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Direct Visualization of Ouzo Zone through Aggregation Induced Emission Dye for the Synthesis of Highly Monodispersed Polymeric Nanoparticles†

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Supplementary Information

1. Calculation of THF composition in mixture

The total amount of THF in mixture (y) changes linearly with the amount of THF in anti-solvent as per Equation S1.

$$\%\, y = \frac{THF\ composition\ in\ anti-solvent\ +\ \ inner\ to\ outer\ ratio}{1+\ inner\ to\ outer\ ratio} \times 100$$

(S1)

where the inner to outer ratio is taken as 0.1. Table S1 shows the volume % of total THF (y) in mixture with respect to change in % of THF in anti-solvent (x).

Table S1: Variation of total volume %THF in mixture (y) with respect to different process by varying %THF in anti-solvent (x).

Process	**%THF in Anti-Solvent (x)**	**% Total THF (y)**
SDM	0	9.09
ESDM_5%THF	5	13.63
ESDM_10%THF	10	18.18
ESDM_15%THF	15	22.72
ESDM_20%THF	20	27.27
ESDM_25%THF	25	31.81
ESDM_30%THF	30	36.36
ESDM_35%THF	35	40.90
ESDM_40%THF	40	45.45
ESDM_45%THF	45	50.00
ESDM_50%THF	50	54.54
ESDM_60%THF	60	63.63
ESDM_70%THF	70	72.72
ESDM_80%THF	80	81.81

2. Calculation of Reynolds number in the mixer

The total flow rate in the system was varied from 1.1 mL min^{-1} to 16.5 mL min^{-1}. The relation between flow velocity (u) and volumetric flow rate (Q) is defined by Equation S2.

$$u = \frac{4Q}{\pi D_i} \tag{S2}$$

where, D_i is the inner diameter of the pipe (1 mm)

Re in the system was varied on the basis of total flow rate using the following Equation S3.

$$Re = \frac{\rho u L}{\mu} \tag{S3}$$

where, μ represents the viscosity of fluid, ρ represents the density of the fluid and L is a characteristic linear dimension (m). For circular pipe, characteristic linear dimension is same as hydraulic diameter (inner diameter of pipe). Equation S4 represents the relation between Re and flow rate.

$$Re = \frac{4\rho Q}{\pi \mu} \tag{S4}$$

Following Table S2 shows the variation of Re with respect to flow rate

Table S2

Flow Rate (mL min^{-1})	1.1	2.2	3.3	5.5	7.7	11	16.5
Reynolds No	23.33	46.67	70	116.67	163.33	233.3	350

3. Effect of polymer to dye ratio (PDR) on distribution

We have investigated the effect of polymer to dye ratio on the intensity distribution of NPs by varying PDR from 0.25 to 2 and keeping total concentration the same (2 mg mL^{-1}) at Re 116 to optimize the dye to polymer loading. For all PDR except 0.5, multiple peaks in intensity distribution can be seen (Figure S1). At low PDR, there is not enough polymer to encapsulate all the dye molecules, which results in further aggregation and non-uniformity in the size of NPs. Whereas, at high PDR, there is formation of large aggregates due to increasing inter-particle collisions.

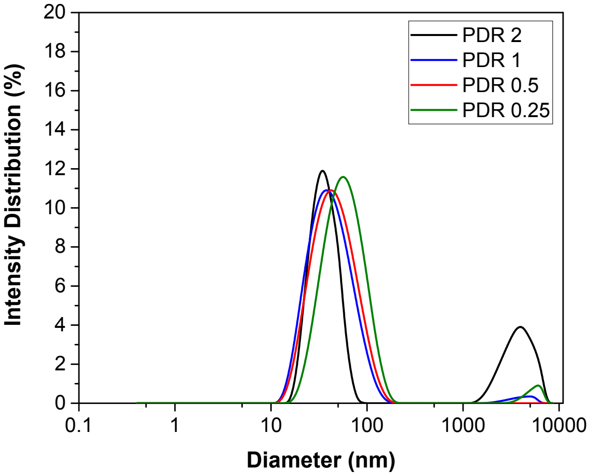


Figure S1. Effect of polymer to dye on intensity distribution (%) for optimization of dye loading

4. Calculation of solubility interaction parameter

The total solubility parameter of semi-solvent can be calculated with numeral fractional addition of particular component (Equation S5).

$$\delta_{ss} = \frac{f_1\delta_1 + f_2\delta_2 + f_3\delta_3 + ..}{f_1 + f_2 + f_3 + ..} \tag{S5}$$

where f_i is the fraction of solvent i present in the mixture and δ_i is the solubility parameter of that particular solvent. δ_{water} is 48.0 and δ_{THF} is 18.5 MPa$^{1/2}$.

The interaction parameter between solvent and antisolvent can be determined by using Equations 1 and Equation S5. Table S3 shows the solvent-antisolvent interaction parameter using different processes.

Table S3. Interaction parameter between solvent and anti-solvent for different processes

% THF in Water (x)	**δ_{as} (anti-solvent)**	**δ_s (solvent)**	**Δδ**	**Interaction Parameter ($\mathcal{X}$)**
0	48.0	18.5	29.5	28.02
5	46.5	18.5	28.0	25.28

10	45.1	18.5	26.6	22.69
15	43.6	18.5	25.1	20.24
20	42.1	18.5	23.6	17.93
25	40.6	18.5	22.1	15.76
30	39.2	18.5	20.7	13.73
35	37.7	18.5	19.2	11.84

5. Computational fluid dynamics (CFD)

To conduct CFD study, we used ANSYS FLUENT 18.2 software to solve laminar model due to low Re. Along with momentum equations models, we included the species transport model for diffusion and convection of a diluted species. Contours of mass fraction of THF with smooth shading and lines were captured to study the mixing process. For Re 350, contour with smooth shading and their corresponding lines were obtained for SDM process (Figure S2).

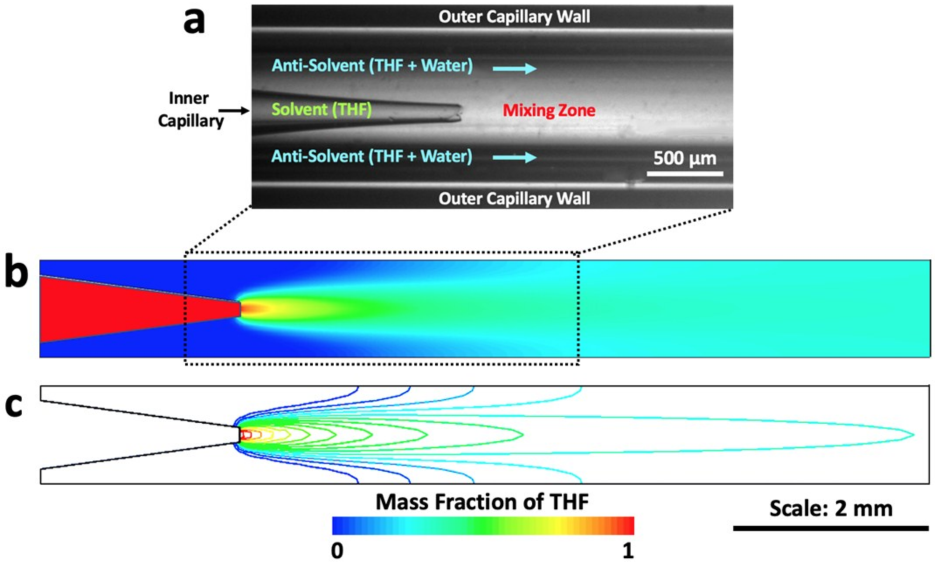
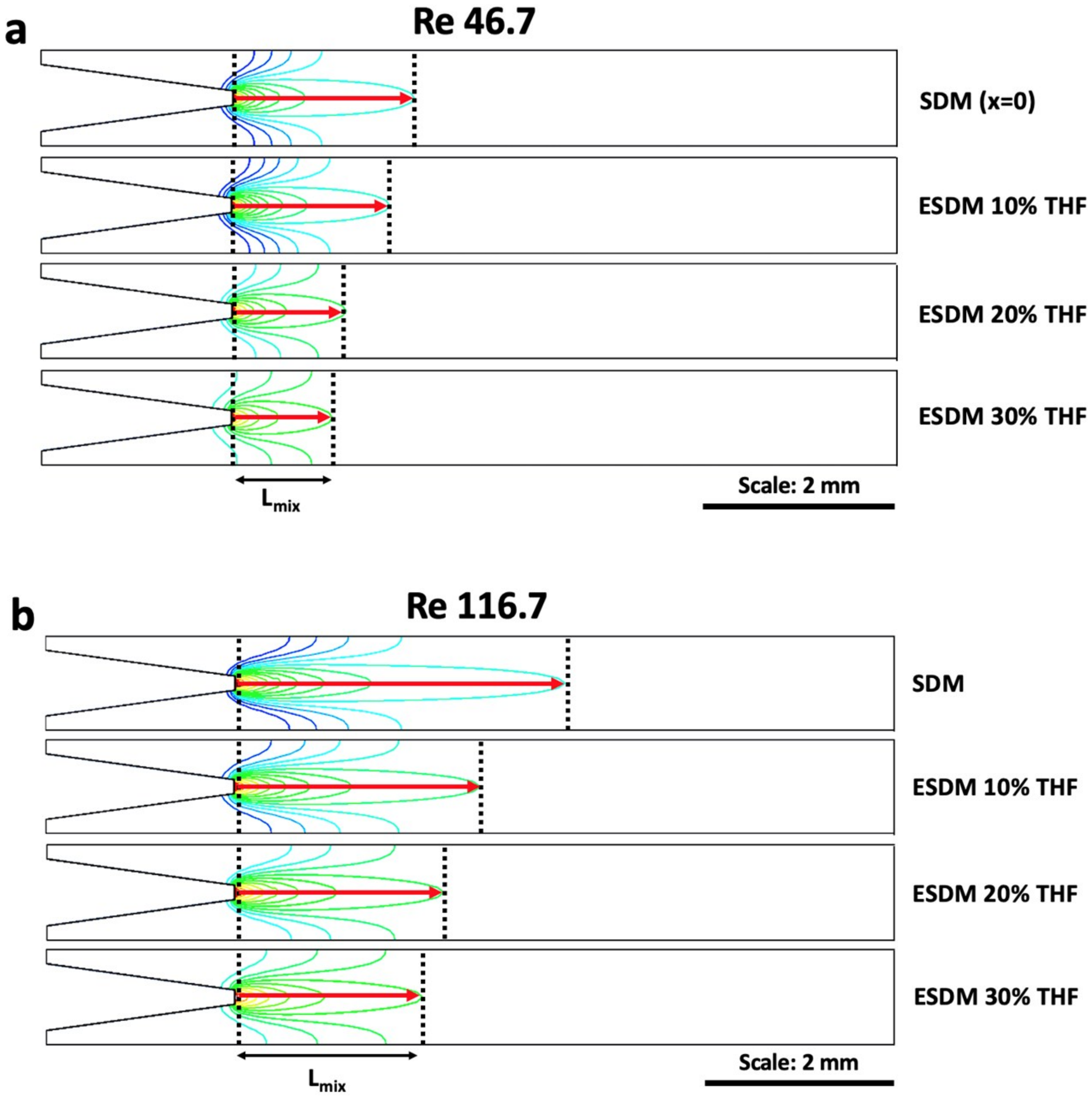


Figure S2. (a) Microscopy image of microfluidic glass capillaries with tip of inner capillary (solvent flow) inside outer capillary (anti-solvent flow). Contour of mass fraction of THF with (b) smooth shading and (c) contour lines after mixing between solvent (THF) and anti-solvent (water) in the process of SDM at Re 350.

6. Mixing length (contour lines)

The mixing of organic solvent THF into the water was studied by estimating mixing length (L_{mix}) for different process at different Re. L_{mix} is taken as the distance between mid-point of inner glass capillary hole and the point where mass fraction of THF achieve saturation into the mixture along the axial direction of pipe by using mass fraction of THF as contour line (no of contour lines: 20) and culling the back faces of image through ANSYS 18.2 software **(**Figure S3).



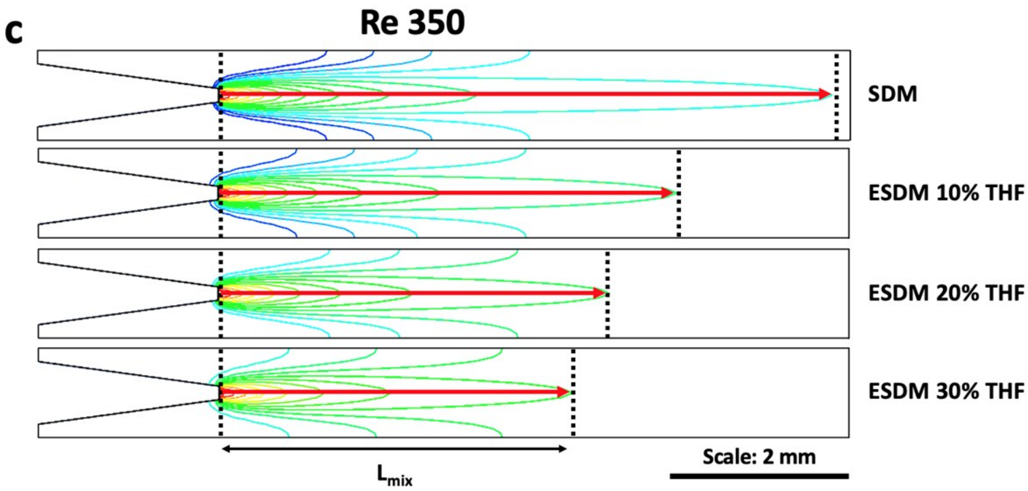


Figure S3. Contour lines of mass fraction of THF to study the effect of variation of %THF (x) in ant-solvent on L_{mix} at (a) Re 46.7, (b) Re 116.7 and (c) Re 350.

7. Mixing time (T_{mix})

With an assumption that fluid flows at an average velocity (u_{avg}) in system, T_{mix} is calculated by quantifying L_{mix} and u_{avg} in the pipe (Figure 2d and Equation S6).

$$T_{mix} = \frac{L_{mix}}{u_{avg}} \tag{S6}$$

8. Reproducibility of ESDM for synthesis of NPs

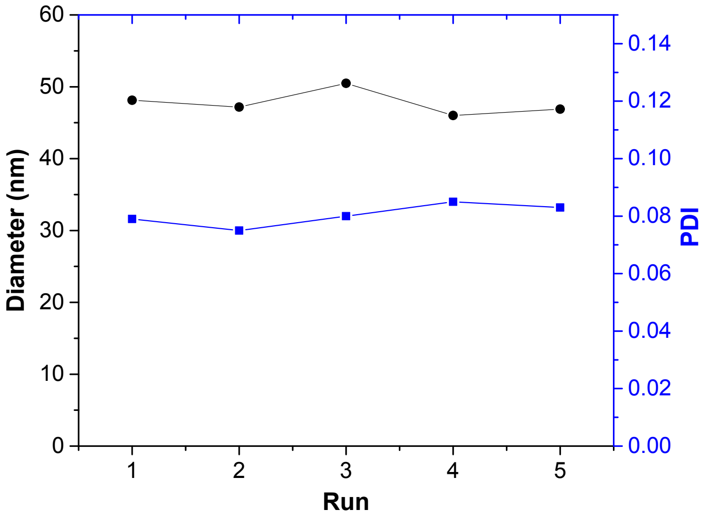


Figure S4. Number average diameter and PDI of BTPEBT loaded DSPE-mPEG$_{2000}$ NPs synthesized at 5 different runs through ESDM 20% THF using same parameters, such as Re 350, PDR 0.5 and WSR 10.

9. Calculation of mass fraction of solute and solvent in final mixture

The mass fraction of solute can be calculated from the concentration of solute taken in solvent for synthesis of NPs. Let's C (mg/mL) be the concentration of solute in organic solvent before synthesis. After mixing of anti-solvent with solvent in mixing zone, the mass fraction of solute (f_{solute}) can be calculated as per below equations.

The mass of solute (M_{solute}) in final mixture (before THF evaporation) per mL is

$$M_{solute} = \frac{(inner\ to\ outer\ ratio \times C \times 10^{-3})}{1 + \ inner\ to\ outer\ ratio}$$

(S7)

where the inner to outer ratio is taken as 0.1 and C is the concentration of solute in solvent (pump B) before mixing.

The mass of solvent (W_{THF}) in final mixture per mL is

$$M_{THF} = \frac{\left(inner\ to\ outer\ ratio \times D_{THF}\right) + \left(\left({}^{x}/_{100}\right) \times D_{THF}\right)}{1 +\ inner\ to\ outer\ ratio}$$

(S8)

where the D_{THF} is the density of THF (0.889 g/mL) at 25°C and x is the % volume of THF in an anti-solvent after pre-mixing step (Figure 1).

The mass of water (W_{THF}) in final mixture per mL is

$$M_{water} = \frac{\left(\left({}^{100-x}/_{100}\right) \times D_{water}\right)}{1 +\ inner\ to\ outer\ ratio} \tag{S9}$$

where the D_{water} is the density of water (0.998 g/mL) at 25°C and (100-X) is the % volume of water in an anti-solvent after pre-mixing step. For SDM x is 0 with anti-solvent as pure water.

The mass fraction of solute (f_{solute}) can be calculated as per below equation:

$$f_{solute} = \frac{M_{solute}}{M_{solute} +\ M_{THF} + M_{water}} \tag{S10}$$

As the mass of solute is very less as compared to solvent (THF) and water present in it. Therefore, the above equation can be approximated as

$$f_{solute} = \frac{M_{solute}}{M_{THF} + M_{water}} \tag{S11}$$

Similar to above, the mass fraction of THF (f_{THF}) can be calculated as per below equation:

$$f_{THF} = \frac{M_{THF}}{M_{solute} +\ M_{THF} + M_{water}} \tag{S12}$$

With approximation, the mass fraction of THF (f_{THF}) can be taken as:

$$f_{THF} = \frac{M_{THF}}{M_{THF} + M_{water}} \tag{S13}$$

To plot the ouzo zone, the different mass fraction of solvent (THF) through SDMx and different mass fraction of solute can be calculated as per above equations. For example, with concentration of 2mg/mL of solute in organic solvent, the mixture synthesized with different f_{solute} and f_{THF} is as per below Table S4.

Table S4. Variation in the mass fraction of THF in final mixture with addition of THF in anti-solvent during pre-mixing step.

Process	**%THF in an anti-solvent (x)**	**f_{THF}**
SDM	0	0.08
ESDM_5%THF	5	0.12
ESDM_10%THF	10	0.17
ESDM_15%THF	15	0.21
ESDM_20%THF	20	0.25
ESDM_25%THF	25	0.29
ESDM_30%THF	30	0.34
ESDM_35%THF	35	0.38
ESDM_40%THF	40	0.43
ESDM_45%THF	45	0.47
ESDM_50%THF	50	0.52
ESDM_60%THF	60	0.61
ESDM_70%THF	70	0.70
ESDM_80%THF	80	0.80

10. Construction of ouzo diagram for DSPE-mPEG$_{2000}$/THF/water system

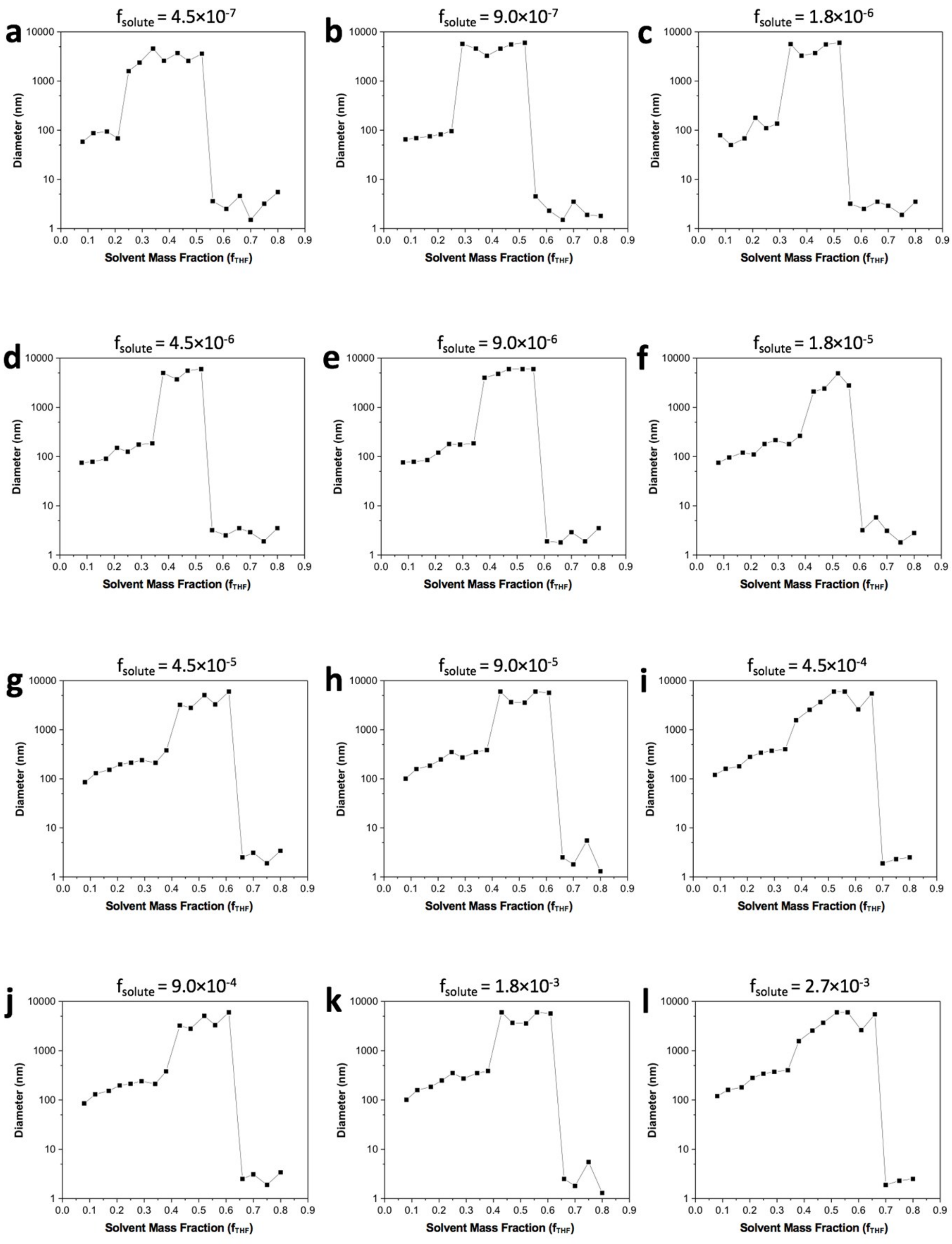
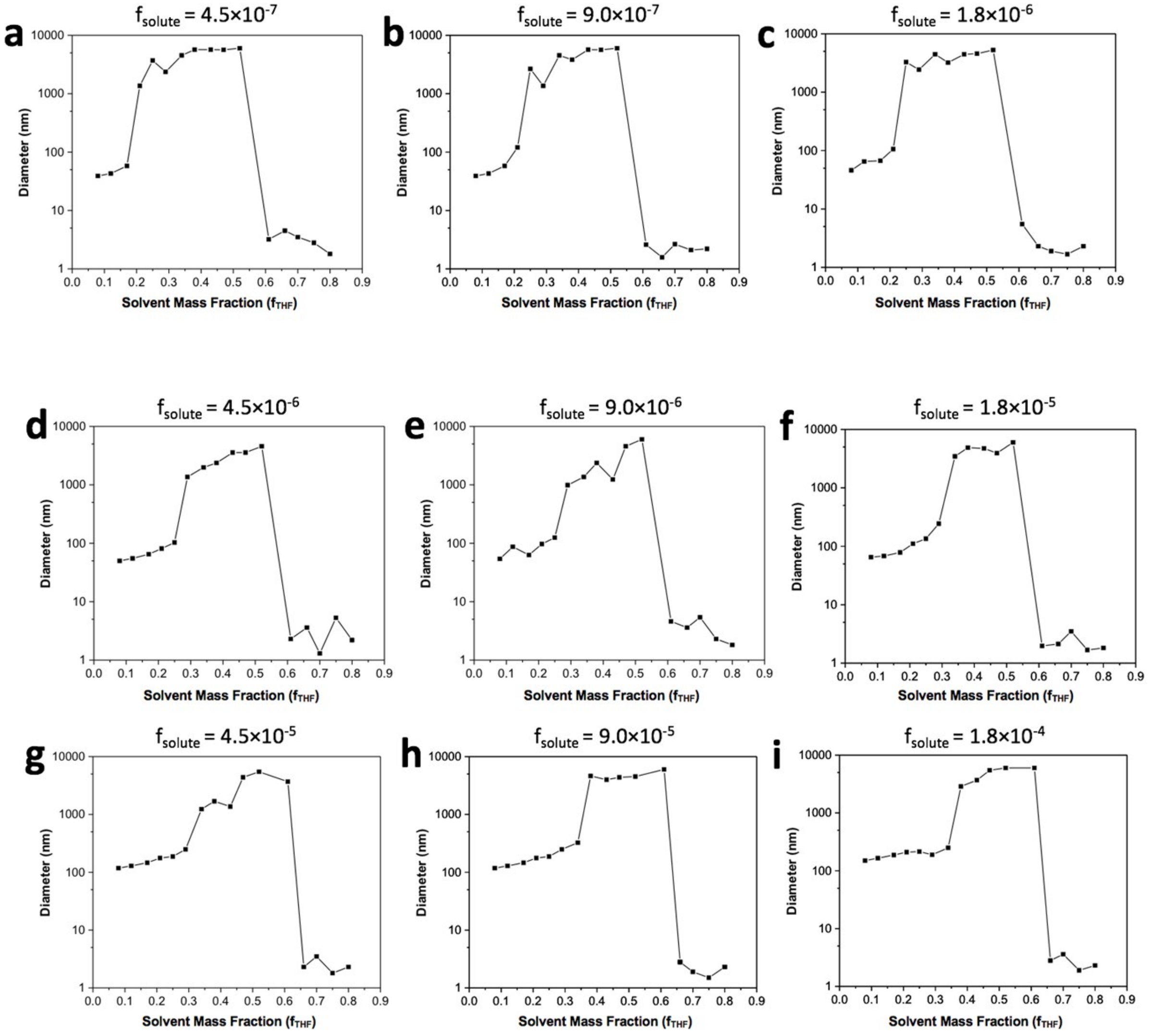

a
$f_{solute} = 4.5\times10^{-7}$
b
$f_{solute} = 9.0\times10^{-7}$
c
$f_{solute} = 1.8\times10^{-6}$
d
$f_{solute} = 4.5\times10^{-6}$
e
$f_{solute} = 9.0\times10^{-6}$
f
$f_{solute} = 1.8\times10^{-5}$
g
$f_{solute} = 4.5\times10^{-5}$
h
$f_{solute} = 9.0\times10^{-5}$
i
$f_{solute} = 4.5\times10^{-4}$
j
$f_{solute} = 9.0\times10^{-4}$
k
$f_{solute} = 1.8\times10^{-3}$
l
$f_{solute} = 2.7\times10^{-3}$
Diameter (nm)
1
10
100
1000
10000
Solvent Mass Fraction (f_{THF})
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9

Figure S5. (a-l) Change in the size of particles formed by empty DSPE-mPEG$_{2000}$ at different solute mass fraction (f_{solute}) by varying solvent mass fraction f_{THF} from 0.08 to 0.80 to determine ouzo zone, non-ouzo zone and binodal point.

11. Construction of ouzo diagram for BTPEBT+DSPE-mPEG$_{2000}$/THF/water system



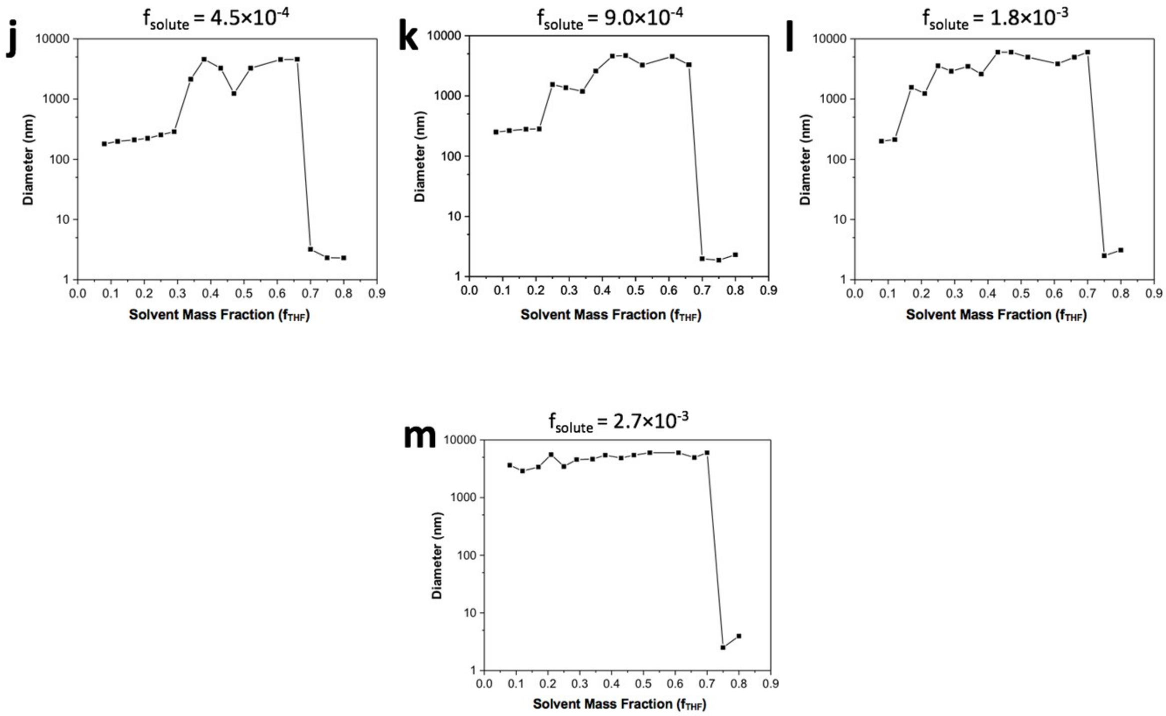


Figure S6. (a-m) Change in the size of particles formed by BTPEBT loaded DSPE-mPEG$_{2000}$ at different solute mass fraction (f_{solute}) by varying solvent mass fraction f_{THF} from 0.08 to 0.80 to determine ouzo zone, non-ouzo zone and binodal point.

12. Standard calibration curve of Tamoxifen

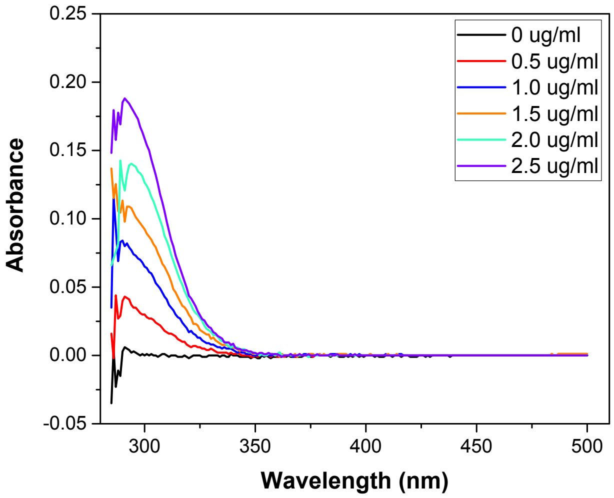


Figure S7. UV absorption of TAM in THF from 280 nm to 500 nm at different concentration.

TAM doesn’t show any UV absorbance peak in the range of 280 nm to 500 nm. Therefore, we have selected the absorbance values at 300 nm as the characteristic UV absorbance value for the construction of standard calibration curve (Figure S8).

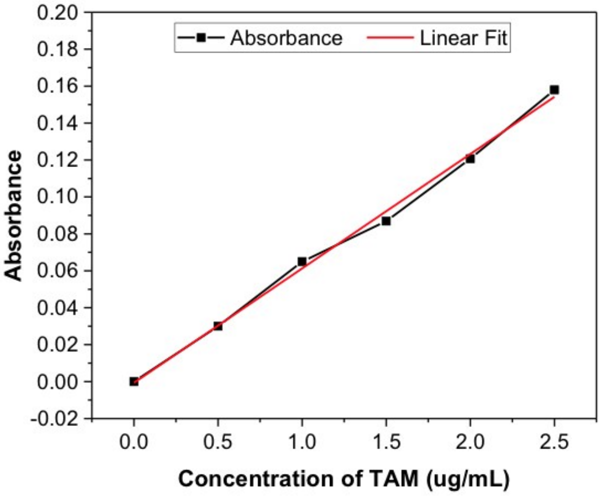


Figure S8. Standard calibration curve of UV absorption of Tamoxifen measured at 300 nm.

13. Encapsulation efficiency and drug loading capacity

Encapsulation efficiency (EE) and drug loading capacity (LC) is determined as per standard procedure.[1, 2] We have synthesized TAM loaded DSPE-mPEG NPs and TAM loaded PLGA NPs using ESDM. To determine EE and LC, we have filtered the solution from 0.44 μm size filter to remove any non-encapsulated TAM, while the TAM loaded NPs solution was collected. 10 μL of NPs solution is mixed into 990 μL of THF and stirred for 2 hours to completely dissolve encapsulated drug into the solution. And, the concentration of encapsulated TAM was determined by using standard TAM calibration curve (Figure).

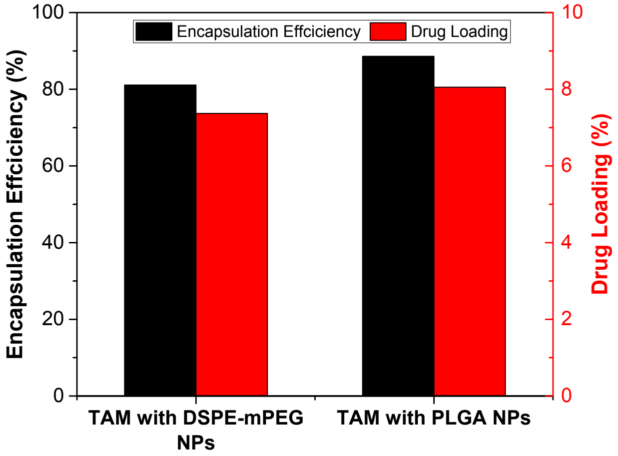


Figure S9. Encapsulation efficiency and drug loading capacity for TAM loaded PLGA NPs and TAM loaded DSPE-mPEG NPs synthesized at Re 350 using ESDM 10% THF in anti-solvent.

14. Determination of ouzo boundary through different approaches of size analysis

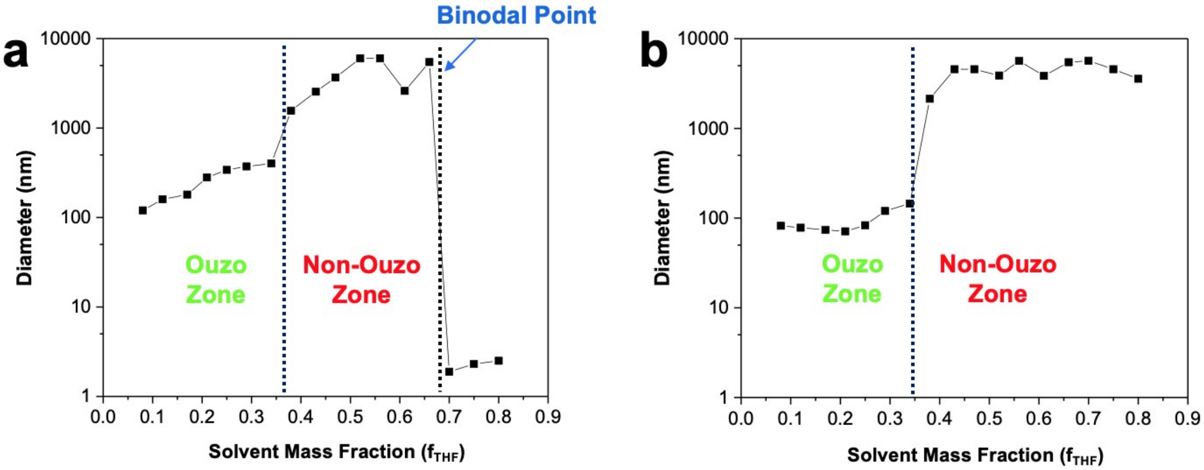


Figure S10. a) Change in the size of particles formed by blank DSPE-mPEG$_{2000}$ with concentration of 2 mg/mL in solvent at various f_{THF} to determine ouzo zone, non-ouzo zone and binodal point without evaporating THF from it. b) Change in the size of particles formed by blank DSPE-mPEG$_{2000}$ with concentration of 2 mg/mL in solvent at various f_{THF} with evaporating THF from it (Augry's method).

15. Synthesis of empty DSPE-mPEG particles

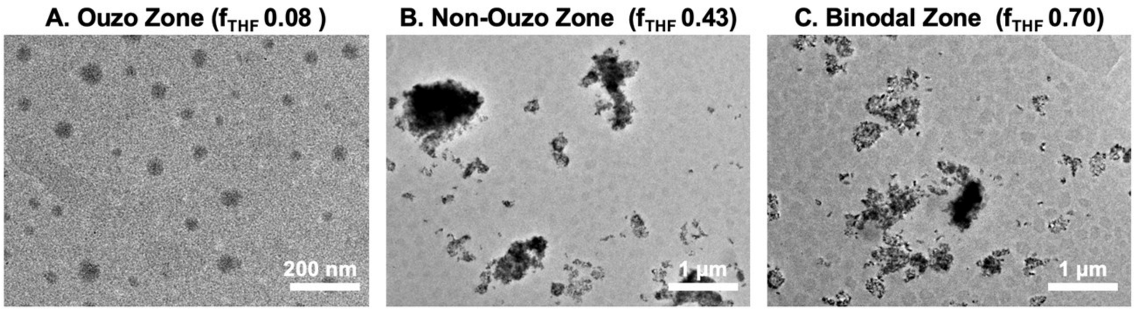


Figure S11. TEM images of DSPE-mPEG particles synthesized under ouzo, non-ouzo and binodal region at 1.8×10^{-4} mass fraction of solute.

16. Stability Analysis of BTPEBT loaded DSPE-mPEG NPs

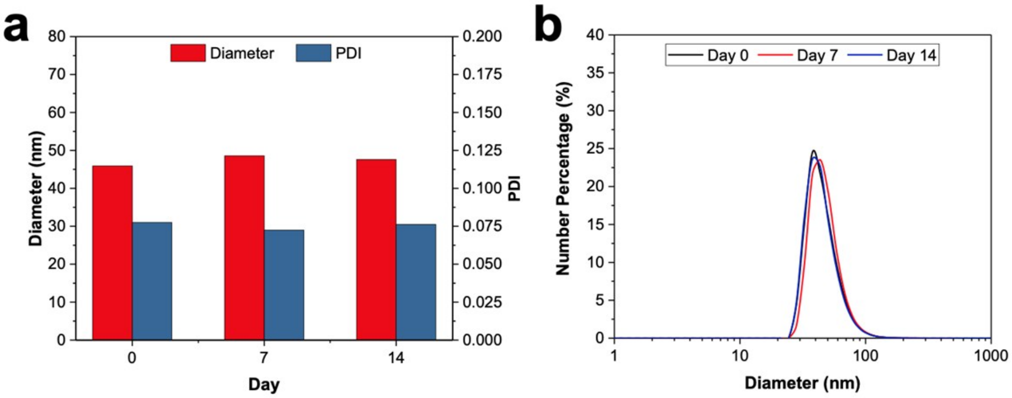
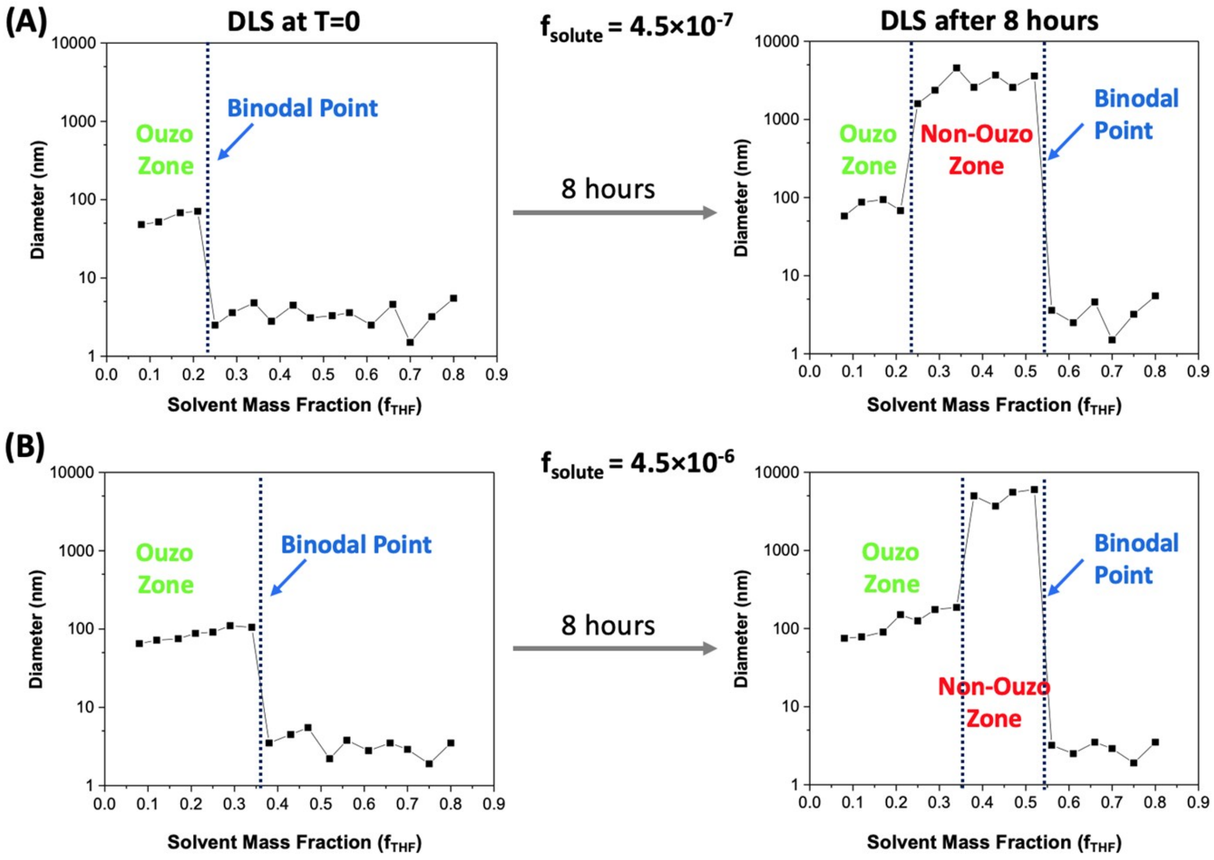


Figure S12. (a) Stability analysis with size and polydispersity (PDI) of BTPEBT loaded DSPE-mPEG NPs synthesized at Re 350 and PDR 0.5 through the process of ESDM 20% THF for 2 weeks. (b) Size distribution comparison of BTPEBT loaded DSPE-mPEG NPs analyzed for 2 weeks. No change in the size and PDI of NPs are noticed for 2 weeks which indicates the high stability of NPs.

17. Shift in Non-Ouzo zone and Binodal line with time

The construction of ouzo diagram with and without AIE in Figure 4-7 were done with analysis after 8 hours of mixing solute, solvent and water (Figure S13). With a solute fraction of 4.5×10^{-7}, the size of DSPE-mPEG is in the range of 45-93 nm under f$_{THF}$ 0.24 at t=0 hour (Figure S13a). Above f$_{THF}$ 0.24, there is a sudden decrease in the size of the particles to a few nanometers, which signifies that the mixture crosses the miscibility limit (binodal curve). However, after keeping the mixed sample for 8 hours, the size of DSPE-mPEG is in the range 50-95 nm with no shift in ouzo zone, but there is formation of large aggregates in the sample with f_{THF} 0.25 to 0.54 (Figure S13a). This is because the mixture entered into surfactant-free micro emulsion domain (SFME).[3] In this domain, the small aggregates sample of few nm size (looks transparent) were formed spontaneously, which increase in the size with time due to Ostwald ripening effect.[4] Above f_{THF} 0.54, the sample was transparent with size of few nm (2-4 nm), which signifies that the mixture is above miscibility limit (binodal curve). Similarly, there is shift in non-ouzo zone and binodal zone for DSPE-mPEG mass fraction f_{solute} 4.5×10^{-7} to 4.5×10^{-4}, which results in the shift of non-ouzo line and binodal line during the construction of small region of the DSPE–mPEG/ THF/water ternary phase diagram (Figure S13b, S13c, S13d).



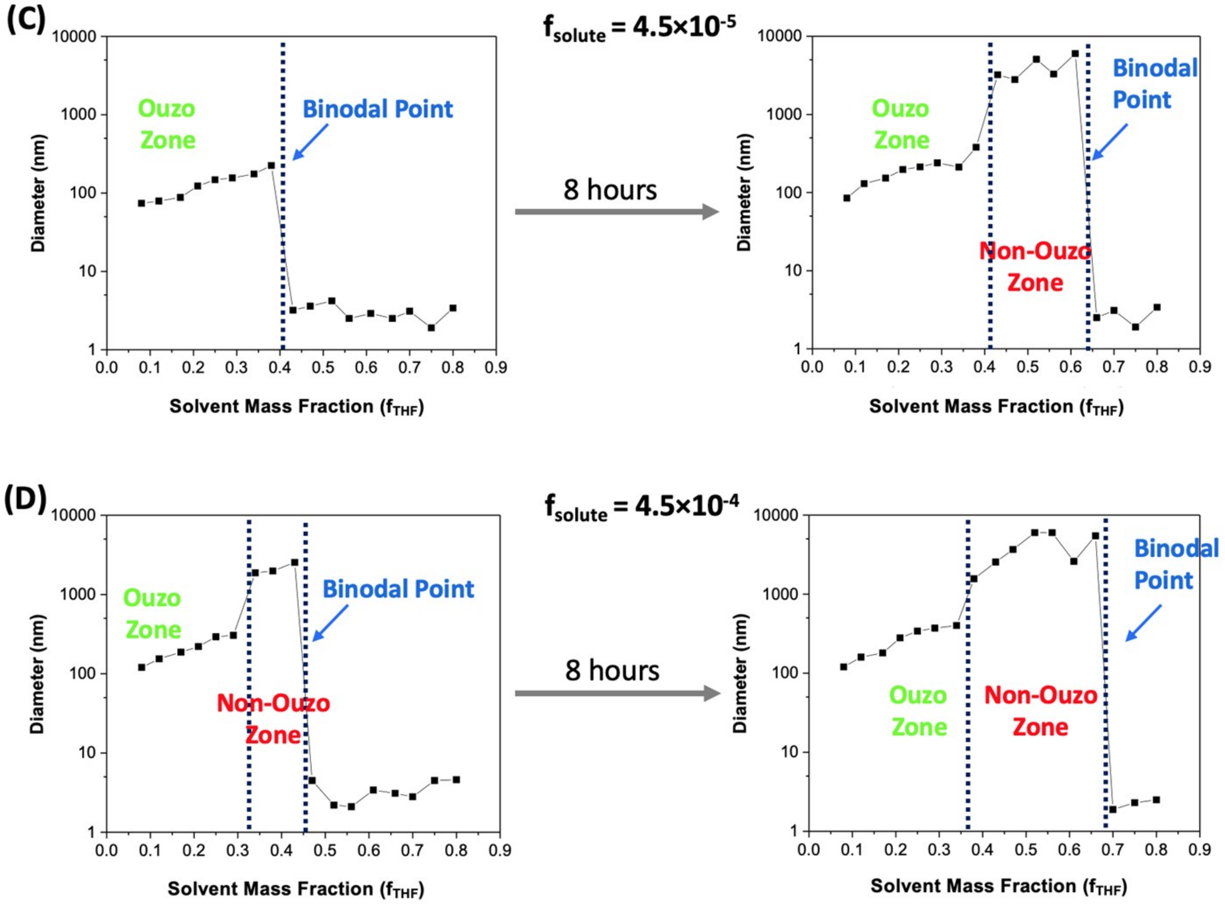


Figure S13. Change in the size of particles formed by blank DSPE-mPEG$_{2000}$ at t=0 and t=8 hours after mixing solute, solvent and water with (a) f_{solute} 4.5×10^{-7}, (b) f_{solute} 4.5×10^{-6}, (c) f_{solute} 4.5×10^{-5} and (d) f_{solute} 4.5×10^{-4}.

We have also varied the mass fraction of PLGA solute (f_{solute}) from 9.0×10^{-5} to 4.5×10^{-3} and the mass fraction of solvent (f_{THF}) from 0.08 to 0.80 by using the microfluidic mixer to construct the BTPEBT + PLGA/THF/water ternary phase diagram (Figure S14b). Similar to DSPE-mPEG, there is shift in non-ouzo zone and binodal line with change in time (0 to 8 hours, Figure S14).

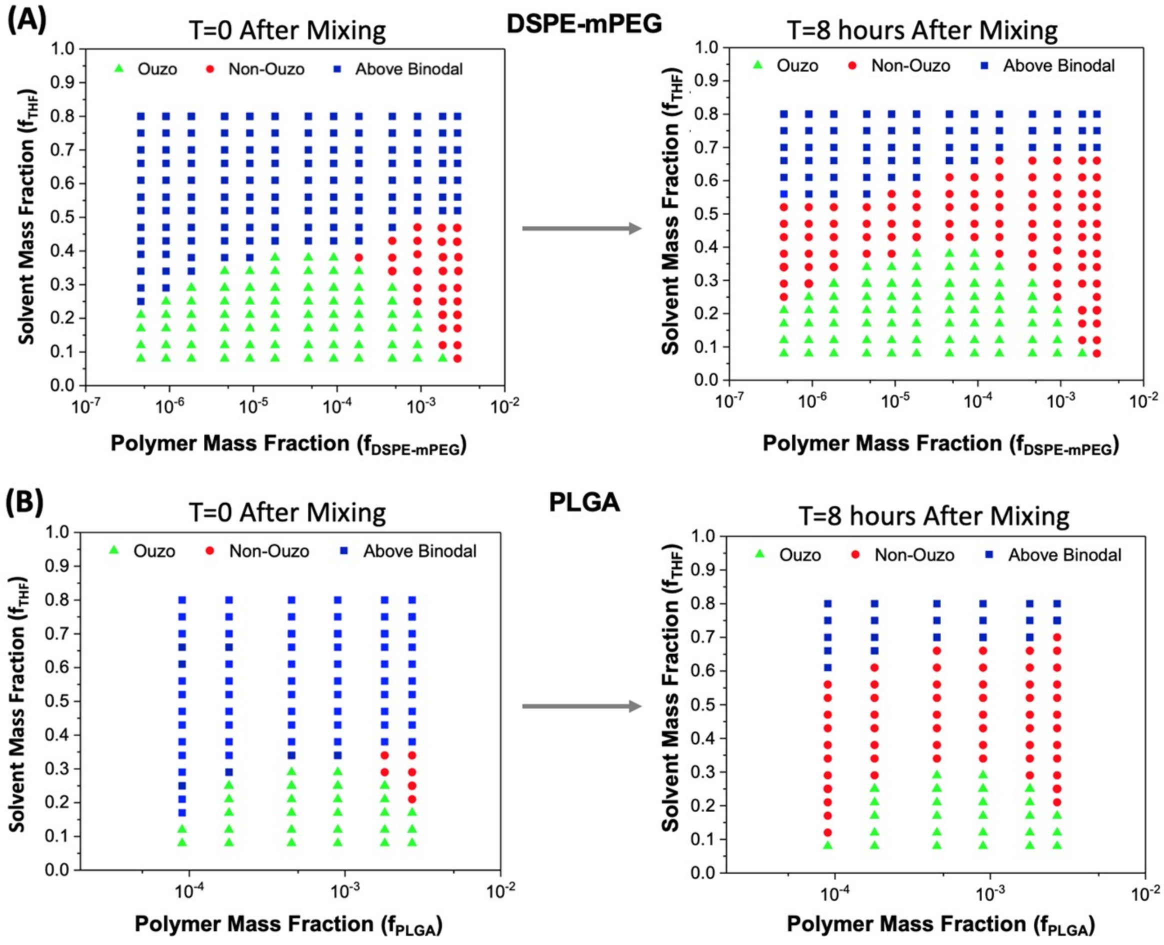


Figure S14. Shift in the ternary phase diagram of the polymers/THF/water combination with respect to time for (a) DSPE-mPEG and (b) PLGA.

References

1. J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer and O. C. Farokhzad, *Biomaterials*, 2007, **28**, 869-876.
2. S. B. Wang, A. Z. Chen, L. J. Weng, M. Y. Chen and X. L. Xie, *Macromol Biosci*, 2004, **4**, 27-30.
3. X. Yan, M. Remond, Z. Zheng, E. Hoibian, C. Soulage, S. h. Chambert, C. Andraud, B. V. d. Sanden, F. i. Ganachaud, Y. Bretonnière and J. Bernard, *ACS Appl. Mater. Interfaces*, 2018, **10**, 25154-25165.
4. E. Lepeltier, C. Bourgaux and P. Couvreur, *Adv. Drug Deliv. Rev.*, 2014, **71**, 86-97.