

Passive mixing enhancement of microliter droplets in a thermocapillary environment

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Abstract Fast and efficient mixing of reagents and bioassays is of great importance in micro total analysis systems and in particular, open-surface microfluidics. This is the first study of droplet collision and mixing phenomenon of levitated droplets on an immiscible thin liquid substrate undergoing Marangoni convection. The mixing percentage was determined by monitoring the changing color during chemical reaction that occurs in the resultant coalesced droplet. The thermocapillary effect of the liquid substrate plays a significant role in mixing in that the liquid surface velocity and the droplet velocity increase with an increase in the surface temperature gradient. This, in turn, increases the mixing of the coalesced droplet, which is further enhanced when the drop size is smaller. Since the collision is convection dominated, an appropriate time scale is proposed, which provides a universal correlation of the non-dimensional mixing time in terms of Reynolds number.

Keywords Mixing enhancement · Droplet merging · Thermocapillary effect · Surface tension · Microliter droplet collision · Microfluidic mixing

1 Introduction

Droplet-based microfluidics with its capability to manipulate discrete units of fluid has many lab-on-a-chip

applications. In such systems, sample transport, filtration, collision and mixing are some of the main operations that should be addressed (Samie et al. 2013). Specifically, the coalescence and mixing of droplets are of great importance in microchemistry, biological applications and inkjet printing (Castrejón-Pita et al. 2013; Beebe et al. 2002). Mixing and internal dynamics of coalescing droplets on solid surfaces with focus on wettability gradient of the substrate have been published in the literature (Yeh et al. 2013, 2015; Castrejón-Pita et al. 2013). In microfluidics, owing to laminar flow conditions the mixing of liquids is difficult to realize (Yakhshi-Tafti et al. 2008; Paik et al. 2003). The two dominant fluid mixing mechanisms in microscale are (a) molecular diffusion and (b) chaotic advection (Yeh et al. 2013; Grigoriev et al. 2006). Generally, molecular diffusion is a slow process, which is governed by viscous forces (Stroock et al. 2002). On the other hand, chaotic mixing is faster, but it requires stirring process that stretches and folds the fluid in the entire domain, which is practically hard to achieve on open-surface platforms (Aref 1984). Wiggins and Ottino (2004) reviewed the mathematical foundation of chaotic mixing in detail. Serpentine channel design is one of the solutions to enhance the mixing phenomenon (Yakhshi-Tafti et al. 2008, 2011; Song et al. 2003; Stone and Stone 2005; Liu et al. 2000); however, such designs have their own challenges in terms of complexity and multiplexity.

So far, fast mixing of droplets have been achieved in different ways. Hosokawa et al. (1999) used the interfacial force during droplet impact to generate internal motions inside droplets. Wu and Nguyen (2005) employed two-phase hydraulic focusing to achieve rapid mixing in microchannels. Electrokinetic mixing in microfluidic devices was applied by Chang and Yang (2007). In this electroosmosis process, for conventional straight

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microchannel devices, they claimed that the mixing time and mixing length required to achieve complete mixing were approximately 1000 s and 0.5 m, respectively. Paik et al. (2003) used electrowetting-based (EWOD) droplet mixers for microfluidic device, where they reported mixing time of 4.6 s for microliter droplets. Dielectrophoresis (DEP) has also been deployed for mixing of droplets (Park et al. 2009). Through this approach, the mixing was enhanced by optical means. However, still 8 s was required for coalescence of a 0.8 μL drop with a 0.5 μL one. Thus, in these different processes, mixing can still take up to few seconds.

Our earlier papers (Yakhshi-Tafti et al. 2009, 2010; Davanlou and Kumar 2015a) have discussed the dual migration of droplet on a free surface of a liquid platform with a temperature gradient. It was shown that when a droplet is released from a height of 1.5–4 times its diameter, it can preserve its spherical shape and stay levitated on the liquid surface. The levitation is possible due to the existence of an air gap between the droplet and the liquid surface. The temperature gradient on the surface causes a surface tension gradient and creates a surface slope. This slope allows the droplet to travel toward the heater despite the countercurrent Marangoni flow underneath. On the other hand, if the droplet submerges into the substrate, displaying a lens-shape configuration on top of the surface, it behaves like a tracer particle and travels with the Marangoni flow away from the heater.

The above concept of dual migration was exploited by the same group (Davanlou and Kumar 2015b) who showed the possibility of natural coalescence of droplets at desired locations by precisely routing them through the Marangoni effect. Because of the applied thermal gradient on liquid layer that is located under the droplets, the surface temperature of the floating droplets increases as they are transported toward the heater. This temperature rise causes an additional internal circulation inside droplet attributed to Marangoni flows. Note that initially upon coalescence of droplets, due to the released surface energy a pair of recirculation cells occurs inside the merged droplets, which improves the fluid mixing (Yeh et al. 2013).

In the current paper, we extend the work performed on coalescing droplets by investigating the influence of thermocapillary flow on two equal-sized colliding droplets that have different compositions. By creating a temperature difference of 0–30 $^{\circ}\text{C}$ between the heater and the droplet loading location, droplets are actuated with different velocities. The mixing progress is quantitatively studied through analyzing the images from merging till the fully mixed state is reached. An appropriate time scale for this problem is developed and implemented to find a universal correlation for mixing time as a function of Reynolds number.

2 Experimental setup

The platform of this experiment, as shown in Fig. 1, is fabricated on a 96×64 printed circuit board (PCB). Copper is used to make the desired heater patterns on top of the board. The rectangular board is sealed with a thin acrylic sheet with height of 11 mm to ensure a sealed environment for the liquid. A power supply (TE, model HC3002) provides the electric potential required for creating certain thermal gradients. A camera (Prosilica, model GE680C) with 388 frames per second (fps) and resolution of 320×240 pixels is connected to a zoom lens (Navitar, model Zoom 7000). This setup is mounted at a 30° angle to the horizon to capture the events from the side. An infrared camera (FLIR, model SC5600) monitors the temperature of the platform. The infrared camera can reach up to 380 fps at full resolution of 640×512 pixels. A combination of precision needles with microsyringe is used to generate droplets (2.5–3.6 mm in diameter). The droplets are made of potassium hydroxide (KOH, 0.01 M; LabChem) and phenolphthalein [$\text{C}_{20}\text{H}_{14}\text{O}_4$, 0.5 % (w/v) in 50 % (v/v) Alcohol, Ricca Chemical Co.]. The potassium hydroxide droplet has a density of 1000 kg/m^3 , viscosity of 1.05 cSt and surface tension of 72.1 mN/m, and phenolphthalein droplet has a density of 1300 kg/m^3 , viscosity of 2.75 cSt and surface tension of 29.1 mN/m. Collision of these two droplets causes a color change due to the chemical reaction that takes place inside the coalesced droplet. Spatiotemporal analysis of this color change through an in-house developed MATLAB code is used to study the progress of mixing phenomenon. The liquid film under the droplets is

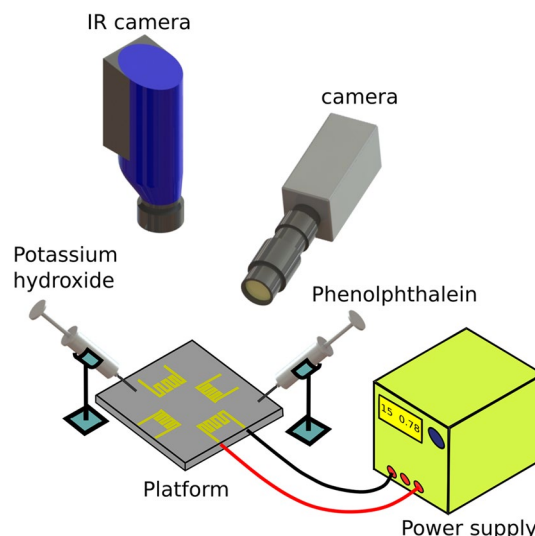


Fig. 1 Schematic diagram of the experimental setup. Phenolphthalein (HIn) is a pH indicator that turns pink in a base solution like potassium hydroxide

fluorinert FC-43 oil. This inert oil has a density of 1880 kg/m³, viscosity of 2.8 cSt and surface tension of 16 mN/m.

3 Results and discussion

We study the head-on collision and the subsequent mixing of levitated droplets due to an imposed thermal gradient on the liquid substrate. The physical principle underlying the thermal actuation of droplets is reviewed here for better understanding of mixing of reagents. By employing thermocapillary effect inside the thin film (here FC-43), the liquid interface experiences a depression at the hot spot. This is due to the imbalance of the tangential surface tension forces. When a droplet is levitated on the liquid interface, it moves down the slope due to gravity toward the heater and in the opposite direction of Marangoni convection below the droplet. Applying the temperature gradient allows us to transport droplets accurately in desired paths. For instance, a 3-mm drop can reach speeds up to 36 mm/s when $\Delta T = 30\text{ }^\circ\text{C}$. In addition, since parallel manipulation of samples (droplets) is feasible, merging and mixing could be realized. The collision kinetic energy of the impact along with the additional convection generated inside the droplet due to its temperature rise leads to efficient mixing schemes. Figure 2 illustrates the sequence of these events in the same order that they occur.

The experiment is devised such that a droplet of potassium hydroxide is dispensed first while enough time is given so that it migrates toward the heater due to liquid

surface deformation (~2.3 s). Then, a second droplet of similar size but made of phenolphthalein is dispensed. The latter droplet moves to the same heater where the first droplet is already trapped (due to surface deformation at the heated spot); hence, the droplets coalesce after 4.1 s from the time the first drop was released, and then, a chemical reaction inside the larger droplet takes place, turning its color pink. The droplets are released from a height ~1.5 times their diameter, a minimum requirement to levitate the droplet with an air gap between the droplet and the liquid underneath (Davanlou and Kumar 2015a; Davanlou 2015). It is interesting to mention that after merging, due to the heat of mixing and heat of reaction the temperature of the droplet rose 3 °C. After reaction, the resultant droplet has a viscosity of 1.8 cSt and surface tension of 29.5 mN/m.

The mixing phenomenon has been quantitatively studied in the literature by employing optical diagnostic tools such as laser-induced fluorescence (LIF) (Carroll and Hidrovo 2013; Yeh et al. 2013), particle image velocimetry (PIV) (Ortiz-Dueñas et al. 2010) and spontaneous Raman scattering (SRS). Here, with the aid of high-speed color imaging and image processing, the mixing phenomenon is investigated by monitoring the color changing reaction that occurs inside the coalesced droplet. Nilsson and Rothstein (2011), and Yakhshi-Tafti et al. (2011) have used a similar approach in order to measure the degree of mixing of two droplets. The process is divided into the following steps: (1) the images undergo a color enhancement process, (2) the image type is converted from RGB to gray scale, (3) suitable filters are applied to cancel or reduce the noise, (4) a new scale is used to determine the image contrast, varying from 0 to 1 and (5) the light reflection from the background surface is eliminated by applying appropriate thresholds. This assures that only the droplet and not the surrounding is analyzed. Figure 3 illustrates these steps. The details of image processing algorithm are explained in Refs (Yakhshi-Tafti et al. 2011; Davanlou 2014).

In our algorithm, I is the measure of mixing, where $I = 0$ is designated for the parts that are fully mixed and $I = 1$ for the non-mixed portions. The deviation of gray intensity around the darkest gray pixel (completely mixed region) is calculated for the pixels of the selected cross section (Stroock et al. 2002):

$$\sigma = \left[\frac{1}{n} \sum_{i=1}^n (I_{\min} - I_i)^2 \right]^{1/2} \tag{1}$$

where n is the number of the cross-sectional pixels, I_i is gray intensity of pixel i of the selected cross section, and I_{\min} is the minimum gray intensity. If there is no mixing, the standard deviation is $\sigma = 1$, while $\sigma = 0$ represents full

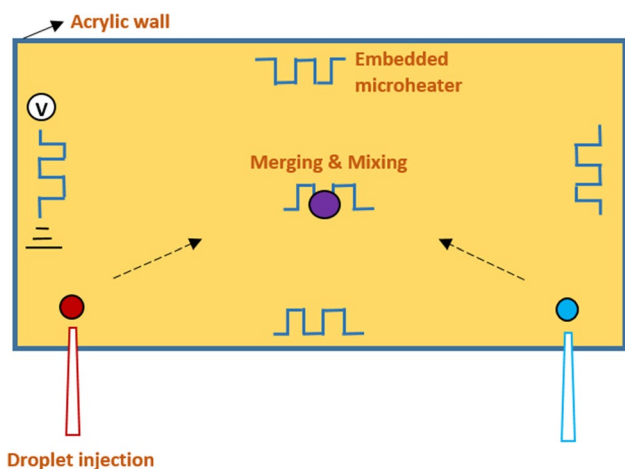


Fig. 2 Schematic of a PCB platform for droplet merging and mixing. Droplets of KOH and phenolphthalein (denoted by different colors) are injected onto the thin liquid film of FC-43 oil. Applying electric potential at the terminals of a microheater, heat is applied due to Joule heating. Consequently, liquid surface deforms and the droplet migrates toward the hot side of the platform where the liquid has the lowest thickness (color figure online)

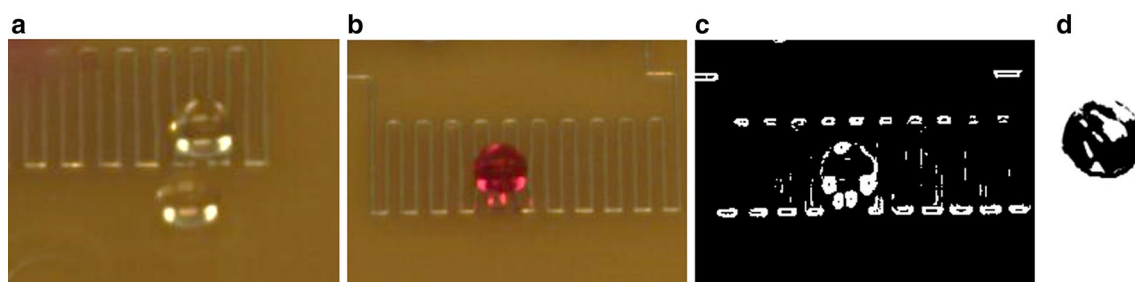
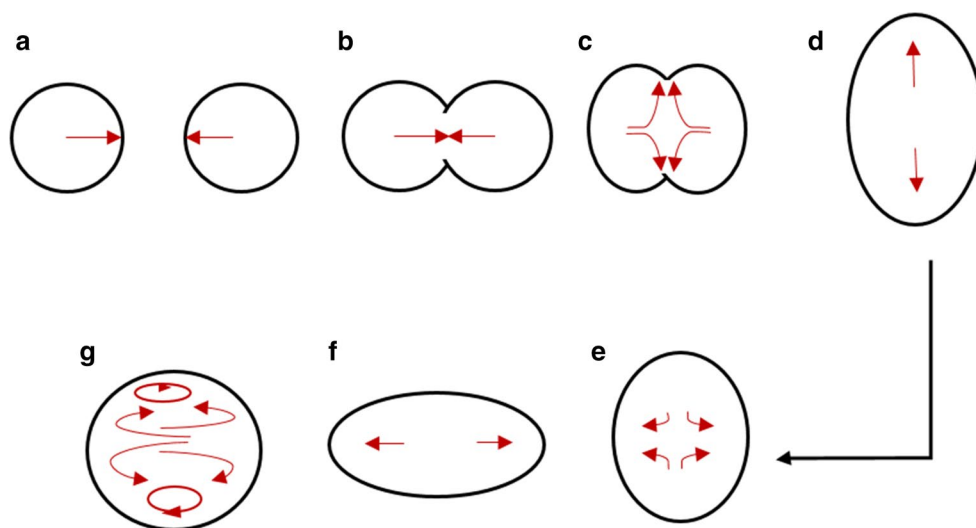


Fig. 3 **a** Head-on collision of droplets. After coalescence due to chemical reaction, the color change occurs. Image processing of the coalesced droplet can be divided into: **b** color image after mixing;

c conversion of the image into a binary image; **d** background subtraction and quality improvement on the binary image (color figure online)

Fig. 4 Schematic diagram of collision and mixing of two equal-sized droplets



mixing as all pixels attain uniform gray intensity. The level of mixing in percentage is calculated as:

$$M(\%) = (1 - \sigma) \times 100 \quad (2)$$

The photographs shown in Fig. 3 are captured schematically in Fig. 4 to illustrate the coalescence and mixing mechanisms. When the droplets collide, the resulting coalesced droplet deforms in the vertical and horizontal directions as shown in Fig. 4d, f, which leads to internal circulation of the fluid inside the droplet. Due to this circulation, the mixing percentage increases and is further enhanced when the droplet temperature rises as it is heated by the liquid surface (Fig. 4g).

Figure 5a illustrates the change in mixing efficiency of droplets when they collide due to a temperature difference. The variation of the liquid film temperature measured by the IR camera is shown in Fig. 5b. Initially, in order to obtain the mixing percentage without a thermal rise (i.e., $\Delta T = 0$ °C), the droplet is placed on the substrate. Then, the second droplet is released on top of it so that coalescence and mixing occur. Also as the droplet size reduces, the mixing

percentage increases. Since a two-dimensional image is analyzed, our technique of mixing analysis uses an arithmetic mean of all the pixels in its depth at a given pixel coordinate. To avoid introducing high uncertainty in measurements, for each case of an imposed temperature drop, ten cases are separately analyzed and averaged. The average value of mixing percentage is plotted against the temperature drop for each experiment in Fig. 5. The repeatability of measurements is above 93 %. It is important to note that the chemical reaction between potassium hydroxide and phenolphthalein is different from normal mixing because of the released heat of reaction as well as the distinct product from the collision, which has its own physicochemical properties (Yeh et al. 2015). Figure 5c schematically shows the Marangoni convection inside a liquid film and the levitated droplet moving against this Marangoni flow. Using infrared thermography, the rise of droplet surface temperature is tracked as it migrates toward the hot region. Figure 5d provides the droplet surface temperature for three drop sizes. As expected, the smaller drop is seen to have a higher temperature in the substrate temperature range of 5 °C $\leq \Delta T \leq 30$ °C.

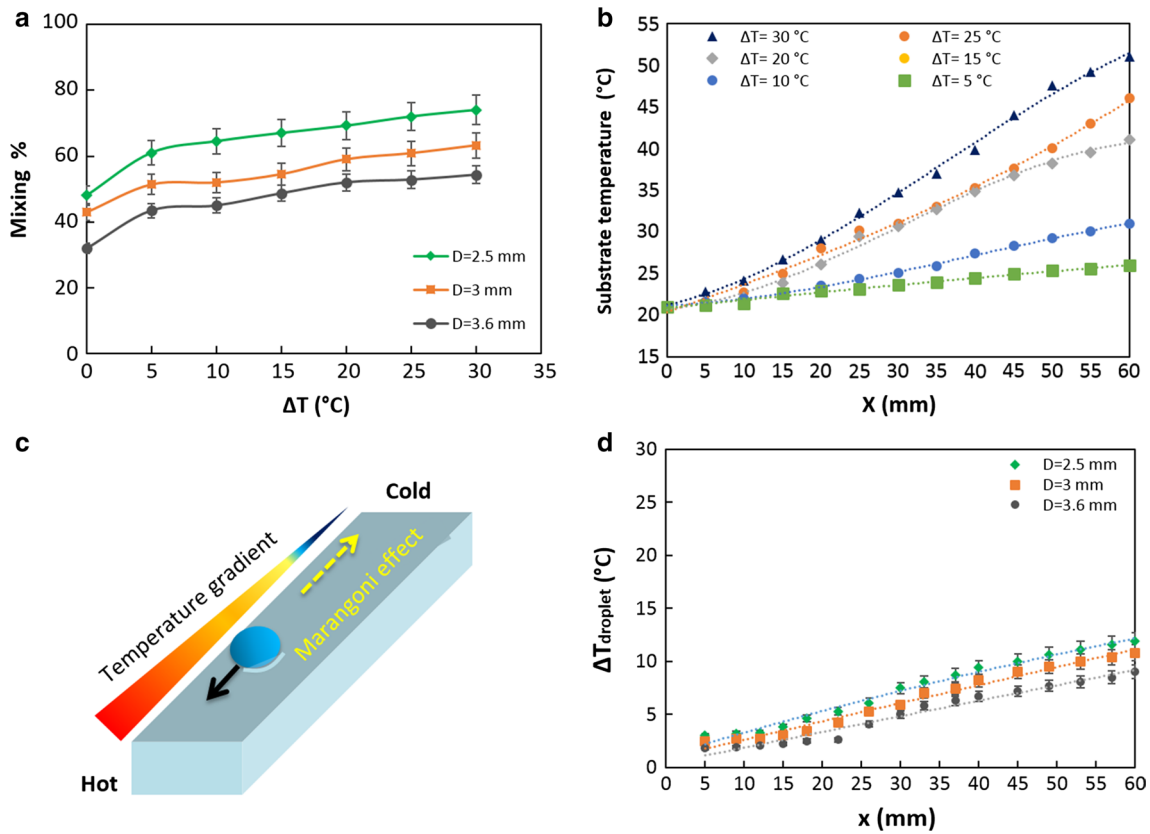


Fig. 5 **a** Variation of mixing efficiency with temperature for three different diameters at 100 ms, **b** platform surface temperature increases almost linearly due to a $\Delta T = 30\text{ }^\circ\text{C}$, **c** the schematic depicts the counter-motion of the droplet toward the heat source

against Marangoni flow in the liquid substrate. The initial temperature of the liquid substrate is measured using the IR camera immediately prior to droplet release, **d** variation of droplet surface temperature as it migrates toward the heater

This problem has several relevant time scales. Schmidt number, $Sc = \nu/D = Pe/Re$, can be used to compare the viscous diffusion with molecular diffusion, i.e., the ratio of Péclet number to Reynolds number. The mass diffusivity of water is equal to $D \approx 10^{-10}\text{ m}^2\text{ s}^{-1}$, and the kinematic viscosity is $\nu \approx 10^{-6}\text{ m}^2\text{ s}^{-1}$, which results in $Sc \approx 10^4$. This shows that the time scale due to viscous diffusion is small. Thermal kinetic energy is the main source of random motion of solute molecules in molecular diffusion. The relevant time scale for molecular diffusion and convection is:

$$\tau_{\text{Diff}} \sim \frac{L_{\text{ch}}^2}{D} \tag{3}$$

$$\tau_{\text{Conv}} \sim L_{\text{ch}}/\tilde{U} \tag{4}$$

where L_{ch} is the characteristic length scale and \tilde{U} is the velocity scale, which will be defined later. The Péclet number can be obtained by dividing the mass diffusion time scale by convective time scale:

$$\frac{\tau_{\text{Diff}}}{\tau_{\text{Conv}}} = \frac{L_{\text{ch}}^2/D}{L_{\text{ch}}/\tilde{U}} = \frac{\tilde{U}L_{\text{ch}}}{D} = Pe = Re \cdot Pr \tag{5}$$

Re and Pr represent Reynolds and Prandtl numbers, respectively, that defined as:

$$Re = \frac{\tilde{U}L_{\text{ch}}}{\nu}, \tag{6}$$

$$Pr = \frac{\nu}{\alpha} \tag{7}$$

where α is the thermal diffusivity. Table 1 summarizes the group of dimensionless numbers for this problem. These values are based on the average of kinematic viscosity of the two drops and droplet diameter.

The change of mixing with respect to time is plotted in Fig. 6a for $\Delta T = 25\text{ }^\circ\text{C}$. It is shown that the mixing advances with time; however, after $\sim 80\text{ ms}$ the curve reaches an asymptotic value (for all cases). A similar trend is observed for different size droplets, while smaller

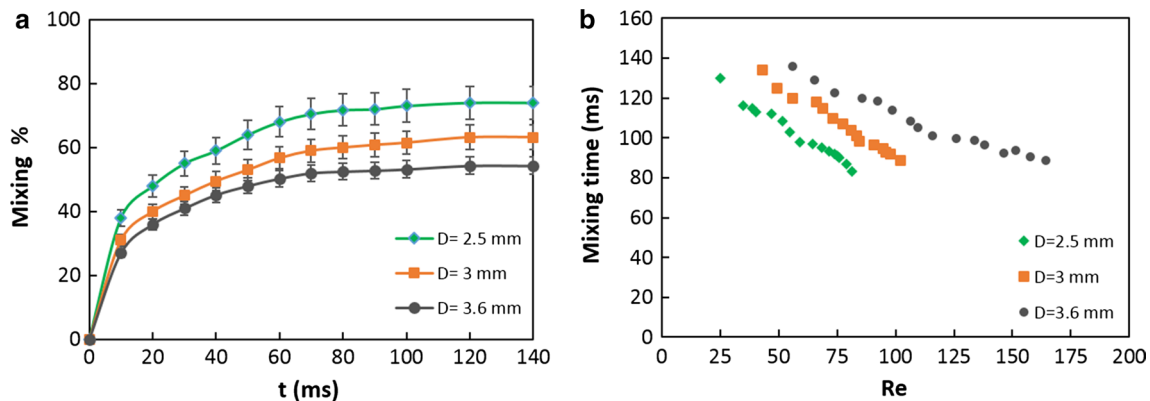
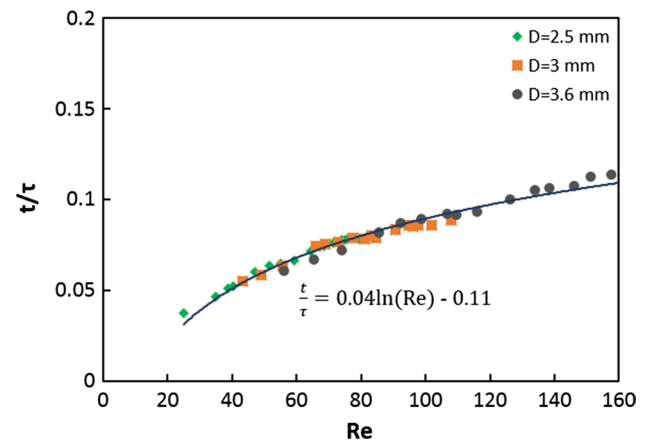
Table 1 Range of dimensionless numbers at different ΔT s

Drop size (mm)	Volume (μL)	Re	Pr	Pe $\times 10^4$	Sc $\times 10^4$
2.5	8.18	25–81.4	6.99	25–83	1
3	14.1	43.2–108	6.99	43.2–108	1
3.6	24.4	56.1–164.1	6.99	56.16–164.2	1

The data are sorted according to the droplet size and volume

droplets undergo higher mixing rates. It is important to note that this graph only represents the mixing progression of the first 120 ms after collision. At longer times, the mixing index is likely to reach 100 % (not shown here). Figure 6b shows that the mixing time decreases with increase in Reynolds number; however, the time required to achieve the final mixing percentage remains almost the same (~ 120 ms) for all cases. In the absence of a thermal gradient, i.e., zero velocity, the mixing time is approximately four times longer (450 ms).

By using Eq. 6 and changing ΔT from 10 to 30 °C, Reynolds number and mixing time for head-on collision of droplets are determined. The results show that regardless of the size, with increase in droplet inertia, the time required for mixing reduces. Mixing is considered to be complete when the time after which the change in mixing percentage is less than 0.1 %. The results indicate a significantly higher Reynolds number for larger droplets due to their larger relative velocity for the same ΔT , despite the fact that the mixing efficiency reduces with size. Note that the mixing time is in the order of milliseconds, which is 8 *order of magnitudes smaller than typical diffusion time scale*. This explains why the mixing mechanism is predominantly governed by convection rather than diffusion. Therefore, it is reasonable to conclude that a higher thermal gradient helps in rapid mixing of droplets as it elevates the kinetic energy of molecules inside the droplet.

**Fig. 6** **a** Mixing progress with time for different drop sizes at $\Delta T = 25$ °C. **b** Dependence of Reynolds number with mixing time**Fig. 7** Normalized mixing time with convection time scale versus Reynolds number

The non-dimensional mixing time versus Reynolds number is plotted in Fig. 7. The mixing time is normalized using a convection time scale $\tau \sim \frac{L}{\tilde{U}}$, where L is the average distance between the drops and the hot spot where they collide and \tilde{U} is the velocity scale casts as $\tilde{U} = \frac{|\sigma_T| |\nabla T| R}{\mu}$ with ∇T be the temperature gradient, R the droplet radius, ν the dynamic viscosity of the film, and σ_T the rate of change of interfacial tension with temperature (at the interface between droplet and thin liquid film). This velocity scale is appropriate since it is based on the thermocapillary flow induced inside the thin liquid film. A logarithmic correlation of mixing time normalized by the convection time scale given as a function of Reynolds number fits the data very well with $R^2 = 0.98$ in the Reynolds number range of 20–160.

$$\frac{t}{\tau} = 0.04 \ln(\text{Re}) - 0.11 \quad (8)$$

4 Summary and conclusions

Recent work by the authors has shown that a droplet can be levitated on an immiscible liquid substrate, and upon application of a temperature drop on the substrate, the droplet travels toward the general direction of the heater and gets pinned at the heater. This work uses this concept to levitate two droplets of equal size at different chemical composition and allows chemical reaction to take place upon collision. The mixing percentage at every instant is calculated by evaluation of color image processing. The temperature gradient on the substrate heats up the droplet. In addition, the heat of reaction as well as the high kinetic energy of droplets due to the applied thermal gradient enhances mixing. Therefore, mixing percentage increases with increasing temperature. Mixing rates as high as 74 % are found to be reachable within 120 ms. For all cases, an increase in droplet inertia (Reynolds number) shortens the mixing time. However, the time required to achieve the saturated level of mixing is found to be nearly the same. The results also suggest that the reduction in droplet size favorably enhances the mixing percentage. Thus, utilizing a thermocapillary environment allows the use of droplets as microreactors in digital microfluidic applications to perform rapid and efficient mixing of reagents.

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