Automated injection from EWOD digital microfluidic chip into HPLC purification system

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ABSTRACT

We report an automated "chip-to-world" interface between an electrowetting-on-dielectric (EWOD) digital microfluidic device and high-performance liquid chromatography (HPLC) system, expanding the application of EWOD chemical synthesis devices to syntheses that require HPLC purification. The interface collects the crude product from the chip without the need for chip disassembly or other manual intervention. We report (a) bubble-free filling of the injection loop; (b) quantification of how much crude product from the chip is loaded into the loop; and (c) successful injection and HPLC purification of a crude product. It should be noted that because of the small chip volume, analytical-scale HPLC could be used, typically leading to 10-20x more concentrated purified product than semi-preparative HPLC.

KEYWORDS

Digital microfluidics, Electrowetting on dielectric (EWOD), world-to-chip interface, High-performance liquid chromatography (HPLC), Purification

INTRODUCTION

Due to its well-controlled volumes, inert surfaces, all-electronic control and flexibility of fluid movement, there has been growing interest in digital microfluidics using electrowetting-on-dielectric (EWOD) for chemical, biochemical and radiochemical synthesis applications [1,5,6]. Several of these require post-synthesis purification, separation and/or analysis. High-performance liquid chromatography (HPLC) is an important and ubiquitous separation technique for both preparative and analytical systems [7]. However, products are often manually pipetted off the chip and introduced into the HPLC [1]. An integrated interface that collects the product from the chip and delivers it to the HPLC system would minimize chemical/radiation exposure, time and effort on part of the operator. In doing so, it is important to inject most of the product from the microfluidic volumes of EWOD chip into the HPLC, and also not introduce air bubbles which could lead to distortion in the chromatograph. Below, we describe our approach to address these challenges and achieve repeatable results.

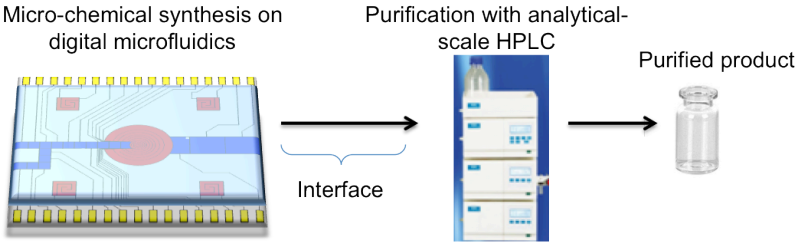

Fig.1: The automated micro-injection interface collects the crude product from the EWOD chip and delivers it to HPLC for purification without manual intervention.

EXPERIMENTAL

The schematic top view of the interface is shown in Fig. 2(a), while its operation is illustrated in Figs. 2(b1-b5). A two-plate EWOD configuration (similar to [2],[8]) is used with the droplet sandwiched between two Teflon®-coated surfaces, with the patterned electrodes on the upper plate and ground plane on the lower plate. This is opposite to the usual orientation of the ground top plate on patterned lower plate. An outlet hole on the lower plate is positioned vertically above a septum-capped glass vial with a flat-bottom insert (400 µL, Agilent, Santa Clara, CA), with a vertical tubing (stainless steel, 3 inch long, 21 ga) connecting this "Extract" vial to the EWOD chip.

First, vacuum is applied to collect product from the chip into the "Extract" vial [2]. The collection of the discrete droplet from the chip tends to occur in spurts, especially towards the end of the transfer, and the process usually leads to bubbles entrapped with the liquid in the Extract vial (Fig. 2(b1)). To eliminate these bubbles that are undesirable in HPLC, the contents are pushed into the "Degas" vial and weak vacuum applied. Liquid is collected at the bottom of the "Degas" vial, while gas bubbles are removed through the top (Fig. 2(b2)).

Next, the degassed liquid is then pulled into the HPLC injection loop (50 µL, PEEK, Idex-HS, Oak Harbor, WA) by applying vacuum at the "Overflow" vial (Fig. 2(b3)). Two tubing-mounted optical liquid sensors (Optek Inc., Carrollton, TX) are placed over transparent tubing – one before and one after the injection loop (LS1 and LS2 respectively) to sense the presence of liquid at these positions. The liquid is pulled in the form of one contiguous segment from the degas vial through connecting tubing into the loop. When both liquid sensors read "high", this indicates the presence of liquid at both the sensors as well as in the loop between them. The rotary valve can then be switched (Fig. 2(b4-b5)) to inject the bubble-free liquid in the loop into the HPLC column to perform the chromatography. Using a combination of timing and feedback provided by the liquid sensors and the electronically controlled gas valves allows the entire operation to be computer-controlled and automated.

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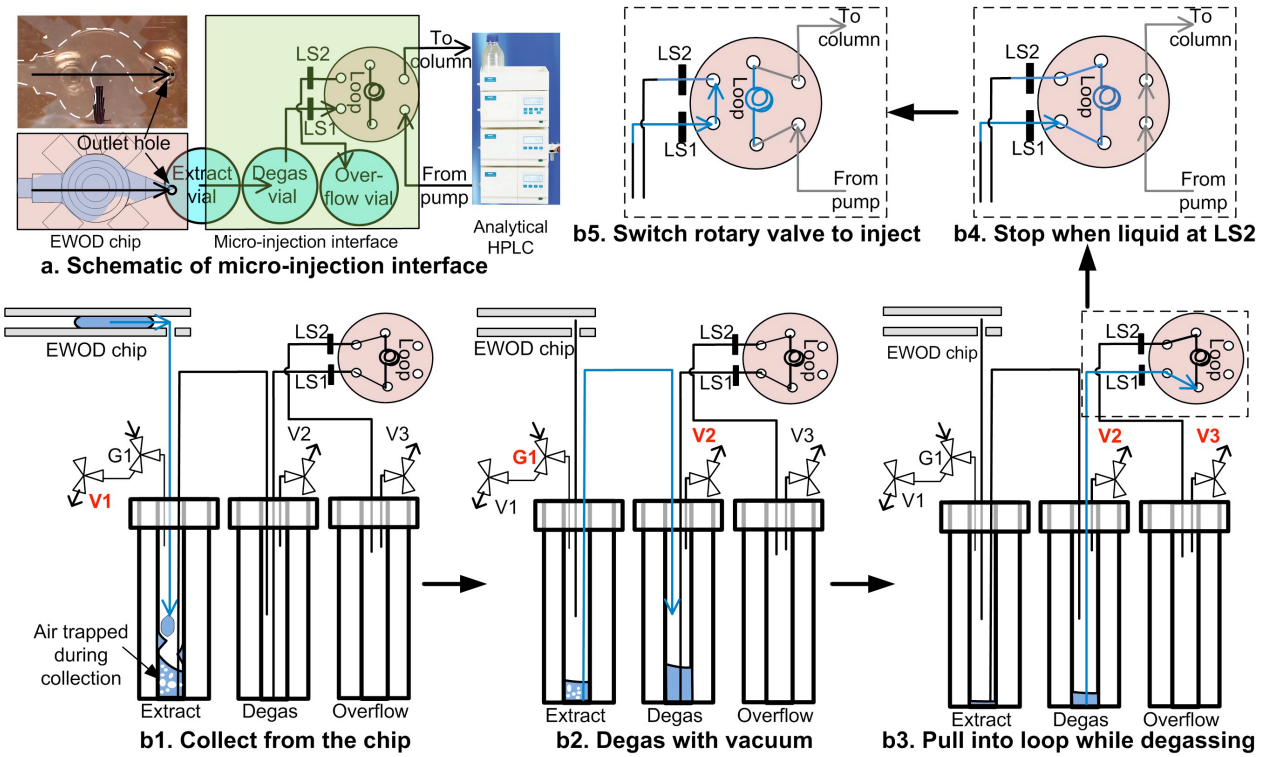

Fig.2: (a) Top view schematic showing the automated micro-injection interface that is comprised of three septum-capped vials, two optical liquid sensors and a rotary injection valve. (Gas and vacuum controls are omitted for clarity.) A droplet (outlined in white) being collected using EWOD and vacuum on an actual device is shown (top left). (b) Sequence of operation of the interface (counterclockwise from bottom left): (b1) Liquid (blue) is moved to the outlet hole using EWOD and collected with vacuum (V1) into "Extract" vial. (b2) To remove air bubbles, liquid is pushed using compressed gas (G1) from Extract vial into the "Degas" vial, where a weak vacuum (V2) applied at the top. (b3) Liquid is pulled from the degas vial using vacuum (V3) applied to the "Overflow" vial, across LS1 into the injection loop on the rotary valve and LS2. (b4) Once liquid fills the loop as indicated by LS1 and 2, V3 is turned off and (b5) the rotary valve is switched to inject the loop's contents into the purification column. The entire process is automated by software-controlled EWOD actuation, vacuum and pressure using timing and electronic feedback.

RESULTS AND DISCUSSION

We used radioactive [^{18}F]fluoride in water to quantify volume loss between the chip and the loop. ~65 µL of radioactive sample was first passed through the interface and loaded until LS1 was activated. (In this state, the Degas vial still contains liquid, and the tubing between the vial and LS1 is completely filled.) Next, ~65 µL of water was loaded onto the chip to rinse out residual activity along the lines and tubing and transfer from the Degas vial was resumed until LS2 was activated. Radioactivity outside the loop (upstream and downstream), and loaded into the loop were separately measured. ~82±4% of the starting activity was found to be loaded into the loop, which is similar or better than typically achieved with manual collection from the EWOD chip. Fig. 3 shows signals from the liquid sensors LS1 and LS2 that flank the injection loop as the liquid is filled. At the inlet side, LS1 first detects liquid and stays activated until the liquid completely fills the loop and reaches LS2.

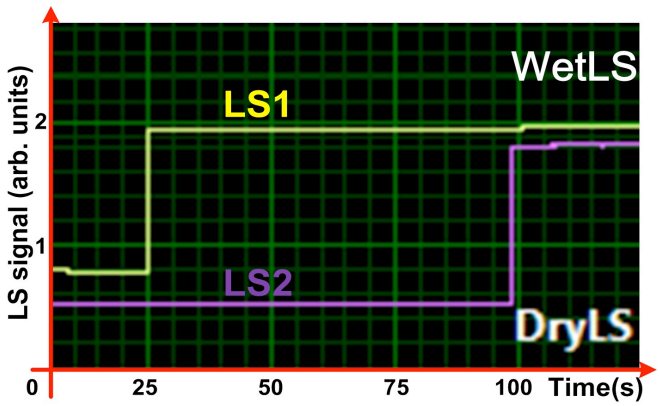

Fig.3: Loading of loop for injection into HPLC: Plot shows signal from liquid sensors 1 and 2 (LS1 and LS2). First, the solution containing crude product is collected from the chip, degassed and transferred towards the loop until LS1 is activated (Fig. 2(b1-b3)) (~25 s on plot). Additional solvent is then added to rinse the tubing and the two vials upstream of the loop, following which transfer is resumed to completely fill the loop such that the liquid reaches LS2 (~100 s on plot) (Fig. 1(b4)). The flat trace of LS1 during loop filling indicates absence of bubbles in the loop.

As proof-of-concept, we injected crude [^{18}F]fallypride made by radiochemical synthesis on EWOD through the interface into the HPLC column and obtained distinct peaks for the main product and side-products (Fig. 4). Peaks were collected in separate fractions enabling isolation of pure [^{18}F]fallypride. The peaks obtained with the injection of radioactive compounds repeatably matched those for the cold standards (separately injected, not shown).

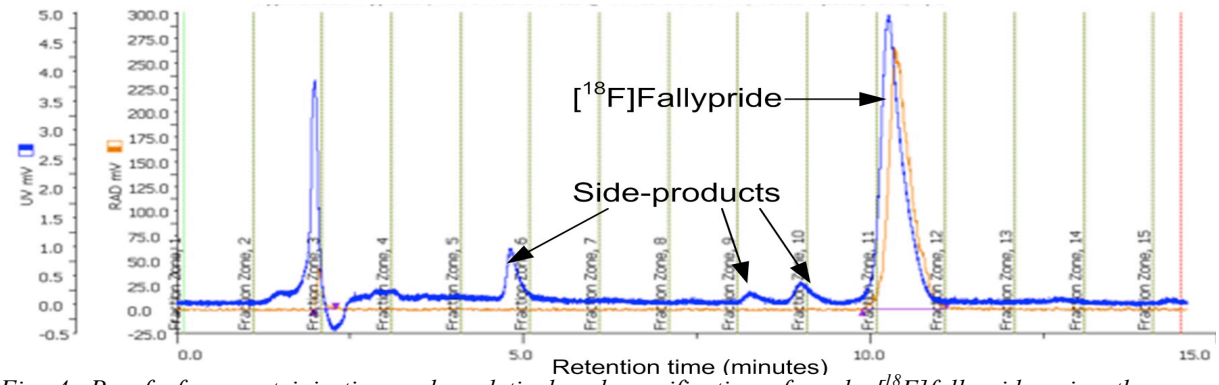

Fig. 4: Proof of concept injection and analytical-scale purification of crude [^{18}F]fallypride using the reported interface. The HPLC chromatograph showing UV absorption (λ = 305 nm, blue trace) and radiation (orange trace) signals shows clear separation of the radiolabeled [^{18}F]fallypride (~11 min) peak from other impurities (e.g. UV peaks around ~5,8,9 min).

CONCLUSIONS

We have demonstrated a practical interface for automated injection from an EWOD chip to an HPLC purification system. Requiring cheap vials, tubing and only gas-valve actuation, the interface contains no expensive wetted components and therefore lends itself well to a disposable cassette-based model. To miniaturize the overall chemical synthesis and purification system, this work could be extended to exploit smaller-scale HPLC columns (e.g. [3]) and systems (e.g. [4]). Although useful for all chemical synthesis due to ease of operation and reduced chemical hazard, the automation has an additional importance for radiochemistry to minimize radiation exposure.

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