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On-chip definition of picolitre sample injection plugs for miniaturised liquid chromatography

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Abstract

The fabrication of components for a miniaturised liquid chromatography system on silicon has recently been reported by our research group [*J. Cap. Electrophoresis Microchip Technol.* 6 (1999) 33; *Analyst* 125 (2000) 25]. To date, inlet and outlet connection ports, separation micro-channels (20–200 μm in width, 0.5–10 μm in depth, 15–60 cm in length), and an intersection for picolitre injection have been etched on a silicon wafer and then sealed with a Pyrex cover plate on which platinum electrodes for on-chip detection have been patterned. The platinum electrodes have been used for the amperometric detection of phenol, using 20 nl off-chip injection. In this work we present our latest results obtained with on-chip pressure driven picolitre injection, designed to realize the full capabilities of this micro-LC system. The injection volume is dependent on the micro-channel depth, width, and also on the intersection length, allowing injection in the low nanolitre to picolitre range. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Interest in microfabricated miniaturised separation systems has grown rapidly in the past decade. The majority of research has concentrated on miniaturised electrophoretic devices due to the ease of application of the method to planar chip formats. There is no need for pressure withstanding bonding technology and as a result a variety of materials and bonding technologies can be used. The separation mechanism does not require a stationary phase and importantly the manipulation of flow on the chip can be controlled without the requirement of mechanical valves. While similar microfabrication techniques are used to make miniaturised high-pressure liquid chromatography systems, the necessity for pressure withstanding bonding technology, the need for stationary phases and mechanical manipulation of liquids on chips has resulted in comparatively slower development of chip based devices. In 1990, Manz et al. [3] reported the design of an open tubular column liquid chromatograph on silicon. The chip consisted of a spiral channel with a microfabricated platinum electrode detection system on silicon, sealed anodically with a Pyrex coverplate. However chromatographic results were not presented for this device. Later in 1995, Ocvirk et al. [4] presented results obtained from a high-performance liquid chromatography (HPLC) device partially integrated onto a silicon chip. This was a packed column device with a microfabricated 'T' injection for split injections and

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an optical detection cell. A microfabricated frit was used to retaining the stationary phase on column. Off-chip injection volumes of 60 nl were performed reducing to 3 nl using a split injection system. Fluorescence detection was performed using optical fibres as a coupling between an Argon ion laser and a microfabricated flow cell. Further work on different facets of HPLC have also been presented. In 1998, Regnier et al. [5] reported the in situ micromachining of monolithic support structures with subsequent derivatisation as an alternative method for achieving a packed bed to traditional packing methods. In 2000, Ceriotti et al. [6] described the packing of a column without the need for a retaining frit. In 2000, McEnery and co-workers [1,2] reported an open tubular liquid chromatograph consisting of a separation column etched in silicon with integrated microfabricated electrodes for amperometric detection. Horizontal attachment of fused-silica tubing was achieved by fixing them into etched v-grooves at the ends of each channel. These connections allowed the introduction of pressures up to 20 MPa to the chip. Fast separation of benzamide and biphenyl using off chip UV detection and on-chip amperometric detection of nitrate and phenol using the device was demonstrated. While the device also contained an integrated sample injection port, the results presented were carried out using off chip injection.

In furtherance of this work we now present the definition and manipulation of sample plugs on chip utilising off chip valves in a manner analogous to the control of sample plugs in on-chip capillary electrophoresis (CE) by off chip power supplies. This method allows us to vary the length and volume of the sample plug, factors which are critical in obtaining high efficiencies with on-chip HPLC.

2. Experimental

2.1. Materials and reagents

Rhodamine 6G with a dye content ~99% (Sigma–Aldrich, Dublin, Ireland) was used as the sample at a concentration of 0.08 m*M* in isopropanol, analytical-reagent grade (Alkem, Cork, Ireland). Polyether Ether ketone (PEEK) tubing, PEEK Microtight sleeves, Microtight nuts, ferrules and zero dead volume fittings were used to make interconnections (Sigma–Aldrich). Fused-silica capillaries (30 μm I.D., Type TSP030150, 20 μm I.D., Type TSP020150) (Composite Metals, Worchester, UK) were used to interface the HPLC chip with the sample reservoirs.

2.2. Instrumentation

Two stainless steel pressure displacement reservoirs built in the laboratory provided sample and mobile phase to the chip at pressures up to 1500 p.s.i. (1 p.s.i.=6894.76 Pa). Four manually actuated, high-pressure, two-way, taper valves (Sigma–Aldrich) were used for definition of the sample plug. The valves were connected to the chip using Microtight PEEK fittings, PEEK Tubing and fused-silica tubing. PEEK tubing connected the reservoirs to the valves. A Shimadzu SPD 6A spectrophotometer (Shimadzu Europe, Duisburg, Germany) fitted with a 3 nl flow cell, built in the laboratory, was used for absorbance detection at 540 nm and was connected to the column outlet valve. Data collection was performed using an ADC 12 voltage input connection and Picolog software (Pico Technology, Cambridgeshire, UK). Illumination and excitation for the Rhodamine 6G sample was provided by a halogen lamp focused onto the injection area. Image collection was performed using a JVC TK-C1381 $\frac{1}{2}''$ CCD camera (Labquip, Dublin, Ireland) mounted on a microscope and capture was performed by Studio MP10 software (Pinnacle Systems, Mountain View, CA, USA). We also used the Flume CAD solver in MemCAD 4.8 software (Coventor, Cary, NC, USA) to simulate the sample plug profile in the silicon micro-channel. The simulation package is based on the finite element method using a user defined mesh to outline the micro-channel structure. The flow of a fluid through a control volume is governed by the Navier–Stokes equation which is solved for each element.

2.3. Experimental set-up

The fabrication of the LC chip has been discussed in detail elsewhere [1,2]. A manually operated valve was connected via fused-silica tubing and PEEK

connections to each connection port on the chip. The mobile phase inlet and outlet valves were connected together in such a manner that they could be actuated simultaneously. The sample inlet and outlet valves were operated in the same manner. The mobile phase outlet valve was connected to the detection cell via fused-silica tubing for UV absorption detection of the sample plug (Fig. 1).

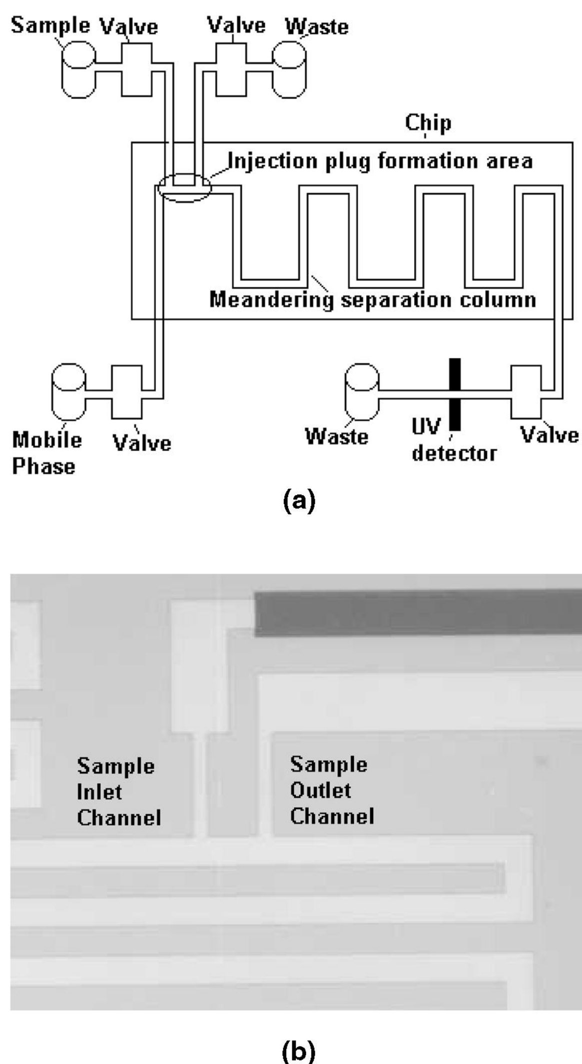

Fig. 1. (a) Schematic of our miniaturised liquid chromatograph system, and (b) microscope image of the sample inlet and outlet – the shaded area is the deep 'v-groove' etch for fused-silica capillary insertion.

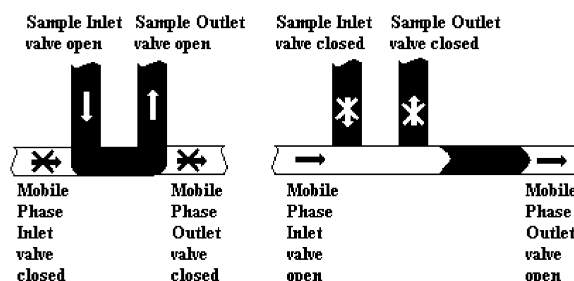

Fig. 2. Schematic of the four valve operation for plug formation.

2.4. Mode of operation

Both sample and mobile phase reservoirs were pressurised to 1000 p.s.i. The mobile phase reservoir contained *n*-propan-2-ol and the sample reservoir contained a 0.08 m*M* solution of Rhodamine in *n*-propan-2-ol. The on-chip injection sequence is as follows. (1) All four valves are opened, priming the connection tubing and chip channels with sample and mobile phase. (2) The sample inlet valve is then shut priming the channels with just mobile phase. At this point an injection plug can be defined. (3) The mobile phase inlet and outlet valves are shut holding the mobile phase on the chip under pressure. (4) The sample inlet and outlet valves are then opened, introducing sample into the sample plug defining area at the top of the separation channel. The sample follows the path of lowest resistance and exits the chip via the sample waste channel. (5) On completion of plug definition, the sample inlet and outlet valves are closed whilst the mobile phase inlet and outlet valves are opened. This causes the defined plug to be swept along the separation channel. The length and volume of the defined injection plug can be altered by varying the length of time the valves are open, the sequence of valve operation and the pressures within the reservoirs (Fig. 2).

3. Results and discussion

3.1. Plug formation

Several interesting features can be observed in the experimental images of the sample plug (Fig. 3). Its

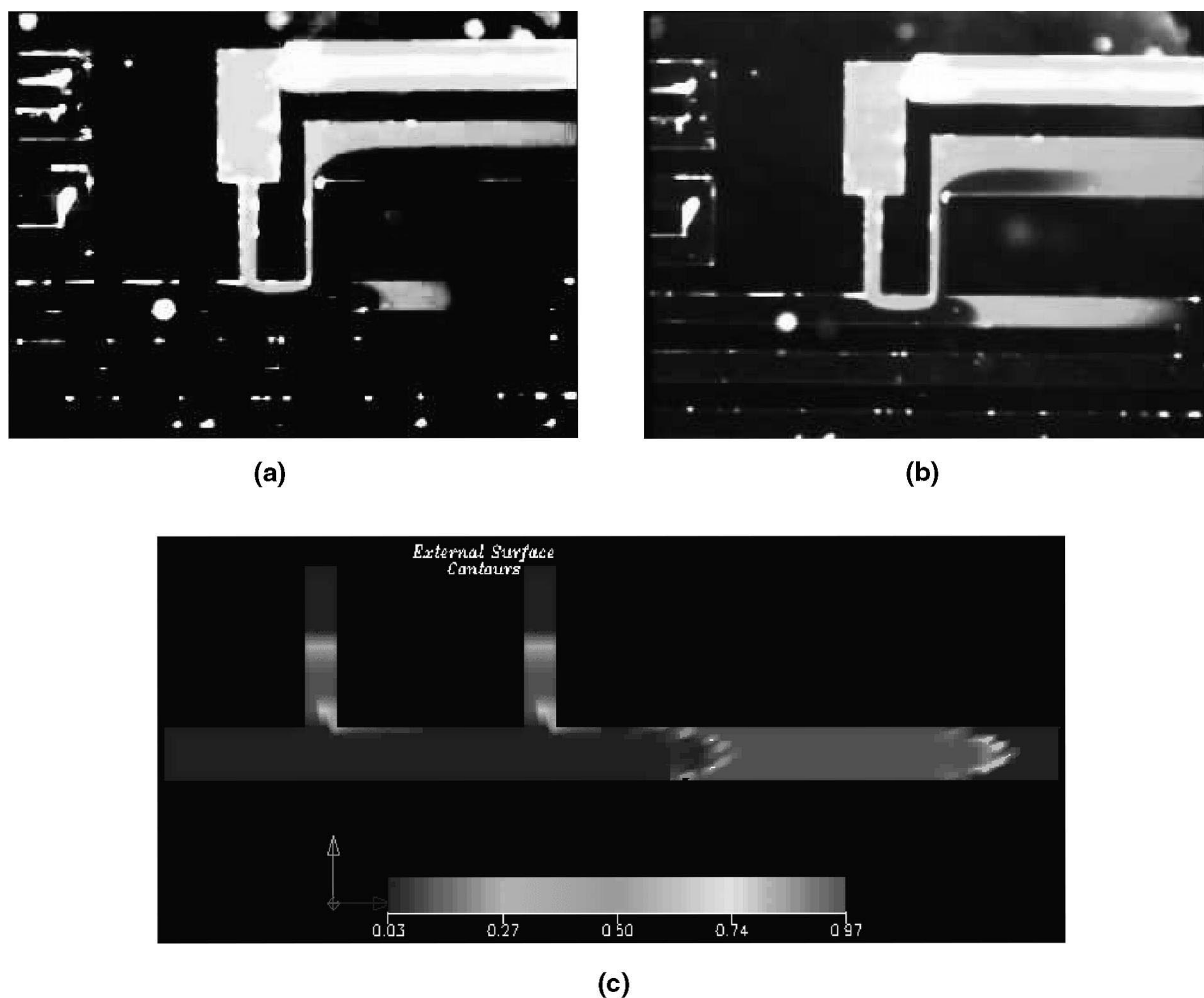

Fig. 3. Experimental images of short (a) and long (b) plugs being swept down the separation channel just after definition. (c) FlumeCAD simulation of the sample plug at a similar position.

shape is somewhat rhomboidal. This is due to the fact that the flow from the mobile phase reservoir has already passed through several 90° turns and the path at the bottom of the channel (with respect to the plan view) is the shorter in term of distance. Thus the plug is slanted as it is pushed due to the difference in time of the arrival of the solvent front at the top and bottom of the channel. This feature was not entered into the simulation and as such a symmetric plug is observed. This highlights the benefits of straight channels over meandering channels. An important difference between the simulated and experimental procedure is that the valves do not deliver absolute on/off control. If this were the case, then a small amount of bleed from the sample inlet and outlet channels could be expected once the sample inlet and outlet valves have been shut off and the mobile phase flow has commenced. This bleed is shown at the bottom right of the sample inlet and outlet channels in the simulation image. If absolute on/off valve were used, this phenomenon would have to be investigated to see if it contributes to significant sample tailing. That the valves do not act as absolute on/off switches yields an advantage that points to

the mode of operation of a system that does have absolute valves. It can be seen from the experimental images that there is no bleed into the top of the separation column once injection has taken place. This is due to the fact that the sample inlet and outlet channels are either not fully closed or the action of closing the valve yields a dead volume that the mobile phase can occupy. As a result, when the sample inlet and outlet valves are shut off and the mobile phase inlet and outlet valves are opened, a portion of the mobile phase enters the sample outlet channels as well as the separation column. A laminar flow of mobile phase separates the sample flow from the top of the separation column thus allowing for the injection of a discrete plug with no tailing from the sample injection channels. Hence in a system with absolute valves the sequence of operation would be designed to mimic this effect. Once the plug has been defined it is transferred to a position just past the sample outlet channel. Then the sample outlet valve is opened to set-up a split flow creating the beneficial laminar division between the column and the injection point.

4. Conclusions

We have demonstrated rapid on-chip injection of picolitre volumes in a HPLC micro-system. Although our system uses four manually operated valves, the system can be readily automated to achieve improved injection volume repeatability. With some modification, the system could be used for hydrodynamic sample introduction on planar capillary electrophoresis chips. Our design enables several variables to be used to delineate the sample plug. These variables include, the injection port design, micro-channel etch dimensions, flow gradients, valve sequencing and injection sequence timing. On-chip injection volumes in the femtolitre range should be achievable by further reducing the micro-channel width, depth or intersection length. By reducing the injection volume and length, the column length can be significantly reduced yielding much faster separations. We are currently designing an on-chip optical detection unit which when combined with our on-chip injection and separation capability will enable our micro-system to compete with conventional HPLC techniques.

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