Microfluidic device coupled with total internal reflection microscopy for in situ observation of precipitation

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Received 12 December 2020 / Accepted 1 April 2021 / Published online 20 April 2021
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Abstract In situ observation of precipitation or phase separation induced by solvent addition is important in studying its dynamics. Combined with optical and fluorescence microscopy, microfluidic devices have been leveraged in studying the phase separation in various materials including biominerals, nanoparticles, and inorganic crystals. However, strong scattering from the subphases in the mixture is problematic for in situ study of phase separation with high temporal and spatial resolution. In this work, we present a quasi-2D microfluidic device combined with total internal reflection microscopy as an approach for in situ observation of phase separation. The quasi-2D microfluidic device comprises of a shallow main channel and a deep side channel. Mixing between a solution in the main channel (solution A) and another solution (solution B) in the side channel is predominantly driven by diffusion due to high fluid resistance from the shallow height of the main channel, which is confirmed using fluorescence microscopy. Moreover, relying on diffusive mixing, we can control the composition of the mixture in the main channel by tuning the composition of solution B. We demonstrate the application of our method for in situ observation of asphaltene precipitation and β-alanine crystallization.

1 Introduction

In situ observation and control of phase separation or precipitation process are important in many areas including growth of nanoparticles [1,2], crystallization [3,4], and biomineralization [5]. A dilution-induced phase separation occurs when the concentration of a solute dissolved in a solvent reaches a critical concentration by the addition of an antisolvent. At the critical concentration, the solute is oversaturated, and the mixture is spontaneously separated into two subphases. Depending on the type of solute, solid phase formation may be formed as precipitates or crystals. In all cases, both the chemical composition of the final mixture (i.e., solute + solvent + antisolvent) and controlled mixing between the solution (i.e., solute + solvent) and antisolvent determine the phase separation outcome. Precipitation is of particular interest during paraffinic froth treatment in oil sand extraction process, where asphaltene precipitates are formed from the addition of antisolvent [6].

Recently, microfluidic devices have been widely used for phase separation of various materials ranging from crystals [7,8], drug nanoparticles [9], and lipid nanoparticles [10]. Phase separation is not only dominated by the composition (i.e., thermodynamic aspect) but also by the mixing dynamics (i.e., hydrodynamic aspect) [11]. In a quasi-2D microfluidic device, the mixing between the solution and the antisolvent is predominantly driven by diffusion. By leveraging control of diffusion, we can disentangle the mixing dynamics and composition to better understand the formation and growth of the new phase. Moreover, the outcome of phase separation can be visualized in situ via optical or fluorescence microscopy. For instance, Sekine et al. were able to observe halite crystal growth in situ using a microfluidic channel [12]. Desportes et al. used fluorescence microscopy coupled with a microfluidic setup to observe the nanocrystallization of rubrene [13]. However, the spatiotemporal resolutions of optical and fluorescence microscopy are not high enough for detailed study of the phase separation dynamics in early stage, due to the scattering of background from nanobubbles, nanodroplets, or nanoparticles. Furthermore, in some cases, an in situ precipitation study is complicated by the strong absorption of light by the dark medium, for example, asphaltene precipitation from toluene solution.

Coupling the advantages of microfluidics with total internal reflection fluorescence (TIRF) microscopy provides an attractive solution to study the phase separation and precipitation dynamics. TIRF is an imaging method that uses the evanescent wave produced at an
interface between two media with different refractive indexes. As the penetration depth of evanescent field is shallow (∼160 nm), combining TIRF and microchamber is a powerful method to study any change that occurs near the chamber surface. Unlike the standard optical microscope in which the scattering of the background may hinder visualization of phase separation, TIRF is capable of following the processes in a scattering medium. For instance, TIRF has been applied to investigate the liquid–liquid phase separation during mixing process [11], early stage diffusive growth of droplets [14], and real-time chemical reactions in surface nanodroplets [15].

In this work, we demonstrate a microfluidic method combined with TIRF for in situ observation of precipitation and phase separation. A bilayer quasi-2D microfluidic device—composed of a shallow main channel and a deeper side channel—is constructed to enable controlled diffusive mixing between two solutions to drive the phase separation. Using fluorescence microscopy, we validate the diffusive mixing process between solutions in the observation area in the main channel. By characterizing fluorescence intensity, we also quantify the change of chemical composition in space and in time in the observation area. We further demonstrate two examples of phase separation using the proposed setup (i.e., coupling the quasi-2D microfluidic chamber and TIRF), namely oiling out using the proposed setup (i.e., coupling the quasi-2D microfluidic device—composed of a shallow main channel and TIRF), namely oiling out crystallization of β-alanine and early stage of asphaltene precipitation. We can decouple the composition and mixing dynamics on oiling out crystallization and better understand the crystal phase formation and growth. Moreover, the early stage of asphaltene precipitation in an opaque medium can be visualized so that asphaltene precipitation in a concentrated solution can be investigated.

2 Experimental

2.1 Device fabrication

The quasi-2D microfluidic device was fabricated via a standard photolithography process followed by wet etching, as shown in Fig. 1. Borofloat glass wafer (81% SiO2, 13% B2O3, 4% Na2O/K2O, 2% Al2O3) was first cleaned with piranha solution (3:1 = H2SO4/H2O2) (Caution: piranha solution is highly caustic!) and rinsed by deionized water. Chrome (20 nm) and gold (150 nm) were sputtered on the metal-masked substrate, which was baked for 1 min. The substrate was exposed under UV light through a photomask with a pre-designed side channel pattern on it. The exposed patterned photoresist was removed by developer. Gold and chrome layers were then etched by gold and chrome etchant. The Borofloat substrate was then etched by HF (hydrofluoric acid) solution (4:1:5 mixture of water/HNO3 (nitric acid)/HF by volume, poured from stock bottles of concentrated nitric acid (69.0–70.0%) and HF (49%) by NanoFab staffs at the University of Alberta) (Caution: HF solution is highly caustic!) to create 240 µm side channel. After etching of the side channel, blue tape was used to protect both ends of the main channel. Photore sist on the main channel was then manually removed by acetone wetted cotton swab. Gold, chrome, and Borofloat etching steps were repeated to get 20 µm etch of the main channel. Borofloat was cleaned by deionized water, and the remaining gold and chrome layers were stripped by etchants. The patterned Borofloat was then bonded with a glass cover slip (24 mm × 50 mm × 170 ± 5 µm, Azer Scientific) by epoxy (WEST SYSTEM 105 epoxy resins). The spacing between the top and bottom glasses was not changed with epoxy, which was proved by changing the focal plane of the microscope from the top glass to the bottom glass. Similar to the structure of microchamber used in our previous work [16], the detailed dimensions of the quasi-2D microfluidic device are shown in Fig. 2a. The narrow main channel is ∼20 µm in depth and 6 mm in width, flanked with two deep side channels with ∼260 µm in depth and 3 mm in width. The lengths of both the main channel and side channel are about 3 cm.

2.2 Visualization of diffusive mixing using the quasi-2D microfluidic device

All of the experiments were conducted at room temperature (∼19–21 °C). To confirm the diffusive mixing inside the microchamber, the mixing process between toluene and n-pentane was followed by using a fluorescence microscope (Nikon ECLIPSE Ni) equipped with a camera (Nikon DS-Fi3). Toluene (ACS grade, Fisher Scientific, 99.9+%), doped with a trace amount of Nile Red (Fisher Scientific), was injected from one end of the side channel at a flow rate of 5 mL/h by a syringe pump (NE-1000, Pumpsystems Inc.) as sketched in Fig. 2. As flowing in the side channel along y-axis, n-pentane diffused transversely into the narrow quasi-2D main chamber to mix with toluene along x-axis. The location of the field of view was set at a distance of 80 µm from the side channel through the transparent top glass of the main channel.

Green laser (559 nm) was used to excite Nile Red, and the emission was monitored at 635 nm. The change in the fluorescence intensity of the dye in the liquid was recorded at 2 frames per second by the fluorescence microscope (Nikon ECLIPSE Ni) equipped with a camera (Nikon DS-Fi3). The fluorescence intensity was converted to grayscale value and read using MATLAB software (The MathWorks Inc.). The fluorescence intensity as a function of time is governed by the mixing process, which is a vital dataset to confirm whether diffusion mixing is dominant and to estimate the chemical composition of solution at a given location.
Fig. 1  
(a) Schematic showing photolithographic procedure for fabricating the channel on glass substrate. (b) The patterned glass substrate is sealed with a blank glass substrate using epoxy. (c) Photograph of the fabricated microfluidic chip.

Fig. 2  
(a) Top view and side view of the fluid chamber. The chamber consists of deep side channel with 260 μm depth (blue) and shallow main channel with 20 μm depth (brown).  
(b) Mechanism of total internal reflection fluorescence (TIRF) microscope. Only the asphaltene in evanescent field (yellow) can be detected.
2.3 In situ observation of precipitation and phase separation using the quasi-2D microfluidic chamber

The microfluidic device was initially filled with asphaltene solution in toluene (solution A). Then, solution B—n-pentane/toluene mixture at various ratios—was injected through the deep side channel with flow rate of 5 mL/h by a syringe pump where n-pentane is an anti-solvent of asphaltene. Part of solution B diffused transversely into the main channel and mixed with solution A. Asphaltene gradually precipitated with n-pentane diffusion in the main channel and could be detected by DeltaVision OMX Super-resolution microscope (TIRF 60×/1.49 NA objective lens) (GE Healthcare UK limited, UK). TIRF was used to detect asphaltene precipitates in the microchannel. The principle of TIRF detection is sketched in Fig. 2b. Light refraction follows Snell’s law, as shown in Eq. (1):

$$n_1\sin\theta_1 = n_2\sin\theta_2$$  \hspace{1cm} (1)



$n_1$ and $n_2$ are incident and refracted index of a given pair of media, separately, and $\theta_1$ and $\theta_2$ are incident and refracted angle of light in the two media, separately. When the light propagates in a denser medium (e.g., the glass substrate) and meets the interface with a less dense medium (e.g., the sample on the substrate) above a critical angle, rays of light are no longer refracted but totally reflected in the denser medium. Total internal reflection (TIR) occurs, generating an evanescent field adjacent to the interface of the media. The electromagnetic wave in the evanescent field can excite the fluorophores in the sample with penetration depth around 100-200 nm, depending on the optical properties of the media and the incident angle [17].

In our measurements of asphaltene precipitation, the refractive index of asphaltene (RI = 1.72) was used based on the literature [18,19], which is different to toluene (RI = 1.50) [20] and n-pentane (RI = 1.36) [21]. An excitation laser with wavelength $\lambda = 488 \text{ nm}$ was used to excite fluorophores, and the emission wavelength detected by the detector was up to 576 nm. The TIRF images were taken on an 82.5 $\mu\text{m}$ length detected by the detector was up to 576 nm. The fluorescence intensity at four locations is plotted as a function of time shown in Fig. 3b. The locations are at 80–140 $\mu\text{m}$ away from the junction of the side channel and the main channel. The decay of the intensity (i.e., fluorescence signal vs. time) at any given location was fitted well by a logistic function with Eq. (2).

$$I = 1 - \left(0.993 + \frac{-1.05}{1 + \exp\left(\frac{-t}{491.5 - A}\right)}\right)$$  \hspace{1cm} (2)

$I$ is normalized intensity, $t$ is time, and $A$ is a constant depending on the location. The delay in the intensity drop of location correlated well with the distance leading to a steady increase of 0.3 per 20 $\mu\text{m}$ distance increment for parameter $A$, as shown in Fig. S1, suggesting that the mixing conditions are identical at different locations in our observation area. The fitting constants 0.993, −1.05, and 0.43 were selected based on error minimization. In our setup, as the diffusion of n-pentane takes place, the fluorescence intensity at a fixed location (80 $\mu\text{m}$) decreases. At early times, the fluorescence intensity decays slowly as the concentration of n-pentane is low. With time, the intensity decreases sharply as the fluorescence probes are diluted by diffusion of n-pentane until the intensity reaches a plateau where all the toluene has been replaced by n-pentane.

In Fig. 3c, the front layer moving distance $d$ normalized by the square root of time $t^{1/2}$ is plotted as a function of the distance $d$ from the side channel. For a constant concentration source, Fick’s diffusion in one-dimensional scenario, the diffusion length $d$, and time $t$ are as follows:

$$\frac{d}{\sqrt{t}} \sim 2\sqrt{D}$$  \hspace{1cm} (3)

$D$ is diffusion coefficient, which is assumed to be constant in our system. The results of constant $\frac{d}{\sqrt{t}}$ suggest...
that mixing at a critical distance (i.e., 80 µm to the side channel) is well described by a diffusion process. A distance from the side channel is less than 80 µm, and the mixing is not well described by diffusion, which could be caused by the influence of the side channel. On the other hand, a longer distance means a longer diffusion time, which is undesirable as the maximal fluorescence intensity is reached in a shorter duration. Therefore, our observation area is kept at 80 µm to the side channel in the following experiments. There is a critical value because of the momentum influence in the side channel, as we do not have a solid boundary such as a membrane to eliminate the influence of the side channel. Based on the observation, at distances longer than 80 µm, the convective effect of the flow is negligible and the transport of the dye is predominantly by diffusion in our system. It worth noting that 80 µm is not a universal value, and it may change if the experimental conditions change such as flow rate in the side channel or liquids in the microfluidic chamber.

The duration time for n-pentane replacing toluene is about 40 s over 491.5 µm distance. Since n-pentane diffusion rate follows Eq. (3), \( \sqrt{\frac{d}{t}} \) is constant, at around 60 µm/s\(^{1/2}\), as shown in Fig. 3c. At 40 s, the calculated travel distance for the front layer is 379 µm, which is comparable with the actual moving distance, i.e., 491.5 µm. The difference between calculated and actual values may be from the non-diffusive behavior below the critical distance of 80 µm.

Given that n-pentane diffusion from the side channel into the main channel is a one-dimensional diffusion in our system, the diffusion is as follows:

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (4)
\]

\( c \) is concentration of n-pentane, \( t \) is time, \( D \) is diffusivity of n-pentane in toluene, and \( x \) is the distance to the side channel. Solving Eq. (4), n-pentane concentration distribution along the x-direction is:
3.2 Applications of the diffusive mixing device

The mixing device was used to observe the precipitation of asphaltene from toluene by n-pentane. As shown in Fig. 5, the precipitation of asphaltene can be observed in situ. Images were taken from 80 µm which is the critical distance needed to achieve diffusive mixing, as also shown in Fig. 3. Up to a distance of 80 µm, mixing may be influenced by the bulk flow of solution B. The high amount of precipitates observed at 80 µm is likely due to the convective mixing. However, at longer distances, the diffusive mixing is established and the amount of precipitates remains similar to distance. With the high resolution of TIRF—i.e., 200 nm—observation of individual asphaltene precipitates may also be possible.

The proposed device is not limited to the in situ asphaltene precipitation, but it can also be applied for studying dilution-induced crystallization from a liquid mixture. The dilution-induced crystallization occurs due to the reduced solubility of the solute in the liquid mixture by the addition of an antisolvent. Using the diffusive mixing device, we can visualize the crystallization process. As an example, we demonstrate in situ growth of β-alanine crystals by dilution of a ternary solution consisting of β-alanine, isopropyl alcohol, and water (solution A) by isopropyl alcohol (solution B). As solution B is diffused through the main channel, β-alanine becomes oversaturated and crystallizes on the surface of the channel, as shown in Fig. 6. Using the device shown here, it is possible to control the crystallization by tuning the mixing between the antisolvent and the liquid mixture.

It is worth noting that although surface properties, including hydrophobicity and roughness, do not affect the diffusion process in the main channel [16], the phase separation may be affected if the surface acts as nucleation sites. For instance, oil droplets from liquid–liquid phase separation may more readily occur on hydrophobic surfaces [22].

4 Conclusions

A method for in situ observation of precipitation and phase separation is shown here via a quasi-2D microfluidic device coupled with total internal reflection (TIRF) microscopy. The device enables controlled diffusive mixing between an asphaltene solution and a mixture of n-pentane and toluene. Using a fluorescent dye, the diffusive mixing front is determined, based on which a critical distance required to achieve fully diffusive mixing is obtained. Moreover, from the intensity profile of the fluorescent dye, the concentration profiles of n-pentane and toluene can be estimated. TIRF image shows the diffusive mixing is not influenced by asphaltene precipitation as well as the quantity of the precipitated particles. The setup shown in this work is useful for high spatiotemporal study of precipitation dynamics for various applications including asphaltene precipitation.

Fig. 4 Change in toluene concentration with time at 80 µm, 100 µm, 120 µm, and 140 µm of distance (l) to the side channel. b) Change in n-pentane concentration with time at 80 µm, 100 µm, 120 µm, and 140 µm of distance to the side channel

\[ c = c_{\text{pen}} \text{erf} \left( \frac{x}{2\sqrt{Dt}} \right) \] (5)

For a given time, n-pentane concentration distribution along x-direction follows error function. The error function distribution is proved in Fig. S2.

Assuming the ratio of the fluorescent dye Nile Red and toluene remains constant, from the intensity with time, we can obtain \( r_{\text{solute/toluene}} \) in the diffusive mixing process. As shown in Fig. 4a and b, the intensity of fluorescence is quantified to obtain the change in the chemical composition in space and in time. Toluene concentration is estimated by \( c \sim I \). Here, \( c \) is toluene concentration, and \( I \) is normalized fluorescence intensity. The diffusion of the probe in different solvents is assumed to be the same, due to similar viscosity. Both n-pentane and toluene are good solvents of Nile red, our probe. At trace concentration (i.e., < 1 µM), the solubility of the dye in both solvents is likely to be negligible for the diffusivity. Toluene concentration decreases with diffusion time, while n-pentane concentration, reverse of the toluene concentration, increases with time.
Fig. 5 Total internal reflection (TIRF) microscopy images of asphaltene precipitation driven by diffusive mixing between an asphaltene solution in toluene as solution A and n-pentane as solution B. More precipitates observed at $l$ of 80 $\mu$m are attributed to the difference in mixing condition near the side channel ($l < 80 \mu m$) and in the inner chamber ($l > 80 \mu m$).

Fig. 6 In situ crystallization of $\beta$-alanine using the diffusive mixing device. With time, crystals—indicated in green line—grow in the direction of diffusive mixing. The red lines indicate the boundary of stationary crystals as reference.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1140/epje/s10189-021-00066-1.

Acknowledgements This work is supported by the Institute for Oil Sands Innovation (IOSI) (project number IOSI 2018-03) and from the Natural Science and Engineering Research Council of Canada (NSERC)—Collaborative Research and Development Grants. The authors are grateful for technical support from IOSI lab, particularly from Lisa Brandt and Brittany MacKinnon. We are also grateful for the technical support of Dr. Xuejun Sun at the Cell Imaging Facility at the Cross-Cancer Institute and Dr. Murray R. Gray in Alberta Innovates for fruitful discussion.

Author contribution statement All the authors were involved in the preparation of the manuscript. All the authors have read and approved the final manuscript.

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