ANALYTICAL SCIENCES NOVEMBER 2015, VOL. 31

A Portable Liquid Chromatograph with a Battery-operated Compact Electroosmotic Pump and a Microfluidic Chip Device with a Reversed Phase Packed Column

Akihiko ISHIDA,*† Mitsutaka FUJII, Takehiro FUJIMOTO,* Shunsuke SASAKI,* Ichiro YANAGISAWA,** Hirofumi TANI,* and Manabu TOKESHI***

**Division of Applied Chemistry, Faculty of Engineering, Hokkaido University, Kita 13 Nishi 8, Sapporo 060–8628, Japan*

***Nano Fusion Technologies, Inc., c/o B-M202 Collaborative Research (CCR) Bldg., Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153–8505, Japan*

A compact and lightweight liquid chromatography system is presented with overall dimensions of 26 cm width × 18 cm length × 21 cm height and weight of 2 kg. This system comprises a battery-operated compact electroosmotic pump, a manual injector, a microfluidic chip device containing a packed column and an electrochemical detector, and a USB bus-powered potentiostat. The pumping system was designed for microfluidic-based reversed-phase liquid chromatography in which an electroosmotically generated water stream pushes the mobile phase *via* a diaphragm for the output. The flow rate ranged from 0 to 10 μL/min and had a high degree of precision. The pumping system operated continuously for over 24 h with dry batteries. The column formed in the microfluidic device was packed with 3-μm ODS particles with a length of 30 mm and a diameter of 0.8 mm. The results presented herein demonstrate the performance of the pumping system and the column using alkylphenols, catecholamine, catechin, and amino acids.

Keywords Electroosmotic pump, liquid chromatography, microfluidic chip, packed column

(Received July 13, 2015; Accepted August 13, 2015; Published November 10, 2015)

Introduction

High performance liquid chromatography (HPLC) is an advanced chemical analysis technique. It plays an important role in separation analysis in scientific research and industry because of its versatility and reliability. However, most conventional HPLC systems are characterized by their bulky size and substantial weight. The use of such systems is therefore limited to special benches in laboratories. Accordingly, samples need to be transferred to laboratories and analytical results are only obtained several days after sample collection. These delays hamper quick recognition and real time monitoring of field situations.

We believe there is a potential need for on-site application of HPLC systems in various fields such as product development, plant management, quality control, food analysis,[1] wastewater management, environmental investigation,[2–4] forensic analysis, clinical diagnosis, veterinary science, and sports science. This wide array of applications suggests both indoor and outdoor uses for HPLC systems. Thus, such systems require compactness, lightness of weight, portability, the ability to be operated with batteries, and robustness. The miniaturization of instruments is expected to reduce the analysis time and cost. Furthermore, miniaturized systems may also offer the benefits of small sample volume, low consumption of solvents and power, and reduced waste. A further potential application is parallel analysis with multiple pieces of equipment, which may only require the same footprint as that of a conventional system.

To date, very little research has been reported on the development of portable chromatographs.[2–5] In 1986, Otagawa *et al.* presented a portable liquid chromatograph with electrochemical detection.[6] The pump was operated by a battery; however, detailed data associated with on-site use including its dimensions and weight were not given. A decade later, Baram reported a portable liquid chromatograph that had dimensions of 530 mm × 200 mm × 300 mm (w × l × h), which are similar to those of a conventional HPLC pump, and weighed 14 kg.[7] This equipment required a regular AC power supply. In 1997, Tulchinsky and St. Angelo designed a portable liquid chromatograph operated by a battery (12 V DC).[8] Although the dimensions (410 mm × 250 mm × 230 mm; w × l × h) were similar to those of the above system, the weight was reduced to approximately 10 kg. Boring *et al.* also presented a portable ion chromatograph that fits in a standard briefcase (280 × 430 × 150 mm) with a weight of 10 kg.[9] Recently, Sharma *et al.* presented a hand-portable liquid chromatograph based on

† To whom correspondence should be addressed.
E-mail: ishida-a@eng.hokudai.ac.jp
M. F. present address: Quality Assurance Department, Takeda Giken Service, Ltd., 4720 Takeda, Mitsui, Hikari, Yamaguchi 743–8502, Japan.
I. Y. present address: Science Solutions International Laboratory, Inc., 2-21-7 Naka-cho, Meguro, Tokyo 153–0065, Japan.

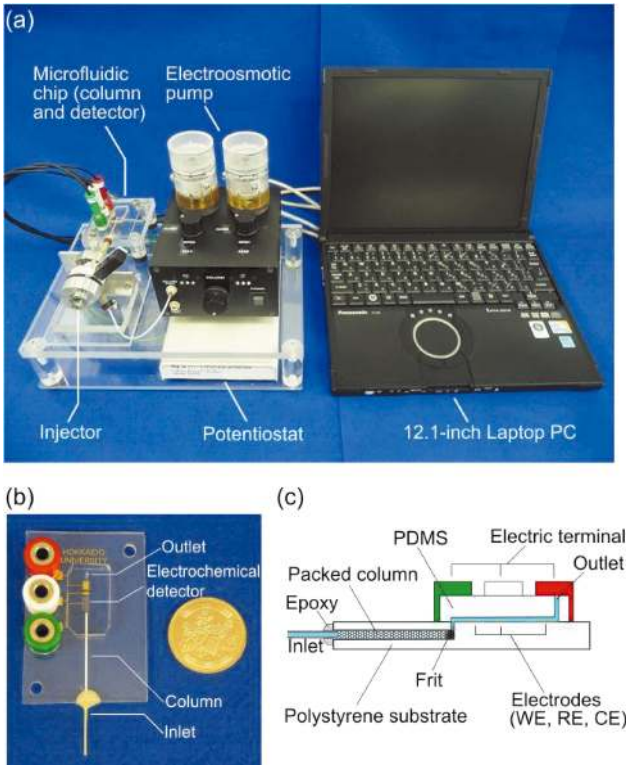

Fig. 1 Photographs of (a) portable liquid chromatograph and (b) microfluidic chip device and (c) schematic side view of the device.

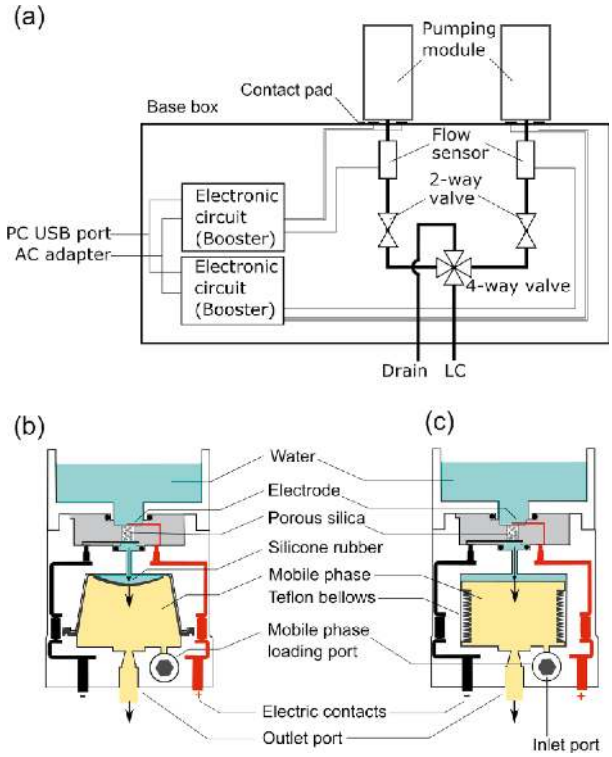

Fig. 2 Schematic diagrams of (a) electroosmotic pumping system and electroosmotic pumping modules with (b) silicone rubber diaphragm and (c) Teflon bellows diaphragm.

a battery-operated lightweight (1.4 kg) pumping system.[10]

Microfabrication or lab-on-a-chip technologies have received considerable attention in miniaturized analytical systems. These technologies are capable of facilitating further system miniaturization. In particular, the resulting miniaturized systems provide short analysis time while minimizing sample volume and solvent consumption. The miniaturization of HPLC systems as per these technologies has been vigorously conducted[11] since the first attempt by Manz *et al.*[12,13] However, very little research has been published on fully miniaturized HPLC systems based on microfabricated devices. The majority of such research has focused on the fabrication of miniaturized columns in microfluidic format.[14-23] These systems still use conventional HPLC pumps and detectors including laser-induced fluorescent microscopes.

To miniaturize pumping systems, some efforts have been made toward a microfluidic format using electrochemistry.[24-30] Xie *et al.* attained the integration of dual pumps and a column on a microchip to conduct gradient elution.[26] In this microdevice, the gas formation resulting from the electrolysis of water at the electrodes was used as a motive force or delivery of the mobile phase. Borowsky *et al.* also fabricated a microfluidic device containing an electroosmotic pump consisting of a microchannel with porous silica beads and a sol-gel monolithic separation column.[29] However, these systems had no flow sensors and the flow velocity of the mobile phase was defined as an applied current or voltage instead of a flow rate, as is commonly used in conventional HPLC. These devices may be expensive because the fabrication process is complicated and the malfunction of just one component may necessitate the replacement of the whole device. Electroosmotic pumps require high-voltage power supplies because of the very high applied voltages (hundreds to thousands of volts), which would be a great disadvantage in on-site analysis.

In this study, we developed a prototype of a portable liquid chromatograph by constructing a battery-operated compact electroosmotic pumping system and a microfluidic chip device containing a low-pressure packed column and an electrochemical detector for on-site use (Fig. 1(a)). This system had dimensions of 260 mm × 180 mm × 210 mm (w × l × h) and a weight of 2 kg. The performance of the pumping system and chromatographic separations were demonstrated.

Experimental

Construction of portable liquid chromatography system

Electroosmotic pump. The pumping system used in this study consisted of twin cylindrical pumping modules and a base box containing flow sensors (LG16-0150A, Sensirion, Switzerland), an electric circuit, and a manifold (Fig. 2(a)). The electric circuit contained a DC-DC power boost converter designed for 12 V DC input to 60 V DC output. The footprint of the base box was approximately 100 mm × 150 mm. The pumping module had dimensions of 40 mm diameter and 68 mm height. The main part of the module was created using polymethyl methacrylate (PMMA). The module contained a water reservoir, a porous silica material (N211, Nano Fusion Technologies, Japan), a lower chamber, a diaphragm, an inlet port, an outlet port, and spring loaded electrical contacts (Figs. 2(b) and 2(c)). Two platinum wire electrodes were attached to the porous silica to generate electric fields inside the silica pores. The porous silica material was retained in a holder created with poly(*p*-phenylene sulfide) (PPS, FORTRON 1130A64, Polyplastics Co., Ltd.) and with sufficient mechanical strength to resist back pressure. The upper reservoir and lower chamber were filled

with the deionized (DI) water and the mobile phase, respectively, and were connected *via* the porous material. The volume of the lower chamber was ~8 mL. The mobile phase was separated from the DI water with a silicone rubber or Teflon bellows diaphragm placed inside the lower chamber, which also prevented the incursion of bubbles into the column.

The pumping modules could be mounted by simply screwing into a fitting on the base box, thus providing electrical contact between the modules and the electric circuit *via* contact pads fixed on the top side of the base box and connections to the manifold. The manifold was built with tubing, two-way valves, and a four-way valve to switch the flow of solvents.

In this pumping system, the electric voltage (ranging from 0 to 60 V) was applied to the porous silica material using a booster circuit, which generated an electroosmotic flow of water in the silica pores. The resulting water stream pressed on the diaphragm, leading to discharge of the mobile phase.

Microfluidic chip device. The fabrication of microfluidic chips was described in a previous paper.[31] Briefly, the microfluidic devices were formed through the assembly of two separate components, an upper polydimethylsiloxane (PDMS) component containing the microchannel and a lower polystyrene (PS) component containing a column and electrodes (Figs. 1(b) and 1(c)). To prepare the PS component, a PS plate (1.7 mm thick, Tamiya Inc., Japan) was cut into rectangular pieces (60 mm × 40 mm). A hole to serve as a column (0.8 mm diameter) was manually drilled into the 1.7-mm-thick side of the PS substrate to the desired length. The drilling was performed using a commercially available drill bit and a drill bit guide prepared in-house. An outlet hole (200 mm) was also drilled into the top face of the PS substrate to access the bottom end of the column. The substrate and hole were rinsed with methanol and water and then dried using an air gun.

After the drilling, detection electrodes were fabricated on the top face of the PS substrate using standard microfabrication technologies. A 100-nm-thick Au film was vacuum-evaporated onto the substrate. A positive photoresist (OFPR-800, Tokyo Ohka Kogyo, Japan) was then spun onto the Au-coated substrate. The resist-coated substrate was exposed to UV light for 10 s through a photomask created by Topic Co., Ltd. (Japan). The substrate was then developed in NMD-3 Developer (Tokyo Ohka Kogyo, Japan). The substrate was post-baked at 90°C for 10 min. After cooling, the Au layer was etched in a 1:1 w/v% I_2/KI solution for approximately 1 min. The remaining photoresist was removed in methanol. The substrate with patterned electrodes was rinsed with water and air dried.

Once electrode fabrication was completed, a small frit of glass wool was placed at the bottom end of the column. Dry C18 silica particles (Wakosil 3C18AR, Wako Pure Chemical Industries, Japan) were placed in a 200-mL plastic pipette tip, which was inserted into the inlet of the column. Using a water aspirator, suction was applied at the outlet hole until the height of the packed bed reached ~30 mm. After removal of the tip, the top end of the packed bed was manually pressed with the flat side of a drill bit. Then, PEEK tubing (1/32 inch o.d., 63.5 μm i.d., 30 mm length) was inserted 5 mm into the inlet of the column and fixed with epoxy glue. No leakage of the particles through the PEEK tubing was observed without a top frit.

The upper PDMS chip containing a microchannel was fabricated by soft lithography. A master was made on a glass substrate with standard photolithography using an SU-8 photoresist and a negative photomask. A degassed 10:1 mixture of PDMS monomer and curing agent (Sylgard 184, Dow Corning Toray, Japan) was poured onto the master and cured at room temperature overnight and at 75°C for 10 min. After peeling the PDMS from the master, it was cut to a chip size of 15 mm × 25 mm and the outlet hole was punched. The PDMS was rinsed with water and methanol and then dried with an air gun. A tube was inserted into the outlet of the microchannel and fixed by pouring uncured PDMS around the periphery of the tubing.

The PDMS layer was bonded onto the PS substrate without any surface treatment, such as O_2 plasma, so that the outlet of the column and the inlet of the microchannel were aligned and the microchannel component was perpendicular to the set of electrodes (Fig. 1(b)).

Assembly. The electroosmotic pump, an injector (Chemco Plus Scientific Co., Ltd., Japan), the microfluidic chip, and an electrochemical analyzer ALS1232A (BAS Inc., Japan) were mounted onto an acrylic base, as shown in Fig. 1(a). The injection volume was 10 or 20 nL. The electroosmotic pump and the electrochemical analyzer were connected to a laptop computer through a USB connection.

Operation of liquid chromatography system

To operate the pumping system, the pump was connected to a 12 V DC power source (AC adaptor or batteries). This operation was accomplished with software written in-house using the programming language C++. Electric voltage (ranging from 0 to 60 V) was continuously applied to the porous silica material according to the flow rate set using the software. The flow rate was monitored with flow sensors and controlled by adjusting the applied voltage using PID algorithm.

Chromatographic measurement was performed using the amperometric *i*–*t* curve mode of the electrochemical analyzer. The operation and data collection were performed with software supplied by the manufacturer. The chromatograms were recorded at previously optimized detection potentials. Sample injection was performed after stabilization of the baseline. All experiments were performed at room temperature. Ultrapure DI water was from a Millipore Milli-Q water purification system.

Results and Discussion

Electroosmotic pump

Commonly, electroosmotic pumps suffer from bubble formation due to electrolysis of water at the electrodes and such bubbles can seriously damage column and detector performance. In addition, flow rate is affected by the composition of the mobile phase, such as its pH and the concentrations of organic solvents and ionic species. In this study, the pumping system was designed to have an indirect delivery mechanism (Figs. 2(b) and 2(c)). In our preliminary study, typical flow rates in the microchip chromatography system that we constructed were 5 – 10 μL/min, and the corresponding maximum back pressure was estimated to be approximately 750 kPa (7.5 bar; 109 psi). The pumping system was then designed to meet these flow conditions.

The flow rate of the electroosmotic flow generated in a porous material at a given pressure difference (back pressure), Δp, can be approximated by[32,33]

$$Q = N_p \left(\frac{\pi \bar{a}^2 \varepsilon \zeta}{\eta \tau L} \Delta V - \frac{\pi \bar{a}^4}{8 \eta \tau L} \Delta p \right), \quad (1)$$

where Q is the flow rate, N_p is the number of pores, $\bar{a}$ is the mean radius of pores, ε is the dielectric constant, ζ is the zeta-potential, η is the dynamic viscosity, τ is the mean tortuosity

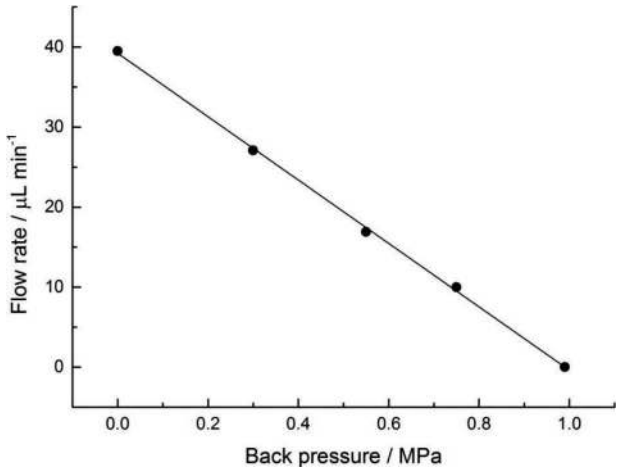

Fig. 3 Plot of maximum flow rate *vs.* back pressure (*Q*–*p* curve) obtained using a porous 4-mm-long material. Solvent: water.

(the ratio of the length of the meandering pore to that of the material), L is the length of the porous material, and ΔV is the voltage applied to both ends of the material.

Equation (1) can also be expressed as a function of Δp at a given length L_0 and voltage ΔV by using Q_{max} and Δp_{max}, where Q_{max} is the maximum flow rate at $\Delta p = 0$ Pa and Δp_{max} is the maximum back pressure that balances with the electroosmotic flow resulting in $Q = 0$ μL/min:

$$Q = -\frac{Q_{max}}{\Delta p_{max}}\Delta p + Q_{max} \quad (2)$$

If the values of Q_{max} and Δp_{max} are known at L_0, this equation can be applied to another length, L:

$$Q = -\frac{Q_{max}L_0}{\Delta p_{max}L}\Delta p + \frac{Q_{max}L_0}{L} \quad (3)$$

In this study, N211 cylindrical porous silica was used (7 mm diameter, 1 mm length, Nano Fusion Technologies Inc.). The optimum length of the porous silica was estimated using Eq. (3). Before this estimation, the values of Q_{max} and Δp_{max} were experimentally obtained at 30 V (L_0 = 1 mm) by pumping water at various back pressures. This experiment was performed by connecting the porous silica pumping system to an in-house pressurizer constructed using an AP100N digital automatic pressure controller (ACE Inc., Japan) and a compressed air cylinder. The following values were obtained: Q_{max} = 9 μL/min and Δp_{max} = 500 kPa. Finally, by substituting values of Q_{max}, Δp_{max}, $Q \geq 10$ μL/min, and $\Delta p = 0.75$ MPa into Eq. (3), the length $L \leq 4$ mm was obtained. In a subsequent study, a shorter length of porous material exhibited poorer flow rate stability, which may have been due to the significant electric field strength ($\Delta V/L$) in the shorter length of pores. Therefore, the length of 4 mm was chosen for further study.

Porous silica material with a length of 4 mm was prepared. With this material, the flow rates were specifically measured by varying the back pressure from 0 MPa to 1.0 MPa at an applied voltage of 60 V. The back pressures were adjusted in the same manner as described above. As shown in Fig. 3, a good linear relationship between flow rate and back pressure was obtained with the regression equation $y = -39.6x + 39.2$ ($r = -0.9996$). This result confirms that a flow rate of 10 μL/min was obtained at 0.75 MPa.

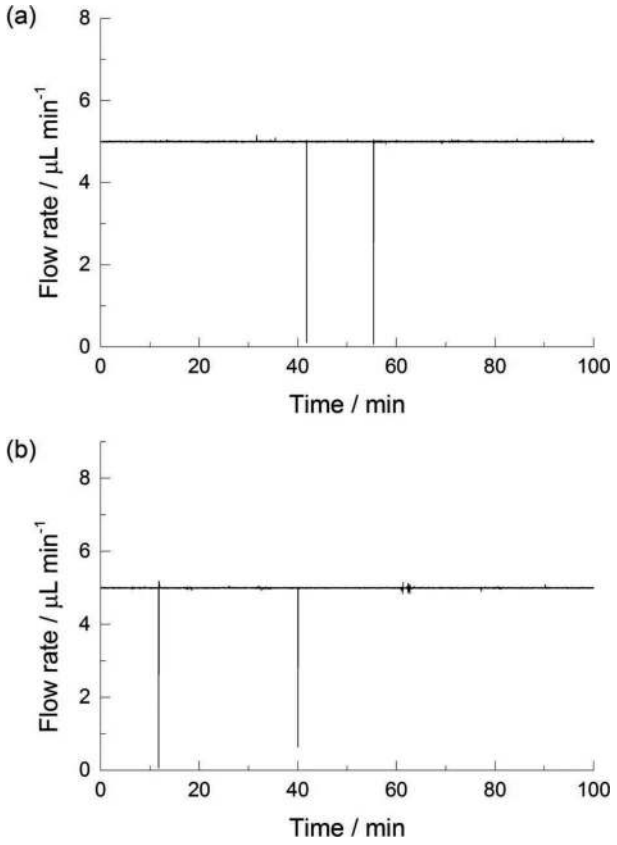

Fig. 4 Flow rate stabilities of electroosmotic pumps with (a) silicone rubber diaphragm and (b) Teflon bellows diaphragm. Tests were performed by connecting the pump to a microfluidic chip device containing a 0.8 mm × 30 mm packed column *via* an injector. Flow rate was set at 5 μL/min for each experiment. Solvent: 20% methanol-water. Several negative peaks were due to external noise.

Next, a pumping system was built with the porous silica material (L = 4 mm). The performance of the pumping system was tested by connecting it to a microfluidic chip. The flow rate reached a maximum of 10 μL/min at a maximum applied voltage of 60 V when using water as the mobile phase. However, the maximum flow rate was approximately 7 μL/min for a 1:2 water/methanol ratio. This flow rate was due to the increased pressure resulting from the viscosity of the mixed solvent being higher than that of water.

The stability of the flow rate was examined by continuous pumping at 5 μL/min for 100 min using a 4:1 water/methanol ratio. Figure 4 demonstrates that a pulse-free continuous flow was produced with a very small variation (RSD, 0.17%) ignoring noise peaks. Next, the reproducibility of the flow rate was examined by performing chromatographic separation of alkylphenols at 5 μL/min for three times using the electroosmotic pump and an MP710i micro HPLC pump (GL Sciences Inc., Japan) because the retention time can be used as the measure of reproducibility of flow rate. Figure 5(a) demonstrates no obvious variations in the retention times of alkylphenols. The RSDs of the retention times were 0.21%, 0.14%, and 0.15% for phenol, 2-methylphenol, and 2-ethylphenol, respectively, with the electroosmotic pump, while the RSDs of the same were 0.44%, 0.22%, and 0.24%, respectively, with the HPLC pump. Moreover, Fig. 5 shows that the respective retention times obtained with the electroosmotic pump are identical to those

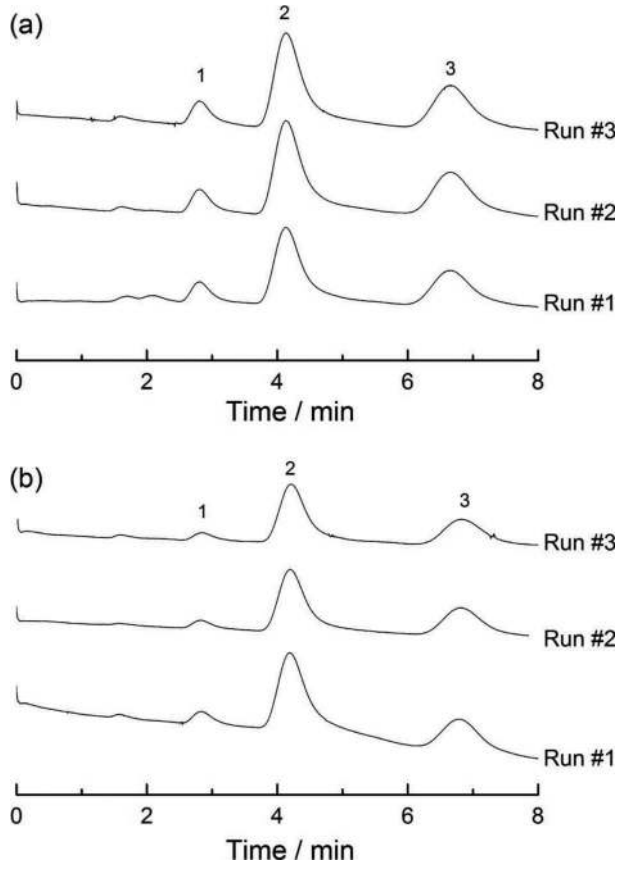

Fig. 5 Reproducibilities of flow rates during chromatographic separation using (a) electroosmotic pump with silicone rubber diaphragm and (b) micro-HPLC pump. A microfluidic chip device with 0.8 mm × 30 mm packed column was used. Model compounds: 1, phenol; 2, 2-methylphenol; 3, 2-ethylphenol. The mobile phase: A: water containing 0.05 M $NaClO_4$, B: methanol; A:B = 1:1 (v/v); flow rate, 5 μL/min; detection, +0.6 V *vs.* Au.

with the HPLC pump with an error of 0.9% (phenol). These results confirm that the present pumping system gives a good reproducibility of flow rate.

The electroosmotic pumping system can be operated by batteries (eight C-size alkaline batteries, 12 V). Under such operation, a durability test was performed with 100% methanol at a flow rate of 5 μL/min. Figure S1 (Supporting Information) shows that the flow rate remains constant for at least 24 h. The pumping system was shut down at approximately 26 h because of voltage depression of the batteries (from an initial voltage of 12.98 to 9.84 V). This result demonstrates the very low power consumption of the pumping system, which represents an advantage over other electroosmotic pumps that require several thousand volts and is useful for on-site analysis. Therefore, the present liquid chromatography system is more portable because the electrochemical analyzer can also be powered by a USB bus.

Microfluidic chip

The configuration of the microfluidic chip minimizes the dead volume between the column end and the detector (10 nL). This device was fabricated using a PS plate that offered ease of fabrication, cost effectiveness, and chemical resistance to organic solvents (*e.g.*, methanol and acetonitrile) typically used for a mobile phase. This device contained a column packed with 3-μm C18 particles. To prepare a column, a dry packing technique previously described was used because the conventional slurry method requires a high pressure that damages the structure of the column. The empty 0.8-mm-diameter column was created manually with a commercially available drill bit as described under the Experimental Section. The length of the column was limited to <30 mm because of the available length of the drill bit.

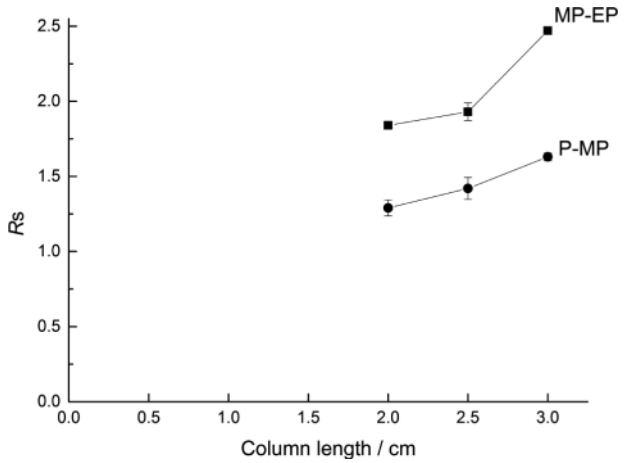

Fig. 6 Plots of alkylphenol resolution *vs.* column length. P, phenol; MP, 2-methylphenol; EP, 2-ethylphenol.

In this study, the effect of column length on its performance was examined. Then, chromatographic separations were performed using the microfluidic chips separately fabricated with different column lengths (20 – 30 mm). Figure S2 (Supporting Information) shows plots of the retention time of alkylphenols *versus* column length. The alkylphenols were well separated by a methylene group in the respective microfluidic chips examined in this study. Figure S2 also displays a highly linear relationship, which is in agreement with the equation given by $t_R = L_c(1 + k')/u$, where L_c is the column length, k' is the capacity factor, and u is the linear velocity of the mobile phase. Therefore, the packing technique used in this study produced a uniform column packing density. However, the plots exhibit a common intersection point at a column length of 7.5 mm, which is possibly due to (i) the length of the net-packed bed being shorter than the nominal length of the column and (ii) the time lag when the solutes transferred to the top of the column after injection.

Figure 6 shows the peak resolutions between phenol and 2-methylphenol and between 2-methylphenol and 2-ethylphenol for the respective column lengths. Each column examined here exhibits a resolution value greater than 1.25 for each of the compounds. In particular, a baseline separation was achieved with a column length of 30 mm. Accordingly, the microfluidic chip device with a column length of 30 mm was used for further studies.

The electrochemical flow cell was formed by bonding a PDMS channel and the PS substrate without any surface treatment. This detector configuration enables easy cleaning of the electrode surface (required due to electrode fouling). For highly sensitive electrochemical detection, a single comb-like array electrode was designed as shown in Fig. 1(b). The electrode array consisted of 24 microelectrodes of 50 μm width and 200 μm spacing. In separate experiments, a detection limit at nanomolar level was obtained. The specific design and performance will be published elsewhere.

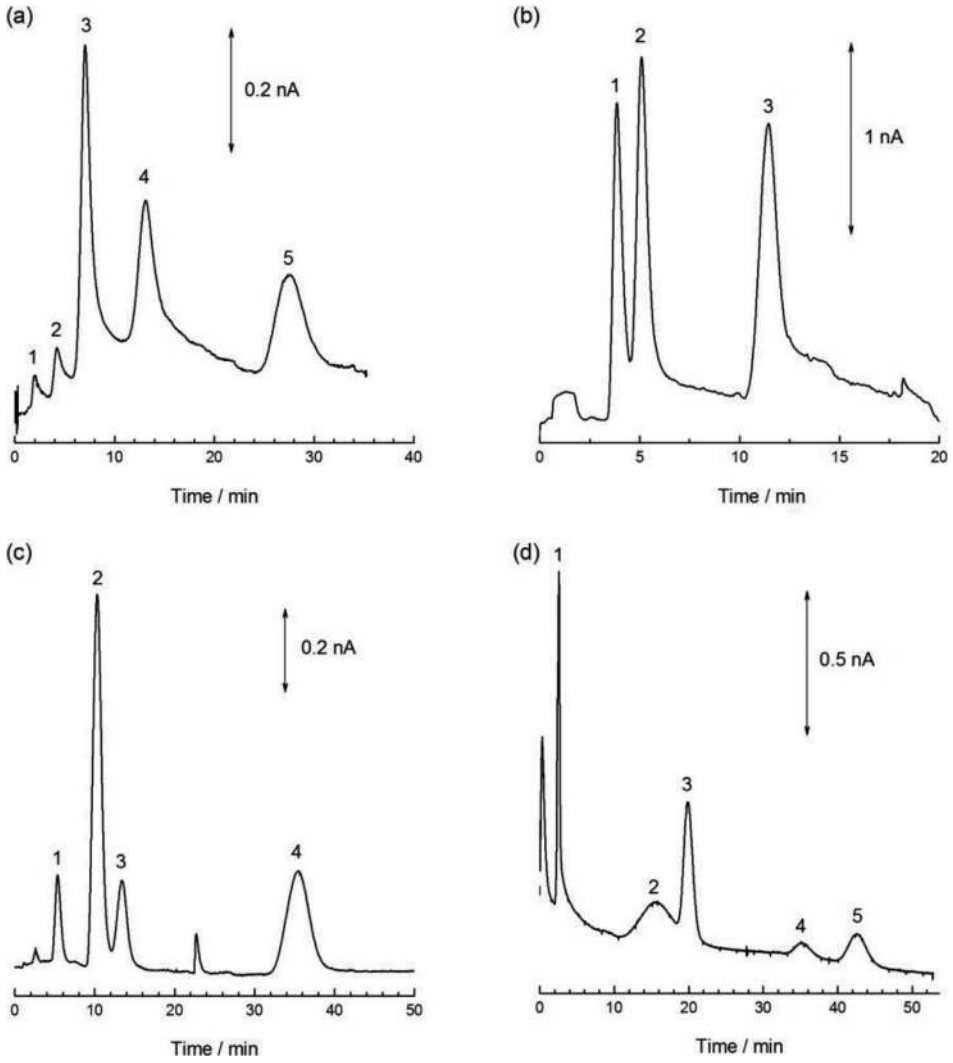

Fig. 7 Separation of (a) alkylphenols, (b) catecholamine, (c) catechin, and (d) amino acids using present liquid chromatograph. Peaks: (a) 1, phenol; 2, 2-methylphenol; 3, 2-ethylphenol; 4, 2-propylphenol. (b) 1, noradrenaline; 2, adrenaline; 3, dopamine. (c) 1, (+)-Catechin; 2, epicatechin; 3, epigallocatechin gallate; 4, epicatechin gallate. (d) 1, glycine; 2 and 3, unreacted derivatization reagent; 4, tyrosine; 5, valine. Analytical conditions: (a) mobile phase: A: 0.1 M $NaClO_4$, B: methanol; A:B = 1:1 (v/v); flow rate: 5 µL/min; detection: +0.6 V *vs.* Au. (b) mobile phase: A: 50 mM phosphate, 50 mM citrate, 100 mg/L octanesulphonate, 40 mg/L EDTA-2Na (pH 3.0) B: methanol; A:B = 95:5 (v/v) ; flow rate: 5 µL/min; detection: +0.6 V *vs.* Au. (c) mobile phase: water-methanol-0.5 M phosphate (7:2:1 v/v/v); flow rate: 5 µL/min; detection: +0.6 V *vs.* Au. (d) mobile phase: A: 50 mM sodium perchlorate; B: methanol; A:B = 1:1 (v/v); flow rate: 4 µL/min; detection: +0.4 V *vs.* Au. Pre-column derivatization of amino acid was performed with NPCA.

Chromatographic separation with the present system

Chromatographic separation with the present miniaturized liquid chromatograph was tested using alkylphenols, catecholamine, catechin, and amino acids. For the electrochemical detection of amino acids, off-line pre-column derivatization was performed with succinimidyl (2*R*)-6-(tetrahydro-2*H*-pyran-2-yloxy)-2,5,7,8-tetramethylchroman-2-carboxylate (NPCA; Tokyo Chemical Industry Co., Ltd., Japan). Figure 7 shows that good separations were achieved for all of the tested compounds. These results indicate that on-site analysis is feasible with the present system.

Our ongoing work involves improvement of the maximum flow rate in the present pumping system and the construction of a microfluidic UV/Vis flow cell. The use of a compact light source and a fiber optic spectrophotometer should provide a portable liquid chromatograph.

Conclusions

We built a portable liquid chromatograph by constructing a battery operated compact pumping system based on electroosmosis and a microfluidic device containing a column and an electrochemical detector. The pumping system exhibited high precision and a long duration of battery operation. The combination of the pumping system and the microfludic device provided good separation for various electroactive compounds, demonstrating its feasiblity for on-site chromatographic analysis.

Acknowledgements

This research was supported by the Adaptable and Seamless Technology Transfer Program through Target-driven R&D, Japan Science and Technology Agency. This study was also partly supported by a Grant-in-Aid for Scientific Research (C) (23550087) from the Japan Society for the Promotion of Science.

Supporting Information

Figure S1 Durability of the electroosmotic pump powered by eight C alkaline batteries (12 V). Figure S2 Plots of retention time of alkylphenols versus column length. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

1. Z. Zhang, X. Tang, D. Wang, Q. Zhang, P. Li, and X. Ding, *PLoS One*, **2015**, *10*, e0123266.
2. N. N. Suscchik, M. I. Gladyshev, G. S. Kalachova, and V. E. Guseynova, *J. Chromatogr. A*, **1995**, *695*, 223.
3. L. Čapka, Z. Večeřa, P. Mikuška, J. Šesták, V. Kahle, and A. Bumbová, *J. Chromatogr. A*, **2015**, *1388*, 167.
4. M. A. Nelson, G. Arther, D. Maud, and D. S. Hage, *Anal. Chem.*, **2004**, *76*, 805.
5. G. I. Baram, M. A. Grachev, N. I. Komarova, M. P. Perelroyzenyu, A. Bolvanov, S. V. Kuzmin, V. V. Kargaltsev, and E. A. Kuper, *J. Chromatogr. A*, **1983**, *264*, 69.
6. T. Otagawa, J. R. Stetter, and S. Zaromb, *J. Chromatogr. A*, **1986**, *360*, 252.
7. G. I. Baram, *J. Chromatogr. A*, **1996**, *728*, 387.
8. V. M. Tulchinsky and D. E. St. Angelo, *Field Anal. Chem. Technol.*, **1998**, *2*, 281.
9. C. B. Boring, B. C. Bradley, P. K. Dasgupta, and S. Anna, *J. Chromatogr. A*, **1998**, *804*, 45.
10. S. Sharma, A. Plistil, R. S. Simpson, K. Liu, P. B. Farnsworth, S. D. Stearns, and M. L. Lee, *J. Chromatogr. A*, **2014**, *1327*, 80.
11. J. P. Kutter, *J. Chromatogr. A*, **2012**, *1221*, 72.
12. A. Manz, Y. Miyahara, J. Miura, Y. Watanabe, H. Miyagi, and K. Sato, *Sens. Actuators, B*, **1990**, *1*, 249.
13. G. Ocvirk, E. Verpoorte, A. Manz, and H. M. Widmer, in Proceedings of the International Solid-State Sensors and Actuators Conference - TRANSDUCERS '95 (1995), doi: 10.1109/sensor.1995.717342.
14. A. Ishida, T. Yoshikawa, M. Natsume, and T. Kamidate, *J. Chromatogr. A*, **2006**, *1132*, 90.
15. J. Liu, C. F. Chen, C. W. Tsao, C. C. Chang, C. C. Chu, and D. L. DeVoe, *Anal. Chem.*, **2009**, *81*, 2545.
16. K. Faure and F. Karine, *Electrophoresis*, **2010**, *31*, 2499.
17. W. De Malsche, D. Clicq, V. Verdoold, P. Gzil, G. Desmet, and H. Gardeniers, *Lab Chip*, **2007**, *7*, 1705.
18. N. V. Lavrik, L. T. Taylor, and M. J. Sepaniak, *Anal. Chim. Acta*, **2011**, *694*, 6.
19. J. Op De Beeck, M. Callewaert, H. Ottevaere, H. Gardeniers, G. Desmet, and W. De Malsche, *Anal. Chem.*, **2013**, *85*, 5207.
20. S. Thurmann, L. Mauritz, C. Heck, and D. Belder, *J. Chromatogr. A*, **2014**, *1370*, 33.
21. S. Thurmann, A. Dittmar, and D. Belder, *J. Chromatogr. A*, **2014**, *1340*, 59.
22. S. Sandron, B. Heery, V. Gupta, D. A. Collins, E. P. Nesterenko, P. N. Nesterenko, M. Talebi, S. Beirne, F. Thompson, G. G. Wallace, D. Brabazon, F. Reganb, and B. Paull, *Analyst*, **2014**, *139*, 6343.
23. M. Araya-Farias, M. Tavernaa, M. Woytasikc, F. Baylec, M. Guerrouached, I. Ayeda, H. H. Caoc, B. Carbonnierd, and N. T. Tran, *Polymer*, **2015**, *66*, 249.
24. S. Liu, L. Shaorong, P. Qiaosheng, and J. J. Lu, *J. Chromatogr. A*, **2003**, *1013*, 57.
25. J. Xie, Y. Miao, J. Shih, Q. He, J. Liu, Y. C. Tai, and T. D. Lee, *Anal. Chem.*, **2004**, *76*, 3756.
26. J. Xie, Y. Miao, J. Shih, Y. C. Tai, and T. D. Lee, *Anal. Chem.*, **2005**, *77*, 6947.
27. H. V. Fuentes and A. T. Woolley, *Lab Chip*, **2007**, *7*, 1524.
28. J. F. Borowsky, B. C.Giordano, Q. Lu, A. Terray, and G. E. Collins, *Anal. Chem.*, **2008**, *80*, 8287.
29. J. Borowsky, B. Joseph, L. Qin, and G. E. Collins, *Sens. Actuators, B*, **2008**, *131*, 333.
30. W. Wang, C. Gu, K. B. Lynch, J. J. Lu, Z. Zhang, Q. Pu, and S. Liu, *Anal. Chem.*, **2014**, *86*, 1958.
31. A. Ishida, M. Natsume, and T. Kamidate, *J. Chromatogr. A*, **2008**, *1213*, 209.
32. S. Zeng, C. H. Chen, J. C. Mikkelsen, and J. G. Santiago, *Sens. Actuators, B*, **2001**, *79*, 107.
33. S. Yao and J. G. Santiago, *J. Colloid Interface Sci.*, **2003**, *268*, 133.