

A MALDI-chip integrated system with a monitoring window

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Received 17th December 2004, Accepted 7th February 2005

First published as an Advance Article on the web 16th February 2005

DOI: 10.1039/b418986h

The integration of a monitoring port along the microfluidic path of a MALDI-chip integrated device is described. Optimization of the microreactor design allows longer reaction and measuring times. The Schiff base reaction between 4-*tert*-butylaniline (1) and 4-*tert*-butylbenzaldehyde (2) in ethanol was carried out on-chip in the MALDI ionization chamber and the formed imine 3 was detected in real time, demonstrating the feasibility of the “monitoring window” approach. This preliminary result opens the way to on-chip kinetic studies by MALDI-MS, by opening multiple monitoring windows along the microchannel.

Introduction

Recently, we reported the first example of a microfluidic system integrated with a matrix-assisted laser desorption ionization (MALDI)^{1,2} time-of-flight (TOF) mass spectrometer (MS).³ Reactions were initiated by using the vacuum of the instrument to actuate solutions within the microreactor. An important novelty of such an integrated microfluidic device is the possibility of analyzing the reaction product “on the spot” by MALDI-MS immediately after the reaction has taken place. After the chip is placed in the MALDI-TOF vacuum chamber, and as soon as the reaction product reaches the outlet, the laser beam ionizes the molecules, which are detected by the TOF analyzer.

In the previous design, due to the channel geometry, very high flow rates were generated using the vacuum-driven actuation, which resulted in very short residence times. Furthermore, the reaction mixture could only be analyzed at one sampling spot (the outlet) where the reaction mixtures were collected. As a consequence no time-dependent experiments could be done. Proper design of the fluidic circuit and the integration of multiple outlet-ports, would allow a quasi-continuous monitoring of the reaction products in time, extending the use of the MALDI-chip to kinetic studies.

In this communication the fabrication of a microreactor that allows longer reaction and measuring times is reported. A novel concept is described consisting of the integration of a “monitoring window” in the chip design, for monitoring reactions by using the MALDI-chip set-up. As a model reaction the Schiff base reaction⁴ between 4-*tert*-butylaniline (1) and 4-*tert*-butylbenzaldehyde (2) in ethanol to give the corresponding imine 3 (Scheme 1) was carried out on the MALDI-chip and the reaction product was detected through the “monitoring window”.

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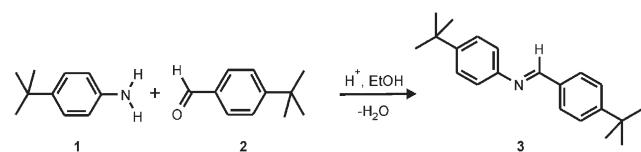
Experimental

Microreactors

Microreactors (Fig. 1) were fabricated in the cleanroom facility of the MESA⁺ Institute for Nanotechnology at the University of Twente. Inlet and outlet holes as well as the “monitoring window” were fabricated in the top silicon wafer by KOH wet-etching⁵ in combination with a boron etch stop.⁶ The reaction microchannel was fabricated in the bottom borofloat wafer by HF etching,⁷ while the triangular reservoirs as well as the bigger inlet channels (going from the inlet holes to the reservoirs) were fabricated by powder-blast micromachining.^{8,9} Square-shaped holes in the monitoring window were fabricated by focused ion beam (FIB). Various combinations of dimensions and number of holes were investigated. The preliminary experiment reported in this paper was carried out using a monitoring window with only one sampling hole of 500 × 500 nm.

Reagents

Reagents were obtained from Aldrich Chemicals, The Netherlands, and were used as supplied commercially without



Scheme 1 Schiff base formation reaction.

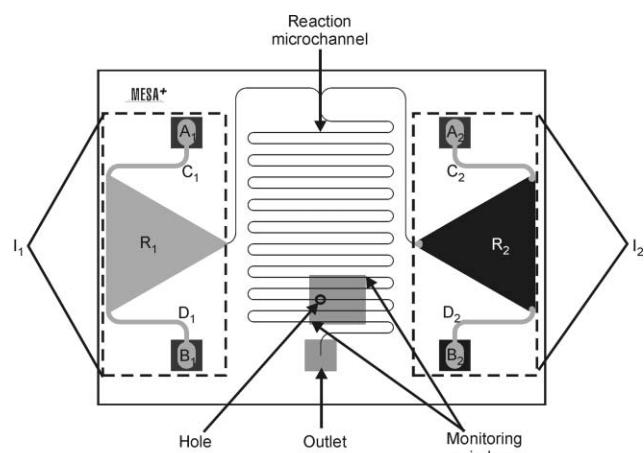


Fig. 1 Outline of the chip used to prove the “monitoring window” principle. Each inlet loading system (I_x) consists of two inlet holes (A_x and B_x) connected to a reservoir (R_x) through channels C_x and D_x (with x = 1 and 2).

further purification. No matrix was added for the MALDI detection of the imine **3**, because of its strong absorption at the laser wavelength ($\lambda = 337$ nm). Reservoir A was filled with a solution of 1×10^{-4} M 4-*tert*-butylaniline (**1**) in ethanol, reservoir B was filled with a solution of 1×10^{-4} M 4-*tert*-butylbenzaldehyde (**2**) in ethanol.

Filling procedure

The reservoirs filling procedure was monitored by mounting the chip, placed on a suitable holder, on an Olympus CK40M inverted microscope.

MALDI-TOF

The reaction product formed in the microreactor channel was identified in real time through a nm size hole fabricated in the “monitoring window” by MALDI-TOF MS using a Voyager-DE-*RP* MALDI-TOF mass spectrometer (Applied Biosystems/PerSeptive Biosystems, Inc., Framingham, MA, USA) equipped with delayed extraction,^{10,11} and a 337 nm UV nitrogen laser, producing 3 ns pulses. The mass spectra were obtained in the reflectron mode.

Focused ion beam (FIB)

Holes in the boron doped silicon membranes (250×250 nm and 500×500 nm) were made using a FEI 200 Focused Ion Beam System. The resulting holes were imaged *in situ* using a Scanning Electron Microscope (SEM).

Results and discussion

Fluid dynamics

A high degree of temporal control over the (micro)fluidics is a prerequisite to carrying out time-dependent studies of (bio)-chemical reactions. In the recently developed³ MALDI-based lab-on-a-chip the fluid is transported through the channels due to a pressure difference between the inlet, where a small air bubble is present (atmospheric pressure) and the outlet (vacuum). The relation between the generated flow rate and the applied vacuum pressure is defined by eqn. (1).^{12,13}

$$\Delta p = R \times \phi_v \quad (1)$$

where Δp (N m^{-2}) is the pressure drop across the channel and ϕ_v ($\text{m}^3 \text{ s}^{-1}$) is the volumetric flow through the channel. The hydraulic resistance R (N s m^{-5}), which is dependent on the channel geometry and the viscosity of the fluid μ (N s m^{-2}), is calculated by integrating the velocity profile over the cross-section area. Based on the channel geometry, a number of design formulae for the calculation of the hydraulic resistance have been derived.¹⁴ For rectangular channels such as that of the MALDI-chip³ the resistance R can be calculated according to eqn. (2).

$$R = \frac{4\mu l}{ab^3} \left\{ \frac{16}{3} - 3.36 \frac{b}{a} \left(1 - \frac{b^4}{12a^4} \right) \right\}^{-1} \quad (2)$$

where a and b are half of the channel width and depth, respectively, l (m) is the channel length, and μ (N s m^{-2}) the

liquid viscosity. Eqn. (2) is valid for low Reynolds numbers (under laminar flow regimes) and long channels such that entrance effects can be neglected. From eqn. (1), and knowing the channel length l of the MALDI-chip, the residence time $t_{(r)}$ (s) can be calculated according to eqn. (3):

$$t_{(r)} = \frac{\ell A R}{\Delta p} \quad (3)$$

where A (m^2) is the channel cross-sectional surface area. In the first reported MALDI-chip microchannel³ ($a = 1.5 \times 10^{-4}$ m; $b = 1 \times 10^{-4}$ m; $l = 8 \times 10^{-2}$ m) the calculated resistance is $R = 1.55 \times 10^{13}$ N s m^{-5} (for water; $\mu = 1 \times 10^{-3}$ N s m^{-2}). The residence time for an applied pressure of 1 bar (10^5 N m^{-2}) is $t_{(r)} = 1.9 \times 10^{-1}$ s.

It is clear that no time-dependent experiments are possible using this microfluidic device, since the residence times are in the order of two hundred milliseconds. Even when opening multiple sampling ports along the channel, the time necessary to move the ionizing laser from one window to the next would be much too long compared to the on-chip residence times. Channels that have a higher hydraulic resistance are a possible solution to the short on-chip residence times. A new microfluidic system was therefore designed to allow a longer residence time.

Microreactor optimization

A microreactor was designed (Fig. 1) having 30 μm wide, 10 μm deep and 125 mm long channels, fabricated in a glass substrate by wet chemical etching using HF. The hydraulic resistance and residence time in this new channel design were calculated using eqns. (2) and (3) to give values of $R = 6.3 \times 10^{16}$ N s m^{-5} and $t_r = 24$ s, respectively.

Due to downscaling, the surface free energy^{15,16} (“surface tension”) becomes considerable. As a consequence, the contribution of the capillary pressure to the MALDI-chip pumping mechanism might be significant. For a flat rectangular channel the capillary pressure P_c (N m^{-2}) is given by eqn. (4).^{17,18}

$$P_c = \frac{2\gamma \cos \theta_c}{h} \quad (4)$$

where γ (N m^{-1}) is the surface tension of the liquid in the channel, θ_c is the contact angle between the edge of the meniscus and the channel wall (which for water in hydrophilic channels is assumed to be between 0° and 30°), and h (m) is the channel depth. However, the capillary pressure P_c (1.3×10^4 N m^{-2}) estimated for water ($\gamma = 0.07 \text{ N m}^{-1}$; $\cos \theta_c \approx 0.9$ with $\theta_c = 25^\circ$) is only about 10% of the pneumatic pressure used to drive the flow in the channel (10^5 N m^{-2}). Therefore, it can be concluded that the hydraulic resistance and residence times calculated according to eqns. (2) and (3) without taking into account the contribution of the capillary pressure to the flow rate are good estimations of the fluid dynamics in the microchannel depicted in Fig. 1.

Although the estimated residence times in the new microchannel are about 2 orders of magnitude higher than those in the previous MALDI-chip design, they are still too short to study reaction kinetics by MALDI-MS. Nevertheless, a considerably longer total measurement time (~ 75 min) can be obtained by the introduction

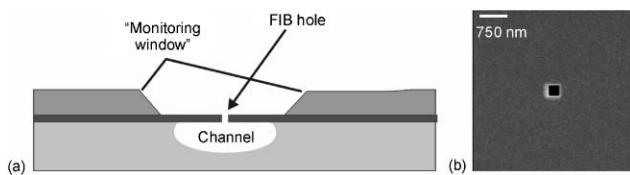


Fig. 2 (a) Cross-section of the silicon–glass hybrid chip with a KOH etched “monitoring window” and a sampling hole above the HF microchannel. (b) SEM image of 250×250 nm sampling holes made by FIB in the doped silicon layer.

of relatively large reservoirs for the reagent solutions (*vide infra*). This chip was therefore used to demonstrate the feasibility of extracting ions from a solution flowing in a microchannel through a hole in a silicon membrane.

The key element of the new microreactor (Fig. 1) is the “monitoring window”. This consists of a freestanding ~ 2 μm thick silicon membrane doped with boron, positioned above the channel (Fig. 2a). Ions can be extracted from the flowing reaction mixture through holes fabricated in the silicon membrane. Holes (250×250 and 500×500 nm), which could be visualized by scanning electron microscopy (SEM), were made by means of a focused ion beam (FIB). A micrograph of the 250×250 nm holes is given in Fig. 2b. The hole dimensions are important, since a pressure drop along the channel may arise from too large holes in the membrane, affecting the microfluidic actuation mechanism. Both hole sizes tested in the experiments here reported were big enough to allow analysis of the reaction mixture without significantly affecting the main flow through the reaction microchannel. A new chip design with multiple “monitoring windows” will require resizing the sampling holes, based on the channel geometry, the number of openings along the channels and the vacuum system used to transport the liquid. The position and dimensions of the “monitoring window” are also important factors. The dimensions of the window in the top silicon wafer must be large enough to let the laser beam, which hits the chip surface under an angle of about 30° , reach the small hole fabricated in the doped silicon layer on the bottom side of the same wafer. In these experiments 2.2 mm by 2.5 mm “monitoring windows” were fabricated, which gave good results.

In the first version of the MALDI-chip,³ reagent solutions were loaded in inlet cups fabricated in the top wafer by powder-blast micromachining. The inlets were then sealed with adhesive tape. A drawback of this inlet system is that the liquid can easily be contaminated by coming into contact with the adhesive used to seal the inlet holes. A new reagent loading scheme was developed (Fig. 1) consisting of two inlet systems (I_1 and I_2). Each system comprises two *via*-holes etched in the top silicon wafer (A_1 , B_1 and A_2 , B_2 , respectively), a triangular reservoir (R_1 and R_2), and two 200 μm wide by 100 μm deep powder-blasted channels (C_1 , D_1 , and C_2 , D_2) that connect each inlet hole with the corresponding reservoir. Flap valves¹⁹ (Fig. 3a) were fabricated in the doped silicon layer at the bottom of the inlet holes (Fig. 3b). These valves open during the filling position upon applying pressure onto the inlet (*vide infra*) and thereby allow the reagent solution to fill the reservoir. When the pressure is released, the valves close, separating the loaded solutions from the glue, and thereby

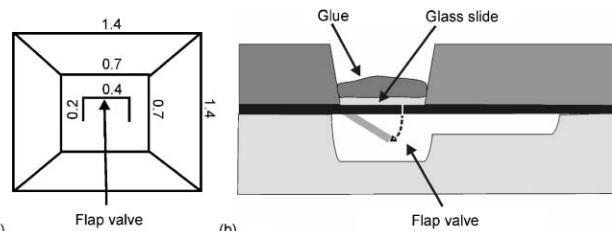


Fig. 3 (a) Top view of the flap valve at the bottom side of each inlet hole (the dimensions are given in mm) and (b) cross-section of the inlet hole with flap valve, glass slide and glue.

eliminating the risk of contamination. The triangular reservoirs (5.4 mm high, 6.2 mm wide, 100 μm deep) were powder-blasted in the bottom glass wafer.

Testing the “monitoring window”

The reservoirs were filled by suction of reagent solutions using a syringe. During the filling procedure the chip was positioned in a suitable holder. About 3 μL of reagent solutions were placed in hole A_1 or A_2 , while the syringe was placed on hole B_1 or B_2 . Each reservoir was filled keeping the corner where the small channel starts dry. This prevented filling of the reaction channel due to capillary forces before the chip was introduced into the ionization chamber (see inlet system I_2 in Fig. 1). During the filling procedure holes A_1 and A_2 were emptied completely, thereby trapping air bubbles in the powder-blasted channels (see inlet system I_2 in Fig. 1). In order to prevent the liquid from filling the reaction channel before entering the MALDI chamber, all other openings (outlet, monitoring window, and second inlet) were sealed with adhesive tape during the filling of each inlet. After both reagents were placed in the reservoirs, the four inlet holes were closed using a UV-curable glue. A glass slide (700 \times 700 μm) was placed in the inlet holes to prevent the glue from entering the inlet channels and polluting the reactants. After introducing the chip into the ionization chamber of the MALDI instrument, the air bubbles trapped behind the liquids expanded, pushing the reagent solutions into the reaction channel.

To prove the principle of the “monitoring window” the Schiff base formation reaction between 4-*tert*-butylaniline (**1**) and 4-*tert*-butylbenzaldehyde (**2**) in ethanol (Scheme 1) was carried out using the MALDI-chip system equipped with the chip in Fig. 1. The chip on the MALDI sample plate was introduced into the vacuum chamber by load lock. The first MALDI-TOF mass spectrum was acquired as soon as the plate reached the right spot in the chamber. The analysis was started after about 1 min. Ions were extracted from the channel through the hole, by hitting the “monitoring window” with the laser. The reaction product, imine **3**, was clearly visible in the mass spectrum at $m/z = 295$ (Fig. 4). Even after about 1 h, imine **3** could still be detected, indicating that reagent solutions were still available for the reaction, as predicted by theoretical calculations.

After the last mass spectrum was recorded (~ 1 h), the chip was examined using an optical microscope. The air bubbles in the reservoirs had expanded into the channel, while

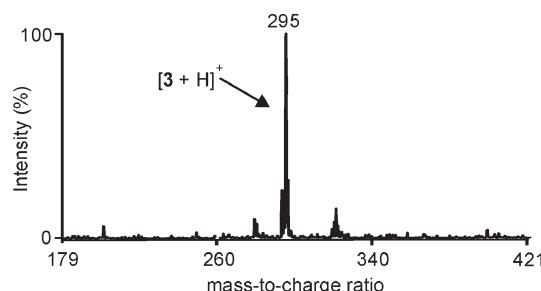


Fig. 4 MALDI-TOF mass spectrum of the imine **3** ($m/z = 295$) formed on-chip within the ionization chamber of the MALDI instrument and detected by extracting ions from the reaction mixture through the monitoring window.

liquid was still present in the portion of the channel close to the outlet.

Conclusions and outlook

A “monitoring window” for real time reaction monitoring was integrated into the design of a microfluidic chip. As proof of the principle, the product (imine **3**) of the on-chip Schiff base reaction of 4-*tert*-butylaniline (**1**) and 4-*tert*-butylbenzaldehyde (**2**) in ethanol was detected by MALDI-TOF mass spectrometry through the “monitoring window”. Although preliminary, this important result clearly demonstrates the feasibility of extracting ions from a flowing solution for analysis of the reaction mixture composition, opening the way to kinetic studies of (bio)chemical reactions by MALDI-TOF MS. The scope of the presented method might be extended to non absorbing analytes by adding a matrix to one of the reagent solutions,³ provided that the selected matrix does not interfere with the chemical reaction under investigation.

Acknowledgements

The authors would like to thank Vishwas Gadgil, who operated the FIB, and the Micro Chemical Systems (MiCS) program of the MESA⁺ Institute for Nanotechnology and the University of Twente for financial support.

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