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

Supplementary Information

In-Line Measurement of Liquid-Liquid Phase Separation Boundaries using a Turbidity-Sensor-Integrated Continuous-Flow Microfluidic Device

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S1 Design and Dimensions of the Turbidity-Sensor-Integrated Continuous-Flow Microfluidic Device with and without Heating Jacket

The dimension of the turbidity-sensor-integrated continuous-flow microfluidic device with and without the heating jacket is shown in Figures S1a and S1B.

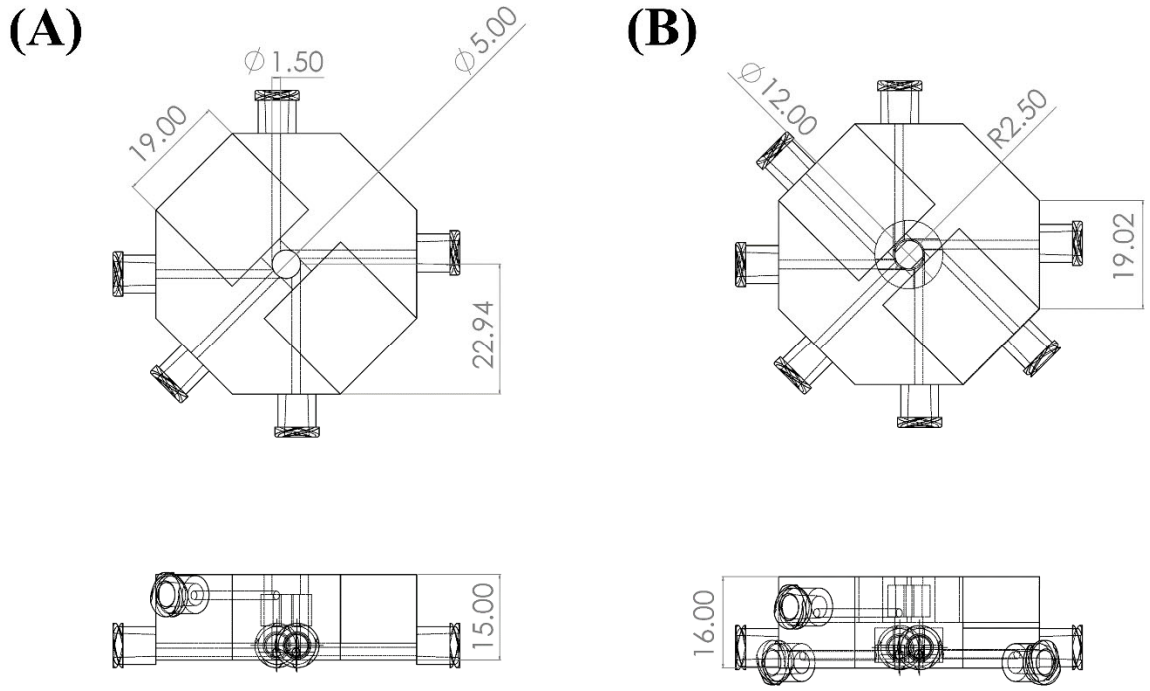

Figure S1: Top view and side view of the turbidity-sensor-integrated continuous-flow microfluidic device **(A)** without the heating jacket and **(B)** with the heating jacket.

S2 Calculation of the Mass Fractions of the Ternary Phase Diagram

Table S1 shows a sample of calculations for converting the flow rates of each stream into the mass fraction of each component.

Table S1: Sample calculation for the mass fraction of each component

Quantity	Description	Value	Unit
Q_1	IPA: Water (90:10)	0.11	$mL.min^{-1}$
Q_2	β-alanine in Water	0.87	$mL.min^{-1}$
Q_3	IPA: Water (10:90)	0.02	$mL.min^{-1}$
$Q_1+Q_2+Q_3$	Summation of flowrates	1	$mL.min^{-1}$
$C_{\beta\text{-alanine}}$	Concentration of the β-alanine	0.5	$g.mL^{-1}$
$\dot{m}_{water}$	Mass of water	0.596286	$g.min^{-1}$
$\dot{m}_{\beta-\text{alanine}}$	Mass of β-alanine	0.435	$g.min^{-1}$
$\dot{m}_{IPA}$	Mass of IPA	0.079386	$g.min^{-1}$
$\dot{m}_{Total}$	Total Mass	1.110672	$g.min^{-1}$
$\boldsymbol{X_{Water}}$	Mass fraction of water	0.536869	-
$\boldsymbol{X_{\beta-Alanine}}$	Mass fraction of β-alanine	0.391654	-
$\boldsymbol{X_{IPA}}$	Mass fraction of IPA	0.071475	-

Table S2 includes the details of the conditions for which oiling out was observed using in-situ optical microscopy. In this table, the volumetric flowrate of each stream is added to obtain the mass flowrate of each species. The mass fractions of each species is obtained from fractional mass flowrates, which are shown in Figure 4. The total volumetric flowrate of the three streams is always maintained at 1 mL/min, and the flowrate of Q_1 streams with 90% of IPA, which is the antisolvent, is increased and the flow rate of the Q_2 stream is decreased to reach a point where the oil droplets are formed. The flowrate of Q_3 is adjusted to a value to maintain the sum of flowrates to 1 ml/min.

Table S2: Details for measurement of the LLPS boundaries using optical microscopy

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8
Q_1(mL.min-1)[IPA: Water (90:10)]	0.11	0.21	0.31	0.41	0.51	0.61	0.71	0.81
Q_2 (mL.min-1) [β-alanine in Water]	0.87	0.55	0.32	0.25	0.19	0.12	0.06	0.04
Q_3 (mL.min-1) [IPA: Water (10:90)]	0.02	0.24	0.37	0.34	0.3	0.27	0.23	0.15
$Q_1+Q_2+Q_3$ (mL.min-1)	1	1	1	1	1	1	1	1
$C_{\beta\text{-alanine}}$ (g.mL-1)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mass of water (g)	0.60	0.60	0.59	0.51	0.42	0.38	0.33	0.22
Mass of beta alanine (g)	0.44	0.28	0.17	0.13	0.08	0.06	0.035	0.02
Mass of IPA (g)	0.08	0.17	0.26	0.32	0.38	0.45	0.5	0.6
Mass fraction of beta alanine	0.54	0.57	0.58	0.54	0.47	0.43	0.38	0.27
Mass fraction of IPA	0.39	0.26	0.17	0.13	0.09	0.07	0.04	0.023
Mass fraction of water	0.07	0.16	0.25	0.33	0.44	0.5	0.58	0.71

S3 Differences Between the Optical Images of the Oil Droplets and Gas Bubbles in the Microfluidic Mixer

To elucidate the differences between the gas bubbles that can potentially form during in-situ mixing and the oil droplets from LLPS, we have performed two control experiments. In the first experiment, streams of IPA and water (without β alanine) were mixed together and the gas bubbles formed were identified with thicker boundaries and bright center (see Figure S2A). The second control experiment involved premix solution of IPA and water was fed along with β alanine solution into the micromixer to induce LLPS. Figure S2B shows the oil droplets of β alanine with characteristic thin boundary and transparent center.

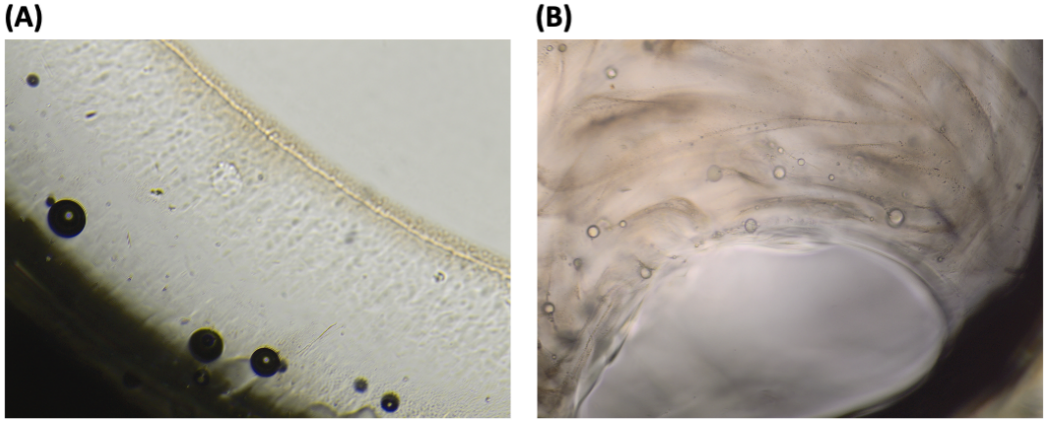

Figure S2: (A) Formation of gas bubbles in the microfluidic mixer when IPA and water are mixed in-situ; (B) Formation of oil droplets in the microfluidic mixer during mixing of β alanine in water with water-IPA mixture.

S4 Size Measurement of the Oil Droplets formed in the Microfluidic Mixer during the Detection of the LLPS Boundary

As shown in Figure S2, the formation of oil droplets can be captured. In Figure S3, we have measured the sizes of several oil droplets using optical microscopy.

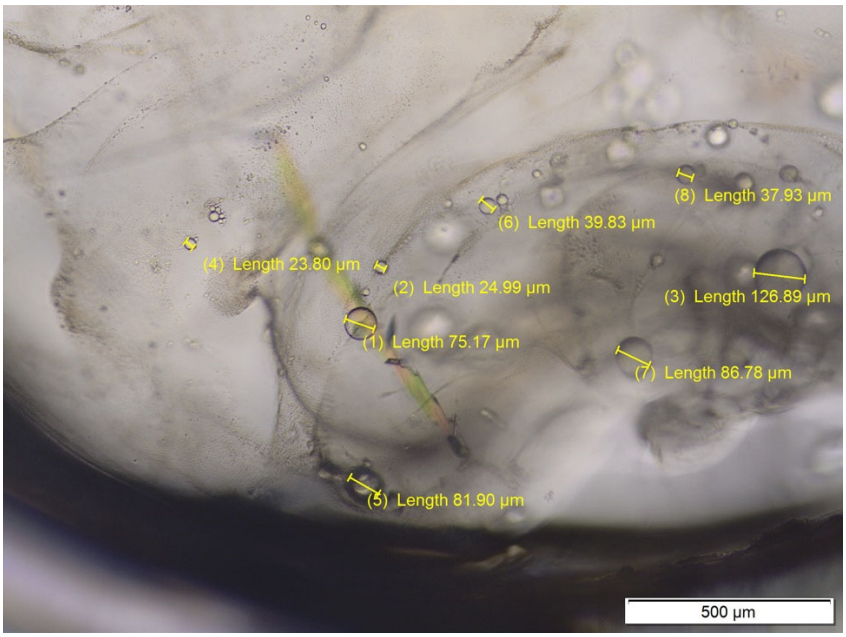

Figure S3: Measurement of the oil droplets in the microfluidic mixer using optical microscopy.

S5 Residence Time Distribution Calculation

In order to calculate the residence time distribution of the merged inlet microfluidic mixer, the concentration at the outlet is recorded from $t = 0$ to $t = 15$ minutes at each flowrate. The flow rate was varied from 0.1 to 1 with increments of 0.1($\frac{ml}{min}$). Using MATLAB, these data were processed to calculate the average residence time and standard deviation at each flowrate.

The outlet concentration at different time $\bar{c}(t)$ is first divided by the initial concentration, which is set as 1 ($\frac{ml}{min}$) :

$$F(t) = \frac{\bar{c}(t)}{c_0} \qquad \textbf{Eq(1)}$$

Here $c(0)$ is the initial concentration and $\bar{c}(t)$ is the average concentration at the outlet boundary. From here, the average residence time $\bar{t}$ and the standard deviation (σ) is calculated as:

$$\bar{t} = \int_0^\infty [1 - F(t)]dt \qquad \textbf{Eq(2)}$$

$$\sigma^2 = 2\int_0^\infty t[1 - F(t)]dt - \bar{t}^2 \qquad \textbf{Eq(3)}$$

The values of $\bar{t}$ and σ are calculated for each flowrate and the results are shown in Figure S4.

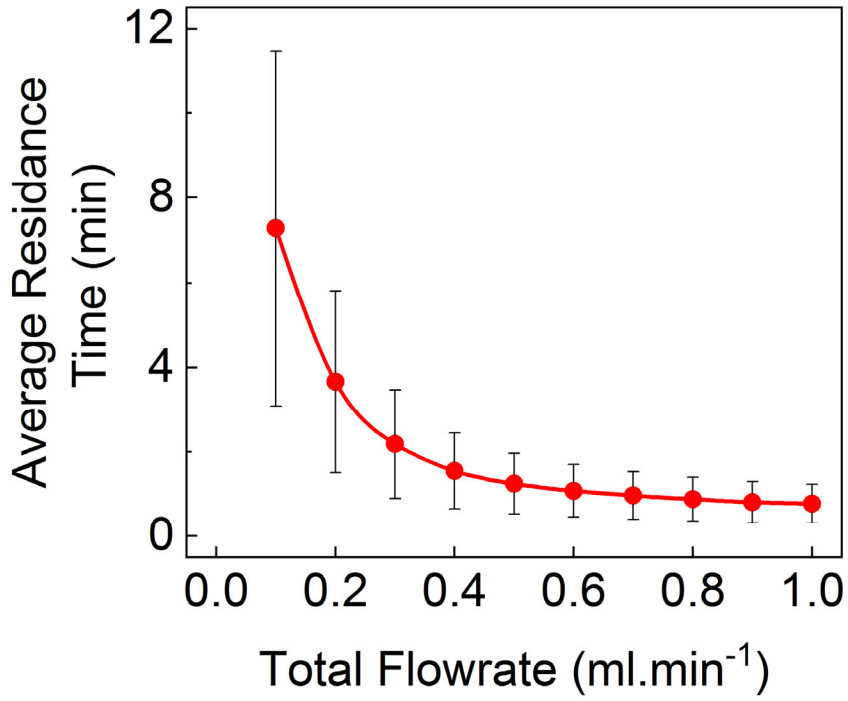

Figure S4: Residence time distribution in the microfluidic mixer device