Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels

Helen Song, Michelle R. Bringer, Joshua D. Tice, Cory J. Gerdts, and Rustem F. Ismagilov[a)]
Department of Chemistry, The University of Chicago, 5735 S. Ellis Avenue, Chicago, Illinois 60637

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This letter describes an experimental test of a simple argument that predicts the scaling of chaotic mixing in a droplet moving through a winding microfluidic channel. Previously, scaling arguments for chaotic mixing have been described for a flow that reduces striation length by stretching, folding, and reorienting the fluid in a manner similar to that of the baker's transformation. The experimentally observed flow patterns within droplets (or plugs) resembled the baker's transformation. Therefore, the ideas described in the literature could be applied to mixing in droplets to obtain the scaling argument for the dependence of the mixing time, $t \sim (aw/U)\log(Pe)$, where w [m] is the cross-sectional dimension of the microchannel, a is the dimensionless length of the plug measured relative to w, U [$\mathrm{m\,s^{-1}}$] is the flow velocity, Pe is the Péclet number ($Pe = wU/D$), and D [$\mathrm{m^2\,s^{-1}}$] is the diffusion coefficient of the reagent being mixed. Experiments were performed to confirm the scaling argument by varying the parameters w, U, and D. Under favorable conditions, submillisecond mixing has been demonstrated in this system. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1630378]

This letter describes an experimental test of a simple argument that predicts the scaling of mixing of solutions by chaotic advection inside droplets moving through winding microfluidic channels.[1] In microfluidic systems[2,3] operating at low values of the Reynolds number *Re*, streams of reagents flow laminarly. Diffusive mixing across laminar streams is slow because the mixing time t_{diff}[s] is proportional to the square of the initial striation length $stl(0)$ [m], the distance over which the mixing occurs by diffusion with a diffusion coefficient D [$\mathrm{m^2\,s^{-1}}$]:

$$t_{\mathrm{diff}} = stl(0)^2/2D. \tag{1}$$

Mixing that occurs purely by diffusion is too slow for many applications of microfluidic systems, including high-throughput analysis and kinetic measurements. Methods designed to accelerate mixing aim to reduce the striation length, and several attractive approaches have been developed and reviewed.[2] Chaotic advection[4,5] enhances mixing by stretching and folding the fluid to give rise to an exponential decrease in the striation length *stl*.[4] In principle, presence of chaos does not guarantee widespread rapid mixing because poorly mixed islands can coexist with well-mixed chaotic regions.[6]

Chaotic advection has been implemented in microfluidic systems both at intermediate[7] and low[8,9] values of *Re*. We recently reported that mixing by chaotic advection may be achieved in droplets moving through winding channels,[1] as opposed to nonchaotic mixing in straight channels.[10] We refer to droplets large enough to be in contact with all four walls of a microchannel as "plugs." Chaotic advection is caused by the time-periodic recirculating flow inside the plugs, caused by shearing interaction of the fluid of the plug with the walls of the microchannel. Previously, we have demonstrated that mixing times of ~1 ms can be achieved for chaotic mixing in plugs and we have used it to perform millisecond kinetic measurements.[11]

Chaotic flow in plugs moving through winding channels may be thought of in terms of the baker's transformation.[4,8,12] The baker's transformation can be described as a series of stretches, folds, and reorientations that decrease the striation length. Experimentally, we observed flow patterns reminiscent of the baker's transformation in plugs moving through channels of a specially designed geometry shown in Fig. 1(a). In general, it was difficult to clearly observe flow patterns that correspond to the baker's transformation, in part because the two-dimensional component of flow responsible for mixing is obscured by the three-dimensional flow inside the plugs. The fluid inside the plug was folded and stretched by the recirculating flow in the straight portions. It was reoriented as it moved around a turn [Fig. 1(b)]. In this hypothetical system, the striation length decreases by a factor of 2 every turn: $stl(n) = stl(0) \times 2^{-n}$. This exponential decrease in striation length is similar to the rolling droplet concept described by Fowler *et al.*[13]

The concept of the baker's transformation has been used to predict the scaling of mixing by chaotic advection $t_{\mathrm{mix}} \sim \log(Pe)$.[4,8,12] Experimentally observed flow patterns [Fig. 1(a)] justify applying this concept to mixing in plugs. To obtain a simple scaling argument, we made two assumptions. First, we assumed that for one cycle of chaotic advection ($n=1$), the plug must travel a distance $d(1) \sim aw$, where w [m] is the cross-sectional dimension of the microchannel and a is the dimensionless length of the plug measured relative to w. Essentially, we assumed that the plug must travel a certain number of its own lengths for each cycle of chaotic advection. The initial striation length $stl(0) \sim w$. After n cycles of chaotic advection $stl(n) \sim w\sigma^{-n}$ (where σ is the Lyapunov

[a)]Electronic mail: r-ismagilov@uchicago.edu

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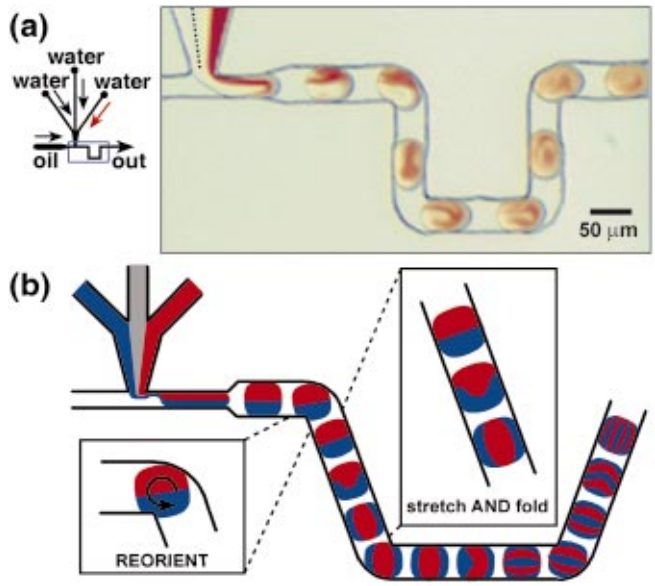

FIG. 1. (Color) Mixing by the baker's transformation in plugs moving through winding microfluidic channels shown (a) experimentally and (b) schematically. (a) Left: a scheme of the microfluidic network. Right: microphotograph of plugs. Solutions were as in Fig. 3 of Ref. 10. Total flow velocity $U=53\ \mathrm{mm\,s^{-1}}$.

exponent), the time scale $t_{\mathrm{diff,ca}}$ for mixing by diffusion [Eq. (1)] becomes

$$t_{\mathrm{diff,ca}}(n)\sim w^2\sigma^{-2n}/2D. \tag{2}$$

For n cycles, the time scale for transport by convection would be

$$t_{\mathrm{conv}}(n)\sim naw/U, \tag{3}$$

where $U\ [\mathrm{m\,s^{-1}}]$ is flow velocity.

Second, in the spirit of the traditional description of chaotic mixing[12] (and also the argument by Stroock *et al.*[8]), we

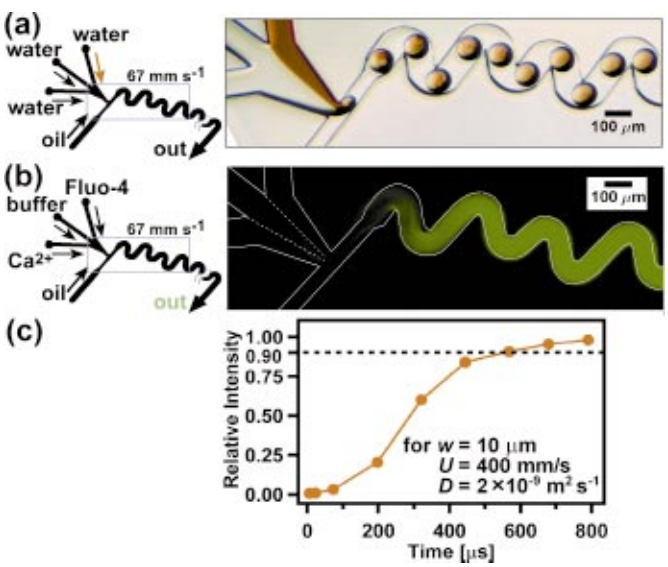

FIG. 2. (Color) An illustration of mixing in plugs moving through microchannels and quantification of the mixing. (a) and (b) Left: a scheme of the microfluidic network. (a) Right: microphotograph showing flow patterns in plugs moving through the microchannels used to test the scaling argument. (b) Right: a false-color fluorescence microphotograph of plugs showing time-averaged fluorescence arising from mixing of Fluo-4 and Ca^{2+} solutions (see Ref. 1). (c) Mixing curve obtained by analyzing intensities of fluorescence in images such as shown in (b). Submillisecond mixing was observed for the specified conditions of w, U, and D.

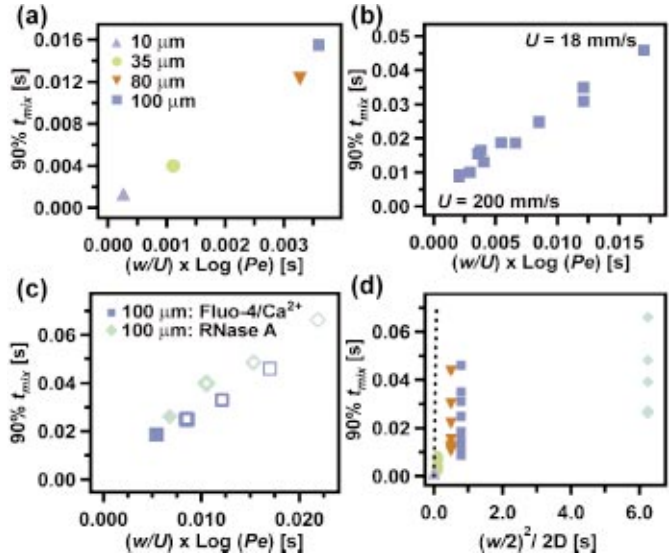

FIG. 3. (Color) Experimental data testing the scaling of chaotic mixing. (a)–(d) 90% mixing time (90% t_{mix} [s]), was obtained from mixing curves such as shown in Fig. 2(c). Mixing time as a function of (a) w (symbols) for constant $U=100\ \mathrm{mm\,s^{-1}}$; (b) U for constant $w=100\ \mu\mathrm{m}$; and (c) D for constant $w=100\ \mu\mathrm{m}$ and varying U from 18 $\mathrm{mm\,s^{-1}}$ (open symbols) to 66 $\mathrm{mm\,s^{-1}}$ (closed symbols). Data are shown for □ $D\sim1.6\times10^{-9}\ \mathrm{m^2\,s^{-1}}$ obtained with the Fluo-4/Ca^{2+} system, and for ◇ $D\sim2\times10^{-10}\ \mathrm{m^2\,s^{-1}}$ obtained with the RNase A system. (d) Entire data set from (a)–(c) is replotted versus $(w/2)^2/2D$, where $stl(0)\sim w/2$. This plot demonstrates that the observed mixing by chaotic advection is much faster than would have been predicted if mixing occurred purely by diffusion (prediction of mixing by pure diffusion is shown by dashed line with a slope of 1).

assumed that the mixing time $t_{\mathrm{mix,ca}}$ is determined by the number of cycles n for which the time scales for convective transport $t_{\mathrm{conv}}(n)$ and diffusive mixing $t_{\mathrm{diff,ca}}(n)$ are matched: $t_{\mathrm{conv}}(n)=n(aw/U)=w^2\sigma^{-2n}/2D=t_{\mathrm{diff,ca}}(n)$. After rearrangement, we obtain $2an\sigma^{2n}=wU/D=Pe$, where the Pe is the dimensionless Péclet number, defined as $Pe=wU/D$. In the limit of large Péclet number, we obtain $n\sim\log(Pe)$. By replacing the derived value of n within the equation for transport by convection, the mixing time $t_{\mathrm{mix,ca}}$ is

$$t_{\mathrm{mix,ca}}\sim t_{\mathrm{conv}}(n)\sim(aw/U)\log(Pe). \tag{4}$$

We emphasize that this result is similar to the one obtained by Stroock *et al.* for chaotic mixing in structured microchannels[8] and to the classic result for chaotic mixing.[12] The derivation of this argument for mixing in plugs is needed to outline the assumptions that we made, and therefore, to show the limits of its applicability.

We conducted a series of mixing experiments to test the scaling argument [Eq. (4)] by independently varying each of

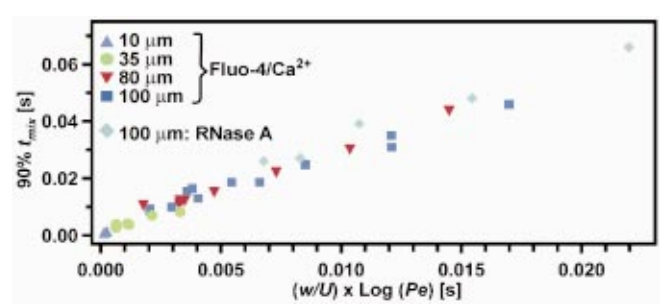

FIG. 4. (Color) Entire experimental data set from Fig. 3 collapses on a line when plotted versus $(w/U)\log(Pe)$, in agreement with the scaling equation.

the parameters w, U, and D. To conduct the mixing experiments, we used a random, general microchannel geometry [Fig. 2(a)] to achieve chaotic flows within plugs. The winding part of the channel has been randomized to exclude the possibility of the formation of poorly mixed islands.[6] The channels shown in Fig. 2(a) have a constriction in the plug-forming region, designed to produce smaller plugs with a short distance between them.[10] We quantified mixing as previously described,[1,14] by using the fluorescence generated upon diffusion-controlled binding of Ca^{2+} ions to a dye Fluo-4 [Fig. 2(b)]. Mixing time $t_{\mathrm{mix,ca}}$ was determined by analyzing fluorescence images as shown in Fig. 2(b). A representative mixing curve is shown in Fig. 2(c), where we have defined the mixing time as the time at which mixing is 90% complete, 90% $t_{\mathrm{mix,ca}}$ [s].[8] We varied w over a tenfold range [Fig. 3(a)] at a fixed flow rate U. In addition, we varied U over a tenfold range at fixed w [Fig. 3(b)]. Results of three experiments performed in three different copies of the devices are shown, these results are in good agreement with Eq. (4). To extend the range of the diffusion coefficients, mixing was measured for the cleavage of a fluorogenic substrate[15] by ribonuclease A (RNase A).[11] To ensure rapid cleavage ($\sim$3 ms) of the substrate, so that mixing and not enzymatic kinetics would be measured, both the substrate (10 μM) and enzyme (50 μM) were used at high concentrations and RNase A was used in a fivefold excess.[11] The diffusion coefficients for RNase A ($D \sim 1.2\times 10^{-10}\ \mathrm{m^2\,s^{-1}}$)[16] and for the fluorogenic substrate ($D \sim 2\times 10^{-10}\ \mathrm{m^2\,s^{-1}}$)[17] are lower than those for Ca^{2+} ($D \sim 1.6\times 10^{-9}\ \mathrm{m^2\,s^{-1}}$). In the Fluo-4/$Ca^{2+}$ system, mixing is dominated by diffusion of Ca^{2+}, which is smaller than Fluo-4 and was used in excess. In the RNase A system, mixing is determined by the diffusion of both the substrate and the larger enzyme used in excess. The mixing times obtained for rapidly diffusing species and the slow diffusing species were similar and scaled in agreement with Eq. (4) [Fig. 3(c)]. We used the diffusion coefficient of the substrate in calculating the Péclet number; using the diffusion coefficient of the enzyme gives an even better fit to Eq. (4). Only slow mixing can be measured with the enzymatic reaction ($\sim$3 ms); therefore, larger channels and slower flow velocities had to be used in this experiment.

The factor by which mixing efficiency is increased by chaotic advection over pure diffusion is: $t_{\mathrm{diff,ca}}/t_{\mathrm{mix,ca}} \sim Pe/a \log(Pe)$, as also shown previously for chaotic mixing.[12] Indeed, the experimental data from mixing by chaotic advection [symbols, Fig. 3(d)] contrast the mixing efficiency that would have been predicted for mixing by pure diffusion, shown in Fig. 3(d) as a dashed line. Chaotic advection was observed to accelerate mixing the most for systems with high Péclet number: for wide channels, high flow velocities, and analytes with low diffusion coefficients. Submillisecond mixing (as rapid as $\sim$500 μs) has been obtained with this system [Fig. 2(b)].

All of the experimental t_{mix} data plotted together versus $(w/U)\log(Pe)$ collapses onto the same line, corroborating the scaling argument [Fig. 4]. It is clear that the dependence of mixing time on Pe is weak, although it would have been useful to obtain data over a larger range of Pe to establish that the dependence is logarithmic and to determine the slope accurately. We could not obtain data at higher Pe by decreasing D (by increasing viscosity μ [$\mathrm{kg\,m^{-1}\,s^{-1}}$]) or by increasing U, because in this system the capillary number ($Ca = U\ \mu/\gamma$, where γ is the surface tension [$\mathrm{kg\,s^{-2}}$]) must be kept low and we were at the upper range of the values of Ca where droplet formation was reliable.[10] We also did not obtain data at lower Pe by reducing U because Eq. (4) assumes a high Pe.

In this study, we chose not to use any specially designed geometry of the channels; for example, the geometry in which baker's transformation is seen. We felt that it was important to quantify scaling in a general (even random) geometry that is more likely to correspond to the geometries used for mixing in real microfluidic devices. The experimental verification of the scaling argument will be useful for choosing proper conditions for microfluidic experiments within a particular geometry (e.g., choosing the proper flow rates for a reagent with certain D to obtain the desired mixing time). However, this scaling argument is too simple to predict the geometry of the microchannels that produces the most rapid mixing, and more theoretical and experimental work towards this goal will be required. The scaling of mixing with a should be investigated when the effect of geometry on mixing is well understood.

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