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Microfluidic mixing through electrowetting-induced droplet oscillations
Magnetic microsphere-based mixers for microdroplets

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While droplet-based microfluidic systems have several advantages over traditional flow-through devices, achieving adequate mixing between reagents inside droplet-based reactors remains challenging. We describe an active mixing approach based on the magnetic stirring of self-assembled chains of magnetic microspheres within the droplet as these stirrers experience a rotating magnetic field. We measure the mixing of a water-soluble dye in the droplet in terms of a dimensional mixing parameter as the field-rpm, fluid viscosity, and microsphere loading are parametrically varied. These show that the mixing rate has a maximum value at a critical Mason number that depends upon the operating conditions. © 2009 American Institute of Physics.

I. INTRODUCTION

Microfluidic devices that enable chemical reactions have a large number of sensor applications such as multistep chemical synthesis,1 bioanalytical diagnostics,2 DNA analysis,3 catalytic hydrogenation of alkenes,4 acid/base titrations,5 etc. Recent advancements in microfabrication technology have led to the development of microtial analytical systems (μ-TAS),6 more popularly known as laboratory-on-a-chip (LOC) devices. These devices have a relatively small size and are capable of performing the sample and reagent handling steps together with analytical measurements.7

Recent developments in producing and manipulating microdroplets in microfluidic environments have increased the use of droplet-based reactors for μ-TAS applications.8 Droplet-based microfluidic system offers an emerging LOC tool for the real-time online analysis of nanoliter biological and chemical agent volumes.9,10 The droplets offer an easy means for compartmentalizing and isolating reactants, reduce the reagent consumption, and eliminate the possibility for cross-contamination due to dispersion, which is a major problem in a continuous flow microchannel. Thus, droplet-based microfluidic systems offer a promising platform for massively parallel DNA analysis and real-time molecular detection and recognition.11-13 They also offer the promise of decoupling a binary yes/no response from the determination of agent concentration.9

Rapid mixing is essential in many microfluidic systems for proper biochemical analysis,13 sequencing or synthesis of nucleic acids,14 and for reproducible biological processes that involve cell activation,15 enzyme reactions,16 and protein folding.17 However, at this small length scale, species transport in fluid flow is dominated by molecular diffusion, which is usually very slow in comparison with the flow residence time. This is particularly important for bioanalytical applica-

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synchronism with the field,\textsuperscript{29,30} causing active mixing in the droplet.

Since the beads can be functionalized with a large variety of antibodies, they can also be used for quick and selective “tagging” biomolecules or cells\textsuperscript{31} within the droplet-based mixer. After the mixing operation, the beads can be easily separated out using a magnetic field gradient.\textsuperscript{32} Thus, bioanalytical diagnostics involving immunomagnetic separation can also be performed in such a droplet.

The field induced dynamic chain formation and rotation of magnetic microspheres due to a rotating magnetic field has been computationally investigated by several researchers using the particle dynamics,\textsuperscript{30,33} direct numerical simulation,\textsuperscript{34} and lattice Boltzmann methods.\textsuperscript{35} Their results predicted that the mixing induced by rotating chains of magnetic microspheres depends on a dimensionless Mason number, which represents the ratio of the viscous torque to the magnetic torque. Although the structure of the predicted rotating magnetic chains has been adequately validated through experimental measurements,\textsuperscript{30,35,36} the corresponding results for a quantitative evaluation of mixing are absent from literature. Therefore, we experimentally demonstrate the mixing enhancement in a microliter-size sessile droplet by rotating chains of magnetic microspheres and quantify the mixing rate in terms of the half-life of decay of a normalized mixing index. We investigate the influence of several operating parameters on the overall mixing rate, e.g., the rotational speed of the magnetic field, droplet liquid viscosity, and the magnetic particle concentration in the liquid.

II. EXPERIMENTAL CONFIGURATION

The experimental configuration is schematically described in Fig. 1(a). A micro- or nanoliter volume water droplet, impregnated with a measured quantity of magnetic microspheres (CMAGPS 2.5 Corpuscular Inc., available in a dense suspension of 25 mg/ml) is deposited on a superhydrophobic substrate using a digital microdispenser. The polystyrene beads, containing magnetic nanoparticles, have a mean diameter of 2.65 m and a mean magnetization of 20 emu at 10 000 Oe. This corresponds to effective magnetic susceptibility of $\chi_{\text{eff}}=0.019$. The droplet-microsphere suspension is subjected to a magnetic field by placing it between two aligned NdFeB permanent magnets (residual magnetism of 1.2 T at the pole faces) that are mounted on a variable-speed turntable. The magnetic field intensity is measured by a Hall probe digital Gaussmeter (DGM 900, Ferrites India). All experiments are performed for magnetic field distribution shown in Fig. 1(b) for a base magnetic field of $B_0=0.123$ T. As seen in that figure, the field gradient at the droplet location is nearly zero.

During an experiment, the droplets are first deposited on a superhydrophobic substrate, following which a chain-forming time of 10 s is allowed for the microspheres to form chainlike structure under the influence of the imposed magnetic field [as shown in Fig. 1(c)]. A dye is then injected into

![Schematic of the experimental setup.](image)

![Variation of the magnetic field strength of the component perpendicular to the pole faces.](image)

![Self-assembly of magnetic microspheres into chains.](image)
the droplet using a hydrophobic needle and the turntable rotated about its axis at a specified angular speed, which produces the rotating magnetic field. Care is taken to isolate the droplet from the convection currents of the surrounding air so as to prevent shear-induced mixing inside the droplet. Images are obtained using a microscope-mounted digital camera (Olympus SP 350) at 25 frames/s. The microscope (Magnus MSZ-TR zoom stereo trinocular) has a $4 \times 10 \times$ optical zoom, while the camera has a $3.5 \times$ optical zoom [an additional $10 \times$ objective is fitted when the chain structures shown in Figs. 1(c) and 2(a) are viewed]. The recorded movies are subsequently split into color bitmap (RGB) hue-intensity histograms and their pixel information is obtained by image processing (using Magnus Image Pro and MATLAB). To eliminate errors induced due to the noise and shadows in the image, the hue-intensity histogram of a base reference image is subtracted from all other images.

The extent of mixing (i.e., the homogeneity with which the dye is distributed across the droplet) is quantified through a mixing index parameter\textsuperscript{21}

$$C' = \sqrt{\frac{1}{N} \sum_{1}^{N} \left[ (P - \bar{P})^2 \bar{P}^2 \right]}, \quad \text{for} \quad 0 \leq P \leq 256,$$

where the mean pixel value $P = (1/N) \sum_{1}^{N} P$ and $N$ denotes the total number of pixels. Initially, when the dye is relatively unmixed, the variance of the pixel density is large, and $C'$ has a correspondingly large value ($C'_0$). As mixing progresses, the hue-intensity distribution becomes more uniform so that, for perfect mixing, the value of $C'$ approaches a steady low value ($C''_0$). When the mixing index parameter is normalized as

$$C = \frac{(C' - C'_0)}{(C''_0 - C'_0)},$$

the $C$ versus $t$ curve varies from unity (unmixed state) to zero (fully mixed state).

Thus, $C(t)$ exhibits a typical exponential time decay that can be approximated as $C(t) \sim \exp(-t/\tau)$, where $\tau$ denotes the characteristic mixing time that is calculated from the hue-intensity histograms. We compare various magnetic chain induced mixing times with a case for purely diffusive mixing (i.e., when the magnetic field does not rotate). Parametric investigations reveal the influence of the rotational speed of the magnetic field (i.e., the rpm of the turntable), the viscosity of the droplet liquid (by premixing a measured amount of glycerol) and the particle number density.

III. RESULTS AND DISCUSSION

A. Behavior of the rotating chains

As soon as the substrate containing the droplet is placed in the magnetic field, the particles tend to form the chains [Fig. 1(c)] and when the magnetic field is rotated, these chains also follow its changing orientation [see Fig. 2(a)]. When this occurs, the chain containing $N$ spherical particles experiences a magnetic torque $M^m$ and an opposing viscous drag $M^v$ as illustrated in Fig. 2(b)\textsuperscript{36,37}

$$M^m = \frac{\mu_0 \mu_r m |\mathbf{m}|^2 N^2}{4 \pi} \frac{2(2\alpha)}{\tau^2} \sin(2\alpha), \quad \text{and}$$

$$M^v = \frac{4}{3} N \pi \sigma^3 \frac{2 N^2}{\ln(N/2)} \eta \omega.$$

Here $\alpha$ denotes the angle between the rotating field and the magnetization vector $\mathbf{m}$ of a particle, $N$ the number of particles in a chain, $\mu_r$ the relative permeability of the medium, $\eta$ the fluid viscosity, and $\omega$ the angular velocity of the chains. Two other forces influence the chain integrity. The tensile force $F_t$ acting on the chain arises due to the centrifugal force on the rotating particles (which is negligibly small as compared to the magnetic and viscous forces for our experiments) promotes chain disintegration. The compressive force $F_c$ occurs due to the magnetic interaction between the particles and tends to hold the chain together. The response of a chain to the magnetic force is characterized through the Mason number\textsuperscript{36}

$$Ma = \frac{\ln(N/2) M^v}{M^m \sin(2\alpha)} = \frac{\mu_0 \sigma^2 \omega \eta}{\mu_r \gamma_{\text{eff}} \mu_0 \sigma^2 \omega \eta},$$

which compares the magnitudes of the viscous and magnetic forces. When $B_0 = 0.123$ T, $\omega = 5$ rpm, $\gamma_{\text{eff}} = 0.019$ and $\eta = 0.0009$ in water (i.e., $\mu_r \approx 1$) the particle chains in Fig. 2(a) correspond to $Ma \approx 0.003$. For these conditions, the microspheres form long unbroken chains that are approximately 10–15 $\mu$m (i.e., 5–7 particles) long, which rotate in synchronism with the imposed magnetic field. The value of $\alpha$ for these chains is observed to be a very small angle, and so they closely follow the orientation of the imposed rotating magnetic field, as shown schematically in Fig. 2(a).
B. Quantification of mixing in the droplet

When a water-soluble blue dye is introduced into the droplet and spurious fluid motion in the droplet (due to the disturbances caused by ambient air currents) is suppressed, mixing of the dye occurs solely through molecular diffusion. However, when the droplet liquid is loaded with magnetic microspheres and a homogeneous rotating magnetic field is applied, rotating chains of magnetic microspheres lead to advective mixing within the droplet. Figure 3(a) shows a time sequence of images that illustrate the magnetically assisted mixing of a dye inside a 500 nl droplet containing a 10% (by volume) glycerol-in-water mixture (i.e., $\eta=1.1 \times 10^{-3}$ Pa s). When the mixing index is calculated as per Eqs. (1) and (2), the normalized $C$ vs $t$ curve shown in Fig. 3(b) is obtained.

The initial time $t=0$ denotes the instant when the injector is completely withdrawn after dye is injected into the droplet. In Fig. 3(a), the dye is observed in its most concentrated form at $t=0.04$ s. With time, as the droplet is rotated clockwise at $\omega=180$ rpm, the dye streak rotates in the same direction due to viscous interaction between the rotating chains and the liquid, and also stretches and diffuses into the host liquid. The stretching of the dye streak increases with time, which also enhances diffusion, leading to a nearly homogeneous final mixture at $t=12$ s. The initial variance of the hue intensity is large and $C \approx 1$, but with progressive mixing in the droplet, its value decreases to zero according to the relation

$$C(t) = \exp(-0.15t).$$

The half-life of mixing $t_{1/2}$ is the time required to attain a value of $C=0.5$, i.e.,

$$t_{1/2} = 0.6932/0.15 = 4.6 \text{ s}.$$

C. Effect of $\omega$ and $\eta$ on mixing

Figure 4 presents the $C$ versus $t$ plots for two cases, one for the purely diffusive mixing ($\omega=0$) and the other with a rotating magnetic field ($\omega=180$ rpm) inside a 500 nl droplet containing a 20% (by vol.) glycerol-in-water solution (for which $\eta=1.55 \times 10^{-3}$ Pa s). The value of $t_{1/2}=20.3$ s for the purely diffusive case, and 7.9 s, i.e., 2.6 times faster, when the field is rotated. Both droplets are produced from the same initial mixture that has a magnetic microsphere concentration of 1.0 mg/ml.

Comparing Figs. 3(b) and 4 shows that when $\omega = 180$ rpm, the value of $t_{1/2}$ increases from 4.6 s to 7.9 s as the liquid viscosity increases from $\eta=1.1 \times 10^{-3}$ to $1.55 \times 10^{-3}$ Pa s. This difference is attributed to several competing factors. While a higher liquid viscosity enhances the momentum exchange between the rotating chains and the droplet fluid, which is favorable for mixing, it also leads to greater viscous dissipation within the droplet (and consequently a larger momentum transfer to the narrow base where the droplet is attached to the superhydrophobic surface), which dominates and reduces the mixing rate.

Figure 5(a) presents the variation of $t_{1/2}$ when $\omega$ and $\eta$ are varied. For pure water ($\eta=0.902 \times 10^{-3}$ Pa s), the mixing half-life decreases from 17.3 s for purely diffusive mixing to 1.7 s at $\omega=210$ rpm as per the relation

$$t_{1/2} = c_0 + c_1\omega + c_2\omega^2$$

(7)

(where $c_0$, $c_1$, and $c_2$ are constants). The fastest mixing occurs when
and then increase with increasing $\omega$. The constants $c_0$, $c_1$, and $c_2$ of Eq. (7) are obtained from polynomial curve fits of the data presented in Fig. 5(a). Here, $c_0$ denotes the mixing time with pure diffusion ($\omega=0$), $c_1$ is negative (see Table I), since an increase in the advective velocity leads to a reduction in the mixing time, and $c_2$ accounts for the effect of chain breakdown. The value of $c_2$ is influenced by several factors such as the particle-particle interaction (magnetic attraction, steric repulsion, and friction) and the viscous force. The second term in Eq. (7) is proportional to $\omega$ and therefore denotes the influence of the advective velocity.

The $t_{1/2}$ versus $Ma$ plots in Fig. 5(b) illustrate the competing effects of $\omega$ and $\eta$ and follow the general relation that is obtained through a rational curve fit,

$$t_{1/2} = \frac{41.97 \; Ma^2 - 6.602 \; Ma + 0.5705}{(Ma^2 + 0.2143 \; Ma + 0.0276)}.$$

The mixing half-life initially decreases with increasing $Ma$ to assume a minimum near $Ma=0.1$, and then increases as the value $Ma$ increases. Similar results have been reported by Calhoun et al.\textsuperscript{35} who determined a critical $Ma=0.12$ through LB simulations, and Kang et al.\textsuperscript{34} who, through a direct numerical method, also established an optimum $Ma=0.002$ (equivalent to $Ma=0.06$ as per our definition), which they correlated with the occurrence of rotating and corotating flows caused by alternating chain breakup and reformations that lead to the fastest mixing. In region A of Fig. 5(b), mixing is limited by the extent of momentum transfer, while in region B, it is limited by chain deformation and breakdown.

The location of $t_{1/2}$ minima in Fig. 5(a) is found to shift toward decreasing $\omega$ as $\eta$ increases, which is shown by the dotted line. The minimum value of $t_{1/2}$ occurs at $\omega = 210$ rpm for pure water, while for a 30% glycerol-water mixture (i.e., $\eta=2.3 \times 10^{-3}$ Pa s), it occurs at $\omega \approx 140$ rpm. Figure 6(a) presents a corresponding plot of $\omega_{crit}$ as a function of $\eta$, which scales as $\omega_{crit} \propto \eta^{0.32}$. For specified values of $\chi_{eff}$ and $B_0$, $Ma \propto \omega \eta$, and the critical Mason number exhibits a weak dependence on the fluid viscosity as $Ma_{crit} \propto \eta^{0.68}$. The $t_{1/2}$ minima values (at the $\omega_{crit}$ conditions) exhibit a linear relationship with respect to $Ma_{crit}$ as shown in Fig. 6(b). Thus, while Fig. 5(b) illustrates whether the mixing within the droplet is limited by momentum transfer or by
chain breakdown over a wide range of Mason numbers, Fig. 6 shows how the optimum mixing condition varies with changing fluid viscosity.

D. Effect of particle loading on mixing

The advective motion in the droplet depends on the relative number of particle chains contained in a unit volume of the droplet liquid, which is a function of the particle loading $N_p$. Figures 7(a) and 7(b) present the $C$ versus $t$ relationships for mixing in pure water (i.e., $\eta = 0.902 \times 10^{-3}$ Pa s) at $\omega = 140$ rpm with a dense (1.5 mg/ml) and a dilute (0.5 mg/ml) particle loadings, respectively. The value of $t_{1/2}$ for the former case is 1.6 s, while that for the latter is 6.9 s. Therefore, a threefold increase in particle loading improves mixing by a factor of 4.3. An increase in particle loading can influence chain formation in two ways. First, enhancing the particle concentration promotes chain formation and growth during the initial incubation period. Second, it also leads to formation of a larger number of chains. Both factors enhance mixing as shown in Fig. 7(c). The $t_{1/2}$ values decrease significantly from 17.7 to 1.6 s as $N_p$ increases from 0 to 1.5 mg/ml, and then marginally as it is increased to 2.0 mg/ml. Higher particle loadings produced dark rotating chain images, which were difficult to reliably analyze.

IV. CONCLUSIONS

We demonstrate a novel approach to enhance mixing in micron-sized droplets through magnetic stirring. Magnetic microspheres, which are added to the droplet form aligned chains in situ under the influence of a homogeneous magnetic field. When the magnetic field is rotated, the chains also rotate synchronously. Viscous interactions between the particle chains and the liquid induce advective motion inside the droplet thereby enhancing mixing, which is otherwise diffusion limited. The mixing of a dye is characterized through a dimensionless mixing parameter that decays from unity (denoting an unmixed state) to zero (denoting a completely mixed state). The influence of other parameters, e.g., the field rpm, fluid viscosity and the microsphere number density, on the half-life of decay for the mixing parameters is also experimentally determined.

For a specified fluid viscosity, the mixing half-life initially decreases as the field rpm is increased, but then increases at higher rotational speeds. For a fixed field rpm, the mixing half-life increases monotonically with the viscosity of the droplet liquid. The mixing rate increases with the Mason number when $Ma < 0.1$, while for greater $M$ values it decreases. The value of $Ma_{crit}$ has a weak dependence on the fluid viscosity and the critical value of the mixing half-life varies linearly with $Ma_{crit}$. The mixing rate increases monotonically with particle loading, but this improvement diminishes at higher particle loadings.

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