A Picoliter-Volume Mixer for Microfluidic Analytical Systems

ARTICLE *in* ANALYTICAL CHEMISTRY · JUNE 2001

Impact Factor: 5.64 · DOI: 10.1021/ac000850x · Source: PubMed

5 AUTHORS, INCLUDING:

Fred E Regnier
Purdue University
354 PUBLICATIONS **17,424** CITATIONS

Anal. Chem. **2001,** *73,* 1942−1947

A Picoliter-Volume Mixer for Microfluidic Analytical Systems

Bing He,† Brian J. Burke, Xiang Zhang, Roujian Zhang, and Fred E. Regnier*

Department of Chemistry, Purdue University, Lafayette, Indiana 47907

Mixing confluent liquid streams is an important, but difficult operation in microfluidic systems. This paper reports the construction and characterization of a 100-pL mixer for liquids transported by electroosmotic flow. Mixing was achieved in a microfabricated device with multiple intersecting channels of varying lengths and a bimodal width distribution. All channels running parallel to the direction of flow were 5 μm in width whereas larger 27-μm-width channels ran back and forth through the parallel channel network at a 45° angle. The channel network composing the mixer was ∼10 μm deep. It was observed that little mixing of the confluent solvent streams occurred in the 100-μm-wide, 300-μm-long mixer inlet channel where mixing would be achieved almost exclusively by diffusion. In contrast, after passage through the channel network in the ∼200-μm-length static mixer bed, mixing was complete as determined by confocal microscopy and CCD detection. Theoretical simulations were also performed in an attempt to describe the extent of mixing in microfabricated systems.

Microfluidic systems of 50−100-μm channel width are now being described that require mixing as part of an analytical protocol.[1−4] Liquid streams generally enter these systems laterally along one or both sides of a central channel, frequently at different points. Channels of this width are perhaps too large for rapid diffusive mixing and too small to allow installation of a dynamic mechanical mixer. At issue is the rate and degree to which mixing occurs in the micromachined systems noted above.

Some type of *static mixer*[5] capable of substantial lateral transport would seem to be a better alternative than either purely diffusive or mechanical mixing. Because the volume of current microfluidic systems is generally in the range of 1−10 nL/cm and it would be desirable to effect mixing within 0.1−1 mm of transport distance along a channel, mixing would have to be achieved in a volume of hundreds of picoliters. The question is how to build a *static mixer* of this volume with a high degree of lateral transport.

Static mixing is frequently accomplished in liquid chromatographic packed beds. The primary mechanism for mixing in this case results from splitting the stream of liquid moving through the bed into a large number of microstreams traveling between particles at slightly different velocities.[6,7] These velocity differences result from heterogeneity in channel dimensions and mix by a mechanism referred to as eddy diffusion in the chromatography literature.[6] It is also known from the chromatography literature that mixing or dispersion in packed beds is further enhanced by poor mass transfer between these streams and stagnant pools of liquid within porous particles in the bed.

The problem with packed-bed static mixers is that they are far more effective in promoting longitudinal than lateral mixing. As a consequence, their mixing efficiency is poor and large volumes of liquid are required for mixing. The mixing requirements for microanalytical devices[8−12] are far smaller than can be met with these packed beds. One solution to this problem is to merge two liquids to be mixed in many microchannels simultaneously.[9] Although the streams laminate at the point of confluence, mixing by lateral diffusion occurs with much higher efficiency than in packed beds. Mixers ranging down to 600 nL have been produced in this way.[9] However, capillary electrochromatography columns (CEC) are now being reported that range down to 15 nL in volume.[13] Solvent gradient formation with columns of this size requires a mixer of a few hundred picoliters or less. To date, a microfabricated mixer has not been reported that can effectively mix volumes needed for these miniaturized CEC systems.

It was the objective of this work to design, fabricate, and test an electroosmotically driven mixer that was at least a 1000-fold smaller than previously reported mixers. Efficacy of the resulting mixer and the extent to which mixing occurs by diffusion alone in microchannel systems was evaluated.

MATERIALS AND METHODS

Materials. Photolithography masks, SL-4006-2C-AR3-AZ1350, and 3-in. quartz wafers, QZ-3W40-225-UP, were purchased from

† Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799.

(1) Jacobson, S. C.; Hergenroder, R.; Moore, A. W., Jr.; Ramsey, J. M. *Anal. Chem.* **1994**, *6*, 4127.
(2) Seiler, K.; Fan, Z. H.; Fluri, K.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 3485.
(3) Hadd, A. G.; Raymond, D. E.; Halliwell, J. W.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **1997**, *69*, 3407.
(4) Fan, Z. H.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 177.
(5) The term *static mixing* is obviously both a misnomer and a contradiction but will be used here because of its broad usage in the literature. Actually, any system in which there is convective transport is in a dynamic state of flux.
(6) Farkas, T.; Guiochon, G. *Anal. Chem.* **1997**, *69*, 4592.
(7) Terry, S. C.; Jerman, J. H.; Angell, J. B. *IEEE Trans. Electron Dev.* **1979**, *ED-26* (12), 1880.
(8) Branebjerg, J.; Birgit, F.; Gravesen, P. *Proceedings of the μ-TAS '94 Workshop*; Kluwer: London, 1994.
(9) Bessoth, F.; deMello, A.; Manz, A. *Anal. Commun.* **1999**, *36*, 213−215.
(10) Havenkamp, V.; Ehrfeld, W.; Gebauer, K.; Hessel, V.; Lowe, H.; Richter, T.; Wille, C. *Fresenius J. Anal. Chem.* **1999**, *364*, 617−624.
(11) Haswell, S.; Skelton, V. *Trends Anal. Chem.* **2000**, *19*, 389−395.
(12) Ameel, T.; Papautsky, I.; Warrington, R.; Wegent, R. *J. Propul. Power* **2000**, *16*, 577−582.
(13) He, B.; Tait, N.; Regnier, F. E. *Anal. Chem.* **1998**, *70*, 3790−3797.

10.1021/ac000850x CCC: $20.00
Published on Web 03/28/2001

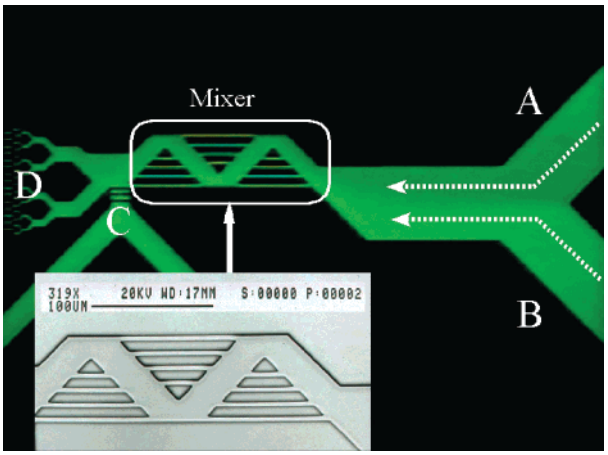

Figure 1. Photomicrograph of the microfabricated mixer. The small image in the lower left corner is the SEM of the mixer. This mixer is about 100 by 200 μm wide and 10 μm in depth. Effect of mixing is evaluated by bringing in two fluids from channels A and B, and flowing to D. Channel C is not used in this work.

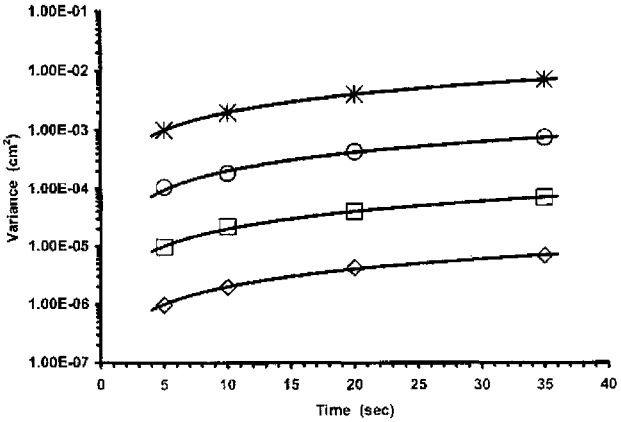

Figure 2. Comparison of the Eistein−Smoluchowski and 3-D random walk methods of evaluating diffusion. The solid line is a plot of variance predicted by the Einstein−Smoluchowski equation. The symbols are values obtained by simulation where the symbol ◇ is for 10^{-7} cm^2 s^{-1}, □ is for 10^{-6} cm^2 s^{-1}, ○ is for 10^{-5} cm^2 s^{-1}, and * is for 10^{-4} cm^2 s^{-1}.

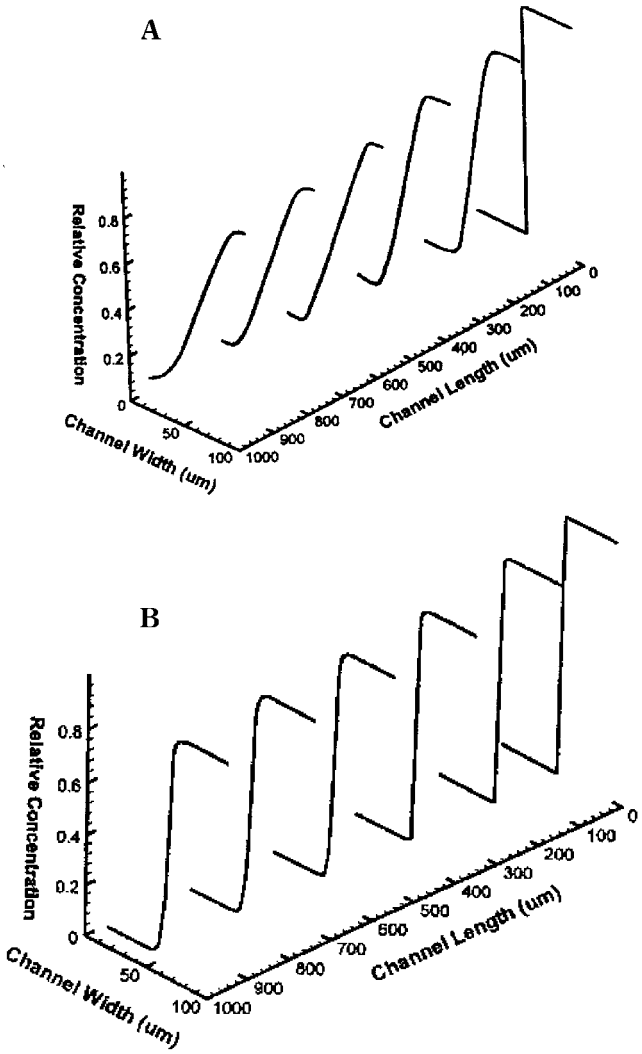

Figure 3. Simulation of two reagents merging together in an open channel, entering as two discrete zones. Mixing begins to occur between the two reagents as they move further down the channel. Mixing is noted in this figure as the one zone begins to move laterally across the channel. (A) shows the mixing for a solution with a diffusion coefficient of 10^{-6} cm^2 s^{-1} and (B) is 10^{-8} cm^2 s^{-1}.

Hoya Corp. (Shelton, CN). This quartz wafer is 400 μm thick, has a coefficient of thermal expansion that is $<5 \times 10^{-7}$/K and has an ultraflat surface (<3-μm variation across the whole wafer surface).

Chip Fabrication. The computer system used for chip layout, transfer of the GDS formated files to the fabrication laboratory, and photomask production has been described previously.[13] The etching of monolith structures in quartz wafers, production of cover plates with access holes, thermal bonding of the cover plate, and chip storage were executed according to the literature. A photomicrograph of this microfabricated device is shown in Figure 1.

Chip Treatment. Internal surfaces of the chip were activated to produce the high density of silanol groups necessary for both wafer surface wetting and electroosmotic pumping. All buffer wells were filled with a mixture of MeOH/H_2O (1:1) with one outlet connected to a Barnant (Barrington, IL) vacuum pump. A 20 mmHg vacuum was applied to wet the internal surface of the microchip. NaOH (1.0 M) was then introduced and retained for 1 h to activate internal silanol groups. The microchip was then rinsed with MeOH/H_2O (1:1). Finally, the mixer was treated with 10 mM, pH 7.0 potassium phosphate buffer.

Microfluidic Voltage Control. During mixing, liquids were moved through the mixer exclusively by electroosmotic flow using a computer-controlled power supply. A National Instruments (Austin, TX) AT-AO-10 analog power output card was used to generate analog voltages (0−10 V, 0−20 mA) in up to 10 channels. This analog voltage was then amplified 1000-fold using a Gamma (Ormond Beach, FL) PMT10-0.1P/M power supply to provide voltage output of 0−10 000 V and current output up to 100 μA for each channel. Voltage output was programmable through an in-house-written LabVIEW program (National Instruments).

Confocal Fluorescence Photomicroscopy. A Nikon Inverted Eclipse TE-300 optical microscope, equipped with a TE-FM epifluorescence attachment, was used to monitor the performance of the mixer. A 10^{-4} M fluorescein solution (Sigma, St. Louis, MO) in sodium phosphate buffer (10 mM, pH 7, with trace of methanol) was used as the test solution to monitor mixing. A Nikon EF-4

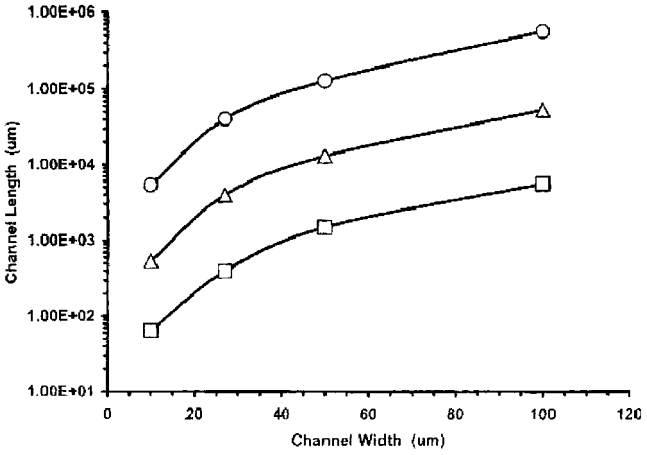

Figure 4. Theoretically predicted channel length needed to allow complete mixing. The values are obtained by simulation where the symbol ○ represents analytes with diffusion coefficients of 10^{-8} cm^2 s^{-1}, Δ is for 10^{-7} cm^2 s^{-1}, and □ is for 10^{-6} cm^2 s^{-1}.

B-2E/C FITC filter cube was used with the fluorescein analyte because of their similar excitation and emission characteristics. This filter cube selectively allows the passage of lines around 488 nm as the excitation beam while blocking their entry at the detection window. A DVC-1310 Mega-Pixel CCD digital video camera (Austin, TX) was used on the side port to collect real-time fluorescence images. A PIXCI PCI imaging board and XCAP image processing program (EPIX Inc., Buffalo Grove, IL) was used to capture and process images. The CCD and peripheral equipment were assembled by Vision 1 (Bozeman, MT).

RESULTS AND DISCUSSION

It is frequently the objective in microfluidic systems to perform analyses quickly. The assessment of mixing in microchannel systems was addressed in two ways. The first was by mathematical modeling of mixing in converging streams. The second method used confocal microscopy to visually and quantitatively measure the extent of mixing in both open channels as well as a microfabricated mixer. The mathematical modeling will be addressed first to describe the mixing problems in microfabricated systems.

Theoretical Assessment of Mixing. A three-dimensional random walk algorithm was developed to study diffusion-controlled mixing phenomena in microchannels. Some assumptions were made in developing this model. First, the assumption was made that liquid transport occurred exclusively by electroosmotic flow (EOF). Second, it was assumed that all analytes and reagents were neutral and moved through the system at an average velocity equal to the EOF. A third assumption was that all analytes and reagents were pointlike in dimensions and did not interact with mobile-phase buffer constituents. Fourth, it was assumed that transport in each dimension within channels was fully independent. Finally, an elastic collision mechanism was applied whenever a molecule collided with a channel wall.

Initially, a predetermined number of molecules were injected into an inlet channel in a random spatial distribution across the

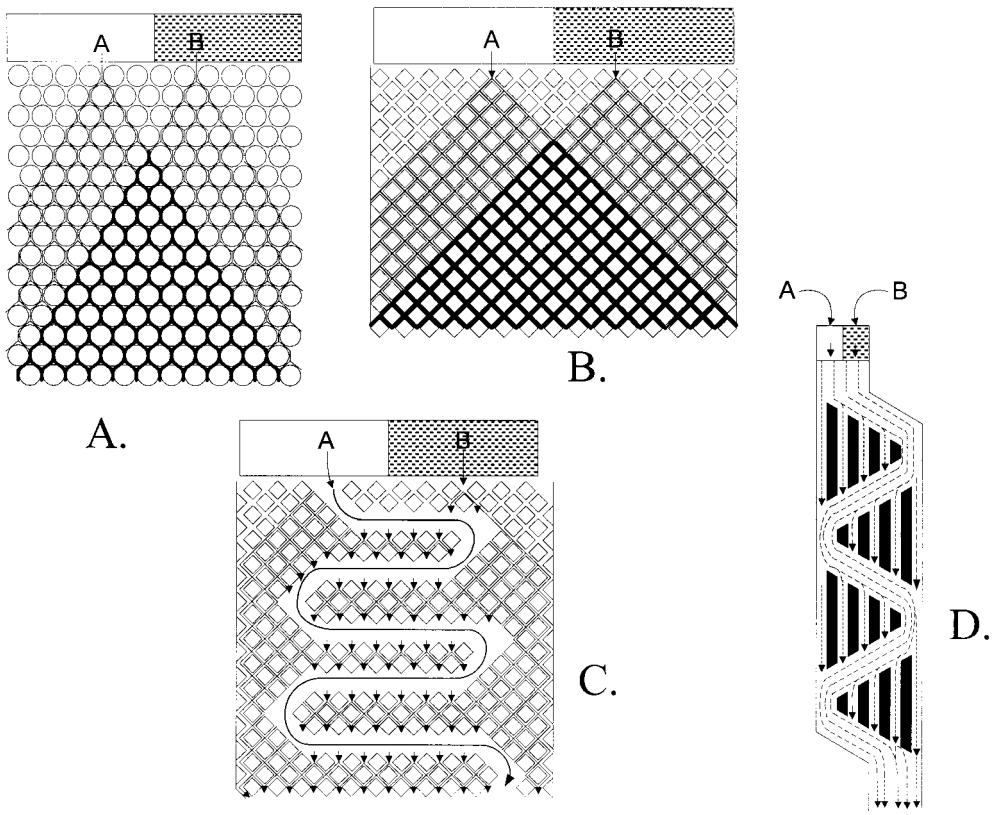

Figure 5. Evolution of the microfabricated mixer. (A) shows a 2-D image of a packed bed and how mixing might occur if two discrete zones of sample were injected. As the analytes move further down the column, the two reagents begin to mix as shown in the darkened regions. (B) is a similar image except a microfabricated column on a chip is seen. (C) shows how flow might occur if some "particles" from this bed are removed. It seems from this diagram as if the analytes will have more area to interact and potentially mix. (D) is the microfabricated mixer used in this work.

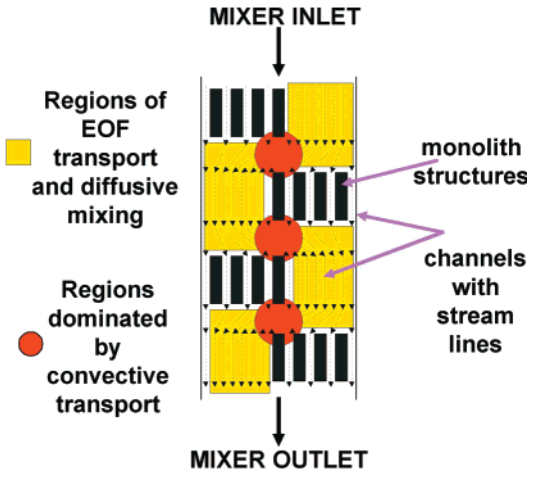

Figure 6. How flow might occur in a mixer similar to the one used in this work. Diffusive mixing is enhanced and convective mixing also takes place, which would not occur in open channels.

lateral plane of the channel. Flow was then initiated in the channel for a fixed time increment. At the end of this increment, flow was interrupted and the position of each analyte molecule within the three-dimensional space of the channel was calculated based on EOF and diffusion. This incremental transport process was repeated until all molecules reached the end of the channel or until complete mixing occurred. After the simulation, the population was summed in discrete segments of distance.

During each time increment, all molecules will move together with different diffusion step lengths. The probability function of molecular diffusion step length of all molecules in each time increment was derived from the Gaussian profile:

$$P = (2\pi\sigma^2)^{-1/2} \exp-(\rho - m)^2/2\rho^2 \qquad (1)$$

where ρ is the diffusion step length in each time increment (δt), σ^2 is standard variance given by the Einstein−Smoluchowski equation,[14] and m is the most probable diffusion step length. The term m is defined as

$$m = (4D\delta t)^{1/2} \qquad (2)$$

where D is diffusion coefficient.[15]

The variance of 10 000 molecules was monitored as a function of simulation time in an effort to verify the diffusion algorithm. Simulated results were compared with predictions based on the Einstein−Smoluchowski equation.[14−15] As seen in Figure 2, good agreement is observed for diffusion coefficients in the range of 10^{-4}−10^{-8} cm^2 s^{-1} using a 1-ms time increment between the simulation and the predicted values.

The impact of analyte or reagent diffusion coefficient on mixing was examined by merging analyte-bearing streams into a central channel from opposite sides at a velocity of 0.25 mm/s. The relative degree of mixing was computed when analyte was injected into the system through a stream at a rate of 200 molecules/ms. The trend in Figure 3 is as expected. Molecules with higher rates of diffusion are easier to mix. This modeling predicts that smaller analytes with higher rates of diffusion will not be fully mixed even 1 mm down the channel (Figure 3A). The mixing problem becomes even worse with larger analytes having lower diffusion rates as seen in Figure 3B. This becomes a serious issue when mixing is needed in a short distance, as is the case in most microanalytical systems.

Obviously, with sufficiently long channels, even molecules with very slow rates of diffusion will mix. The issue is whether mixing occurs within a sufficiently short distance to be practical in terms of time and architecture of the analytical system. Figure 4 shows the channel length that will be needed to achieve complete mixing in the "Y"-type architecture illustrated in Figure 1. Linear velocity was taken to be 0.25 mm/s, and mixing was defined as complete when the difference in analyte concentration varied less than 5% between opposite sides of the channel. Mixing was poorest with substances that diffuse slowly, such as macromolecular species. It is also seen that channel width must be decreased to less than 50 μm to have any substantial impact on mixing efficiency in short distances. Although it is possible to prepare and operate microfluidic systems with channels of 10-μm width, they are less desirable from an operational standpoint. The conclusion drawn from these theoretical calculations is that full mixing is difficult to achieve in short distances when solutions are pumped by EOF. The problem becomes significantly worse as the size of the analytes increase.

In an attempt to compare some of the theoretical predictions to experimental results, a chip with a simple cross on it was used with dimensions of 100 μm wide and ~20 μm deep. Buffer was brought in from one channel and a solution of fluorescein (representing a high diffusion coefficient) flowed in from the other side. Full mixing was determined using the CCD camera when the fluorescence profile across the channel was completely flat. The theoretical calculations predicted that a small molecule, like fluorescein, in an open channel of 100 μm, would take ~3 mm to fully mix. The experimental results showed that it would take between 2.5 and 3 mm to fully mix. Because the method of determining the channel length was of fairly low resolution, it was felt by the authors that the theoretical predictions compared nicely with the experimental results at this flow rate and channel length.

Design of a Static Mixer. Recent reports[13] have shown that particle-type beds of 0.1−10-nL volume can be microfabricated. The ease of miniaturization in this process led to the selection of a design that mimicked a packed-bed mixer. Mixing in particle beds, as in a typical LC column, is strongly dependent on transchannel coupling[16] as represented in Figure 5A. Transchannel coupling is the process by which liquids mix when adjacent streams merge after passing around particles in a bed. Lateral mixing occurs gradually, as the liquid streams pass along the principal flow axis of the bed. These microfabricated beds are essentially one particle deep. Particles in the literature[13] are 5 × 5 × 10 μm cubes micromachined into a quartz substrate that is covered with a quartz wafer by thermal fusion after micromachining. Channels between the particles were 1.5 × 10 μm. Transchannel coupling would be responsible for mixing in this bed as illustrated in Figure 5B.

(14) Einstein, A. *Ann. Phys.* **1905**, *17*, 549.
(15) Hopkins, D. L.; McGuffin, V. L. *Anal. Chem.* **1998**, *70*, 1066−1075.
(16) Giddings, J. C. *Dyn.amics of Chromatography*; Dekker: New York, 1964

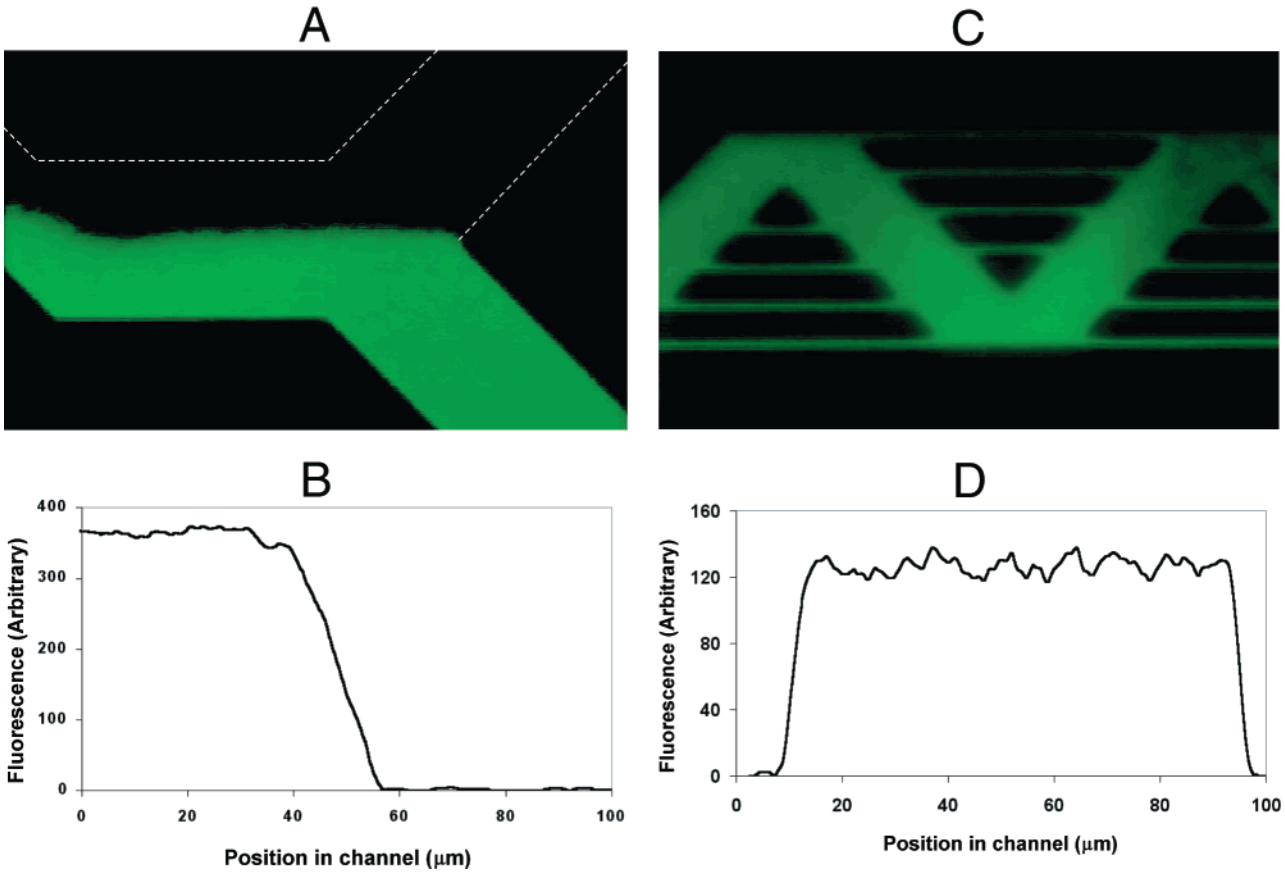

Figure 7. Two solutions merging together into one larger channel. The top solution is buffer and the bottom one is fluorescein in buffer. (A) shows mixing of the solutions before entering the mixer. The distinct interface between the fluorescein solution and the buffer shows lack of mixing at the inlet to the mixer, although they flow together over a length of ~300 μm. (B) is the fluorescence profile of (A). Little mixing is occurring as noted by two discrete areas in the graph. If the solutions are mixed, the profile would be flat. (C) shows mixing at the outlet of the mixer. Complete mixing seems to be achieved by visual inspection. (D) is the fluorescence profile of (C). Complete mixing has occurred as evidence by the flat fluorescence profile.

The problem with both of these systems is that mixing is heavily dependent on transchannel coupling. It is known that heterogeneity in packing density and particle size increase both lateral and longitudinal mixing in pressure-driven systems by providing a range of channel sizes that further enhance mixing.[16] This concept could be further amplified by arranging these voids in a pattern (Figure 5C and D) that would introduce still greater lateral transport into the migration of liquid streams through the bed. This strategy particularly addresses the issue of mixing confluent streams that are spatially heterogeneous across the lateral axis of a channel. Obviously, many other patterns of channel architecture would be equally effective in promoting lateral flow. The most critical elements of the selective void strategy illustrated in Figure 5C and D are the much larger degree of lateral transport and the fact that there are a large number of flow paths through the bed of differing length, both of which enhance mixing. The channel architecture in Figure 6 is a simplified version of the mixer used in this work. The purpose of this figure is to rationalize how flow might occur in these types of channel networks. Unfortunately, EOF in beds of tortuous channels has not been modeled to the level that exact flow profiles can be predicted.

This research used the diagram shown in Figure 5D as the model for the micromachined mixer. A picture of this mixer is seen in Figure 1. Major features of this design that should be noted are (i) the distribution of channel size is bimodal, (ii) the smaller 5-μm-width channels are parallel to the flow axis and vary in length, and (iii) the larger 27-μm-wide channels weave across the bed at a 45° angle to the longitudinal flow axis. The logic behind this design is that the bulk of the flow will travel through the larger channels and move both longitudinally and laterally across the system during transport through the bed. Although electroosmosis drives liquid through the system longitudinally, the alternating side-to-side distribution of large channels causes substantial longitudinal convective flow through the bed as illustrated in Figure 6. Smaller volumes of liquid leave the major semilateral stream and enter the minor 5-μm-width channels where migration is longitudinal and of varying path length. Upon leaving the minor channel, liquid again merges into the major channel. The channel network portion of the mixer is roughly 200 μm in length and has a computed volume of ~100 pL.

Assessment of Mixing by Microscopy. Mixing efficiency in this static bed was assessed at EOF rates of ~300 μm/s (very similar to the rate used in the theoretical model). This velocity was taken to be an average flow in many microfabricated systems. In Figure 7A, two streams merge into one larger stream and flow together over a length of ~300 μm. From visual inspection, it seems as if very little mixing is occurring between the fluorophore and the buffer as was predicted by the theoretical calculations. This visual assessment was experimentally confirmed using a CCD camera that can convert a fluorescence image into digital data.

Therefore, one can monitor the fluorescence in a channel, and essentially the mixing profile, at any area on the chip. The fluorescence profile for 7A is seen in Figure 7B. It is obvious from these data that little mixing is occurring. If the solutions were mixed, one will see a homogeneous, flat fluorescence profile. In Figure 7B, two distinct zones are seen with very little interaction between them.

The two streams in Figure 7A merge together, flow into and through the microfabricated mixer as seen in Figure 7C. It seems visually as if by the end of the mixer that the fluorophore and buffer are fully mixed. Our visual assessments were experimentally confirmed in Figure 7D, suggesting that the microfabricated mixer presented here will mix these two solutions. The interesting thing to note is that this mixing was accomplished over a length of ~200 μm, while in open channels, it was experimentally found that it would take ~3000 μm under the same conditions to fully mix these solutions.

CONCLUSIONS

Based on data presented in this paper it is concluded that when two, or perhaps more, electroosmotically pumped liquid streams enter a microchannel of ≥50-μm width at velocities around 0.3 mm/s from spatially different points (i) little mixing occurs within the first 300 μm of transport distance after contact and (ii) the small amount of mixing that does occur is by diffusion alone. This dilemma is most serious with macromolecular analytes when analysis times of less than 10−20 s are desired and is probably the Achilles heel of "lab-on-a-chip" systems of >50-μm channel width that depend on diffusive mixing.

It is concluded that static mixing systems consisting of a microchannel complex of crossing channels that encourage lateral transport can partially, or perhaps totally solve this problem. At highest velocity, mixing can be achieved in a 100-pL system in less than 1 s. Mixers of this type should be of broad utility in all microfluidic systems where rapid mixing is required to either initiate a chemical reaction or prepare mobile phases in liquid chromatography.

ACKNOWLEDGMENT

The authors gratefully acknowledge support from the National Institute of Health (Grants 25431 and 035421) and PE-Biosystems. The Jonathan Amy Facility Center for Instrumentation is thanked for their time and helpful conversations (Purdue University, West Lafayette, IN).

Received for review July 27, 2000. Accepted December 31, 2000.

AC000850X