A Simple Microfluidic Chip Design for Fundamental Bioseparation

Alan S. Chan, 1,2 Michael K. Danquah, 2,3 Dominic Agyei, 2 Patrick G. Hartley, 1 and Yonggang Zhu 1

1CSIROMaterialsScience andEngineering, Highett, VIC 3190, Australia
2Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia
3Department of Chemical Engineering, Curtin University of Technology, Sarawak 98009, Malaysia

Correspondence should be addressed to Michael K. Danquah; mkdanquah@curtin.edu.my
and Yonggang Zhu; yonggang.zhu@csiro.au

Received 23 October 2013; Accepted 12 December 2013; Published 8 January 2014

Academic Editor: Ravichandra Potumarthi

Copyright © 2014 Alan S. Chan et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A microchip pressure-driven liquid chromatographic system with a packed column has been designed and fabricated by using poly(dimethylsiloxane) (PDMS). The liquid chromatographic column was packed with mesoporous silica beads of Ia3d space group. Separation of dyes and biopolymers was carried out to verify the performance of the chip. A mixture of dyes (fluorescein and rhodamine B) and a biopolymer mixture (10kDa Dextran and 66kDa BSA) were separated and the fluorescence technique was employed to detect the movement of the molecules. Fluorescein molecule was a nonretained species and rhodamine B was attached onto silica surface when dye mixture in deionized water was injected into the microchannel. The retention times for dextran molecule and BSA molecule in biopolymer separation experiment were 45s and 120s, respectively. Retention factor was estimated to be 3.3 for dextran and 10.4 for BSA. The selectivity was 3.2 and resolution was 10.7. Good separation of dyes and biopolymers was achieved and the chip design was verified.

1. Introduction

High-performance liquid chromatography (HPLC) is a widely used separation technique with numerous implementations in both preparative and analytical systems [1–4]. A wide variety of chromatography media available provides different requirements for various molecular separation modes. The miniaturized HPLC system would offer the advantage of smaller sample size, reduction of dead volume, lower solvent consumption, faster, higher-throughput analysis, and portability of the analytical system, enabling on-site and remote analysis [5, 6]. Despite these advantages, miniaturization of chromatographic systems needs to address some technical issues such as fabrication of chip-based chromatographic systems without compromising separation efficiency [6]. One such challenge is the introduction of stationary phase materials into a microfabricated microchannel [7].

Numerous examples of chip-based chromatographic systems in pharmaceutical and biomedical applications have been reviewed extensively [6, 8–10].

Open-tubular liquid chromatography microchips integrated with a sample injector and electrode demonstrated low chromatographic efficiency [11]. The low efficiency could be attributed to small surface area and relatively large injection volume of the system. A microfabricated device with C18 coated channels was used to demonstrate on-chip phase extraction [12]. However, using a separation column packed with beads may yield better separation efficiency because of higher available surface area per unit volume and reduced diffusion distances through the narrow fluid paths between neighbouring particles [13].

Several microchips with porous polymer monoliths formed in channels via photoinitiated polymerization have been reported [14–18]. Reversed-phase silica particles are also widely used as the stationary phase in HPLC and solid-phase
Figure 1: Schematic design of a poly(dimethylsiloxane) microfluidic chip with integrated HPLC column using mesoporous silica. The dimension of the channel cross-section is 70 \( \mu \text{m} \) depth and 100 \( \mu \text{m} \) width. The diameter of reservoirs and PE membrane port is 2 mm. The silica column dimension was 5 mm long, 70 \( \mu \text{m} \) deep, and 100 \( \mu \text{m} \) wide.

Figure 2: Schematic drawing of fabrication of nickel shim for microfluidic chip manufacturing.

2. Microchip Design and Fabrication

The proposed microfluidic chip design is aimed at improving the separation efficiency and resolution for macromolecules by integrating a HPLC column with mesoporous silica as the stationary phase. The designed chip consists of a mobile-phase channel, an injection channel, a packed silica column, and detection channel. The design consists of three layers of microstructures shown in Figure 1. Various microfluidic ports and separation capillary were constructed in the top layer of the PDMS substrate. Two polyethylene membranes were integrated into the second layer of the chip. These membranes serve as porous frits that keep the silica particles inside the HPLC column at the bottom layer and prevent it from leaking into the microchannels.

In this paper, the fabrication of a cost-effective and easy structured multilayered pressure-driven microchip for reversed-phase liquid chromatography in poly(dimethylsiloxane) (PDMS) is presented. Poly(dimethylsiloxane) (PDMS) is the most dominant polymeric material for microfluidics. This is due to its unique properties such as elastomeric properties, biocompatibility, optical transparency down to 280 nm, hydrophobic surface chemistry, pliability and ease of molding into micron size, and low manufacturing costs [10]. Reversed-phase mesoporous silica was used as a stationary phase in the bottom layer of the microchip liquid chromatographic system. The silica was trapped in between two polyethylene membranes acting as porous frits. Injection chamber and separation channel were fabricated on the top layer of the chip. The performance of the microchip was demonstrated by the separation of the dye mixture, fluorescein and rhodamine B, and the biopolymer mixture of 10 kDa Dextran and 66 kDa bovine serum albumin (BSA).
photoresist (Shipley 5038) that was laminated on a polished stainless steel. The microfluidic chip design was patterned lithographically onto the photoresist by UV illumination ($\lambda = 350–450$ nm) through photomask. The UV source was operated at 20 mJ/cm$^2$. The exposed pattern was then developed in a 20% Na$_2$CO$_3$ solution and replicated as a nickel shim by a two-stage nickel deposition process. A thin nickel layer of 100 nm in thickness was first coated onto the patterned surface by sputter deposition. Secondly, a nickel layer of 150 $\mu$m in thickness was electrodeposited in a nickel sulphamate bath. A mixture of PDMS prepolymer base and curing agent with a weight ratio of 10 to 1 was poured over the shim and then degassed under vacuum before curing at 60$^\circ$C for 2 h. A PDMS substrate with the desired features was obtained and the thickness of each layer of PDMS substrate was kept at ~2 mm. The dimension of the channel cross-section is 70 $\mu$m of depth and 100 $\mu$m of width. The diameter of the reservoirs and PE membrane port is 2 mm. The silica column dimension is 5 mm long, 70 $\mu$m deep, and 100 $\mu$m. About 2 mm holes were punctured through the reservoirs in the top layer and PE membrane ports in the middle layer of the PDMS substrate. The middle and bottom layers of PDMS substrate were irreversibly bonded after pretreatment by oxygen plasma and curing at 60$^\circ$C for 2 h. A 2 mm PE membrane with pore size of 10 $\mu$m was placed on one end whilst silica particles were packed by pulling vacuum on top of the membrane. Another PE membrane was then put on the other end after the surface of the combined substrate was cleaned. Finally, the combined substrate was irreversibly bonded with the top layer of the substrate through pretreatment by oxygen plasma and curing at 60$^\circ$C for 2 h. After chip fabrication was completed, the external tubing was put into the reservoirs and sealed with glue.

3. Experimental

3.1. Materials. Sylgard 184 PDMS prepolymer base and curing agent were purchased from Dow Corning. Mesoporous silica was supplied by CSIRO Material Science and Engineering Division. The properties of mesoporous silica were reported as follows: pore geometry—cubic (Ia3d space group); pore size—10.5 nm; porosity—0.945 cm$^3$/g, and surface area—731 m$^2$/g. Fluorescein sodium salt, rhodamine B, and methanol were purchased from Ajax Fine Chemicals. Dextran Alexa Fluor 568, 10 kDa and BSA Alexa Fluor 488, 66 kDa were purchased from Invitrogen. Deionized water was prepared using Milli-Q system from Millipore. All reagents were of analytical grade.

3.2. Experimental Setup. In the present work, a dye mixture (fluorescein and rhodamine B) and a biopolymer mixture (10 kDa dextran and 66 kDa BSA) were used to demonstrate the applicability of a chip-based reversed-phase liquid chromatographic separation. Figure 3 shows a schematic diagram of the chip LC system. The ends of the microchannels in the microchip were connected to four two-way stop cork valves (V1, V2, V3 and V4) via a separate plastic tube. The valves V1 and V3 were connected to a syringe pump and sample injection syringe pump, respectively. The chip LC system was placed on an inverted microscope with appropriate lens and UV band pass filters. Fluorescence technique with UV
illumination was used to detect molecules. The incoming UV light beam is focused onto the separation channel. Emitted light from sample molecules is then directed to the photomultiplier (PMT) tube for experimental monitoring.

3.3. Methodologies

3.3.1. On-Chip Injection. The mobile-phase inlet and outlet valves, V1 and V2, were opened while the sample inlet and outlet valves, V3 and V4, remained closed. The separation channel was flushed with the mobile-phase (DI water) until the baseline of the PMT tube output was stabilized. The sample plug was then introduced into the separation channel after the mobile-phase inlet and outlet valves (V1 and V2) were closed. Once the mobile-phase flow was halted, the sample inlet and outlet valves (V3 and V4) were then opened to load the sample using a syringe connected to the sample inlet valve V3 for 5 s after which the sample inlet and outlet valves were closed. The mobile-phase valves were then opened and the syringe pump for the mobile-phase was started.

For dye separation experiments, the sample containing 0.05 g/mL of fluorescein and 0.05 g/mL rhodamine B in deionized water was injected into the separation channel after the channel had been flushed with the mobile-phase. Deionised water was replaced by methanol as mobile-phase to elute the molecule attached onto the silica particles.

For biopolymer separation experiments, the sample mixture containing 0.25 mg BSA and 0.03 mg dextran in 1 mL of 0.01 M phosphate buffer saline (PBS) was prepared. The sample was loaded onto the silica column and the microchip was flushed with 0.01 M PBS buffer for 30 s to remove the remaining sample. The buffer was then replaced by a mobile-phase composition of methanol to elute the bounded biopolymers from the silica column.

4. Results and Discussion

4.1. Dye Separation. After the mixture of dyes in deionised water was injected into the separation channel, only one peak was detected as shown in Figure 4. Methanol was then used to elute the retained dye from the silica, and this resulted in a single peak as shown in Figure 5. Microscopic analysis performed in between sample injection and elution showed that the silica particles turned red corresponding to the emission spectrum for rhodamine B. Thus, fluorescein molecules passed through the channel while rhodamine B molecules were retained in the silica packing. Hence, dyes were separated completely. The separation results, as expected, correspond to a conventional reversed-phase chromatography mechanism in which neutral molecules are separated in solution on the basis of their hydrophobicity [36, 37]. Solute retention increases with increasing polarity of the mobile-phase. A polar solvent, such as water, interacts preferentially with basic solutes such as amines. A comparison of the chemical structures rhodamine B \((C_{28}H_{31}ClN_2O_8)\) and fluorescein \((C_{20}H_{12}O_5)\) is shown in Figure 6. rhodamine B is therefore more likely to be retained on the surface of silica compared to fluorescein molecules.

4.2. Biomolecule Separation. Reversed-phase mode HPLC was performed on a chamber packed with mesoporous silica equilibrated with the PBS buffer. The biomolecule mixture sample was loaded onto the silica column. Both molecules were retained and Figure 7 shows the elution of the 10 kDa...
5. Conclusions

A pressure-driven liquid chromatography microfluidic device integrated with mesoporous silica as HPLC stationary phase on PDMS chip has been developed. This LC chip system illustratively provided favorable chromatographic separation for a dye mixture of fluorescein/Rhodamine B and biomolecule mixture of 10 kDa dextran and 66 kDa BSA. Hence, applications previously developed for a conventional HPLC system can potentially be adapted to the microchip LC system. The design of this microfluidic device can also be adapted for various biomolecular separation systems, such as capillary electrophromatography. This becomes feasible by choosing a suitable stationary and mobile-phase and can find use in applications such as point-of-care diagnostics and residue or pathogen detection in food and water.

Conflict of Interests

The authors declares that there is no conflict of interests regarding the publication of this article.

Acknowledgments

The authors gratefully acknowledge Monash University for NSMRF Fund and CSIRO for OCE postgraduate scholarship of A. S. Chan.

References

[1] F. Morales-Trejo, S. V. y. León, A. Escobar-Medina, and R. Gutiérrez-Tolentino, “Application of high-performance liquid chromatography-UV detection to quantification of clenbuterol in bovine liver samples,” Journal of Food and Drug Analysis, vol. 21, no. 4, pp. 414–420, 2013.

[2] T. P. Lee, R. Sakai, N. A. Manaf, A. M. Rodhi, and B. Saad, “High performance liquid chromatography method for the determination of patulin and 5-hydroxymethylfurfural in fruit juices marketed in Malaysia,” Food Control, vol. 38, pp. 142–149, 2014.

[3] C. Czerwenka, M. Lämmerhofer, and W. Lindner, “Micro-HPLC and standard-size HPLC for the separation of peptide stereoisomers employing an ion-exchange principle,” Journal of Pharmaceutical and Biomedical Analysis, vol. 30, no. 6, pp. 1789–1800, 2003.

[4] T. Nema, E. C. Y. Chan, and P. C. Ho, “Applications of monolithic materials for sample preparation,” Journal of Pharmaceutical and Biomedical Analysis, vol. 87, pp. 130–141, 2014.

[5] Y. Shintani, K. Hirako, M. Motokawa et al., “Development of miniaturized multi-channel high-performance liquid chromatography for high-throughput analysis,” Journal of Chromatography A, vol. 1073, no. 1-2, pp. 17–23, 2005.

[6] F. Foret, P. Smekal, and M. Macka, “Chapter 20-Miniaturization and microfluidics,” in Liquid Chromatography, S. Fanali, P. R. Haddad, C. F. Poole, P. Schoenmakers, and D. Lloyd, Eds., pp. 453–467, Elsevier, Amsterdam, The Netherlands, 2013.

[7] J. W. Jorgenson, “Capillary liquid chromatography at ultrahigh pressures,” Annual Review of Analytical Chemistry, vol. 3, no. 1, pp. 129–150, 2010.

[8] M. Borecki, M. L. Korwin-Pawlowski, M. Beblowska, J. Szmidt, and A. Jakubowski, “Optoelectronic capillary sensors in micro-fluidic and point-of-care instrumentation,” Sensors, vol. 10, no. 4, pp. 3771–3797, 2010.

[9] Y. C. Lim, A. Z. Kouzani, and W. Duan, “Lab-on-a-chip: a component view,” Microsystem Technologies, vol. 16, no. 12, pp. 1995–2015, 2010.

[10] J. Zhou, A. V. Ellis, and N. H. Voelcker, “Recent developments in PDMS surface modification for microfluidic devices,” Electrophoresis, vol. 31, no. 1, pp. 2–16, 2010.

[11] M. M. McEnery, J. D. Glennon, J. Alderman, and S. C. O’Mathuna, “Liquid chromatography on-chip,” Biomedical Chromatography, vol. 14, no. 1, pp. 44–46, 2000.

[12] J. P. Kutter, S. C. Jacobson, and J. M. Ramsey, “Solid phase extraction on microfluidic devices,” Journal of Microcolumn Separations, vol. 12, no. 2, pp. 93–97, 2000.

[13] E. Verpoorte, “Beads and chips: new recipes for analysis,” Lab on a Chip, vol. 3, no. 4, pp. 60N–68N, 2003.

[14] X. Chen, H. D. Tolley, and M. L. Lee, “Weak cation-exchange monolithic column for capillary liquid chromatography of peptides and proteins,” Journal of Separation Science, vol. 34, no. 16-17, pp. 2063–2071, 2011.

[15] X. Chen, H. D. Tolley, and M. L. Lee, “Polymeric cation-exchange monolithic columns containing phosphoric acid functional groups for capillary liquid chromatography of peptides and proteins,” Journal of Chromatography A, vol. 1217, no. 24, pp. 3844–3854, 2010.

[16] X. Chen, H. D. Tolley, and M. L. Lee, “Monolithic capillary columns synthesized from a single phosphate-containing dimethacrylate monomer for cation-exchange chromatography of peptides and proteins,” Journal of Chromatography A, vol. 1218, no. 28, pp. 4322–4331, 2011.

[17] Z. Walsh, P. A. Levin, S. Abele et al., “Polymisation and surface modification of methacrylate monoliths in polyimide channels and polyimide coated capillaries using 660 nm light emitting diodes,” Journal of Chromatography A, vol. 1218, no. 20, pp. 2954–2962, 2011.

[18] E. S. Sinitsyna, E. G. Vlakh, M. Y. Rober, and T. B. Tennikova, “Hydrophilic methacrylate monoliths as platforms for protein microarray,” Polymer, vol. 52, no. 10, pp. 2132–2140, 2011.

[19] K. Yao, Y. Xue, Q. Wu, J. Li, Y. Wang, and C. Yan, “Preparation of zirconia silica core shell microspheres and its application for...
highly specific phospholipids enrichment,” *Chinese Journal of Analytical Chemistry*, vol. 41, no. 8, pp. 1214–1219, 2013.

[20] C. G. A. da Silva, C. H. Collins, E. Lesellier, and C. West, “Characterization of stationary phases based on polysiloxanes thermally immobilized onto silica and metalized silica using supercritical fluid chromatography with the solvation parameter model,” *Journal of Chromatography A*, vol. 1315, pp. 176–187, 2013.

[21] S. A. Schuster, B. M. Wagner, B. E. Boyes, and J. J. Kirkland, “Optimized superficially porous particles for protein separations,” *Journal of Chromatography A*, vol. 1315, pp. 118–126, 2013.

[22] X. Zhang, Z. Zhang, L. Wang et al., “Chromatographic evaluation of octadecyl-bonded SiO$_2$/SiO$_2$-based stationary phase for reversed-phase high performance liquid chromatography,” *Journal of Inorganic and Organometallic Polymers and Materials*, vol. 23, no. 6, pp. 1445–1450, 2013.

[23] H. M. Tan, X. Wang, S. F. Soh et al., “Preparation and application of mixed octadecyl- and (3-(c-methylcalix 4 resorcinarene)-hydroxypropoxy)-propylsilyl-appended silica particles as stationary phase for high-performance liquid chromatography,” *Instrumentation Science & Technology*, vol. 40, no. 2-3, pp. 100–111, 2012.

[24] Q. Liu, L.-T. Wang, S.-Q. Dong, Z.-X. Zhang, and L. Zhao, “Preparation and characterization of SiO$_2$/SiO$_2$ core-shell microspheres as RP-HPLC stationary phase,” *Journal of Inorganic and Organometallic Polymers and Materials*, vol. 21, no. 4, pp. 941–945, 2011.

[25] K. Hormann and U. Tallarek, “Analytical silica monoliths with submicron macropores: current limitations to a direct morphology-column efficiency scaling,” *Journal of Chromatography A*, vol. 1312, pp. 26–36, 2013.

[26] K. Liu, P. Aggarwal, J. S. Lawson, H. D. Tolley, and M. L. Lee, “Organic monoliths for high-performance reversed-phase liquid chromatography,” *Journal of Separation Science*, vol. 36, no. 17, pp. 2767–2781, 2013.

[27] M. Iwasaki, N. Sugiyama, N. Tanaka, and Y. Ishihama, “Human proteome analysis by using reversed phase monolithic silica capillary columns with enhanced sensitivity,” *Journal of Chromatography A*, vol. 1228, pp. 292–297, 2012.

[28] C. M. Olivia, P. G. G. Burnett, D. P. Okinyo-Owiti, J. Shen, and M. J. T. Reaney, “Rapid reversed-phase liquid chromatography separation of cyclolinopeptides with monolithic and microparticulate columns,” *Journal of Chromatography B*, vol. 904, pp. 128–134, 2012.

[29] J. A. Wolfe, M. C. Breadmore, I. P. Ferrance et al., “Toward a microchip-based solid-phase extraction method for isolation of nucleic acids,” *Electrophoresis*, vol. 23, no. 5, pp. 727–733, 2002.

[30] A. Ishida, T. Yoshihawa, M. Natsume, and T. Kamidate, “Reversed-phase liquid chromatography on a microchip with sample injector and monolithic silica column,” *Journal of Chromatography A*, vol. 1132, no. 1-2, pp. 90–98, 2006.

[31] J.-W. Choi, C. H. Ahn, S. Bhansali, and H. T. Henderson, “New magnetic bead-based, filterless bio-separator with planar electromagnet surfaces for integrated bio-detection systems,” *Sensors and Actuators B*, vol. 68, no. 1–3, pp. 34–39, 2000.

[32] G.-L. Lettieri, A. Dodge, G. Boer, N. F. De Rooij, and E. Verpoorte, “A novel microfluidic concept for bioanalysis using freely moving beads trapped in recirculating flows,” *Lab on a Chip*, vol. 3, no. 1, pp. 34–39, 2003.

[33] M.-I. Aguilar, “HPLC of peptides and proteins,” in *HPLC of Peptides and Proteins: Methods and Protocols*, pp. 3–8, 2003.

[34] S. H. Hwang, Y. Maitani, K. Takayama, and T. Nagai, “High entrapment of insulin and bovine serum albumin into neutral and positively-charged liposomes by the remote loading method,” *Chemical and Pharmaceutical Bulletin*, vol. 48, no. 3, pp. 325–329, 2000.