ELECTROKINETICALLY ACTUATED, HEATED MICROREACTOR FOR METABOLOMICS

Tiina Sikanen[1]*, Maria-Elisa Moilanen[1], Susanna Aura[2], Tapio Kotiaho[1], Sami Franssila[2], Risto Kostiainen[1]

[1] University of Helsinki, Helsinki, Finland, [2] School of Chemical Technology, Aalto University, Espoo, Finland

ABSTRACT

In this study, we present thermal and fluidic characterization of an electrokinetically actuated, heated microreactor made of Ormocomp® polymer and monolithically integrated with a capillary electrophoretic (CE) separation unit. A thin film heating element patterned at the bottom of the reactor part allows accurate control of the temperature in the reactor without heating the separation part. Thus, stable electroosmotic flow is maintained enabling high CE separation efficiency and operation of the chip by electrokinetic flow alone.

KEYWORDS

Microreactor, microchip electrophoresis, fluorescence detection, Ormocomp

INTRODUCTION

Metabolomics is an emerging research area which is focused on measuring small molecules in biological samples. Lately, microfluidics has been successfully applied to metabolomics research. Most studies have focused on either analyzing off-chip produced metabolites or optimizing the procedures for enzyme immobilization [1], but coupling of microreactors to on-line, on-chip separation systems remains rare. This is likely because enzyme reactors often require precise heating to 37-40°C in order to mimic physiological conditions, but at the same time unsufficient elimination of heat in the separation part may easily result in temperature gradients and thus distortion of the flow profiles and deterioration of the separation efficiency [2,3]. In this study, we demonstrate successful coupling of a locally heated microreactor to an electrophoretic separation system which is efficiently cooled and can thus be fully operated by electrokinetic flow. The lack of external pumps allows precise valving of minute sample volumes so that consumption of expensive reagents, such enzymes, is reduced.

EXPERIMENTAL

The microreactor chip was fabricated from Ormocomp® polymer using UV-embossing and adhesive bonding as described earlier [4,5]. As depicted in Fig. 1, the microreactor (500 µm×20 µm×5 mm, w×h×L) incorporated three inlets and outlets for sample loading as well as an integrated CE channel (50 µm×20 µm×50 mm, w×h×L) for separation of the reaction products. In addition, a thin-film (100 nm) platinum resistor was patterned as a heating element to the bottom of the reactor part (Fig. 1). Mixing of the reactants occurs in a narrow channel joining the three inlets to the reactor.

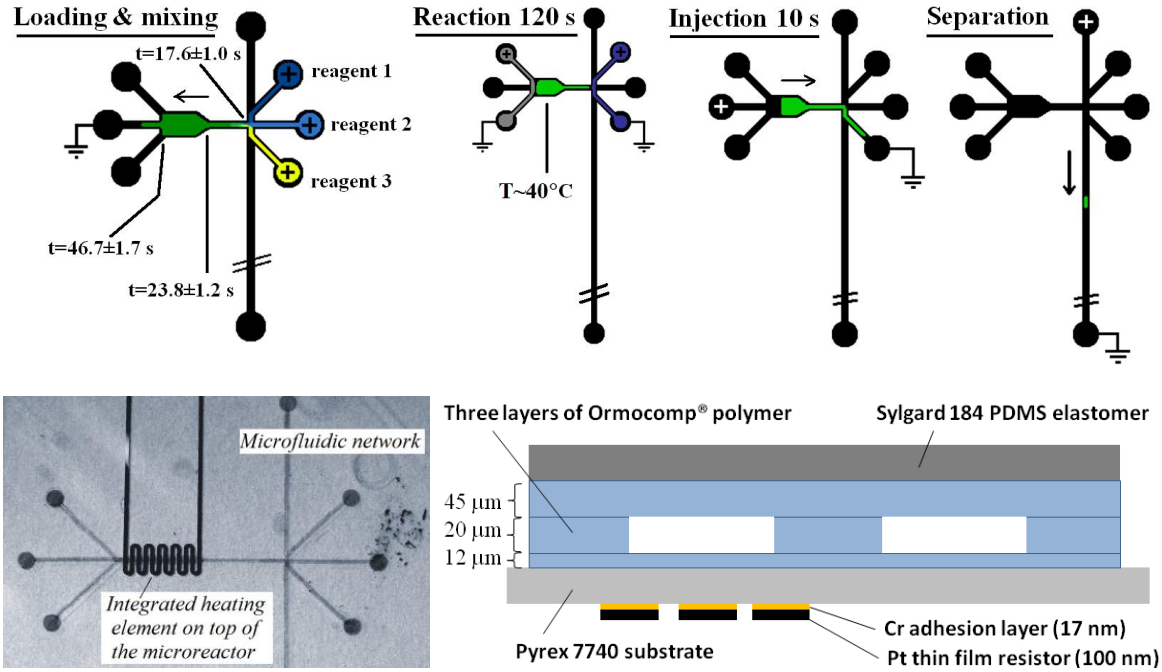

***Figure 1**. Schematic presentation of the operation cycles of the integrated microreactor-electrophoresis chip (top) and top and side views illustrating the thin-film (100 nm) platinum resistor patterned at the reactor part (bottom). The loading times (top-left) were determined from ten repeated runs by visualizing the liquid flow using 5 µM fluorescein in 20 mM sodium phosphate buffer (pH 12.0) containing 10% glycerol.*

The microreactor loading and injection times and voltages were optimized by visualizing the liquid flow with 5 µM fluorescein in 20 mM sodium phosphate buffer (pH 12.0) containing 10% glycerol (Fig. 1). In this study, the reaction time was set to 120 s and the reactor part was heated to approximately +40°C with constant applied power of 10 Wm^{-1} applied to the platinum resistor. The temperature change (ΔT) on the microchip surface (above the reactor) was measured with a thermocouple and the temperature inside the reaction chamber was estimated based on thermoresponsive behavior of rhodamine B (added to a 5 µM concentration to the buffer solution) as described elsewhere [6]. After the reaction step, small proportion (100 pL) of the reaction solution was injected (10.0 s) to the CE separation channel in pinched mode and the sample components were detected by laser-induced fluorescence (LIF) microscopy (excitation 488 nm, emission 500-700 nm). The effective separation length was 45 mm and the applied electric field strength 600 Vcm^{-1}. The repeatability of the reaction/separation cycle was determined using 5-(4,6-dichlorotriazinyl)aminofluorescein (5-DTAF) as the model compound.

RESULTS AND DISCUSSION

The performance of the integrated, Ormocomp-based microreactor-CE chip was characterized with respect to its fluidic and thermal stability. First, the repeatability of the electroosmotic flow (EOF) was determined in Ormocomp® microchannels and was $(4.13\pm0.04)\times10^{-4}$ $cm^2V^{-1}s^{-1}$ (0.88% RSD, n=4) for 20 mM sodium phosphate buffer (pH 12.0) containing 10% glycerol. Thanks to the good stability of the EOF, the microreactor loading times given in Fig. 1 were also very well repeatable, i.e., within 3.7-5.8% RSD for ten repeated loading cycles. Addition of glycerol to the buffer solution was found critical in reducing the evaporation rate and thus improving the stability over time. On the basis of in-house performed enzyme incubations, the activity of for instance human cytochrome P450 recombinant enzymes was not affected by the addition of glycerol up to 15% (data not shown) supporting the envisioned use of the microreactor in metabolism research.

Next, the temperature changes (ΔT) both on the microchip surface (above the reactor) and inside the reaction chamber were determined as a function of power applied to the Pt thin film resistor. Linear response between the applied power and the temperature reached was observed up to at least 12 Wm^{-1} (Fig. 2a). With constant applied power of 10 Wm^{-1}, the temperature of the reaction solution inside the microreactor reached +40°C within approximately 2 minutes (Fig. 2b). The surface temperature was on the average 1-2°C lower than that inside the reactor, which is in accordance with the numerically derived data on (vertical) thermal gradients of a similar polymer-based CE chip in a previous work [7].

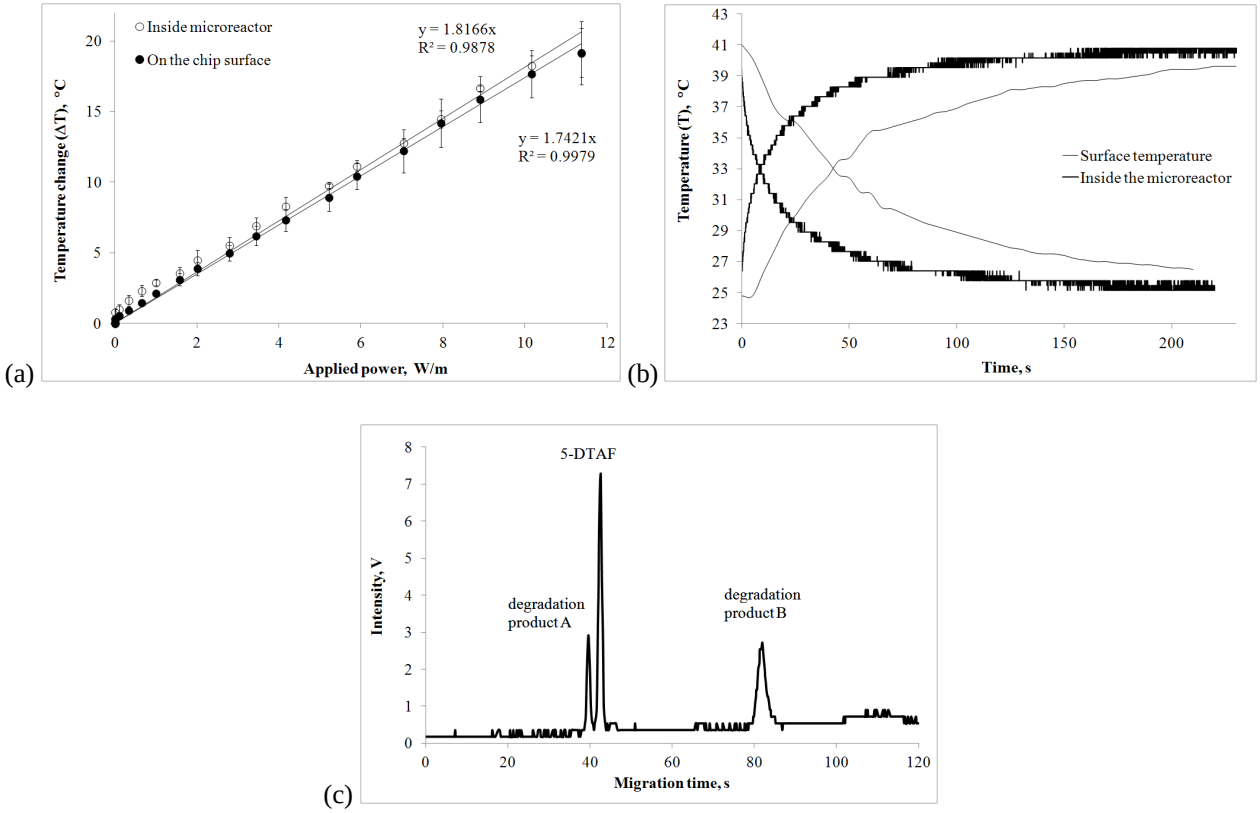

Figure 2. *(a) Temperature change (ΔT) on the microchip surface and inside the reaction chamber as a function of power applied to the resistor (n=3). (b) The heating and cooling times of the absolute temperature on the microchip surface and inside the reaction chamber with constant applied power of 10 Wm^{-1}. (c) Separation (left) of 20 µM 5-DTAF from its degradation products in 20 mM sodium phosphate (pH 12.0) containing 10% glycerol following 120-s-long incubation at 40°C (applied heating power 10 Wm^{-1}). The separation was performed under cathodic EOF at E=600 Vcm^{-1}.*

The effect of thermal gradient (between the reactor and the separation parts) on the separation efficiency was examined by performing an online CE-LIF analysis of 5-DTAF and its degradation products following 120-s-long incubation at 40°C (Fig. 2c). Since the Pt heating element was precisely confined only at the reactor area, the reaction solution was effectively cooled down in the narrow microchannel joining the reactor to the separation channel during the injection step. Thus, the heating power could be kept on during the separation step which allowed one to perform multiple reaction/separation cycles repeatedly without time-lag due to microreactor heating/cooling. Typically, one cycle from microreactor loading to sample detection took less than 4 minutes. Finally, the good repeatabilities of both the migration time (0.24% RSD, n=3) and the peak area (3.9% RSD, n=3) of 5-DTAF evidenced that heating of the reactor part had negligible effect on the separation efficiency.

CONCLUSIONS

The thermal and fluidic characterization of the developed chip demonstrate successful coupling of a heated microreactor to an on-line, thermally isolated electrophoretic separation unit. No problems related to distortion of the flow profile because of unsufficient elimination of heat were observed in the separation part as evidenced by the good figures of merit of the CE-LIF separation of the model compound 5-DTAF. Future work includes numerical modeling of the thermal gradients of the microreactor chip as well as implementation of enzyme assays onto the developed microchip using immobilized cytochrome P450 enzymes.

ACKNOWLEDGEMENTS

Financial support was provided by the Academy of Finland (projects 121882, 251629) and Orion-Farmos research Foundation. Ms Petra Kuisma is gratefully acknowledged for her help with the graphics.

REFERENCES

[1] J.R. Kraly, R.E. Holcomb, Q. Guan, C.S. Henry, Anal. Chim. Acta 2009 (653) 23.
[2] N.J. Petersen, R.P.H. Nikolajsen, K.B. Mogensen, J.P. Kutter, Electrophoresis 2004 (25) 253.
[3] G.Y. Tang, C. Yang, J.C. Chai, H.Q. Gong, Anal. Chim. Acta 2004 (507) 27.
[4] S. Aura, T. Sikanen, T. Kotiaho, S. Franssila, Sens. Actuators B 2008 (132) 397.
[5] T. Sikanen,S. Aura, L. Heikkilä, T. Kotiaho, S. Franssila, R. Kostiainen, Anal. Chem. 2010 (82) 3874.
[6] D. Ross, M. Gaitan, L.E. Locascio, Anal. Chem. 2001 (73) 4117.
[7] T. Sikanen, T. Zwinger, S. Tuomikoski, S. Franssila, et al., Microfluid. Nanofluid. 2008 (5) 479.

CONTACT

*Tiina Sikanen, tel. +358-9-19159169, tiina.sikanen@helsinki.fi