Published in final edited form as:

J Micromech Microeng. 2014 March ; 24(3): 034006–. doi:10.1088/0960-1317/24/3/034006.

Dispersion of a Nanoliter Bolus in Microfluidic Co-Flow

A J Conway¹, W M Saadi¹, F L Sinatra¹, G Kowalski², D Larson³, and J Fiering³

¹Bioengineering Center, Charles Stark Draper Laboratory, Tampa, Florida 33612, USA

²Department of Mechanical and Industrial Engineering, Northeastern University, Boston, Massachusetts 02115, USA

³Charles Stark Draper Laboratory, Cambridge, Massachusetts 02139, USA

Abstract

Microfluidic systems enable reactions and assays on the scale of nanoliters. However, at this scale nonuniformities in sample delivery become significant. To determine the fundamental minimum sample volume required for a particular device, a detailed understanding of mass transport is required. Co-flowing laminar streams are widely used in many devices, but typically only in the steady-state. Because establishing the co-flow steady-state consumes excess sample volume and time, there is a benefit to operating devices in the transient state, which predominates as the volume of the co-flow reactor decreases. Analysis of the co-flow transient has been neglected thus far. In this work we describe the fabrication of a pneumatically controlled microfluidic injector constructed to inject a discrete 50nL bolus into one side of a two-stream co-flow reactor. Using dye for image analysis, injections were performed at a range of flow rates from 0.5-10 μ L/min, and for comparison we collected the co-flow steady-state data for this range. The results of the image analysis were also compared against theory and simulations for device validation. For evaluation, we established a metric that indicates how well the mass distribution in the bolus injection approximates steady-state co-flow. Using such analysis, transient-state injections can approximate steady-state conditions within predefined errors, allowing straight forward measurements to be performed with reduced reagent consumption.

Keywords

Dispersion; Microfluidic Devices; Microfluidics; Co-Flow Reactor

1. Introduction

Microfluidic technologies are widely used for delivering small samples to both reactors and sensors. Because laminar flow typically prevails, predictable distributions of solutions can be established within microchannels. These distributions can be exploited for novel applications such as, on the one hand, continuous co-flow devices with steady-state concentration gradients usually transverse to flow, and, on the other hand, bolus or slug

injectors, which are of interest for their time-dependent concentration gradients along the flow direction. [1]

Continuous co-flow devices have been used in many applications. In these, two or more input channels merge into a single wider channel at constant velocity, creating a laminar flow interface where diffusion is usually the primary means of mass transport between them (figure 1a). This arrangement has been used in a number of examples. Yager and colleagues employed the pioneering “T-sensor” configuration and utilized diffusion at the interface to measure molecular properties of reacting species [2, 3]. Numerous groups have developed microfluidic devices that rely on diffusion between co-flowing laminar streams to generate concentration gradients for parallel parameterized experiments, with notable examples reported by the Whitesides and Toner groups [4, 5]. Moreover, gradients between co-flowing streams of different ionic strength have been used to modulate electric field for continuous separation of proteins and peptides [6, 7]. Theoretical descriptions of mass distribution in co-flow on the microscale are available and agree closely with experimental measurements[8-10].

Meanwhile, bolus injections into single streams are well known in other examples (figure 1a), where the solution of interest occupies only part of a channel, and flows in series with a leading and trailing fluid. In pressure-driven flow, diffusion plays less of a role at short time scales, but Taylor-Aris dispersion has a major impact on the mass distribution in pressure-driven flow[11]. The theory for dispersion in single channels has been studied extensively for both transient and steady-state regimes, and numerous equations have been derived to describe the effect of dispersion on bolus profile[11-14]. Many microfluidic systems for electrophoretic separations of molecules must also account for dispersion. In one such example, investigators applied a Taylor-Aris dispersion model to optimize the design of an electrokinetic injector in a capillary electrophoresis chip, in order to optimize resolution and signal-to-noise ratio of the separation [15]. While sample dispersion during injection often results in non-ideal concentration profiles, some researchers have taken advantage of axial bolus dispersion to produce concentration gradients for novel applications in microchannels [16, 17].

Here we characterize a less familiar case, in which a finite bolus of solution is injected into one side of a two-stream co-flow reactor (figure 1b). In this case, both dispersion and diffusion are significant, and the mass distribution is time-dependent and asymmetric with respect to the flow direction. The system has practical utility for delivering reagents to a microcalorimeter (figure 1c), which we describe elsewhere[18, 19]. The calorimeter uses a co-flow configuration such that the reaction under measurement occurs at the laminar flow interface between the two streams. It requires a predictable mass distribution across its many embedded sensor sites in order to calculate enthalpy of reaction, because species concentration data must be deconvolved from temperature data. While the calorimeter can operate in continuous steady state co-flow, new applications of the calorimeter can proliferate as the required sample size is reduced. Therefore, there is motivation to perform measurements with volumes of reagents less than that required to establish steady-state co-flow. As a result, the transient “quasi-co-flow” discussed below is a valuable mode of operation for the instrument.

In this paper, we present a detailed experimental investigation of the development of a bolus in co-flow. To our knowledge, this configuration has not been studied previously. We show results using dye and image analysis to monitor the mass distribution of a 50nL bolus, and we compare these results against simulations and single-channel dispersion theory at a range of flow rates. To simplify utility of bolus injections in co-flow reactors, we establish regimes in which mass distribution in the bolus injection can be approximated by single-channel injections or steady state co-flow, and we compare these for several cases. In addition, we describe the pneumatically controlled, integrated sample injector constructed for the test.

2. Methods

2.1. Device Fabrication

To inject a discrete 50nL bolus for analysis, a microfluidic injector using a pneumatically controlled valve system was fabricated (figure 2a). The volume of the bolus was selected arbitrarily as twice the nominal volume of the reactor. The injector scheme was similar to others reported previously.[20, 21] The device consisted of three layers shown in figure 2(b): a glass slide at the bottom and two polydimethylsiloxane (PDMS) (Dow Sylgard 184, Midland, MI) layers. The bottom of the two PDMS layers contain the control channels for the valve system (250 μ m wide $\times$ 30 μ m tall) as well as the co-flow reactor (500 μ m wide $\times$ 30 μ m tall), and the top PDMS layer (4mm thick) holds the reagent channels (250 μ m wide $\times$ 30 μ m tall). To fabricate the bottom PDMS layer, a Si wafer was patterned with SU-8 (Microchem Co, Newton, MA) using standard photolithography techniques to create a mold for forming the channels. After patterning SU-8, tridecafluoro-1,1,2,2-tetrahydrooctyl trischlorosilane (Gelest Inc, Morrisville, PA) was vapor-deposited on the patterned wafer for ease of PDMS removal. Uncured PDMS (10:1 base to curing agent weight ratio) was spun on top of the silane treated Si wafer at 1500 RPM for 1 min and cured for 1 hour at 65°C. This layer formed the pneumatic control lines and the co-flow reactor, had rectangular cross section and a 10 μ m PDMS film spanning the top. Using an X-acto knife, a 250 μ m by 1mm through hole was cut out at the entrance of each arm of the co-flow reactor. To fabricate the top PDMS layer, a Si wafer was patterned with AZ40-XT (AZ Electronic Materials) using standard photolithography techniques. This wafer was the mold for making 30 μ m tall channels that have rounded profiles. The AZ40-XT patterned Si wafer was also silane treated using the described method above. PDMS was cast on top of the wafer at a 10:1 base to curing agent weight ratio followed by degassing and curing at 65°C for 2 hours. The cast top layer was peeled off and permanently bonded to the bottom PDMS layer using oxygen plasma (Plasma Etch PE-100 Asher, Carson City, NV at 100mTorr, 10 sec, 200W). Fluidic ports were punched using a 1.5mm biopsy punch. The bonded PDMS stack was bonded to the glass slide using oxygen plasma (100mTorr, 10sec, 200W).

This system was designed to inject a 50nL bolus on demand during the course of an experiment without interrupting flow, switching connections, or introducing air bubbles. The pneumatic valve design was similar to others reported in the literature.[22]. As shown in figure 2(c), by pressurizing the control channel, the thin film of PDMS that separates the two chamber layers deflects upward and closes off the reagent channel. The control lines crossed the fluid channels at right angles resulting in a diaphragm size of 250 μ m $\times$ 250 μ m. After

assembly of the device as shown in figure 2(d), the injection of reagent sample was performed with the fluidic network as shown in figure 2 (a) and (c). All flow channels (both for reagent and reaction) were first primed with deionized water using a syringe pump. The injection loop was then primed with deionized water by closing valves 1 and 2 and filling the injection loop. The pressure on the control line to close the valves was set to 69kPa. Once primed, the contents of the injection loop were released by closing valves 3, 4, and 5 and opening valves 1 and 2. The syringe pump pushed the 50nL bolus out of the injection loop and into the reaction chamber. Switching of all 5 valves between the two states was instantaneous using electronically controlled solenoid valves external to the chip.

2.2. Intensity Measurements and Data Analysis

We monitored downstream development of the bolus by image analysis. A qualitative demonstration of an injection using colored dyes is shown in figure 3a-c. The injector's sample loop was primed with Reagent B while Reagent A flowed through the other channels (figure 3a); actuation of the valves diverted flow through the loop, injecting the bolus into the reactor (figure 3b-c). The valve state was then reconfigured for a new cycle of loading an injection.

For data acquisition, the injector's sample loop was primed with 0.04 wt% fluorescein (EMD Chemicals, Billerica, MA). Injections were performed at flow rates of 0.50, 1.0, 2.5, 5.0, and 10.0 $\mu\text{L}/\text{min}$ using a dual syringe pump (Harvard PHD2000). We captured images of the bolus injections using an inverted microscope (Leica DMI 6000B) with a monochrome camera (Leica DFC 340 FX) operating at 8-bit mode with a resolution of 800 $\times$ 600 pixels and 0.100 second exposure time at the co-flow reactor. ImagePro Plus software started acquiring the images of injections at a capture rate of five frames per second as soon as the injection loop valve was opened. Timing of the valve actuation was identified by an indicator lamp momentarily visible in the images.

We analyzed the images using ImageJ and Matlab software at 3 locations, designated as entrance, middle, and exit, located at 0.11, 1.54, and 3.09 mm from the Y junction (figure 3d) to extract the intensity measurements of the pixels at these respective locations. Note that the bolus travels an additional 6.73 mm in the inlet channel before reaching the Y junction. The intensity measurements at these locations were measurements from a top view across the channel width (x axis); hence intensity is summed through the channel height (y axis). For comparison with the bolus injections, we also imaged continuous co-flow of fluorescein and water without using the sample loop (figure 3e) at a series of flow rates. Consideration was taken for potential non-uniformities across the microscope's field of view by also capturing dark field images (only water in coflow reactor) and flat field images (fluorescein flooding the entire co-flow reactor). The image intensity data was adjusted for this by first subtracting the dark field intensity data from the raw data at each of the three locations. This value was then divided by the flat field intensity data at again these respective locations. "Difference maps" were generated by subtracting the normalized intensity at a given location in the steady-state co-flow from the corresponding location in the bolus co-flow, and plotting the difference over time and across the channel width. In other words, at a given time point and for a given z , we calculate the difference value, ΔI_x ,

using the expression $I_x = (B_x - C_x)/F_x$, where B_x , C_x , and F_x are the raw intensity values for the bolus co-flow, steady-state co-flow, and flat field images, respectively at a given position x across the channel width.

2.3. Simulation

Computational modeling (COMSOL Inc., Burlington, MA) of an approximated reactor geometry was performed in 2 dimensions in order to simulate dispersion and diffusion of a reagent within the co-flow channel (figure 4). For this initial exploration, 2-dimensional modeling served as an efficient trade-off between computing time and model accuracy.

To assess the effect on the concentration distribution from 2D and 3D geometry, we constructed plots of the average concentration (across width and height) versus time. These plots were compared at a flow rate of 1 $\mu\text{L}/\text{min}$ for a single channel using 2D (250 μm by 1mm channel) and 3D geometry (30 μm by 250 μm by 1mm channel). The R-Pearson value was calculated between the concentration plots for the 2D geometry and the 3D geometry, and was found to be 0.9982. This indicates that, at least for the single channel case, 2D simulations result in reasonable and efficient approximations for dispersion on this scale.

A Newtonian fluid dynamic module coupled with transport of diluted species module solved the Navier-Stokes and transport equations respectively. Continuity, momentum and convection-diffusion equations were solved simultaneously in the entire modeling domain, using the steady state form of the continuity and momentum equations for the laminar flow, and steady state convection-diffusion when simulating the continuous flow scenario. The transient convection-diffusion was used to simulate the injection scenario. Table 1 summarizes the equations, where $\mathbf{u}$, ρ , P , μ , C and D are the velocity, density, pressure, viscosity, concentration and diffusivity respectively. The time to reach steady state for flow in the co-flow reactor is almost instantaneous ($\sim 0.003\text{s}$ to $\sim 0.006\text{s}$) for the flow rates studied, confirmed by independent transient laminar flow simulations using the same geometry and flow rates studied in the co-flow reactor; hence solving the laminar flow as a stationary phenomenon is a reasonable approximation. The density and viscosity values were specified for water at room temperature. The diffusivity of fluorescein in water was taken from available values in the literature[23], and used for transport calculations in the co-flow reactor. The modeling parameters are listed in Table 2.

Channel layout and dimensions were taken from the device design. The geometries of primary interest to simulate were the co-flow reactor and the injector channel; both of which were included in the modeling domain. The length of the inlet channel of Reagent B (fluorescein) was determined by the location of valve 2, and a Poiseuille boundary condition was applied to solve the fluid dynamics. In the experimental device, the Reagent A (water) channel had a length of 3mm before merging into the co-flow reactor; meeting the fully developed condition $x/D_h > 10$ [24], where x is the channel length and D_h the hydraulic diameter of the channel cross-section. In the simulation, the inlet of Reagent A was arbitrarily specified to be 1mm from the Y junction, with a fully developed boundary condition. Zero-pressure outlet and no-slip fluid—wall interface boundary conditions were imposed to solve for the laminar flow.

A parametric study was performed for flow rates of 0.5, 1, 2.5, 5, and 10 $\mu\text{L}/\text{min}$ ($0.012 < \text{Re} < 1.186$), where the flow rate applied to Reagent A and B were equal to each other for each simulation. Anormalized concentration was applied to the inlet of Reagent B; zero inlet concentration was specified for Reagent A. During steady-state simulations the inlet concentration was continuous, whereas in transient simulations the inlet concentration was multiplied by a step function to control the injection duration of Reagent B. The injection duration was calculated for each flow rate assuming a 50nL volume. No-Flux and species outflow boundary conditions were imposed to the remainder of the boundaries. Delauney triangulation in addition with a boundary layer defined at the inlet of Reagent B set the mesh generation.

Mesh refinements were performed to minimize the difference between inlet mass flux and outlet mass flux. After two successive refinements, resulting in 569335 mesh elements, the error in mass flux ranged from 1.39% to 4.80%, increasing in error with higher flow rate. Further mesh refinements were tested on simulations using flow rates $> 1\mu\text{L}/\text{min}$; only a decrease in error of 0.5% was achieved leading to a more than 3-fold computation time increase. An iterative geometric multigrid (GMRES) algorithm solved the equations for the steady-state condition, while a direct backward differentiation formula (BDF) algorithm was required for the transient study. Time steps of 0.01s and 0.05s were also evaluated, showing no significant difference on the output of the simulations; hence 0.1s was chosen as the preferred time step which allowed for effective data processing. Data analysis for the computational model was performed at the same locations (entrance, middle and exit) as in the experimental data. Figure 5 shows the Reagent B concentrations for steady-state (a) and transient (b) simulations at a flow rate of 0.5 $\mu\text{L}/\text{min}$.

3. Results and Discussion

3.1 Traverse Profile Analysis

The experimental image intensity and simulated concentration profile across the channel width reflects the concentration of the fluorescent dye over time and shows the progression of the bolus. Figure 6 shows the detailed results at one representative flow rate. In this example, each stream is flowing at a rate of 1.0 $\mu\text{L}/\text{min}$. The profile data was collected at flow rates of 0.5, 1, 2.5, 5, and 10 $\mu\text{L}/\text{min}$. These rates correspond to an average velocity ranging from 1.11—22.22 mm/sec and Péclet numbers (with respect to channel width) from 1305 to 26117. Similar to single-stream dispersion, the concentration rises faster in the center of the bolus compared to its edges, until a nearly uniform profile is achieved, followed by a decline (again faster in the center) towards baseline. These results show that in order to extract information from an injection in a co-flow reactor in this transient regime, one must account for effects of dispersion.

Although the simulation captures the general trends of the experimental data, noticeable disagreement is observed. Two main discrepancies exist between 2D and 3D models for the channel aspect ratio (width to height) of 50/3 encountered in the tested device. First the flow profile across the channel width is parabolic for a 2D channel, but is almost uniform for a 3D channel [3]. Secondly, the thickness of the interfacial diffusion layer for a co-flow reactor is dependent on the channel height if $\text{Pe} > z/h > 1$, meaning that gradients generated

by the fluid boundary layer in the y -direction create a “butterfly” concentration along the channel height [8, 5]. Here, Pe is the Peclet number (with respect to channel height), z is the position along the channel length and h is the channel height. For cases where $z/h > Pe > 1$, the thickness of the interfacial diffusion layer is independent of the y -direction, because diffusive transport along the height of the channel eliminate the effects from the non-uniform velocity profile [5]. In the tested device at the locations examined, the transition between these two regimes occurs at about 1 $\mu\text{L}/\text{min.}$, .

3.2 Analytical Approximation

In addition to tracking the transverse profile, a common analysis of dispersion in single channels examines the longitudinal spreading of a bolus as it progresses along the channel, as viewed from a fixed point in the channel[25]. In this approach, the transverse distribution is averaged across channel height and width, and in many cases an expression can be found for this average concentration as a function of time at any desired downstream position. Most relevant to our device, Goulpeau et al. and Bontoux et al.[16, 11], developed analysis for a shallow microchannel. As discussed in the previous section, they identify a “short time regime”, in which enough time has elapsed such that the species is assumed to be uniformly distributed across the small height dimension but is still nonuniform across the much larger width dimension, due mainly to advection. The short time regime is characterized by $h^2/D \ll t \ll w^2/D$ and applies marginally to our experimental parameters for the lower flow rates. Here, h , w , and t , are height, width and time respectively.

In this approach, a Péclet number $Pe(x) = v(x)h/D$ is defined such that it varies across the channel width, according to the corresponding velocity field $v(x)$ across the channel width. The Péclet number is then used in an effective diffusion coefficient

$$D_{eff}(x) = D \left(1 + \frac{1}{210} Pe(x)^2 \right) \quad (1)$$

Goulpeau et al. [ref] further show that the evolution of the average concentration of the trailing end of an infinitely long bolus may then be written

$$c(z, t) = \frac{1}{2w} \int_{-\frac{w}{2}}^{\frac{w}{2}} \left(1 + \text{erf} \left(\frac{z - z_0 - v(x)t}{2\sqrt{D_{eff}(x)t}} \right) \right) dx \quad (2)$$

where we have adapted the expression for the case of a rectangular channel of uniform height h and normalized the initial concentration to unity. The position z_0 is the location of the concentration step at time $t=0$ and erf is the error function.

As a first step in analyzing the dispersion of a bolus in co-flow configuration, we sought to compare this analysis, which had been developed for a single channel, with the experimental data from our case of a bolus in co-flow. We therefore defined for the finite length bolus

$$c_{net}(z, t) = c(z, t, z_0 - l) - c(z, t, z_0 = 0) \quad (3)$$

with l equal to the length of the region where the sample is held before injection, between valves 1 and 2 in Figure 2 (nominally 6.67mm). We assumed an arbitrary velocity function of the form $v(x) = v(\bar{a} - (\beta 2x/w)^2)$, with v the average flow velocity based on volumetric flow and channel cross section, and α, β , and l used as fitting parameters. The values of the fitting parameters were adjusted by comparing the result of Eqn. (3) at a location $z = 6.7\text{mm}$, just prior to the merging of the two inlet streams, to image analysis at the same location. At this position, the bolus should behave as in the standard single channel case, and ideally Eqn (3) would apply exactly. The numerical evaluation of c_{net} was performed with Mathcad software.

Figure 7 shows the results of the above analysis compared with the experimental results at positions downstream of the merge point, with the bolus evolving in coflow at two flow rates. We found that the analytical result agrees reasonably well with the experiment at the “entrance” position, but that the agreement deteriorates further downstream. This result would be expected since at the entrance the bolus has had little exposure to the co-flow conditions. Furthermore, the result suggests that the velocity function $v(x)$ likely needs to be adapted to the coflow case, but this is not undertaken in this paper.

3.3 Application of Difference Maps

Another type of analysis that can be implemented, for practical purposes in sensor applications, is to compare the concentration profile in the bolus to that of steady-state continuous co-flow and to define regions where the transient state approximates that of the steady state. To perform this comparison, we generated maps of the fluorescence intensity across the channel width, versus time, at each location in the channel (entrance, middle, and exit, see figure 3(d), for both the bolus and a steady-state co-flow (not shown). We then subtracted these two maps and generated a “difference map,” (see Methods, section 2.3 for a detailed explanation) showing the experimentally observed percent difference between the bolus and steady state throughout the duration of the injection (figure 8a-c). For comparison, we also generated difference maps from the simulation at flow rates ranging from $0.5\mu\text{L}/\text{min}$ to $10\mu\text{L}/\text{min}$.

The difference maps in figure 8 permit visualization of the interval within an injection that could be used to approximate continuous co-flow. As expected, the length of the dispersed bolus as represented in the difference maps is affected by its residence time in the co-flow reactor, with increased dispersion at the exit compared to at the entrance. In contrast to single-channel plug dispersion, the shape of the dispersed plug is further distorted due to the nonuniform velocity distribution across the width of the co-flowing streams, with the interior side of the plug (at the laminar flow interface) advancing faster than the side at the channel wall. This effect becomes most evident at the downstream end (exit) of the channel.

For the most part, the concentrations within the bolus were lower than those in the steady-state co-flow, resulting in mostly negative values in the difference maps. However, we observed that the maps, particularly at the lower flow rates, produced high concentration areas (red areas, corresponding to positive difference values) near the interface between the bolus and the adjacent co-flowing stream, and that these are not observed in the simulation difference maps. While not shown, the high concentration areas increased at this interface as

the flow rate decreased. We attribute this to the inequalities in the flow rate of the two incoming streams. This may be because of the syringe pump's inability to maintain uniform flow at low flow rates which causes the streams to oscillate in the x direction or because of other deviations from identical flow rates at the two inlets. To investigate this, we increased the velocity of the injection channel stream in the simulation to generate unequal flow rates between the reagent and injection channel streams. The resulting difference maps produced similar results to our experimental difference maps at low flow rates and are shown in figure 9.

Having mapped out the deviations of bolus mass distribution relative to steady-state co-flow, it is useful for our co-flow reactor application to define regions of the bolus that approximate steady-state within a specified tolerance. One can use these regions to perform measurements while ignoring the effects of dispersion and the transient regime, as long as the pre-defined tolerance level is appropriate for the measurement. To illustrate this point, we defined a rectangular window whose width (x, transverse to flow) is a predetermined portion of the reactor (figure 10). For our calorimeter applications, we selected $125 < x < 375 \mu\text{m}$ as the active region of the device, which also ensures that any edge effects are eliminated. From the difference maps (figure 8), we then determined a window in the bolus (within the active region) that approximated steady state within predefined tolerances (10, 20, and 30%). Since the difference maps are a direct representation of the percent difference between the pixel intensities of the bolus and steady state, we were able to determine the window by examining the maps. For example, for a 10% tolerance, any pixels having a difference value greater than 10% were excluded, and the largest contiguous area was selected among the remaining pixels. This contiguous area was termed the “window in spec.” Since the maps plot the evolving reagent concentration at a fixed z location over time, the “length” of the window is a time measurement. We repeated this analysis at the three locations we examined (entrance, middle, and exit) for the five flow rates tested experimentally (0.5 to 10 $\mu\text{L}/\text{min}$) plus one obtained from simulation (0.25 $\mu\text{L}/\text{min}$), and plotted the duration of the window in spec for each of these conditions (figure 10). These tolerance plots show the windows in terms of time duration, not absolute times. The exact beginning and end of each window was omitted for the sake of simplicity, but can be readily determined from the maps. For example, for 1.0 $\mu\text{L}/\text{min}$ at the exit, the window in spec lies between 6.6 seconds and 8.4 seconds, and these time points can be correlated back to Figure 6.

By examining these tolerance maps for different conditions, we see that the window becomes longer with increasing tolerance level and with decreasing flow. Both trends make sense and correspond well to intuitive predictions. In the latter case, the decrease in flow rate affects both bolus residence time (which impacts the extent of “smoothing” of the bolus due to diffusion) and dispersion. We also notice that the window is longer at the middle and exit compared to the entrance, which reflects effective diffusion of the fluorescent dye as the bolus travels down the channel. However one discrepancy is that, from experimental data, the window is slightly smaller at the exit than at the middle for the lowest flow rate, while modeling shows that both locations should result in nearly the same window. We interpret these errors as the inability of our apparatus to maintain accuracy at low flow rates. In fact, all conditions showed larger deviations between experiments and modeling at the low flow

rates. As we discussed above, we observed while performing the experiments that the lower flow rates had noticeable oscillations of the interface between the two streams, indicating a fluctuating imbalance of the input stream flow rates. We expect this problem to be minimized by using higher precision syringe pumps, or by adding high-resistance tubing segments to the chip inlets and outlets to filter or suppress these oscillations.

With the tolerance plots, we can begin to define useful operating parameters for performing measurements in this transient co-flow regime for our channel geometry. First, it may be preferable to perform measurements at locations that are toward the downstream end of the reactor, which yields a window that is $\sim 2\times$ longer than that obtained $100\mu\text{m}$ from the entrance. Second, the modeling data suggest that lower flow rates are most useful, if longer measurement times are desirable. For example, a 10% tolerance level and a $1\text{-}\mu\text{L}/\text{min}$ injection provide a window of <1 sec when evaluated at the middle or exit locations, which may be too short for useful measurements. At this tolerance, $0.5\text{ }\mu\text{L}/\text{min}$ is a better choice and results in a 2-sec window, which is potentially more useful for measurements and corresponds to $\sim 33\%$ of the injected bolus volume. With a 30% tolerance, a $2\text{ }\mu\text{L}/\text{min}$ may still be useful, providing a 1-second window that corresponds to $\sim 67\%$ of the bolus volume.

While the experiment and simulation show reasonable agreement, there are numerous factors that might account for their differences. We have already discussed the impact of using a 2D, rather than 3D simulation, and we have noted the irregularities that arise from inequalities in the flow rate of the two merging streams. Another difference is that the experimental device has an out-of-plane through hole, or “fluidic via” that the bolus passes through, from upper to lower layer, before reaching the co-flow region (see figure 2). The through hole was used to meet the competing demands of having a curved channel cross section for good valve sealing but a rectangular channel cross section for the reactor application. As a result of this architecture, it is likely that the dispersed bolus shape is distorted from the ideal Taylor-Aris shape before it enters the reactor channel.

This paper addresses only a part of a potentially large parameter space, and does not undertake a complete theoretical description. A full treatment would consider not only variations in flow rate, but also channel aspect ratio, diffusivity, and length of injection channel. Nevertheless, because the bolus in co-flow configuration has not been previously examined, the results here are useful as a preliminary investigation, giving insight into the general behavior and, we hope, stimulating further work on a problem of both practical and theoretical interest.

4. Conclusion

A microfluidic injector has been fabricated, motivated by the need to use transient co-flow injections for sample analysis. As a first step in analyzing the evolution of the bolus in co-flow, we investigated the feasibility of approximating the transient co-flow bolus by comparing it to transient single-channel bolus injections and steady-state continuous co-flow. Using such analysis, we found that the behavior of the bolus in co-flow agrees reasonably with single-channel theory at upstream locations of the reactor channel (closer to where the reagent channels have merged), where the bolus shape has not been altered

significantly by the co-flow. By the same token, portions of the bolus begin to resemble steady-state continuous co-flow at downstream locations, after diffusion has had time to counteract some of the effects of dispersion. This analysis indicates that straight forward measurements could be performed with reduced reagent consumption in co-flow reactors, given an awareness of how the mass distribution differs from conventional cases. Our study represents a step towards a more detailed description of bolus development in co-flow in the transient regime. With an improved understanding of dispersion effects in a co-flow transient, our future work will include implementing the microinjector for delivering small reagent samples to our existing microcalorimeter platform. We expect to be able to investigate the heat release due to transient injections to calculate enthalpy of reaction with radically reduced reagent volume. This will considerably add to the utility of the device.

Acknowledgments

The project described was supported by Award Number R21CA131884 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institute of Health. We are grateful to Mehmet Sen for development of the Nanocalorimeter application.

References

1. Atencia J, Beebe DJ. Controlled microfluidic interfaces. *Nature*. 2004; 437:648–55. [PubMed: 16193039]
2. Weigl BH, Yager P. Microfluidic diffusion-based separation and detection. *Science*. 1999; 283:346–7.
3. Kamholz AE, Weigl BH, Finlayson BA, Yager P. Quantitative analysis of molecular interaction in a microfluidic channel: the T-Sensor. *Analytical Chemistry*. 1999; 71:5340–7. [PubMed: 10596213]
4. Irimia D, Geba DA, Toner M. Universal Microfluidic Gradient. *Generator Analytical Chemistry*. 2006; 78:3472–7.
5. Jeon NL, Dertinger SKW, Chiu DT, Choi IS, Stroock AD, Whitesides GM. Generation of Solution and Surface Gradients Using Microfluidic Systems. *Langmuir*. 2000; 16:8311–6.
6. Song Y-A, Hsu S, Stevens AL, Han J. Continuous-Flow pI-Based Sorting of Proteins and Peptides in a Microfluidic Chip Using Diffusion Potential. *Analytical Chemistry*. 2006; 78:3528–36. [PubMed: 16737204]
7. Janasek D, Schilling M, Manz A, Franzke J. Electrostatic induction of the electric field into free-flow electrophoresis devices. *Lab on a Chip*. 2006; 6:710–3. [PubMed: 16738720]
8. Kamholz AE, Yager P. Theoretical analysis of molecular diffusion in pressure-driven laminar flow in microfluidic channels. *Biophysical Journal*. 2001; 80:155–60. [PubMed: 11159391]
9. Ismagilov RF, Stroock AD, Kenis PJA, Whitesides G, Stone HA. Experimental and theoretical scaling laws for transverse diffusive broadening in two-phase laminar flows in microchannels. *Applied Phys. Lett.* 2000; 76:2376–8.
10. Oak J, Pence D, Liburdy J. FLOW DEVELOPMENT OF CO-FLOWING STREAMS IN RECTANGULAR MICRO-CHANNELS. *Microscale Thermophysical Engineering*. 2004; 8:111–28.
11. Ajdari A, Bontoux N, Stone HA. Hydrodynamic Dispersion in Shallow Microchannels: the Effect of Cross-Sectional Shape. *Analytical Chemistry*. 2006; 78:387–92. [PubMed: 16408918]
12. Dutta D, Ramachandran A, Leighton D Jr. Effect of channel geometry on solute dispersion in pressure-driven microfluidic systems. *Microfluidics and Nanofluidics*. 2006; 2:275–90.
13. Vedel S, Bruus H. Transient Taylor–Aris dispersion for time-dependent flows in straight channels. *Journal of Fluid Mechanics*. 2012; 691:95–122.
14. Datta S, Ghosal S. Characterizing dispersion in microfluidic channels. *Lab on a Chip*. 2009; 9:2537–50. [PubMed: 19680578]

15. Bharadwaj R, Santiago JG, Mohammadi B. Design and optimization of on-chip capillary electrophoresis. *Electrophoresis*. 2002; 23:2729–44. [PubMed: 12210178]
16. Goulpeau J, Lonetti B, Trouchet D, Ajdari A, Tabeling P. Building up longitudinal concentration gradients in shallow microchannels. *Lab on a Chip*. 2007; 7:1154–61. [PubMed: 17713614]
17. Ramji R, Roy P. Microfluidic bolus induced gradient generator for live cell signalling. *Microfluidics and Nanofluidics*. 2013; 15:99–107.
18. Kowalski GJ, Talakoub A, Ji J, O'Connell JG, Sen M, Larson D. Fast temperature sensing using changes in extraordinary optical transmission through an array of subwavelength apertures. *Optical Engineering*. 2009; 48:104402.
19. Sen, M.; Fiering, J.; Kowalski, GJ.; Larson, D. Optimum sensor placement in microchannel reactors: design tool applications.. In: Becker, H.; Gray, B., editors. *Microfluidics BioMEMS and Medical Microsystems IX*. San Francisco: 2011. p. 79290K-1-K-13.
20. Leach AM, Wheeler AR, Zare RN. Flow Injection Analysis in a Microfluidic Format. *Analytical Chemistry*. 2003; 75:967–72. [PubMed: 12622393]
21. Nguyen TV, Duncan PN, Ahrar S, Hui EE. Semi-autonomous liquid handling via on-chip pneumatic digital logic. *Lab on a Chip*. 2012; 12:3991–4. [PubMed: 22968472]
22. Unger MA, Chou H-P, Thorsen T, Scherer A, Quake SR. Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography. *Science*. 2000; 288:113–6. [PubMed: 10753110]
23. Culbertson CT, Jacobson SC, Michael Ramsey J. Diffusion coefficient measurements in microfluidic devices. *Talanta*. 2002; 56:365–73. [PubMed: 18968508]
24. Schetz, A.; Fuhs, A. *Fundamentals of Fluid Mechanics*. John Wiley & Sons; 1999.
25. Probstein, RF. *Physicochemical Hyrdrodynamics*. John Wiley & Sons; Hoboken, NJ: 2003.

Figure 1.

(a) Previous studies investigated diffusion between co-flowing laminar streams. Other studies examined dispersion and other related transients in single stream devices. (b) Our investigation examines experimental and simulation analysis of bolus development in a co-flow reactor. (c) Co-flow microcalorimeter principle, showing two laminar reagent streams (A and B) reacting over the temperature sensor array. The reaction product (AB) distributes spatially in a predictable region (shown bounded by the red lines). Each temperature sensor is a simple array of nano-apertures in a gold film.

Figure 2.

a) Schematic channel layout (top view) of the on-chip injection system, drawn to scale. Top layer is shown in red and bottom layer is shown in green. Note that reagents are in the upper layer as they enter the device but flow down to the lower layer just prior to merging at the reaction chamber. (b) By pressurizing the control channel, the thin film of PDMS deflects upward and closes off the reagent channel. (c) Detail of the channel cross sections (side view). (d) Photograph of microinjector microfluidic chip; device area is approximately 25×15mm.

Figure 3.

Micrographs of (a) microfluidic injector and reactor with pneumatically controlled microvalves and filled with dye to illustrate function. (b-c) Fluid path of a 50nL bolus of Reagent B being delivered to the co-flow reactor in parallel with the continuously flowing Reagent A. (d) Injection of 50nL fluorescein bolus through the co-flow reactor. (e) Continuous flow of water (Reagent A) and fluorescein (Reagent B) through co-flow reactor.

Figure 4.
Modeling domain and dimensions

Figure 5. Normalized concentration for steady-state (a) and a selected timepoint in the transient (b) simulations at a flow rate of $0.5\mu\text{L/min}$.

Figure 6.

Development of 50nL bolus over time at 1.0 $\mu\text{l/min}$ for experimental intensity data (Left) and simulation concentration data (Right). Plots are normalized intensity or concentration profiles across the channel over time, at channel entrance, middle, and exit. The data was normalized to the maximum intensity or concentration value that occurred at these three respective locations. Note that the time values for the simulation concentration profiles were shifted by - 1.4 seconds to correlate with the experimental intensity profiles.

Figure 7.

Evolution of normalized intensity data vs time for the co-flow condition, averaged across channel width ($x = 0\text{--}500\mu\text{m}$), compared with the calculated result for a single channel at (a) $1.0\mu\text{m}/\text{min}$ and (b) $10.0\mu\text{m}/\text{min}$ at the channel entrance, middle, and exit. Experimental measurements are shown as symbols and calculation as solid lines. The fitting parameters α , β , and l , were 0.88, 0.7, and 6 mm, respectively for all cases.

Figure 8.

Comparison between bolus injection and steady-state co-flow for flow rates of (a) 0.5 $\mu\text{L}/\text{min}$, (b) 1.0 $\mu\text{L}/\text{min}$, and (c) 2.5 $\mu\text{L}/\text{min}$. Difference maps plotted as x vs time, obtained by subtracting steady-state normalized intensities from bolus normalized intensities, at channel entrance, middle, and exit. Note the progression of the co-flow interface from a no-slip boundary to a velocity maximum.

Figure 9.

Impact of unequal inlet flow rates on difference map from figure 8a. (a) Experimental intensity difference map at nominal 0.50 μL/min at both inlets. (b) Simulation with the injection channel flow rate increased to 0.69 μL/min to simulate unequal flow rates between the reagent and injection channel streams. (c) Simulation difference map at 0.50 μL/min at both inlets.

Figure 10.

Tolerance plots. Using the difference maps shown in Fig 8, we calculated the time window “in spec” (i.e., the duration that the transient concentration profile differed from steady state profile, for a region of interest from $x = 125$ to $375\mu\text{m}$, by less than the specified tolerance) with 10%, 20%, and 30% tolerance, at the three channel locations. Dashed lines on difference map (upper left) illustrate the window in one representative case.

Table 1

Equations employed during computational model

	Name	Equation
Steady State	Continuity Equation	$\rho(\nabla \cdot \mathbf{u}) = 0$
Steady State	Momentum Equation	$\rho(\mathbf{u} \cdot \nabla)\mathbf{u} = -\nabla P + \nabla \cdot (\mu \nabla \mathbf{u})$
Steady State	Convection-Diffusion Equation	$\mathbf{u} \cdot \nabla C = D \nabla^2 C$
Transient	Convection-Diffusion Equation	$\frac{C}{t} = D \nabla^2 C - \mathbf{u} \cdot \nabla C$

Table 2

Modeling Parameters

Parameter	Value	Description
ρ	998.2 kg/m ³	Density of water
μ	1.003e-3 Pa*s	Dynamic viscosity of water
D	4.2e-10 m ² /s	Diffusivity of fluorescein in water