SHRUNK TO NANO: A NOVEL APPROACH FOR FEMTOLITER COMPARTMENTALIZATION USING W/O EMULSION

Tianzhun Wu1*, Hiroaki Suzuki1,2 and Tetsuya Yomo1,2,3
1 ERATO, JST, JAPAN
2 Graduate School of Information Science and Technology, Osaka University, Japan
3 Graduate School of Frontier Biosciences, Osaka University, Japan

ABSTRACT

A novel method is proposed to generate monodisperse femtoliter compartmentalization using water-in-oil (W/O) emulsion by controlled water diffusion. The shrinkage dynamics is modeled by blocked diffusion originated from the accumulation of solute or surfactant molecules at the W/O interface. W/O droplets generated by vortex and microchannels are used with addition of solutes and surfactants to control the shrinkage rate and droplet sizes. It is experimentally verified that shrinkage can be slowed and stopped by increasing solute or surfactant concentration, and sub-femtoliter W/O emulsions have been achieved. Typical linear and nonlinear shrinkage curves are well fitted by the proposed model.

KEYWORDS: Femtoliter compartmentalization, Water-in-oil emulsion, Diffusion, Modeling

INTRODUCTION

Water-in-oil (W/O) emulsion attracts much research interests for various physical, chemical, biological and medical applications [1], and is often used as the simple but effective compartmentalization for artificial cell-sized microreactors [2]. Typical volumes of bacterial cells are only several femtoliters (fLs), and their sizes are around 1 μm or less. However, it is difficult to produce batch and monodisperse W/O emulsion down to fL volume or less by widely used microchannel technologies like flow-focusing or T-junction, since their emulsion sizes are typically larger than 10 μm. Two technologies are reported to produce monodisperse nanosize W/O emulsion by using nanochannel [3,4] or electrospray [5], both of which need dedicate fabrication and high pressure or voltage to breakup droplets of nanosize directly from liquid mass, due to the extreme high surface/volume ratio of nano emulsion.

Instead of atomizing droplets from liquid bulk with high energy, we take a novel approach to shrink droplets by diffusing water into oil in easy-control manners, which can be simple, effective and high throughput. It has been known that aqueous droplets shrink due to the slight solubility of water in some kinds of oil, whereas ions, solutes or biomolecules can remain in the water phase. [6] However, this phenomenon is often used for concentrating solute [6] or assembling nanoparticles [7,8] without attempting to predict and control final droplet sizes. Here we describe the method to shrink W/O droplets into fL compartments, model the droplet shrinkage dynamics, and control the shrinkage rate and the size for experimental verification.

METHOD AND THEORY

It is known that deionized (DI) water droplets will diffuse into some kinds oil and quickly disappear, [6,8] nevertheless their shrinkage rate and final size can be tuned by adding sufficient solute (Fig. 1a) or surfactant (Fig. 1b). It is assumed that solute or surfactant molecules accumulating along the W/O interface hinder the water diffusion by reducing the diffusion area, and finally totally block the diffusion process as the droplet shrinks to a threshold size.

To quantitatively indentify the influence of solute or surfactant accumulation, a new parameter \( f_S \in [0,1] \) is introduced as the block fraction, defined as the ratio of occupied area of solute or surfactant along W/O interface to its apparent area \( S \). Assume the droplet is a free-floating sphere and the solute is uniformly distributed inside the droplet with volume density \( \rho = 6N/\pi d^3 \), where \( N \) is the number of solute molecules and \( d \) is the droplet diameter. Then the occupied area of solute is expressed as \( S_f = \rho \pi d^2 \phi^* \pi (\phi/2)^2 = 3\pi N\phi/2d \), where \( \phi \) represents the diameter of a single solute molecule. Hence the formulation of the block fraction for solute is \( f_S(d) = S_f/\pi d^2 = 3N(\phi/d)^2/2 \). Similarly, the block fraction for surfactant is \( f_S(d) = N(\phi/d)^2/4 \), where \( \phi \) here represents the diameter of the single surfactant molecule at the interface.

Suppose the water concentration is \( c \) and the initial value inside and outside the droplet is \( c_0 \). By assuming the water diffusion into bulky oil is a steady process obeying the Fick’s first law, we obtain

\[
J = -D \frac{\partial c}{\partial r} = \frac{\partial m}{\partial t} = \rho_c JS \left[ 1 - f(d) \right] = -\rho_c D \pi d^2 \left[ 1 - f(d) \right] \frac{c_0 - c}{r^{d/2}},
\]

Figure 1. Nano W/O droplet generation method by controlled shrinkage. Solute (a) and surfactant (b) molecules in the diffusion block layer reduce the diffusion flux area when concentrated, and finally totally stop the diffusion process.
where \( D \) and \( J \) are the water diffusion constant and mass flux respectively on W/O interface, \( \rho_w \) is the water volume density, and \( r \) is the arbitrary radius starting from the droplet center. By substituting \( \partial m / \partial t = \rho_w \pi d^2 / 2 \partial d / \partial t \) into Eq. 1, we achieve the following equation for droplet surface shrinkage rate

\[
\frac{dS}{dt} = 2\pi D(c_s - c_0) \left[ 1 - f \left( \frac{d}{d} \right) \right] \left[ 1 - \frac{b}{d^2} \right], \quad n=2,3.
\] (2)

In Eq. 2, \( a, b \) are fitting parameters representing \( a \approx dS/dt \bigg|_{d=0} \) and \( b \approx d^2 \bigg|_{d=\infty} \), where \( n=2 \) for solute and \( n=3 \) for surfactant. Eq. 2 predicts the surface shrinkage is linear for water with dilute solute or surfactant on W/O interface, and it becomes nonlinear when \( d \) further reduces, because \( f \) abruptly increases and approaches towards 1. Hence \( D, c_0, c_s, \) and \( N \) (corresponding to solute or surfactant concentration) can be used for shrinkage control, and hereafter we demonstrate the controlled shrinkage of aqueous droplets in various oils to examine the method and theory above.

**EXPERIMENTS AND SIMULATION**

Micro droplets with sizes range from 5~50 \( \mu m \) were generated by using vortex then observed under an inverted microscope (Olympus IX 71) (Fig. 2a). Monodisperse W/O emulsions were generated by flow-focusing devices fabricated with the standard soft lithography (Fig. 2b) to study effects of solute/surfactant concentration with the same initial droplet size. Hydrodynamic trap array [9] with 20 \( \mu m \)-width and 25 \( \mu m \)-height was designed to trap some droplets for shrinkage observation, while other droplets enter the output well and suspend in the oil reservoir.

**References**

[9] Figure 2. Experimental setup for the droplet generation and observation (not to scale). (a) W/O emulsions prepared by vortex. (b) Monodisperse droplets generated by flow focusing microchannels are observed in traps or the output well.

**Figure 3.** Snapshots of the droplet shrinkage in soybean oil. (a) DI water (b) Water containing 7 \( \mu M \) fluorescence-tagged dextran (MW=40,000).

**Figure 4.** Droplet size history for different oils, surfactant and buffer solution. Diffusion is found affected by oil types and solute/surfactant existence. HEPES KOH buffer PH=7.6.

**Figure 5.** DI water droplets shrinkage in soybean oil on a microfluidic chip. Snapshots (1)–(3) show droplets with initial diameter 26 \( \mu m \), 30 \( \mu m \) and 20.9 \( \mu m \) respectively.
Figure 3a shows snapshots of a DI water droplet which shrinks quickly in soybean oil and finally disappears. When solute like fluorescence-tagged dextran is included in water (Fig. 3b), fluorescence intensity is found to increase while concentrating, indicating that dextran does not diffuse into oil. As shown in Fig. 4, it is confirmed that diffusion process can be controlled by the oil type (with different D and c0), and/or slowed down by adding solute or oil-soluble surfactant. Droplets with the diameter ~1 μm or volume ~0.5 fL can be readily achieved.

Diffusion block due to reduced diffusion area is also confirmed in Fig. 5 where droplets significantly larger than their trap size have less W/O diffusion area or higher f, leading to slower surface shrinkage ratio (defined as S/S0, where S0 is the initial surface area) than free-floating droplets in channels or the well. However, if the droplet diameter (20.9 μm) is slightly larger than trap size, f is small enough and diffusion rate is almost not affected.

Figure 6 shows the influence of concentration of dextran and span 80 on shrinkage in soybean oil. With the same initial droplet size, shrinkage is slower for higher surfactant/solute concentration. Initially linear shrinkage is observed, but it sharply stops for higher concentration when S/S0<10%. Four typical shrinkage curves (i.e., pure water in soybean oil, water with solute, buffer solution and oil with surfactant) are fitted with Eq. 2 with proper a and b. As shown in Fig. 7, all linear and nonlinear curves can be well fitted, showing the present model can be used to explain and predict shrinkage in various conditions. In a well controlled environment for diffusion, it is believed that the same shrinkage can be well repeated and predicted with the two fitting parameters.

To generate monodisperse sub-micrometer W/O droplets in microfluidics, we have designed static droplet array based on microchannels and successfully achieved uniform W/O array. Further progress will be reported elsewhere.

CONCLUSION

We presented a novel, simple but effective method for femtoliter compartmentalization by shrinking W/O emulsion towards nano scale. We proposed the diffusion-block model, in which the water diffusion is limited by the accumulation of solute or surfactant molecules, experimentally demonstrated the controlled W/O emulsion shrinkage to femtoliter by vortex and microfluidics, and showed that this model was useful for prediction. With the present method W/O emulsion with nanosize should be readily achieved by adjusting the concentration of solute or surfactant.

REFERENCES


CONTACT
*Tianzhun Wu, tianzhun-wu@bio.eng.osaka-u.ac.jp