Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supplementary Information

**Formation of water-in-water-in-oil double emulsion by triphasic co-flow step emulsification**

**Methods**

Hydrophobic treatment of PDMS microchannels: Aquapel (PPG, USA) is injected into the channel to render the PDMS channel hydrophobic, and later expelled by using the compressed air. Since the channel has a double-layer structure, if the entire device is subjected to the hydrophobic treatment, Aquapel gets trapped in the corners at the step and is hard to drive it out by blowing the air. Therefore, only the Hele-Shaw channel is subjected to the hydrophobic treatment. Air is injected into the channel from the inlet of the outer dispersed phase by a pressure pump (Flow EZ Fluigent), the air pressure is adjusted to avoid Aquapel entering the upstream channel. Aquapel is injected into the channel from the inlet of the continuous phase for 30 seconds, before increasing the air pressure to remove the Aquapel. Finally, we apply heat to the microchannel at 90℃ for 1 hour.

Preparation and characterization of the solutions: Polyethylene glycol (PEG, $M_w$=8,000 g mol$^{-1}$, Sigma-Aldrich, USA) and Dextran (DEX, $M_w =$ 15,000 g mol$^{-1}$, D&B Biological Science and Technology Co. Ltd, China) polymers are dissolved in deionized water, both at 10% (w/w). We centrifuge the solution at 300 rpm for 2 hours and let it settle overnight for phase separation. We then collect PEG-rich and DEX-rich aqueous phases to be further used as disperse phases of the w/w/o double emulsion. A fluorinated oil (HFE7500, 3M, USA) is mixed with a surfactant Krytox 157 FS(L) (Dupont, USA) at concentration of 1% (v/v). The PEG-rich solution, the DEX-rich solution and the HFE7500 oil have viscosity at 8.3 mPa s, 20.7 mPa s, and 1.4 mPa s, respectively, measured by a rheometer (Anton-Paar MCR 302, rotor CP50-1, with 50 mm diameter and cone angle of 1°). The surface tension between HFE7500 oil and PEG-rich solution is $\gamma_{23} = 6.2$ mN m$^{-1}$, and between PEG-rich solution and DEX-rich solution $\gamma_{12}$~0.1 mN m$^{-1}$. [1]

**Water-in-water-in-oil double emulsion generation**

The HFE7500 oil with surfactant is injected into the channel as a continuous phase. DEX-rich solution and PEG-rich solution are used as inner (core) and outer (shell) disperse phase, respectively. W/w/o double emulsion droplets are formed at the step, as shown by Fig. S1-a in the synchronous step-emulsification regime. Experiments with different flow rates of the inner disperse phase $q_1$ at a fixed $q_2$ and $q_3$ are performed.

Scaled droplet outer diameter $D/b$ is plotted as function of $k_1$ at fixed $k_2$ in Fig. S1-b, a good agreement is found with the theoretical prediction in Eqn (12) up to a multiplicative factor 1.92. The prediction for scaled diameter of inner droplet, $d/b = uD/b$, is shown to agree closely with the experimental measurements in Fig. S1-b.

The shell thickness is plotted in Fig. S1-c. As described by Eqn (11), the shell thickness is controlled by the flow rate ratio of two disperse phases. In these set of experiments the scaled shell thickness

is not small, therefore that the full expression, $\frac{e}{D} = \frac{1}{2}\left[1 - \left(1 - \frac{q_2}{q_1+q_2}\right)^{1/3}\right]$, obtained from Eqn (11) is used for comparison. As in Fig. S1-c, the agreement is found between the experimental results (symbols) and this theoretical prediction (solid curve) for almost the whole range of flow rate ratios. Notice a discrepancy in Fig. S1-c between the analytical prediction for and the experimental results for the shell thickness: the theory (which relies solely on mass conservation) somewhat overestimates the shell thickness and it is particularly visible at low values of $q_2/(q_1 + q_2)$. This discrepancy may be due to incomplete phase separation between the PEG-rich and DEX-rich solutions, as tiny droplets of DEX can be observed at large enough magnification in PEG-rich solution. As the two aqueous solutions co-flow in the Hele Shaw channel, tiny droplets of DEX merge within the stream of the DEX-rich solution, leading to an effective reduction in flow rate of the outer disperse phase, $q_2$, and resulting in a somewhat lower value of $e/D$.

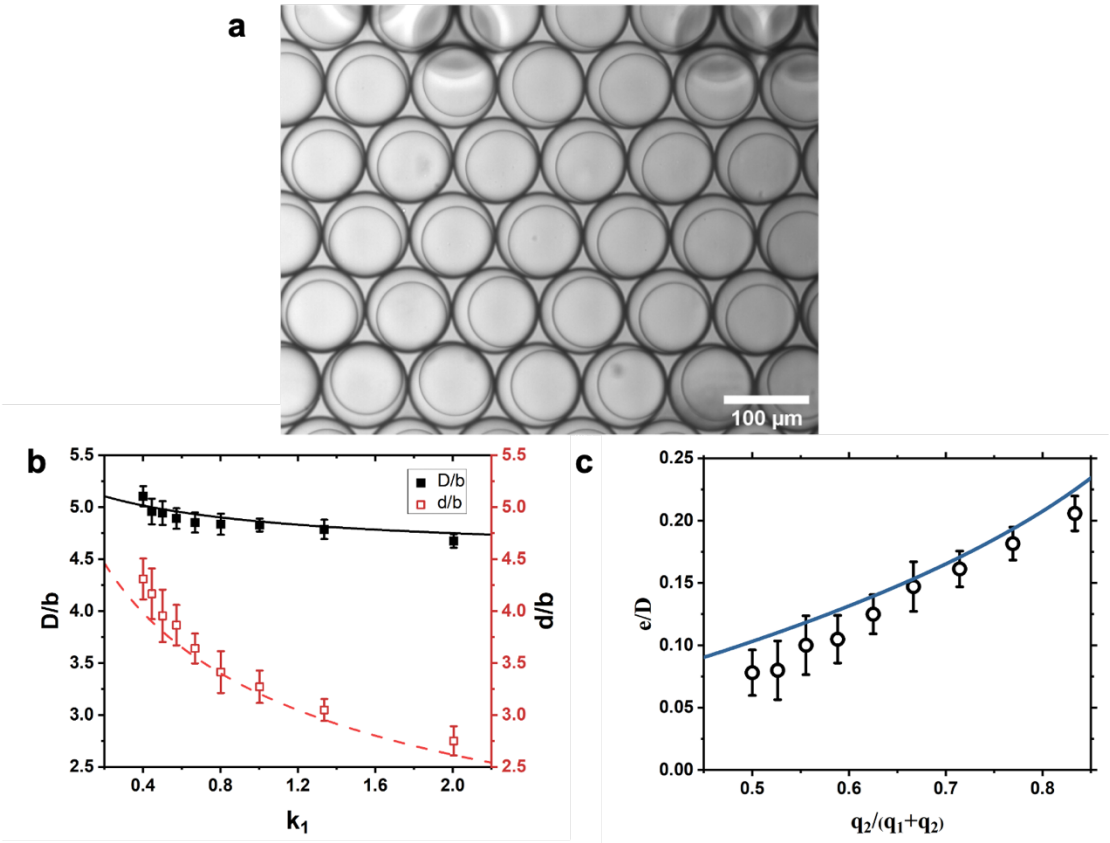


**Figure S1:** W/w/o double emulsion droplets formed in triphasic co-flow step emulsification device with aspect ratio $\beta = 20$ of the inlet channel. a) Double emulsion droplets formed at the flow rates: $q_1 = 4$ μl h$^{-1}$, $q_2 = 5$ μl h$^{-1}$ and $q_3 = 50$ μl h$^{-1}$; b) Scaled outer, $D/b$, and inner, $d/b$, droplet diameters vs. $k_1$ at fixed $k_2 = 1.69$, and fixed $q_3 = 50$ μl h$^{-1}$. Black solid line and red dashed line are the theoretical predictions, for $D/b$ and $d/b$, respectively; c) Dimensionless shell thickness, $e/D$, vs. the flow rate ratio $q_2/(q_1 + q_2)$; blue solid line is the analytical prediction.

Reference:

1 L. Nan, Y. Cao, Y. Shuai, H.C. Shum, *Microsyst. Nanoeng.* 2020, **6**, 70.