acting on charged ions or particles. Depletion force is the force acting on charged species as a result of ICP (ion depletion), which is electrostatic in nature. In the concentrator device, the ICP boundary is generated along the Nafion stripe with a fixed designed distance, and charged species are only accumulated at this zone.

Fig. 2 shows the two fabrication processes that can be used to implement the device concept depicted in Fig. 1. Most of the previously reported ICP-based devices used Nafion solution to deposit a thin nanoporous membrane with a thickness varying from 191 nm using micro flow patterning method² to 125–200 μm by filling a laser-cut micro channel.⁷ To simplify the fabrication process, we used an off-the-shelf Nafion membrane (NR-212, DuPont Co.) with a thickness of 50 μm instead of using the Nafion solution to deposit a thin cation-selective membrane on a glass substrate. This off-the-shelf membrane provides a better mechanical durability and longer working time thus more efficient. Moreover, a thicker membrane generates a stronger electrical repulsive force around the membrane.⁴ The silicon wafer was silanized before placing the Nafion membrane, in order to ensure that the Nafion membrane does not stick on the master mold surface when peeling off the PDMS. The microchannels of the device were fabricated following a general PDMS chip fabrication process. The master mold was made with SU-8 photore sist (MicroChem Corp.) and standard photolithography process.

Sylgard 184 and a curing agent (Dow Corning Inc.) were mixed in a 10 : 1 ratio by weight. The mixture was degassed for
1 hour in a vacuum chamber. After that, the mixture was poured onto the silicon master and cured at 80 °C for 2 hours. Then the PDMS was peeled off from the SU-8 mold and bonded to the PDMS-glass (Fig. 2(a)) or PDMS-only (Fig. 2(b)) substrate integrated a Naﬁon membrane with the help of oxygen plasma treatment and under a precise alignment system, Fig. 3(a) and (b).

As shown in Fig. 2, the device can be fabricated with a glass support or directly on a PDMS substrate. The advantage of the first case is the rigid support of the glass substrate. Fig. 4(a) and (b) shows the layers of the device with glass support and the final device, respectively. To make sure that a thin PDMS is coated, the PDMS mixture can be spin coated before curing in an oven. Fig. 4(c) shows the height if the Naﬁon membrane relative to the PDMS surface.

**Experimental setup**

In our experiment, all liquids were kept in 1 ml glass syringes, which were driven by syringe pumps (neMESYS, Cetoni) to deliver the required flow rates to the device. A high-speed CMOS camera (Phantom Miro eX4) attached to a microscope was used to capture the grayscale images. To demonstrate the sample concentration and droplet generation, 1 μM ﬂuorescein sodium salt (Sigma Aldrich, St. Louis, USA) was used as the inlet sample. We also prepared another concentration of sample 100 μM to measure the reference ﬂuorescence intensity value. Mineral oil (330760, Sigma Aldrich, St. Louis, USA) mixed with 0.5% wt surfactant Span 80 (S6760, Sigma Aldrich) work as the continuous phase for droplet formation. The phosphate buffer solution (PBS) with the concentration of 100 μM was prepared for the buffer channel with the electrical ground. Platinum wires (Sigma-Aldrich, 0.1 mm diameter) were used as electrodes. The electric ﬁelds were provided by a high voltage DC power supply (Model PS350, Stanford Research System, Inc). After recording the images with the high-speed camera, we measured the droplet diameter using a custom-i-ﬁed MATLAB program. For each data point, a total of 20 droplets were measured. However, as the resultant droplet has an apparent diameter bigger than the depth of the channel, it assumes a discoid shape. Hence, the measured result slightly overestimates the actual droplet diameter. The ﬂuorescence intensity of the formed droplet and the distance measurement were processed using another customized MATLAB program.

It is important to control the overall reference pressure of the channels system. To control the effect of the back pressure, the outlets pressure of both concentrated outlet and diluted outlet...
were compensated using a precision z-movement system. Each opening end of outlet tubing was attached to a dial height gage (Series 192 with Digital Counter, Mitutoyo, Japan). During the experiment, one can adjust the pressure difference between two outlets to compensate the backpressure effect.

Results and discussions

Concentration with ICP

We characterize the fluorescence intensity at the area located in front of the Naion membrane to demonstrate the continuous concentration of the device. Once the ICP triggered, all the charged ions will be repelled away from the Naion membrane and formed a boundary to prevent any charged species to enter this region. The concentration of the fluorescein salt was calibrated against its concentration. The relative intensity ratio between the concentrated sample and the inlet sample of 1 μM allowed the concentration to be determined. Fig. 5 shows a representative result of the sample concentration. At 10 μL h⁻¹ and 30 V a 100-fold concentration from 1 μM to 100 μM can be achieved.

We first fixed the input flow rate and varied the applied voltage to understand the interaction between the hydrodynamic force and the depletion force and the associated location of the preconcentrated zone. Once the ICP is triggered, a preconcentrated zone will appear and stabilise based on the balance between the hydrodynamic force and depletion force. A high applied voltage shifts the preconcentrated zone further away from the Naion membrane, because the depletion force overcomes the hydrodynamic force and pushes the charged ions away from the nanoporous membrane.

Fig. 6 shows the measured distance between the ICP boundary and the edge of the Naion membrane. In this range, the distance is almost linear to the applied voltage. However, we observed that the ICP boundary fluctuated significantly and may disappear if the applied voltage is too high. These effects could presumably be explained by the breakdown of the PDMS substrate under the threshold voltage. In this situation, short circuit occurred and caused the significant increase in the current, which was monitored continuously by an ammeter (Keithley). In our experiments, the current was limited to 1 μA by the DC power supply. Therefore, the applied voltage can only be varied in a limited range.

More details are shown in the ESI† (details in S1-video, recorded at a rate of 30 frames per second). After the initial test to establish the operation point (10 μL h⁻¹, 30 V) where the preconcentrated zone is shifted exactly to the concentrating inlet of the droplet formation part, the flow rates were varied from 10 to 50 μL h⁻¹ to tune the concentration. The concentration at the preconcentrated zone depends on the mass transport of the ions or the supply flow rate at the inlet and the time they are allowed to accumulate. Increasing the inlet flow rate requires a larger voltage to maintain the position of the ICP.
boundary. In our device, the applied voltage should fall in the range from 10 V to 100 V with a flow rate ranging from 10 to 30 μL h⁻¹. Fig. 6(b) shows the operation line of our device. Since the applied voltage vary linearly with the inlet flow rate, flow rates used in the experiments of 10, 15, 20, 25, 30 μL h⁻¹ correspond to the applied voltages of 30, 45, 60, 75, 90 V, respectively. Comparing with the reference operation point of (10 μL h⁻¹, 30 V, Fig. 7(a)), we assign each operation point a gain factor of 1, 1.5, 2, 2.5 and 3.

At a constant oil flow rate, the size of the formed droplets depends on the inlet sample flow rate. Fig. 7 shows the representative droplet formation process at different flow rates and applied voltages. At the reference operation point Q = 10 μL h⁻¹, V = 30 V, the ICP boundary is located at the inlet of the droplet formation part, Fig. 7(a). With an oil flow rate of Qoil = 2 μL h⁻¹, stable droplets with concentrated sample are formed, Fig. 7(b). At a gain factor of 2, the flow rate and the voltage increase to Qin = 20 μL h⁻¹ and V = 60 V, respectively. Since both hydrodynamic force and depletion force increase, the droplet formation also increases while the oil flow rate remains at Qin = 2 μL h⁻¹, resulting a larger droplet, Fig. 7(c). At again factor of 3 (Qin = 30 μL h⁻¹, V = 90 V) the formed droplets become even larger, Fig. 7(d). Fig. 8 shows the measured droplet diameter as function of the gain factor.

A higher sample inlet flow rate supplied more charged ions that are accumulated at the ICP boundary and transferred to the droplets. Thus, a higher inlet flow rate or a higher gain factor leads to a higher sample concentration in the formed droplet. Fig. 8 shows that the concentration jumps to more than 100-fold at the gain factor of 2.5, corresponding to the flow rate of 25 μL h⁻¹ and the applied voltage of 75 V. Increasing the gain factor further, the fluorescence intensity increases slowly and may reach a plateau. This behaviour may be caused by the saturation of the fluorescent intensity.

**Conclusion**

We demonstrated a continuous droplet-based generator based on the phenomenon of ion concentration polarization. This device can both concentrate and generate the droplets continuously and controllably. The sample can be concentrated by 100-fold with droplet size changing from 25–50 μm. The fabrication process for making this device is relatively simple. The use of off-the-shelf Nafion membrane leads to a better durability and a longer lifetime of the device. This concept can be applied for assays with low-abundant input analytes. The target analytes can be concentrated to a desired concentration for the subsequent analysis processes.

**Acknowledgements**

D.T.P acknowledges the support from the Nanyang Technological University PhD Scholarship via Nanyang Environment & Water Research Institute (NEWRI).

**Notes and references**