

ON-CHIP NANOFILTERS FOR BIOLOGICAL SAMPLE PRE-TREATMENT FOR ELECTROPHORETIC ANALYSIS OF SMALL MOLECULES IN WHOLE BLOOD

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ABSTRACT

We present a new method for on-chip sample pretreatment based on size and charge exclusion in nanochannels formed by simple electric breakdown of poly(dimethylsiloxane) (PDMS). In contrast to previously published methods, control over pore size was achieved by setting a current limit during the breakdown step, allowing selective transport through the nanopores of different size molecules. Finally, we demonstrate the application of this method for the direct analysis of quinine in whole blood samples at clinically relevant concentrations.

KEYWORDS

Nanochannels, sample pre-treatment, blood, pharmaceuticals.

INTRODUCTION

Unless an enzyme specific immunoassay is utilized, direct measurement of pharmaceuticals in whole blood requires sample pretreatment to remove blood cells, plasma proteins, and endogenous macromolecules. On-chip sample pretreatment include solid-phase extraction, cross flow filtration, and ultrafiltration. Filtration through nanochannels under an applied electric field offers greater selectivity than the other techniques [1]. The downside of nanofabrication is the high cost of fabrication, which limits its applicability in disposable devices. Nanochannels can also be formed by PDMS electric breakdown [2]. This technique was used to concentrate proteins on one side of the membrane, and can thus be useful for sample treatment if the transport of smaller molecules can be maintained. However, care must be taken as the movement of smaller ions can be restricted through ion permselectivity [3].

The nanochannels formed by PDMS breakdown are usually irregular in shape and have varying diameter along their length. While fabrication of straight nanochannels with defined diameter is required for certain applications, this is not necessary for the purpose of our work. Permeability of the nanochannels for a certain molecule is determined by its size and charge relative to that of the nanochannel. Hence, the smallest diameter along the channel length is the critical factor irrespective of the channel length or shape. Control over the average pore size can be achieved by changing the applied electric field strength or the time of the voltage application. The main drawback of these approaches is the poor reproducibility between devices due to the inherent inhomogeneity of PDMS.

Here we applied much lower electric field using low ionic strength electrolyte and set a current limit to control pore size. Once the specified current is achieved, the voltage is restricted to prevent further electric breakdown of PDMS. This approach is based on the fact that no current can be measured before nanochannel formation and the current increases with the number and diameter of nanochannels formed during the breakdown.

EXPERIMENT

The microfluidic device is shown in Figure 1. It consists of a PDMS layer that contains the microchannels and is irreversibly bound to a flat glass slide to form the enclosed structure. The V-shaped sample compartment has its tip separated by 100 μm -thick PDMS membrane which will act as nanofilter after the electric breakdown.

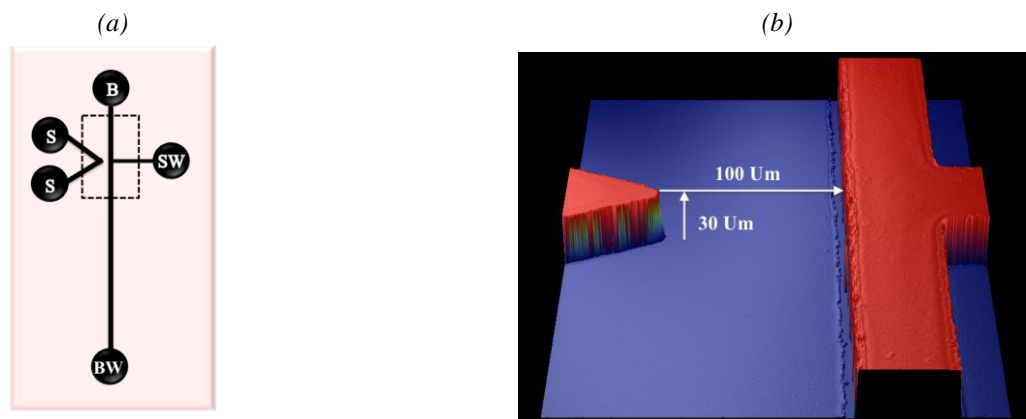


Figure 1. Microfluidic device. (a) Schematic diagram of the microchip design (not to scale). Sample (S), sample waste (SW), buffer (B), and buffer waste (BW). (b) Profilometer image of the PerMX template used for rapid prototyping.

Another design with wider PDMS filter and U-shaped sample compartment (not shown here) was tested in an attempt to enhance extraction rate by increasing the surface area available for formation of nanochannels. However, we found that as soon as one point starts to break in the PDMS, the current flow is focused through this point making it less likely to have other breakdown points. Based on this, experiments were continued with the design presented in Figure 1. Also, the length of PDMS gap across which the breakdown is performed was chosen to be 100 μm instead of the reported 20 μm [4] or 40 μm [5] to allow higher voltages to be used during extraction for long time without the risk of secondary breakdowns.

When reversibly bound devices were used, the breakdown happened between the PDMS and glass and the pore size continued to increase during the subsequent runs. This change in pore size means that increasing amounts of the plasma proteins will continue to leak into the separation channel compromising the separation efficiency. Using irreversibly bound devices solved this problem. PDMS and glass layers were exposed to air plasma for 15 s using a handheld corona discharge device. The treated surfaces are then brought into conformal contact and left to bind overnight at 60°C. It is important to keep the binding process consistent in order to obtain reproducible results.

To form the nanochannels, an electric field of 2200 V (22 V/ μm) was applied across the PDMS gap. This voltage is slightly above the dielectric strength of PDMS (21 V/ μm). Reported electric fields were 25 V/ μm [5] to 40 V/ μm which is almost double the dielectric strength of PDMS [6]. Factors that affect pore size by PDMS breakdown include ionic strength of the electrolyte, applied voltage, and time of application. Different experimental conditions were studied for their effect on PDMS permeability as in Table 1. The key point to control nanochannel pore size is to set a current limit to adjust the applied voltage. A LabView program was used to control the power supply. Once a pre-set current threshold is reached, applied voltage is restricted to prevent further breakdown.

Two ionic strengths were tested: 1 and 10 mM disodium hydrogen phosphate solution, pH 9. Different current limits were set from 1 to 10 μA . The permeability of the formed nanochannels was tested using inorganic cations and anions, small molecular weight organic acids (negatively charged), slightly larger basic dyes (positively charged), bovine serum albumin (BSA) labeled with fluorescamine, and blood cells. When the current limit was set to 1 μA , the same results were obtained at both ionic strengths, with both inorganic cations and anions passing freely through the formed nanochannels as indicated by formation of red complex on both sides as shown in Figure 2. Small molecular weight organic acids, namely pyruvate, lactate, and 3-hydroxy butyrate, were also able to pass. However, negatively charged fluorescent dyes like fluorescein and 5(6)-carboxy naphthofluorescein were blocked, as the passage of co-ions was not favored through the negatively charged nanochannels even with prolonged injection times.

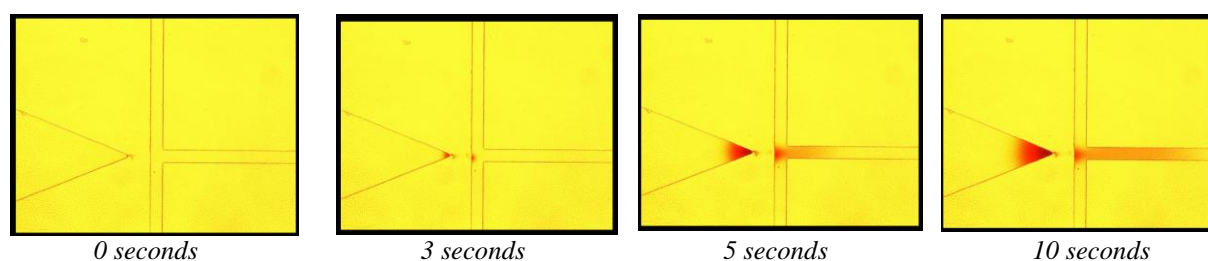


Figure 2. Screen shots showing the bidirectional transport of both inorganic anions and cations through nanochannels visualized by the formation of red coloured iron (III)-thiocyanate complex at different times after voltage application.

Accordingly, we increased the current limit to 3 μA while still using 1 mM electrolyte solution. Figure 3 shows that while the membrane can still hinder the passage of labeled BSA, the negatively charged dye 5(6)-carboxynaphthofluorescein passed through the nanochannels. Next, permeability was tested with positively charged molecules. As the nanochannels favor the passage of counter-ions, positively charged rhodamine 6G passed freely. Although exact estimation of pore size is not feasible, according to the results obtained we expect them to be only few nanometers in diameter. The mechanism involves charge and size exclusion. Additionally, the balance between electrophoretic mobility and the EOF play an important role in membrane permeability for a specific analyte.

Through our experiments, we noticed that using current limits up to 3 μA during breakdown hindered the movement of BSA as long as the electrolyte concentration is 1 mM. Using 10 mM electrolyte solution resulted in larger pores even at low current limit. The same was observed when using 1 mM electrolyte concentration at high current limit (5 μA). An extreme case is setting high current limit (10 μA) and using 10 mM electrolyte solution. The resulted channels allowed the passage of red blood cells which suggests channels that are few μm in diameter.

Table 1. Permeability of nanochannels produced with different breakdown conditions

Nanochannels Permeable to...	Breakdown Conditions	
	current limit (μA)	ionic strength (mM)
Organic acids (<150 g/mol)	1	1
		10
Basic compounds (<1000 g/mol.)	2	1
	3	1
Labeled BSA	2	10
	5	1
Blood cells	10	10



Figure 3. Screen shot showing (on right hand side) the extraction of the red coloured 5(6)-carboxynaphtho fluorescein from a mixture of labeled BSA and free dye (on the left hand side).

The applicability for using the nanochannels to create a sample-in/answer-out analytical device for pharmaceuticals was demonstrated with the isolation and separation of the antimalarial drug quinine sulfate from whole blood without any other sample processing (figure 4) with the entire process requiring less than 190 s. The method consists of extraction step through electrokinetic injection for 100 s followed by ITP step in peak mode for 90 s. We use the same ITP system reported by Mikus *et al.* [7] with leading electrolyte of 10 mM sodium acetate, 20 mM acetic acid, 1 mM NaH_2PO_4 , and 0.1% HPMC (pH 4.34) and terminating electrolyte of 10 mM β -alanine and 10 mM acetic acid (pH 4.20). Linear range is from 0.5 to 25 $\mu\text{g}/\text{ml}$ with regression coefficient (R^2) of 0.999. This covers the clinically useful range (3 – 10 $\mu\text{g}/\text{ml}$).

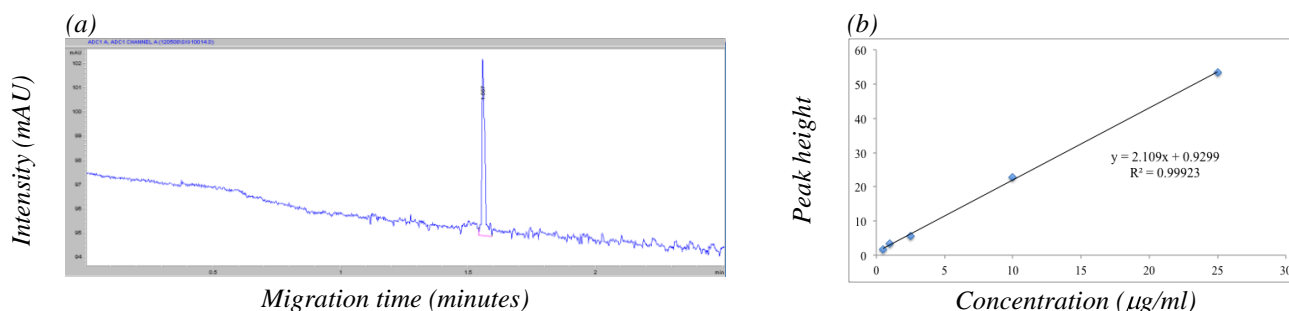


Figure 4. Method application for quinine determination. (a) Isotachopherogram for blood sample spiked with 2.5 $\mu\text{g}/\text{ml}$ quinine sulfate. (b) Calibration curve from spiked blood samples covering the range 0.5 – 25 $\mu\text{g}/\text{ml}$.

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