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Microfabricated liquid chromatography columns based on collocated monolith support structures

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Abstract

There is great interest today in massively parallel analytical strategies as a way to accelerate the rate of discovery in biological research; among them being 'biochips' and 'laboratories-on-a-chip'. The concept in the 'chip' approach is that minaturization will allow large numbers of operations to be performed in parallel in a small space, as in electronics. Proceeding with the semiconductor analogy, this paper demonstrates that in situ micromachining can be used to simultaneously fabricate millions of micrometer size, particle like structures in multiple liquid chromatography columns on a single wafer. Reduction of this widely used bioanalytical tool to the nanoliter volume, parallel processing, chip format is a significant step toward laboratories-on-a-chip.

Keywords: Biochips; Laboratories-on-a-chip; Semiconductor analogy; In situ micromachining

1. Introduction

New paradigms in the study of genomics [1], proteomics [2], immunology [3], neuroscience [4], and drug discovery [5] require that ever smaller quantities of analytes be separated in increasingly large numbers of samples. For example, more than 10000 separations/day on nanogram quantities of material will be needed in the pharmaceutical industry to support drug discovery based on combinatorial chemical libraries and compound banks. At issue is how this will be done. Microseparation systems are of great interest because minaturization has the great advantage (i) of increasing the probability of recovering microfractions for further analysis and characterization and (ii) allowing multiple, parallel processing separation systems to be built that could increase the rate of research and discovery.

Attention is being focused on creating microfabricated systems on quartz or silicon wafers [6–10] that are capable of executing large numbers of integrated analytical operations, including capillary electrophoretic (CE) separations [11–14]. Although CE is a powerful separation tool, it is far from being the universally best separation technique. Chromatography is of much broader utility but it is difficult to fabricate and pack multiple, parallel processing columns on wafers [15]. Continuous polymer rod columns [16] have

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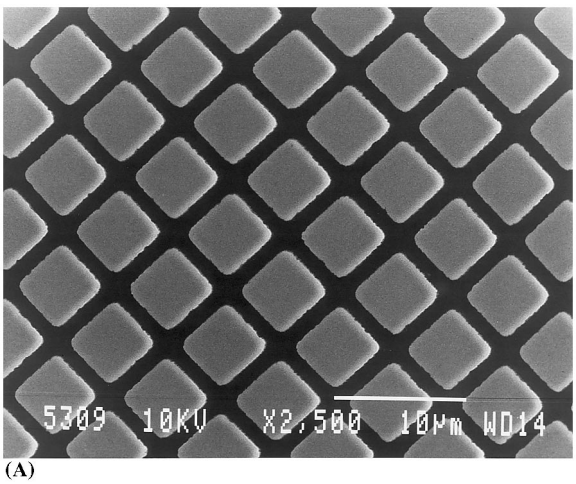
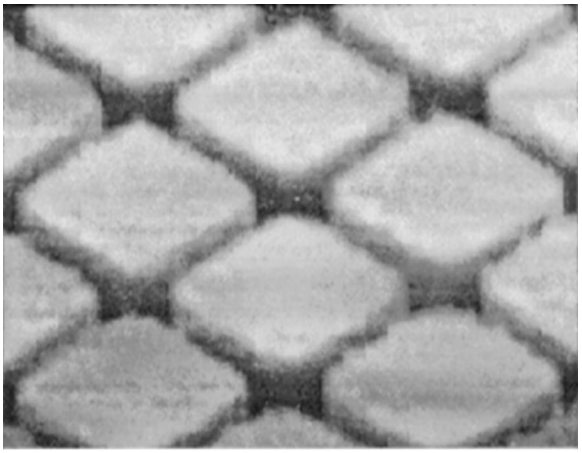


(A)



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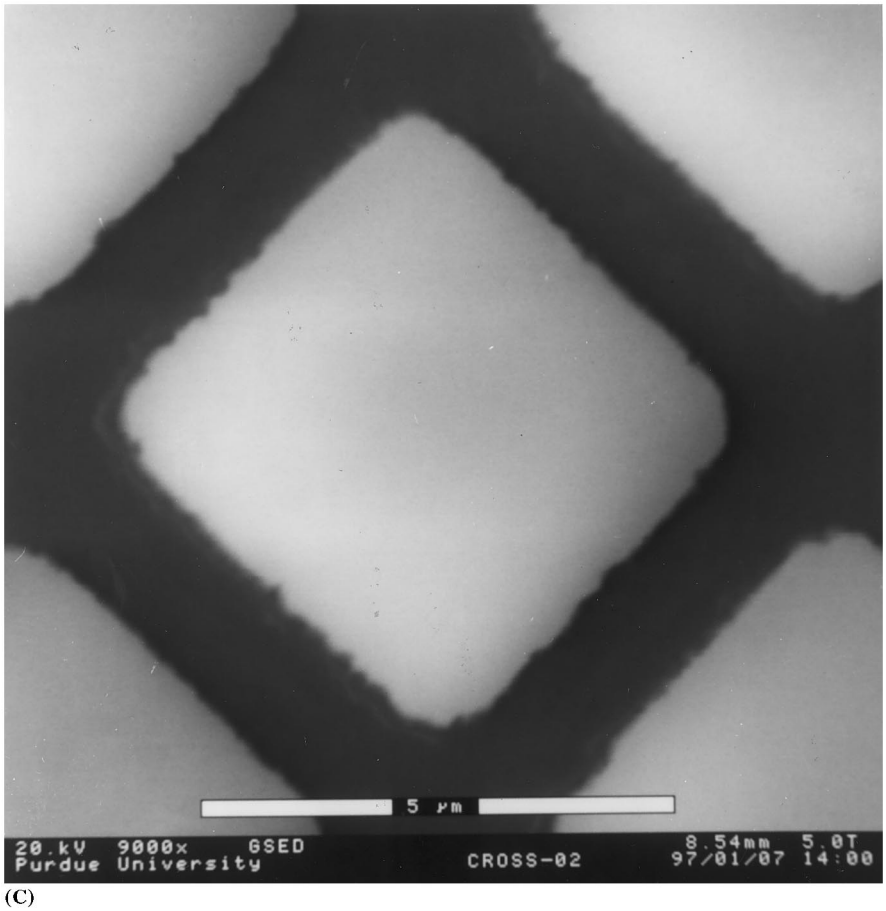

(C)

Fig. 1. Scanning electron micrographs of 5 × 5 μm collocated monolith support structures (COMOSS) with 1.5 μm channels between the support structures. Panel A: top view of a section of column showing collocated support structures and high degree of spatial symmetry in their distribution. Magnification × 2500. Panel B: angled view of support structures showing 1.5 μm channels. Magnification × 5000. Panel C. A single monolith support structure showing roughness in the channels walls at the level of 1000 Å. Magnification × 9000.

also been considered, but it is difficult to restrict polymerization to specific channels on a wafer. Open tubular columns are yet another option. The problem with this approach is that the requisite 2 μm channels for the rapid mobile phase mass transfer [17] are easily plugged.

The difficulty involved with producing chromatography columns on wafers leads to the question, why not form all the components of the column by micromachining them into the wafer substrate? Unfortunately, microfabrication does not easily produce the spherical structures generally used in chromatography. The nearest geometric approximation that can be easily fabricated is either a cylinder or a cube. Scanning electron micrographs of 5×5 μm collocated monolith support structures (COMOSS) cubes micromachined into a quartz wafer (Fig. 1A, B, respectively), show that they might actually be superior to conventional spherical particles for chromatography, particularly with regard to uniformity of support distribution, regularity of support particles, and the congruity of channels between them. Surface heterogeneity observed at the level of 1000 Å (Fig. 1C) is unlikely to impact analyte dispersion in channels. When a second wafer is thermally bound to the micromachined wafer (Fig. 1A) in such a manner as to enclose this 'bed' of microfabricated support structures and channels, a chromatography column is formed.

The major differences between columns COMOSS and packed bed chromatography columns are that, (i) supports fabricated in situ are bonded to the walls of the column at both the top and bottom of the monolith, (ii) the supports are more uniform in size, (iii) touching is not required for mechanical stability in monolith support beds, (iv) channel dimensions do not depend on the packing process in COMOSS columns, (v) channel width can be varied independent of monolith size, (vi) mobile phase velocity between structures will be more uniform in fabricated channels, (vii) there are no unswept lateral channels, and (viii) the bed and channels, as a whole, are more uniform.

Packing columns with particles allows wide variations in column volume. This raises the question of size limits in columns of microfabricated monolith structures. The maximal linear length of a microfabricated column is determined by the diameter of the wafer. Columns of 20 cm in length can be fabricated on an 8″ wafer. Width along the major lateral axis is easily increased by lateral addition of monolith structures. Columns of a few cm in width are probably possible, although only 150 μm wide columns have been made. (This assumes that the sample could be uniformly distributed across the column inlet, which will be addressed below). Increasing column width along the minor lateral axis was achieved by etching deeper channels (Fig. 2). The elongated $5 \times 5 \times 9$ μm monolith is roughly equivalent to the height of two particles. Using deep reactive ion etching [18,19] or LIGA [20,21], it will be possible to fabricate even deeper 1.5 μm width channels anisotropically, perhaps of 50 μm depth. This would increase the volume of columns from $\sim$20 to 100 ηl. Based on the various models described above, it may be calculated that the total volume of micromachined columns will generally not exceed 10 ηl.

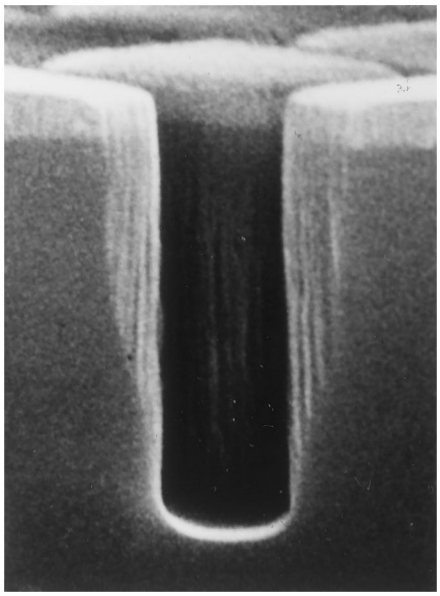

Fig. 2. Scanning electron micrograph showing side view of transport channels and COMOSS. Magnification $\times$5010. The lighter, domed objects on the top of monolith structures are residual photoresist. Prior to bonding the cover wafer, this photoresist is removed by exhaustive solvent washes.

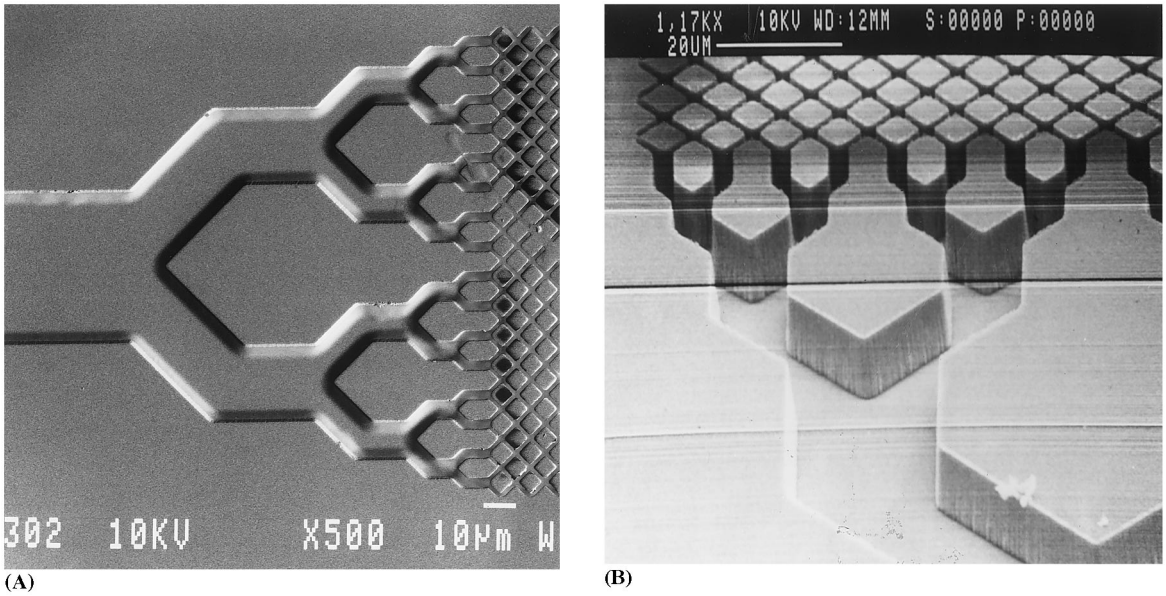

Fig. 3. Scanning electron micrographs of column inlet distributor and outlet collector. Panel A: inlet distributor. Magnification $\times 500$. Panel B: outlet collector. Magnification $\times 1170$.

Uniform distribution and collection of analytes at the column inlet and outlet, respectively, is essential for high column efficiency. This problem was addressed using the micromachined network seen in Fig. 3(A) to form a column inlet distributor. Mobile phase streams were merged at the column terminus with a system that is the reverse of the inlet distributor (Fig. 3B). With this architecture (i) columns have 2^n channels, (ii) all channels in a plane perpendicular to the separation axis are of the same width and height, and (iii) the distance for the center of the fluid distributor inlet to any channel at the head of the column is equal. The channel network in the distributor and column were also designed to minimize solvent degassing when using electroosmotic pumping [22]. It was reasoned that if the combined cross sectional area of channels at the head of the column is greater than at any point in the inlet distributor, electroosmosis will create a negative pressure in the inlet and possibly cause solvent out-gassing. This was addressed by designing a network in which the combined lateral cross sectional area of channels was the same at all points along the separation axis in the inlet, the column, and the outlet. Columns were operated at up to 5000 V cm^{-1} without apparent out-gassing.

An issue today in microfluidic analytical systems is how liquids can best be aliquoted and transported [23]. Although a wide variety of mechanical micro-pumps have been described, none produce the pressure required to drive high performance liquid chromatography columns. COMOSS columns were evaluated exclusively with electroomostically driven liquid transport [24,25] for this reason. There were sufficient ionized surface silanols in these silicon dioxide columns to cause formation of the requisite double layer for electroosmotic pumping above pH 4, even when the capillary walls were derivatized with organosilane stationary phases. Channels filled with either 10 mM buffer or some combination of buffer and acetonitrile transported liquid toward the cathode when potential was applied. At 1700 V cm^{-1}, neutral analytes were transported through the channel network in Fig. 1(A) at a velocity of 4.5 mm s^{-1} using 10 mM phosphate buffer at pH 7.0.

Electroosmotically driven liquid chromatography in capillaries has come to be known as capillary electrochromatography (CEC) [26,27]. The

attractive features of electroomotically driven flow with multiple nanoliter volume columns on a wafer are that (i) the mobile phase is driven by charged surfaces within each column (ii) flow can be induced in all columns simultaneously by voltage alone, and (iii) there are no mechanical parts used for pumping. The requisite charge necessary for electrosomotic pumping in CEC is provided either by (i) residual silanol groups left after organosilane derivatization of the surface of silica supports with stationary phase or (ii) charged groups in the stationary phase. The 32 channel column of $5 \times 5 \times 9$ μm COMOSS separated by 1.5×9 μm^2 rectangular channels shown in Fig. 1A, was chosen for CEC. The combined cross sectional area of channels across the width of this column was 500 μm^2. Two stationary phases were used in this study; siloxane coupled dimethyoctadecyl silane and electrostatically bonded polystyrene sulfate. Silylation of monolith support structures was achieved by a procedure porous silica [28]. Polystyrene sulfate (70 kD) was adsorbed from water to channel surfaces subsequent to silylation of the channel walls with γ-aminopropyl trimethoxysilane [29,30]. Sample introduction was achieved through a 'cross-type' inlet microfabricated into the system before the inlet distributor [12]. Isocratic elution of Rhodamine 123 from a 4.5 cm column with a mobile phase consisting of 50% aqueous acetonitrile (Fig. 4) showed an efficiency of ~ 35000 plates (777000 plates m^{-1}). This is equivalent to efficiencies achieved in CEC using columns packed with particles ≤ 1 μm in diameter [31]. When this 4.5 cm column was operated in the capillary electrophoresis (CE) mode without a bonded phase at 1700 V cm^{-1}, the separation efficiency for Rhodamine 123 (Fig. 5) was 76500 plates (1.7 million plate m^{-1}). Based on the fact that the separation efficiency in the CEC mode is roughly half that in

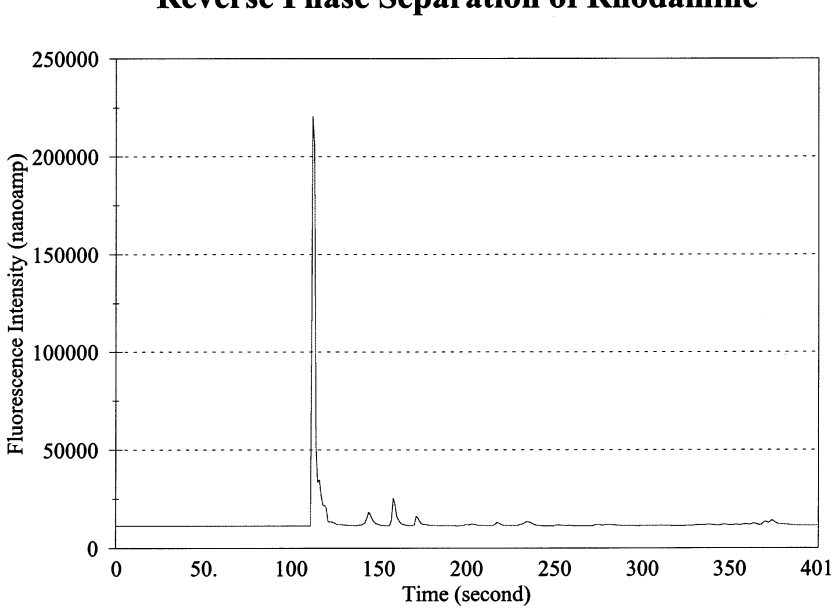

Fig. 4. Chromatogram of a Rhodamine 123 sample from a microfabricated reversed phase column operated in the capillary electrochromatography mode. The separation was achieved with a 4.5 cm long, 32 channel column of $5 \times 5 \times 9$ μm COMOSS separated by 1.5×9 μm rectangular channels. The stationary phase was 70 kD polystyrene sulfate which has been adsorbed electrostatically to the walls of a γ-aminopropyl silane derivatized capillary [29,30]. The column was operated isocratically at 1700 V cm^{-1} with a mobile phase consisting of 1/1 mixture (v/v) acetonitrile and 0.01 M phosphate buffer at pH 7. Detection was achieved by laser induced fluorescence using an argon-ion 488 nm laser. The excitation and emission wave lengths were 488 and 546 nm, respectively. Samples were introduced into the column through a microfabricated 'cross-injector' as described in the literature [12].

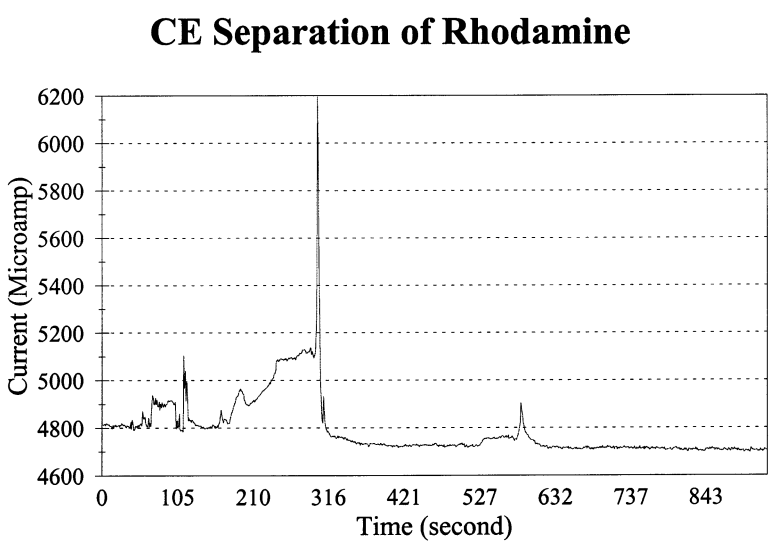

Fig. 5. Electropherogram of a Rhodamine 123 sample from a microfabricated column operated in the capillary electrophoresis mode. The column, inlet, and laser induced fluorescence detection system were the same as used in Fig. 4, except that the column had no stationary phase. The column was operated at 1700 V cm^{-1} with 0.01 M phosphate buffer, pH 7.

the CE mode, it is apparent that there are mass transfer limitations in the chromatography mode. It is not clear whether this is due to diffusion limitations in the mobile or stationary phase.

It is known that separation speed and efficiency in CEC are dominated by mass transfer and heat dissipation [26,27]. Theory tells us that the way to deal with these problems is to minimize the column diameter to increase heat transfer and reduce the transport channel size to enhance mobile phase mass transfer. It is in this context that micromachined columns provide a great advantage. In the case of heat transfer, COMOSS columns were operated at 2500 V cm^{-1} without overheating much higher than normally used with CEC columns and is attributed to three unique features of these columns [26,27]. The fact that it is < 5 μm from any point in the column to a heat dissipating column wall enhances heat transfer. The unique architecture of the column also contributes to heat dissipation. Because monolith structures have higher thermal conductivity than water, they act as cooling fins. Heat transfer to the column walls is also facilitated by the fact that monolith structures are attached to the column walls at both the top and bottom of the monolith. This enhances heat transfer away from the separation channel to the face of the wafer. The difficulty in packing the requisite small particles for liquid chromatography is another problem that in situ micromachining circumvents. Although it is possible to produce submicrometer chromatography support particles, it is difficult to pack uniform, stable beds and retain particles in columns [31]. Chromatography columns based on COMOSS, on the other hand, could be easily fabricated with high quality and uniformity as demonstrated in Fig. 1. More importantly, multiple columns and column networks could be fabricated without significantly increased difficulty and cost in comparison to single columns, which is decided by the nature of microfabrication. This paper examines how uniformity issues associated with channel dimension and support size along with problems of packing conventional chromatography columns may be addressed with alternative technology in the specific case of producing multiple high resolution chromatography columns on wafers. In situ micromachining technology was used to simultaneously place and define, to

the level of ± 1000 Å, all the components of multiple separation columns on a single wafer. Moreover, the channel network architecture described allows high rates of mass transfer and heat dissipation while affording frequent interchannel mixing to compensate for interchannel heterogeneity. At an operational level this allows one to enjoy the resolution and transport properties of 1.5×9 μm channels in multiple columns and escape the catastrophic failure issue associated with plugging single channel columns.

How will this impact chromatography? The explosive growth of microfabrication techniques that triggered the semiconductor and micro-electromechanical systems (MEMS) revolutions will continue. Reductions in minimum structure size are projected to continue at the rate of roughly 20–25% per year from the current limit of 3000 to 500 Å by 2017 [32]. Particle size decreases in liquid chromatography, in contrast, have occurred at less than half this rate and are currently at slightly less than 1 μm. These facts and the data presented in this paper make it clear that the increased speed, resolution, and throughput life scientists desire in analytical liquid chromatography systems are more likely to evolve from in situ microfabrication than an extension of existing packed column technology. This could have a significant impact on the way liquid chromatography columns are produced in the future.

Probably a more important question is, how will this impact life science? The substantial attention being focused today on massively parallel bioanalytical strategies and very high throughput analytical systems as a way to accelerate discovery and diagnostics suggests we are entering a biometric revolution. The essential feature of this revolution is that 'one-at-a-time' experimentation will give way to protocols in which hundreds or thousands of analytical measurements are made in parallel. The success of this revolution depends on minaturization and integration of all aspects of measurement systems; including the separation component. The broad significance of this work to the biometric revolution is that a widely used bioanalytical tool has been reduced to the nanoliter volume, laboratory-on-a-chip format necessary for high throughput, parallel processing.

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