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Rapid mixing using two-phase hydraulic focusing in microchannels

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Abstract. Rapid mixing is important in biomedical analysis. In this study, rapid mixing is obtained through two-phase hydraulic focusing in microchannels. Two mixing streams are focused by two sheath streams. Assuming a laminar flow in the channel, the spreading behavior of the two immiscible fluids is modeled and solved analytically. The results show that both viscosity ratio and flow rate ratio between the sheath flow and the sample flow can affect the focusing ratio. Thus, the mixing path of the sample flows can be adjusted by either viscosity ratio or flow rate ratio. Furthermore, an analytical model was proposed and solved for convective/diffusive mixing between the sample streams. According to this model, the focusing ratio is a key parameter for rapid mixing. A fully polymeric micro mixer was fabricated and tested for verification of the presented analytical models. The micromixer was fabricated by laser micromachining and adhesive bonding. The characterization results show the promising potential of mixing in microscale using two-phase hydraulic focusing.

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1. Introduction

Rapid mixing is essential in many microdevices used in biomedical analysis, drug delivery, sequencing or synthesis of nucleic acids. Biological processes such as cell activation, enzyme reactions, and protein folding often involve reactions that require mixing of reactants for initiation. Thus, micromixer is a key component in a lab-on-a-chip (LOC) platform.

In general, micromixers can be categorized as passive micromixers and active micromixers (Nguyen, 2002). Passive micromixers do not require external energy, the mixing process relies entirely on diffusion or chaotic advection. Active micromixers use disturbance generated by an external field for the mixing process. Due to the required external disturbance, active micromixers often are complex and need a complicated fabrication process. Furthermore, the actuators often lead to a relatively large size of active mixers. The integration of active mixers in a microfluidic system is both challenging and expensive. In contrast, passive micromixers do not require external actuators except those for fluid delivery. The often simple passive structures are robust, stable in operation and easy to be integrated in a more complex system.

In the past few years, many passive designs were reported. Due to the dominating laminar flow in microscale, mixing in passive micromixers relies mainly on molecular diffusion (Wong, 2003), (Gobby, 2001), (Veenstra, 1999), (Jackman, 2001), (Moebius, 1995), (Koch, 1999), (Haverkamp, 1999), (Knight, 1998), (Branebjerg, 1996), (Schwesinger, 1996), (Miyake, 1993), (Wang, 2002), (Lin, 2003) and chaotic advection (Liu, 2000), (Vijayendran, 2003), (Chen, 2004), (Johnson, 2002), (Stroock, 2002), (Tice, 2003). Increasing the contact surface between the different fluids and decreasing the diffusion path between them could improve molecular diffusion. Chaotic advection can be realized by manipulating the laminar flow in microchannels. The resulting flow pattern shortens the diffusion path and thus improves mixing.

Among the above designs, the simplest way to obtain rapid mixing is to have a narrow mixing channel such as the one proposed in (Veenstra, 1999). However, due to the extra requirements of microtechnology, decreasing the channel width means increasing the fabrication cost. An alternative method for a short mixing path is hydraulic focusing (Knight, 1998). But in this case, the sample fluid would diffuse into the sheath flows. Furthermore, the focusing ratio only can be adjusted by changing the flow rate or pressure, which further limits the implementation of hydraulic focusing.

This paper presents analytical models and experimental results of rapid mixing in a microchannel using two-phase focusing. First, an analytical model of two-phase hydraulic focusing in a rectangular microchannel is presented. The model considers a system of four inlet streams. The two sheath streams are identical. The sample streams are sandwiched between the sheath streams, which are immiscible to the sample streams. The model results to the velocity distribution inside the microchannel. Next, an analytical model for the convective/diffusive effect between the two mixing streams is presented. This model assumes a uniform velocity in the mixed sample streams. In

Figure 1. Two-dimensional physical model of hydraulic focusing in a rectangular channel.

addition, non-linear effect of diffusive mixing is considered in the analytical model. A fully polymeric micromixer was fabricated to investigate the mixing effect of two-phase hydraulic focusing.

2. Theories

The microchannel under consideration has a rectangular cross section and four inlet streams, Fig. 1. The middle two are sample streams consisting of a solvent and a solute. The other two are sheath streams for focusing the sample streams. The sheath flow and the mixing flow are immiscible. Due to the small Reynolds numbers in microfluidics, the flow can be considered as laminar. The velocity distribution in a cross section can be considered as invariable along the flow direction. For this reason, the problem reduces to a two-dimensional model in the y - z -plane.

2.1. Two-phase hydraulic focusing

The two-phase focusing model consists of four streams: the two mixing streams sandwiched between two identical sheath streams. With the same viscosity, the solvent and the solute can be treated as a single phase, Fig. 2. Figure 2a shows the actual geometry of the channel cross section with the two immiscible phases. The width and height of the channel are $2W$ and H , respectively. The position of the interface is rW . Since the model is symmetrical regarding the y -axis and the z -axis, only $1/4$ of the cross section needs to be considered. The velocity distribution u_1 and u_2 in the channel can be described by the Navier-Stokes equations:

$$\begin{aligned} \frac{\partial^2 u_1}{\partial y^2} + \frac{\partial^2 u_1}{\partial z^2} &= \frac{1}{\eta_1} \frac{\partial p}{\partial x} \\ \frac{\partial^2 u_2}{\partial y^2} + \frac{\partial^2 u_2}{\partial z^2} &= \frac{1}{\eta_2} \frac{\partial p}{\partial x} \end{aligned} \quad (1)$$

where indices 1 and 2 denote the sample flow and the sheath flow, respectively. In (1), η_1 and η_2 are the viscosities of the sample fluid and of the sheath fluid. Non-dimensionalizing the velocity by a reference velocity u_0 and the spacial variables by W

Figure 2. Models for two-phase hydraulic focusing: (a) actual geometry, (b) the dimensionless model.

leads to the dimensionless model (Fig. 2b):

$$\begin{aligned} \frac{\partial^2 u_1^*}{\partial y^{*2}} + \frac{\partial^2 u_1^*}{\partial z^{*2}} &= \frac{W}{\eta_1 u_0} \frac{\partial p}{\partial x^*} \\ \frac{\partial^2 u_2^*}{\partial y^{*2}} + \frac{\partial^2 u_2^*}{\partial z^{*2}} &= \frac{W}{\eta_2 u_0} \frac{\partial p}{\partial x^*} \end{aligned} \quad (2)$$

With $P' = \frac{W}{\eta_1 u_0} \frac{\partial p}{\partial x^*}$, $\beta = \eta_2/\eta_1$, and $\theta = (2n-1)\pi/h$, the solutions of (2) have the forms ($0 < y^* < 1$, $0 < z^* < h/2$):

$$\begin{aligned} u_1^*(y^*, z^*) &= P' \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_1 \cosh \theta y^* + B_1 \sinh \theta y^*) \right] \\ u_2^*(y^*, z^*) &= \frac{P'}{\beta} \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_2 \cosh \theta y^* + B_2 \sinh \theta y^*) \right] \end{aligned} \quad (3)$$

The non-slip condition at the wall results in:

$$u_2^*(1, z^*) = 0. \quad (4)$$

The symmetry condition at z^* -axis is:

$$\frac{\partial u_1^*}{\partial y^*} \Big|_{y^*=0} = 0. \quad (5)$$

At the interface between the sample flow and the sheath flow, the velocity and the shear stress are continuous:

$$\begin{aligned} u_2^*(r, z^*) &= u_1^*(r, z^*), \\ \frac{\partial u_1^*}{\partial y^*} \Big|_{y^*=r} &= \beta \frac{\partial u_2^*}{\partial y^*} \Big|_{y^*=r}. \end{aligned} \quad (6)$$

For a flat channel ($h \ll 1$), the position of the interface can be estimated as:

$$r = \frac{1}{1 + 2\beta\kappa} \quad (7)$$

Figure 3. The dimensionless velocity profile ($h=0.14$, $\kappa = 1$): (a) $\beta = 0.5$, (b) $\beta = 1$, (c) $\beta = 2$.

where $\kappa = Q_2/Q_1$ is the flow rate ratio between the the sheath streams and the mixing streams. The variable r can be considered as the focusing ratio. A Fourier analysis with the above boundary conditions results in the coefficients of (2):

$$\begin{aligned}
 A_1 = D_n [& \beta \sinh \theta r \cosh^2 \theta r \cosh \theta - \sinh^3 \theta r \cosh \theta - \\
 & (\beta - 1) \sinh^2 \theta r \sinh \theta \cosh \theta - (\beta - 1) \sinh^2 \theta r (\cosh \theta - \cosh \theta r) + \\
 & (\beta - 1) \sinh \theta r \cosh \theta r \cosh \theta (\cosh \theta - \cosh \theta r)] / \\
 & [\beta \cosh^2 \theta r \sinh \theta r \cosh^2 \theta - \sinh^3 \theta r \cosh^2 \theta - \\
 & (\beta - 1) \sinh^2 \theta r \cosh \theta r \sinh \theta \cosh \theta]
 \end{aligned} \tag{8}$$

$$\begin{aligned}
 A_2 = D_n [& \beta \cosh^2 \theta r \cosh \theta - \sinh^2 \theta r \cosh \theta - (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta - \\
 & (\beta - 1) \sinh \theta r (\cosh \theta - \cosh \theta r)] / \\
 & [\beta \cosh^2 \theta r \sinh^2 \theta - \sinh^2 \theta r \cosh^2 \theta - \\
 & (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta \cosh \theta]
 \end{aligned} \tag{9}$$

$$B_1 = 0 \tag{10}$$

Figure 4. Models for convective-diffusive mixing of the sample streams: (a) the physical model, (b) the dimensionless model.

$$B_2 = D_n [(\beta - 1) \sinh \theta r (\cosh \theta - \cosh \theta r)] / [\beta \cosh^2 \theta r \cosh \theta - \sinh^2 \theta r \cosh \theta - (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta] \quad (11)$$

where $D_n = (-1)^{n+1} \frac{4h^2}{(2n-1)^3 \pi^3}$. Figure 3 shows the typical velocity field inside the flow channel with a flow rate ratio of $\kappa = 1$. The velocity of the sample flow is lower, if the sample flow is more viscous than the sheath flow ($\beta < 1$), Fig. 3a. If the viscosities are equal ($\beta = 1$), the flows behaves like a single phase, Fig. 3b. If the sheath flows are more viscous ($\beta > 1$), the sample flow is faster, Fig. 3c.

2.2. Convective-diffusive mixing

The convective/diffusive mixing effect is characterized by the Peclet-number:

$$Pe = Uw/D, \quad (12)$$

where U , $w = 2rW$, and D are the uniform velocity, the width of the focused sample streams, and the diffusion coefficient, respectively. The velocity of each stream is important for the convective transport. Assuming a uniform velocity U across the sample streams, no diffusion flux exists in the z direction. Thus, with $h \ll 1$ the problem can be reduced to a two-dimensional model as depicted in Fig. 4. The mass conservation equation including both diffusion and convection is formulated as:

$$U \frac{\partial c}{\partial x} = D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) \quad (13)$$

where D is the diffusion coefficient of the solute. The mixing ratio of the solute and the solvent is 1 : 1. By introducing the new dimensionless variables for the coordinates

system $x^{**} = x/2rW$, $y^{**} = y/2rW$, the dimensionless concentration $c^* = c/C_0$ and the Peclet number (13) has the dimensionless form:

$$\text{Pe} \frac{\partial c^*}{\partial x^{**}} = \frac{\partial^2 c^*}{\partial x^{**2}} + \frac{\partial^2 c^*}{\partial y^{**2}} \quad (14)$$

The corresponding boundary conditions of the inlets are:

$$f_{(I)}(y^{**}) = \begin{cases} c^* \Big|_{(x^{**}=0, -1/2 \leq y^{**} < 0)} = 1 \\ c^* \Big|_{(x^{**}=0, y^{**}=0)} = 1/2 \\ c^* \Big|_{(x^{**}=0, 0 < y^{**} \leq 1/2)} = 0 \end{cases} \quad (15)$$

Full mixing is achieved for the outlet in a semi-infinite channel:

$$\frac{\partial c^*}{\partial x^{**}} \Big|_{(x^{**}=\infty, 0 \leq y^{**} \leq 1)} = \frac{1}{2} \quad (16)$$

The interfaces between the sample streams and the sheath streams are immiscible. Thus, the flux at the wall ($y^* = 1$) and the symmetry line ($y^* = 0$) should be zero:

$$\frac{\partial c^*}{\partial y^{**}} \Big|_{y^{**}=0,1} = 0 \quad (17)$$

Equation (14) is a second order linear partial differential equation. Separating the variables in (14) and applying the corresponding boundary conditions (15-17), the concentration distribution in the channel is:

$$\begin{aligned} c^*(x^{**}, y^{**}) &= \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin n\pi/2}{n} \cos[n\pi y^{**} + \pi/2] \\ &\quad \times \exp \left[\left(\text{Pe} - \sqrt{\text{Pe}^2 + 4n^2\pi^2 x^{**}} \right) / 2 \right] + \frac{1}{2} \\ n &= 1, 2, 3 \dots \end{aligned} \quad (18)$$

According to (18), the Peclet number (12) determines the mixing result. Since both the velocity U and the width of the streams w depend on the mixing ration r , the mixing ratio is the key parameter for rapid mixing.

3. Experiments

3.1. Materials and Methods

A polymeric micro mixer was fabricated and tested. The device is made of two 1-mm-thick polymethylmethacrylate (PMMA) layers and an acrylic double-sided adhesive layer (Adhesives Research, Inc., Arclad 8102 transfer adhesive). PMMA is a polymer with a low self-fluoresce and a good optical transmittance of 92% in the visible spectrum. Thus, the test device is suitable for optical detection in measurements of velocity fields and concentration fields.

The fabrication of the test device is based on laser micro machining and adhesive lamination. The test device measures 25 mm $\times$ 75 mm. The device consists of three layers: the top PMMA layer for optical access, the middle layer defining the channel

Figure 5. Fully polymeric device for mixing with two-phase hydraulic focusing.

structure, and the bottom PMMA layer with position marks and fluidic interconnects. The middle layer is a double-sided adhesive tape. Thus, all three layers are bonded and hermetically sealed. Each layer of the device was cut and engraved with CO₂-laser, which has a characteristic wavelength of 10.6 μm . Alignment holes are drilled by the same laser in all three layers. Position marks are ablated on the bottom layer for positioning the area of interest in the later measurements. The three layers are laminated using alignment pins. The adhesive layer thickness of 50 μm defines the channel height. With this technique a 52-mm long mixing channel with 900- μm width and 50- μm height was fabricated for the experiments, Fig. 5.

The details of our image acquisition system were reported in (Wu, 2004). The set-up consists of three main components: an illumination system, an optical system, and an image acquisition system. The illumination system consists of a Mercury lamp used in the florescent measurement and a laser system used in the velocity measurement. An optical switch selects one of the two light sources using a total reflection mirror. The optical system consists of an inverted microscope (Model ECLIPSE TE2000-S) and a CCD camera (Sony ICX 084). The image acquisition system, its corresponding control and analysis software are implemented in a personal computer (PC).

The measurement consists of two parts. The first part is the characterization of the velocity profile in the microchannel using micro particle image velocimetry (micro-PIV). The second part is the characterization of mixing behavior between the sample flows.

The micro-PIV experiments uses a 4 $\times$ objective. DI-water works as the sample flow. Diluted glycerol with a volume ratio of 1:4 (glycerol to DI-water at 25 $^{\circ}\text{C}$) were used as the sheath flow. Measurement of the two liquids with a viscometer (Brookfield LVDV-I) results to a ratio of $\beta = 1.8 \pm 0.1$. Although glycerol and water are miscible, cross mixing between sample flow and sheath flow is negligible at high velocity and large channel widths. Microspheres of 3 μm in diameter were added to DI-water and then to diluted glycerol with the same volume ratio of 1:4, so that the above measured viscosity

ratio can be assumed. Two 30 mJ laser pulses were used as illumination sources. The interrogation window and the evaluation grid were both $32 \text{ pixels} \times 32 \text{ pixels}$. That means, the measured velocity is averaged by 32 pixels.

The measurements of the concentration fields were carried out with a $20\times$ objective. The same CCD-camera of the micro-PIV measurement was used. The CCD-sensor has a size of $6.3 \text{ mm} \times 4.8 \text{ mm}$ and a resolution of $640 \text{ pixels} \times 480 \text{ pixels}$. Thus, the size of an image pixel is $0.495 \text{ }\mu\text{m}$, while the total size of the measured area of interest is $316.8 \text{ }\mu\text{m} \times 237.6 \text{ }\mu\text{m}$. In this experiment, a filter cube was used (Nikon B-2A, excitation filter for 450 nm to 490 nm, dichroic mirror for 505 nm and an emission filter for 520 nm). To distinguish of the interface between the liquid phases, an extra lamp with phase contrast condenser was used to illuminate the whole area of interest. The measurement used fluorescein disodium salt ($\text{C}_{20}\text{H}_{10}\text{Na}_2\text{O}_5$) diluted in water. This dye is also known under trade names such as Acid Yellow 73 or C.I. 45350. The fluorescent dye has an excitation wavelength and an emission wavelength of 490 nm and 520 nm, respectively. The two sample fluids (dye and DI-water) were primed into the microchannel using two identical precision syringes (Hamilton GasTight 500 μl). Silicone oils of different viscosities were used as sheath fluids. The silicone oils are filled in two identical precision syringes (Hamilton gas tight 1000 and 250 μl). All syringes are driven at the same velocity by a syringe pump (Cole-Parmer 74900-05, 0.2 $\mu\text{L/h}$ - 500 ml/h, accuracy of 0.5%).

3.2. Results and Discussions

Figure 6 shows the measured velocity profiles of the same position ($x^* = 22.2$) in the microchannel, but at different flow rates. The y-axis was normalized by the channel width $2W$. The flow rates of the sample flow (water) and the sheath flows (diluted glycerol) were kept equal ($\kappa = 1$). It's clear from Fig. 6a that the higher flow rates cause a higher velocity in each stream. Normalizing by the average velocity across the channel u_0 , the velocity profiles and the interface position agree well with the theory ($\beta = 1.8$, $\kappa = 1$, $r = 0.22$), Fig. 6b. However, the smaller gradients at the interface (Fig. 6c) were caused by diffusive mixing of glycerol and water. Furthermore, the evaluation grid of $32 \text{ pixels} \times 32 \text{ pixels}$ may decrease further the actual velocity gradients. The results show a good agreement between the theory and the measurement.

Figure 7 shows a typical image from the measurement of the concentration fields. After recording the intensity images on the PC, the concentration profiles were evaluated using a customized program written in MATLAB. First, the program removes the noise in the measured image with an adaptive noise-removal filter. For each pixel, a local mean value is calculated for a window of $5 \text{ pixels} \times 5 \text{ pixels}$. The noise is assumed to have a Gaussian distribution. Subsequently, a path with the known position across the focused mixed streams is evaluated. The position across the channel was normalized against the width of the focused stream, while the measured pixel intensity I was normalized

Figure 6. Micro-PIV results with water in the sample stream and diluted glycerol in the sheath streams at different stream flow rates ($\beta = 1$, $\kappa = 1.8$, $x^* = 22.2$): (a) Velocity profiles, (b) Normalized velocity profiles, (c) Normalized velocity gradients.

Figure 7. Typical intensity distribution along the channel: (a) two dimensional gray scale image, the darker areas are sheath flows and the lighter area contains the two sample flows; (b) three-dimensional color mapped intensity image.

against the maximum $I_{\max}$ and minimum $I_{\min}$ of the intensity at the inlet:

$$I^* = \frac{I - I_{\min}}{I_{\max} - I_{\min}} \quad (19)$$

Figure 8. Typical normalized concentration distribution across the focused streams along the microchannel (Pe=1111, Re=0.76): (a) measurement results; (b) theoretical results.

The dimensionless concentration of the fluorescent dye is assumed to be equal the dimensionless intensity ($c^* = I^*$).

To evaluate the extent of the mixing process, the concept of mixing index (M.I.) or degree of mixing was introduced:

$$\text{M.I.} = 1 - \frac{\sqrt{\frac{\sum_{i=1}^n (I_i^* - \mu)^2}{n}}}{\mu} = 1 - 2\sqrt{\frac{\sum_{i=1}^n (I_i^* - 0.5)^2}{n}} \quad (20)$$

where I_i^* is the local intensity values, i is the pixel index, μ is average value of the local normalized intensity. Due to the mixing ratio of 1:1, μ is equal 0.5 in our experiments. The diffusion coefficient of the dye/water solution was determined in our previous works as $D_0 = 1.5 \times 10^{-9} \text{m}^2/\text{s}$. The non-linear effect caused by the different diffusion coefficients in each stream is considered in the analytical model by the concentration dependent coefficient:

$$D(c^*) = D_0[(1 - a)c^* + a] \quad (21)$$

where $D_0 = 1.5 \times 10^{-9} \text{m}^2/\text{s}$ and $a = 0.4$ (Wu, 2004). A constant average velocity was assumed for both sample streams. The width of mixing stream was evaluated based on (7). Figure 8 shows a typical concentration distribution along the channel and the corresponding theoretical results.

Two sets of mixing experiments were carried out. In the first set, the flow rate of the sample streams is kept at 150 $\mu\text{l}/\text{h}$. The width of the sample flow is adjusted by the flow rates and the viscosities of the sheath flow. Silicone oils with different viscosities of 100 cSt and 10 cSt were used. For both silicone oils, different flow rates of 300 $\mu\text{l}/\text{h}$ and 75 $\mu\text{l}/\text{h}$ were applied. In the second set, only silicon oil with a viscosity of 10 cSt was used. The flow rate ratio between the sheath flow and the sample flow is kept at a constant value of 2. The flow rates of the sample stream is then varied for investigating the convective effects.

Figure 9. Mixing index along the microchannel for different focusing ratios at the sample flow rate ($150 \mu\text{l/h}$, $\text{Pe}=1111$): (a) experimental results; (b) the corresponding theoretical results.

Figure 9a shows the measured mixing index along the channel. Using different flow rate ratios and viscosity ratios, different focusing ratios r can be achieved. The results show clearly that a small focusing ratio improves the mixing effect significantly, even at high Reynolds numbers. The theoretical results from the two models presented in the previous section can predict well this mixing behavior.

Figure 10a shows the measured mixing index along the channel at different flow rates, but with the same focusing ratio of $r \approx 0.1$. The results show clearly that a

Figure 10. Mixing index along the microchannel with the same focusing flow rate ratio ($r \approx 0.1$) and different sample flow rates: (a) experimental results; (b) the corresponding theoretical results.

lower Peclet number leads to faster mixing. However, the differences in Fig. 10 are not significant. Results in Fig. 9 and Fig. 10 show that the focusing ratio r has a big impact for rapid mixing.

4. Conclusions

This paper presented a rapid mixing concept in microchannels utilizing two-phase hydraulic focusing. Analytical models are first established for the effect of hydraulic focusing as well as the effect of convective/diffusive mixing. The models show that the width of the sample flow can be controlled either by the flow rate ratio or by the viscosity ratio. Besides the flow rate, the viscosity ratio also determines the velocity of the sample flow, which in turn has an impact for the convective transport. The convective/diffusive model shows that a small width of the sample flow leads to a small Peclet number and thus improves mixing significantly. Measurement of the velocity profile was carried out with micro-PIV. Focusing effects and the corresponding velocity profile predicted from the analytical model was verified. Fluorescent measurement of the sample flows allows the evaluation of mixing performance by varying different parameters. The measured mixing indices show clearly that for a constant sample flow rate a small focusing ratio improves mixing significantly. At a constant focusing ratio, a low flow rate or a smaller Peclet number leads to slightly better mixing as predicted with the convective/diffusive model.

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