

PORTABLE LIQUID CHROMATOGRAPHY SYSTEM BASED ON BATTERY-POWERED ELECTROOSMOTIC PUMP AND MICROCHIP WITH PACKED COLUMN AND ELECTROCHEMICAL DETECTOR

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ABSTRACT

We present a compact and lightweight liquid chromatography system. This system consists of a battery-powered electroosmotic flow pump, a 20-nL injector, a microchip contains a reversed phase packed column and an electrochemical detector, and a handheld potentiostat powered by a universal serial bus (USB) connected to a laptop computer. The system has overall dimensions of approximately 26 cm wide × 18 cm deep × 21 cm high and its weight is 2 kg. With the present system, various electroactive compounds, such as catecholamine, were successfully separated.

KEYWORDS

Electroosmotic pump, chromatography, packed column, separation, electrochemical detection, digitate electrode.

INTRODUCTION

High performance liquid chromatography (HPLC) plays crucial role in many scientific researches and industries because it is versatile and reliable separation technique. Ultrafast separation with high efficiency is a recent trend in HPLC, however, compactness and portability of HPLC has been potential and latent demands of researchers. The conventional HPLC is usually set up at a specific place in laboratories because of bulky and complicated instrumentation. The miniaturization of HPLC system allows to use readily at any place in laboratories as well as the field. This feature is essential for environmental analysis and point-of-care diagnosis in order to avoid sample decomposition during transportation, and to reduce the time and the cost of analysis. The miniaturized system also offers many applications with small sample, low consumption of solvents and power, reduced amount of waste. The further application is parallel analysis with multiple instruments in the same space as the footprint of conventional system. However, few portable, compact and lightweight chromatographs are commercially available although microchip-based chromatography and related technologies are vigorously presented as several reviews have been reported [1, 2]. Microfluidic and related technologies can realize a fully miniaturized system for liquid chromatography. Recently, we fabricated a microfluidic chip for reversed phase liquid chromatography [3]. One of the authors (I.Y.), on the other hand, produced a small ideally pulse-free pumping system based on electroosmotic flow (EOF) generated in porous silica using low applied voltages (< 100 V). In this study, the electroosmotic pump was designed to drive mobile phases Also, a microchip integrates a column and electrochemical detector with higher performances was developed. Then we here present a portable liquid chromatography system.

EXPERIMENTAL

Chip fabrication. A polystyrene (PS) plate (1.7mm thick, Tamiya, Japan) was used as a microchip substrate. The hole served as a column tube 0.8 mm in diameter was drilled on the side of the PS substrate to a length of 30 mm using a drill bit and then an exit hole (200 μm) was drilled on the surface of the substrate toward the end of the column. The substrate and holes were rinsed with methanol and water and then dried using air gun. Next, detection electrodes were fabricated on the surface of the substrate using Au deposition, standard lithography, and wet chemical etching. For the preparation of a column, a small amount of glass wool was placed at the end of the hole as a frit. Dry C18 silica particles were stored in a 200 μL plastic pipette tip as a reservoir, which was inserted into the top end of the column hole. To introduce the particles into the column, the suction was applied from the access hole by using a water aspirator until the height of the packed bed reached ~ 30 mm. After the reservoir was removed, the top end of the bed was smoothed with a flat side of the drill bit. Then, a PEEK tubing (1/32 inch O.D., 63.5 μm I.D., 30 mm in length) was inserted into the top end of the column by 5 mm and fixed with an epoxy glue. The leakage of the particles thorough the PEEK tubing was not observed without a top frit. The PDMS chip containing a channel was reversibly sealed onto the substrate so that the channel covered the access hole and electrodes.

System construction. An electroosmotic pumping system was built at Nano fusion Technologies (Japan). An injector with sample volume of 10 or 20 nL was purchased from Chemco Scientific (Japan). A potentiostat ALS1232A was received from BAS (Japan). These apparatus were mounted onto an acrylic base, as shown in Figure 1a. The pumping system and the potentiostat were connected to a laptop computer through USB connection, each of which was controlled respectively with a dedicated software.

RESULTS AND DISCUSSION

Figure 1a shows a portable liquid chromatography system developed in this study. This system contains all

requirements for liquid chromatography, including pump, sample injector, column, and detector. The dimensions are 26 cm wide, 18 cm deep, and 21 cm high and the total weight of the entire system is approximately 2 kg. The pumping system can run using dry batteries and the potentiostat can be driven through USB cable. These features display higher portability of the current liquid chromatography system.

The pumping system contains twin cylindrical modules to generate electroosmotic flow, allowing gradient elution and solvent switching. The module was 40 mm diameter and 68 mm high. In general, electroosmotic pumps have a common disadvantage of bubble formation resulted from electrolysis of water. Bubbles can seriously damage column and detector performance. Therefore, the pump module was designed as shown in Fig. 1b. The individual module has vertically two compartments filled with DI water and a mobile phase, respectively, between which compartments a porous silica rod (3 mm diameter, 4 mm high) pinched with two platinum wire electrodes was placed. The mobile phase is separated from DI water with a silicone rubber diaphragm. The chamber volume of mobile phase is ~8 mL. The voltages of 0 – 60 V were applied to the porous silica using a booster circuit, resulting in an electroosmotic flow of water in the pores. The water stream generated presses the diaphragm, which discharges the mobile phase toward a column at flow rate ranging from 0 to 10 $\mu\text{L}/\text{min}$. The flow rate was controlled with PID algorithm and flow sensors. The pumping system can be driven with AC adaptor or DC dry battery (12 V). The advantage of the current pumping system is low power consumption over the other electroosmotic pumps require several thousand volts. This pumping system produced a pulse-free continuous flow with $\pm 1.8\%$ at a flow rate of 5 $\mu\text{L}/\text{min}$ over at least 5000 s. It also continuously transferred a 100% methanol over 24 hours at a flow rate of 5 $\mu\text{L}/\text{min}$ with dry batteries. The pumping system performed up to ~1 MPa.

Figure 2 shows a microchip for chromatographic separation and detection. The chip configuration minimizes dead volumes between column and detector (Figs. 2b and 2c). The microchip device was fabricated using a polystyrene substrate which offers ease of fabrication, a cheap device, and chemical resistance to organic solvents typically used for mobile phase, such as methanol and acetonitrile. For the preparation of a column on a chip, a packing method was studied because a high pressure used in the common slurry method may damage the polymeric substrate. In this study, a dry technique was developed under experimental section. The column was created by packing 3- μm C18 particles into a drilled hole (0.8 mm diameter and 30 mm long). With this column, the pressure drop was tested by using an conventional HPLC pump with pressure monitoring. When 100% methanol was pumped

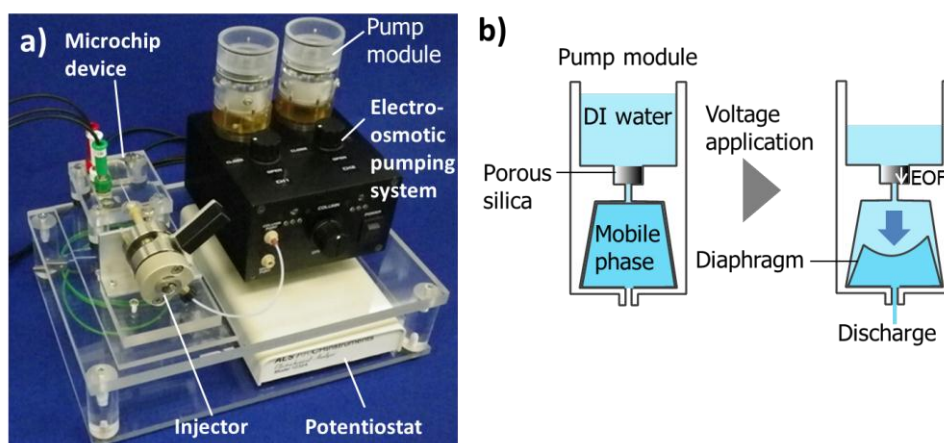


Figure 1: a) Photograph of a portable liquid chromatography system and b) schematic diagram of an electroosmotic pump module.

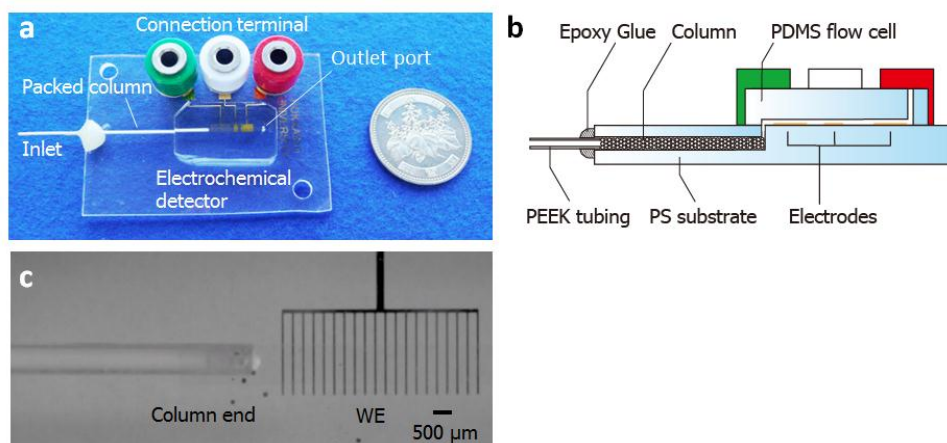


Figure 2: a) Photograph and b) schematic diagram of a microchip with a packed column and an electrochemical detector. c) microphotograph of the end of the column and working electrode

at a flow rate of 4 $\mu\text{L}/\text{min}$, the pressure was 0.3 MPa. This packing method provided higher packing density and thus column efficiency, compared with the column on a chip that we previously reported [3].

For more highly sensitive detection on a chip, a single digitate working electrode was designed as shown in Figure 2c. In separate experiments using a continuous flow FIA mode without the column, microelectrode width, gap, and number of microelectrode were optimized. Then, when a single digitate working electrode with 24 microelectrode 50 μm in width and 200 μm gap, we obtained the detection limit of nM level for catechin. The detection limit was superior to those of previously presented microfluidic separation devices with electrochemical detector [4].

Figure 4 shows chromatographic separations of catecholamine and catechin using the current chromatography system powered by dry batteries. This demonstrates that the present system was successfully applied to the separation of electroactive compounds. The use of higher flow rate shortened the analysis time nearly keeping separation efficiency.

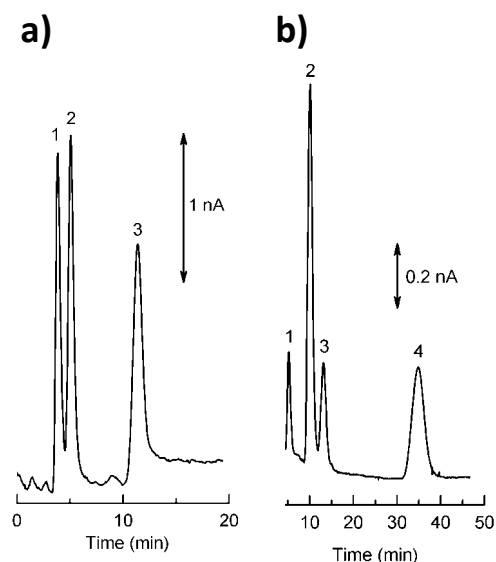


Figure 3: Chromatograms of catecholamine and catechin obtained with the present liquid chromatography system. Peaks: a) 1 noradrenaline, 2 adrenaline, 3 dopamine; b) 1 (+)-Catechin, 2 epicatechin, 3 epigallocatechin gallate, 4 epicatechin gallate. Chromatographic conditions: a) A: 50 mM phosphate, 50 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 $\mu\text{L}/\text{min}$; Detection: +0.6 V vs. Au. b) Mobile phase: Water-methanol-0.5 M phosphate (7 : 2 : 1 v/v/v) ; flow rate: 5 $\mu\text{L}/\text{min}$, Detection: +0.6 V vs. Au.

CONCLUSIONS

We have presented a portable device for reversed phase liquid chromatography. The electroosmotic pumping system was driven by dry batteries with high precision for many hours. This chromatography system provided good chromatographic separation and detection performance for electroactive compounds. This portable chromatography system will allow on-site analysis as well as new applications take advantage of compactness.

ACKNOWLEDGEMENTS

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

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