# RP-HPLC MICROCHIP SEPARATIONS WITH SUBNANOLITER ON-CHIP PRESSURE INJECTIONS

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## ABSTRACT

On-chip HPLC separations require a fluidic control system that can reliably deliver low-volume, high-pressure fluidic injections. We present a valving scheme that allows for metered, diffusionless, on-chip pressure injections. Microvalves are fabricated using *in-situ* photopolymerization of a fluorinated acrylate polymer that moves in response to pressure differentials. The valves are inert and can withhold high pressures. In a similar manner, a reversed-phase separation column is fabricated using phase-separation polymerization of a stearyl acyrlate polymer covalently bonded to the microfluidic substrate. The performance of the column is demonstrated by a rapid HPLC separation of a peptide mixture. The on-chip injector is actuated via a pressure differential and can meter sample volumes less than 1nL.

## KEYWORDS

phase-separated polymer, polymer monolith, microvalve, HPLC, pressure injections

## INTRODUCTION

Planar microchips have been used as sophisticated substrates for chemical separations due to a number of advantageous properties; one prominent improvement over capillary techniques is the ability to conveniently inject small volumes of liquid. Most microchip separations have been electrokinetic in nature, due to the ease of implementing electrokinetic injections with cross configurations and variations thereof. Despite this, HPLC separations remain the most flexible and reliable separation techniques.

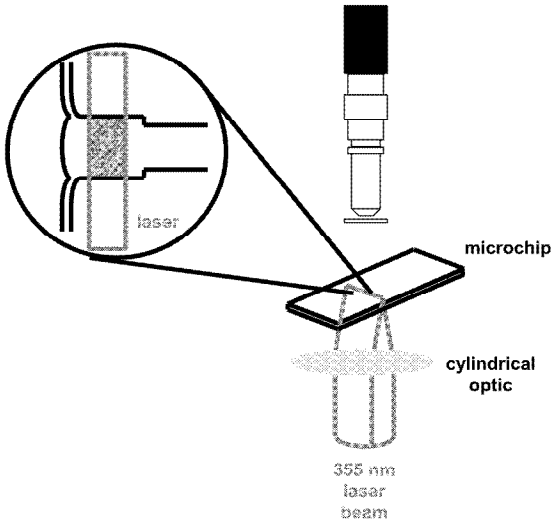


Figure 1. Projection lithographic setup used for patterning valve elements. Separation media requires less resolution, and is patterned using contact lithography.



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HPLC implementation in chip format to date has been limited by the absence of appropriate pumps and fluidic control schemes to allow for reliable, low-volume pressure injections.

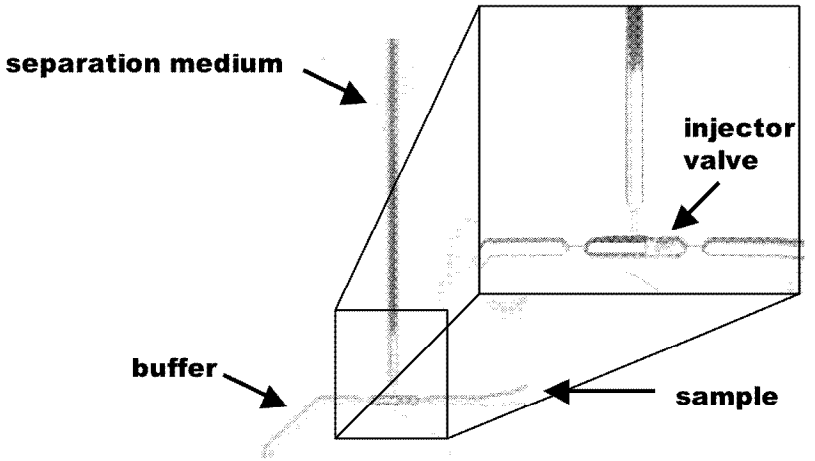


**Figure 2. Sample injection/separation geometry. Separation medium is patterned throughout separation column; valve element is localized at interface between sample and buffer lines.**

Here we present a microchip valving scheme[1] that allows for metered, diffusionless, on-chip pressure injections with volumes below 1 nl. Mobile polymer elements are fabricated in planar silica microchips to allow two input lines (buffer, sample) to be separated from each other while each in turn is in contact with a separation column, also fabricated *in-situ*. By allowing subnanoliter pressure injections, these valving schemes open up numerous possibilities for reaction of small liquid volumes with subsequent HPLC analysis on chip.

## MICROVALVE AND SEPARATION COLUMN FABRICATION

Microvalves consisting of a mobile polymer element formed within in a three-dimensional glass microstructure are fabricated using laser-induced phase-separation polymerization. Localized exposure from a 355 nm output of a frequency-tripled Nd:YAG laser (Figure 1) leads to precipitation of a ~60 μm fluorinated, crosslinked acrylate polymer valve element from a monomer/solvent/ photoinitiator solution. The polymer valve elements are inert and can be used with all normal HPLC solvents and at high pressure. Previously reported data indicate that these valve elements can modulate flow over nine orders of magnitude [1]. In a similar fashion, a ~3 cm RP HPLC column with pore size near 1 μm is fabricated by phase-separation of a crosslinked hydrophobic acrylate polymer (stearyl acrylate crosslinked with 1,6 hexanediol diacrylate). Similar acrylate formulations have been used for RP-CEC of PAHs, amino acids, and peptides [2].

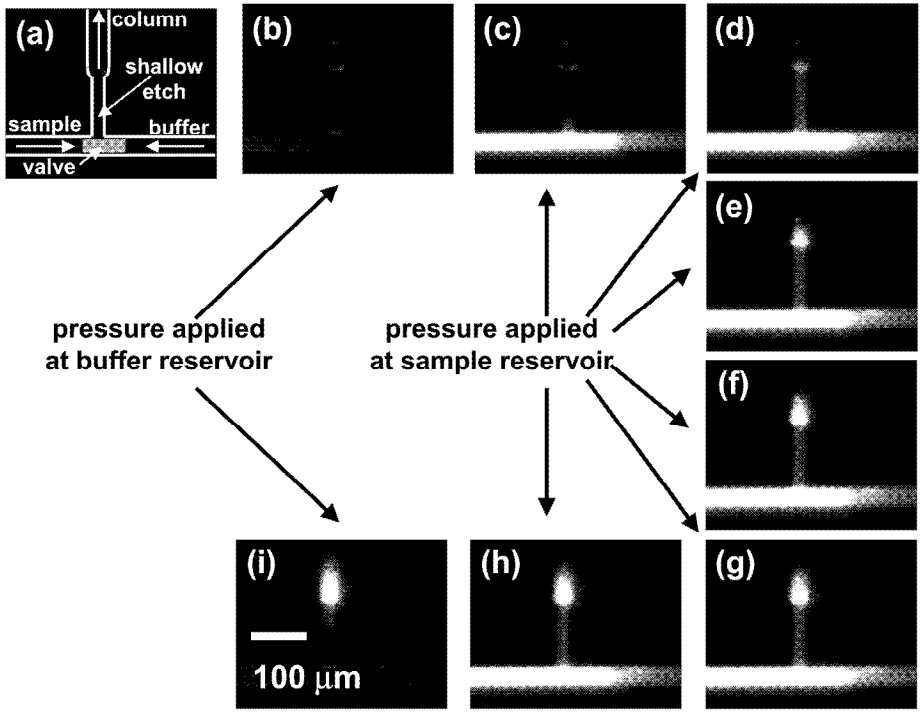


Figure 3. Time history of representative sample injection. Injection is diffusionless, lossless, and approximately 500 pl in volume. Apparent disconnects in the channels are due to shallow regions, which appear dark. (a): location of sample line, buffer line, separation column, and injection valve. (b)-(i): injection time history. (b) pressure applied at buffer line, and buffer (no signal) fills the imaged region. (c)-(h): when pressure is applied at sample line, sample flows into the separation column. (i): pressure is once again applied at buffer line, and sample flows down separation column.

## METERED MICROCHIP PRESSURE INJECTIONS

The microvalve architecture (Figure 2) for pressure injections consists of a zero dead volume, two-input, one-output valve. The mobile polymer element moves in response to differential pressure, allowing the input with higher pressure to proceed down the separation column. The polymer element prevents diffusion between sample and buffer, and repeated injections can be made without wasting any sample. Injections are made by briefly applying pressure at the sample line; while overpressure at sample is applied, the valve opens and connects the sample line to the separation column. When pressure at sample is released, buffer continues down the separation column. An example is shown in Figure 3, in which a 500 pl sample is injected down the separation column.

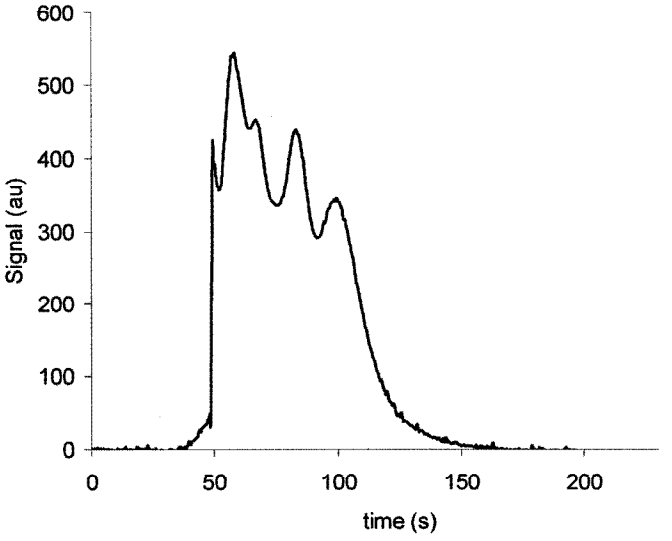


Figure 4. Separation of fluorescamine-labeled 5-component HPLC standard. LIF detection used at 405 nm.

## SEPARATIONS

Preliminary RP-HPLC separations of a fluorescamine-labeled HPLC standard (glycine-tyrosine; valine-tyrosine-valine; met-enkephalin; leu-enkephalin; angiotensin II) on the photopolymerized acrylate column are shown in Figure 4. Gradient elution was used with ACN:phosphate pH 6.8 ratios between 5:95 and 30:70. LIF detection was used at 405 nm.

## REFERENCES

1. B.J. Kirby, T.J. Shepodd, E.F. Hasselbrink, *J. Chrom A* **979** (2002) and refs therein.
2. D.J. Throckmorton, T.J. Shepodd, A.K. Singh, *Anal Chem* **74** (2002) and refs therein.