

## Supplementary information

### Evaluating the effect of synthesis, isolation, and characterisation variables on reported particle size and dispersity of drug loaded PLGA nanoparticles

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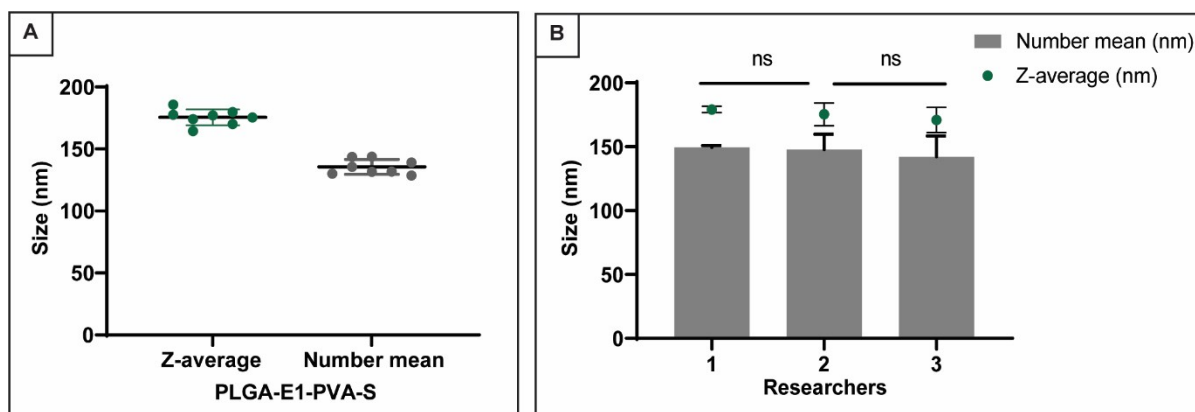
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**Figure S1:** Study of 2Rs of PLGA NPs. (A) Repeatability of PLGA-E1-PVA-S NPs in z-average, n = 8 (A), and (B) replicability of PLGA-A1-PVA-S NPs. The number weighted mean is represented by columns (■) and z-average is represented by ●.

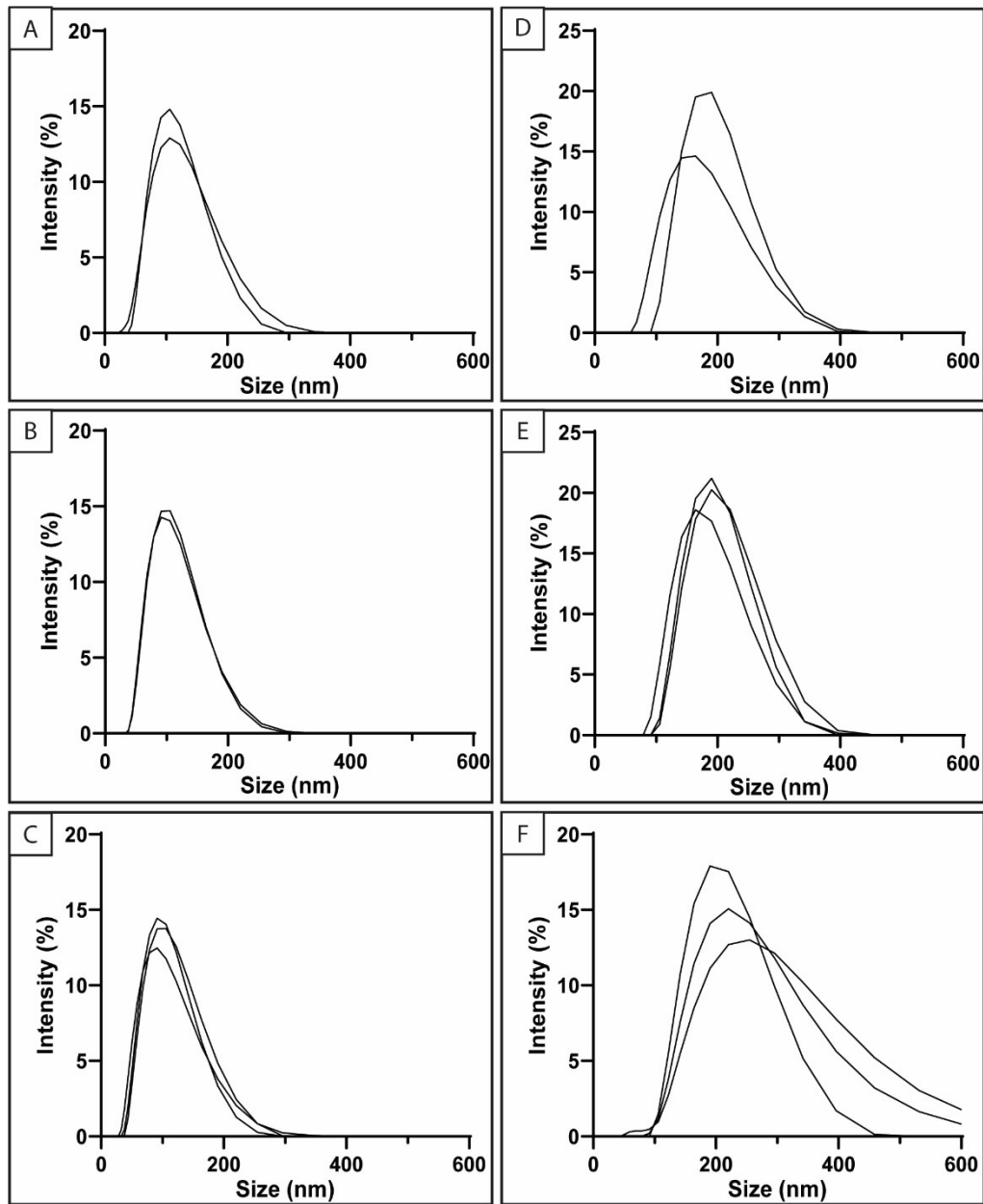


**Table S1.** Particle sizes obtained for various PLGA NP formulations using DLS. n = 3 unless otherwise stated.

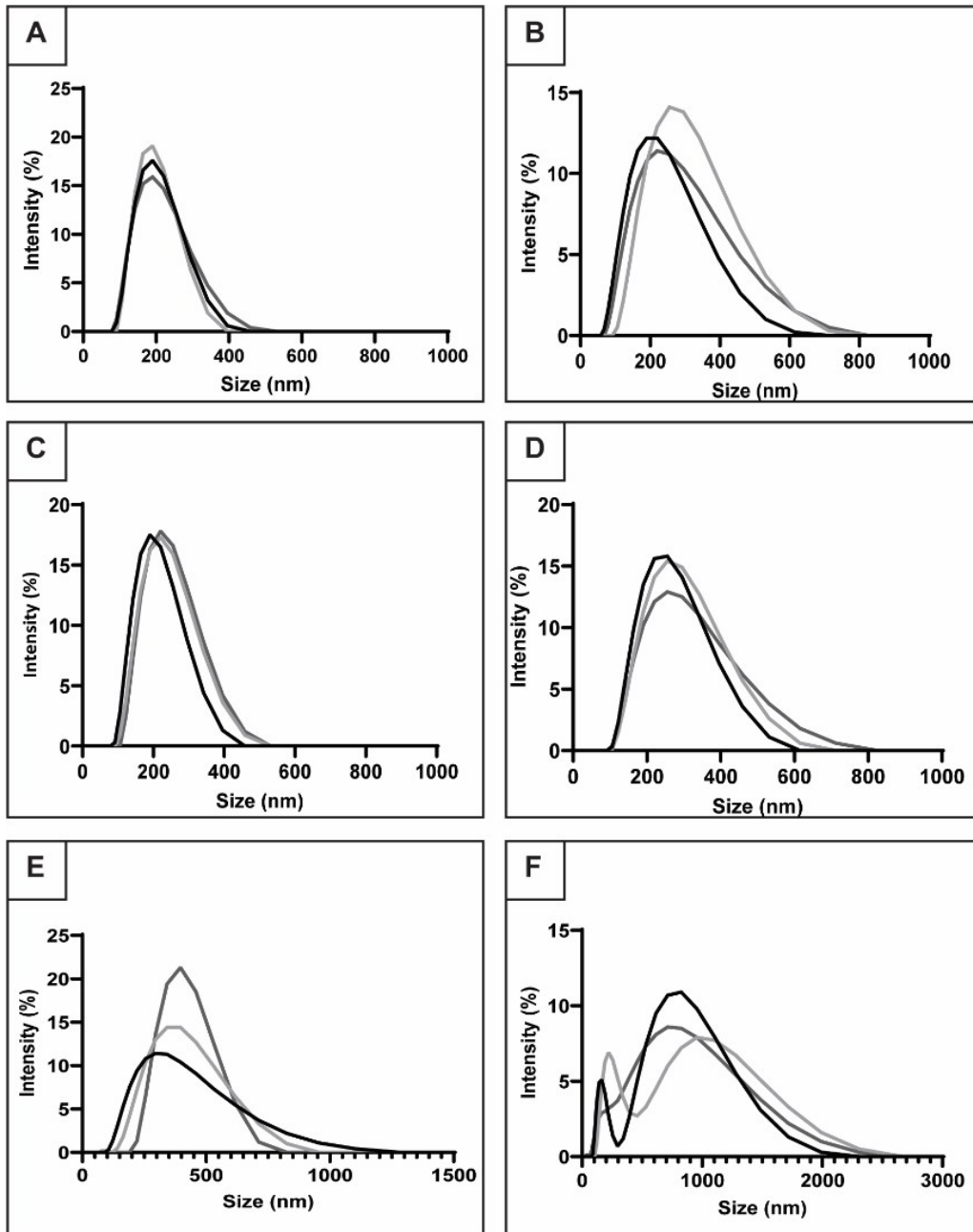
Sample	Number mean (nm)	Z-average (nm)	PDI
PLGA-E1-PVA-S (Fig 3C)*	144 ± 8	186 ± 5	0.12 ± 0.01
PLGA-A1-BSA-S (Fig 3F)*	77 ± 10	137 ± 8	0.23 ± 0.01
PLGA-A1-BSA-S-80s (Fig 4B)	73 ± 31	109 ± 20	0.14 ± 0.02
PLGA-A1-BSA-S-120s (Fig 4B)	74 ± 24	103 ± 19	0.14 ± 0.01
PLGA-A1-BSA-S-240s (Fig 4B)	54 ± 6	88 ± 9	0.15 ± 0.02
PLGA-A1-PVA-S-80s (Fig 4B)	121 ± 27	197 ± 41	0.24 ± 0.11
PLGA-A1-PVA-S-120s (Fig 4B)	140 ± 22	172 ± 15	0.09 ± 0.01
PLGA-A1-PVA-S-240s (Fig 4B)	157 ± 19	225 ± 25	0.17 ± 0.05
PLGA-E1-PVA-H (Fig 5A)	177 ± 19	258 ± 5	0.19 ± 0.02
PLGA-E2-PVA-H	149 ± 3	317 ± 59	0.11 ± 0.02
PLGA-E1-PVA-S	139 ± 1	176 ± 2	0.08 ± 0.02
PLGA-E2-PVA-S	169 ± 7	205 ± 18	0.11 ± 0.01
PLGA-E1-PVA-S-cur	139 ± 19	215 ± 32	0.17 ± 0.05
PLGA-E2-PVA-S-cur	171 ± 15	237 ± 19	0.23 ± 0.09
PLGA-E1-PVA-S-unwashed (Fig 6B)	136 ± 18	175 ± 8	0.16 ± 0.07
PLGA-E1-PVA-S-washed (Fig 6B)	124 ± 15	173 ± 10	0.21 ± 0.06
PLGA-A1-PVA-S-unfiltered (Fig 6C)	142 ± 16	171 ± 10	0.07 ± 0.03
PLGA-A1-PVA-S-filtered (Fig 6C)	141 ± 12	171 ± 11	0.06 ± 0.02

\*n=1

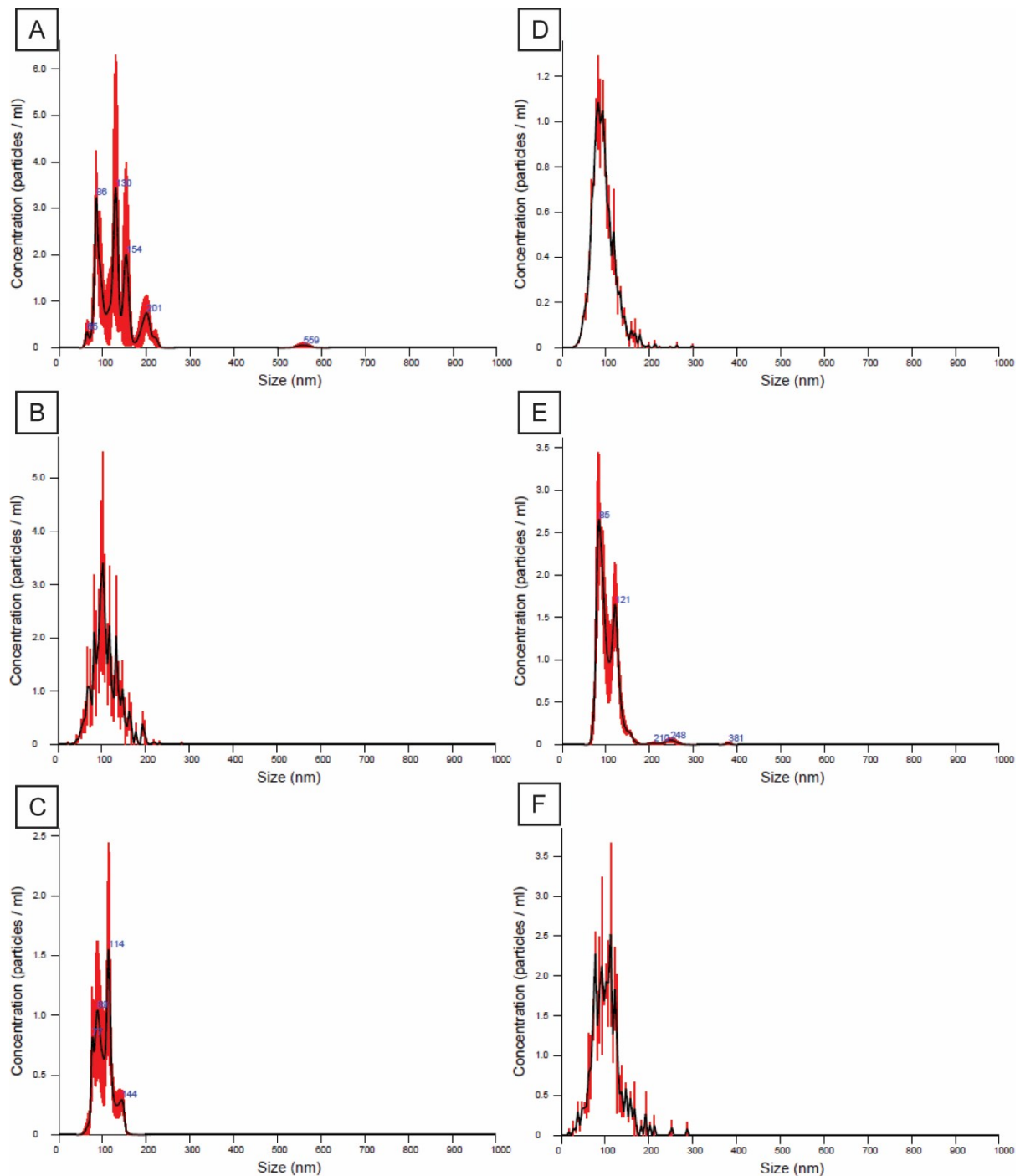
**Figure S2A:** Size distribution curves of all samples not shown in main manuscript. Samples: PLGA-A1-BSA-S-80s (A), PLGA-A1-BSA-S-120s (B), PLGA-A1-BSA-S-240s (C), PLGA-A1-BSA-S-80s (D), PLGA-A1-PVA-S-120s (E), PLGA-A1-PVA-S-240s (F). Each size distribution curve represents a different sample.



**Figure S2B.** Size distribution by intensity of all samples not shown in the main manuscript. Samples: PLGA-E1-PVA-S (A) and PLGA-E1-PVA-S-cur (B); PLGA-E2-PVA-S (C) and PLGA-E2-PVA-S-cur (D); PLGA-E2-PVA-H (E) and PLGA-E2-PVA-H-cur (F). Each size distribution curve represents a different sample.



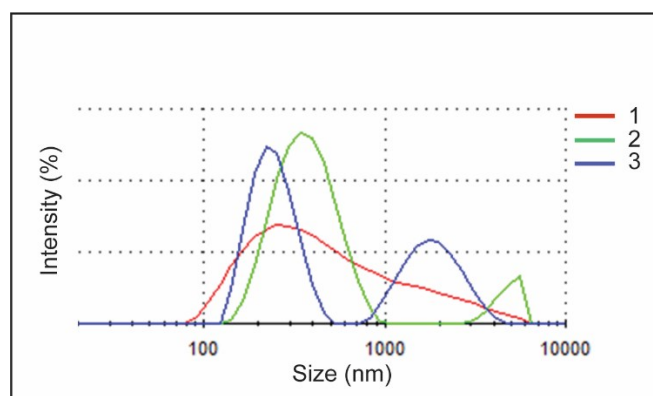
**Figure S3.** The particle size distribution curve measured by nanoparticle tracking analysis (NTA) after emulsification via ultrasonication for (A) 20 s, (B) 40 s, (C) 60 s, (D) 80 s, (E) 100 s and (F) 120 s. The size distribution is given as a population values across 5 nm sized bins. The red curve represents the standard deviation within each size bin's population.



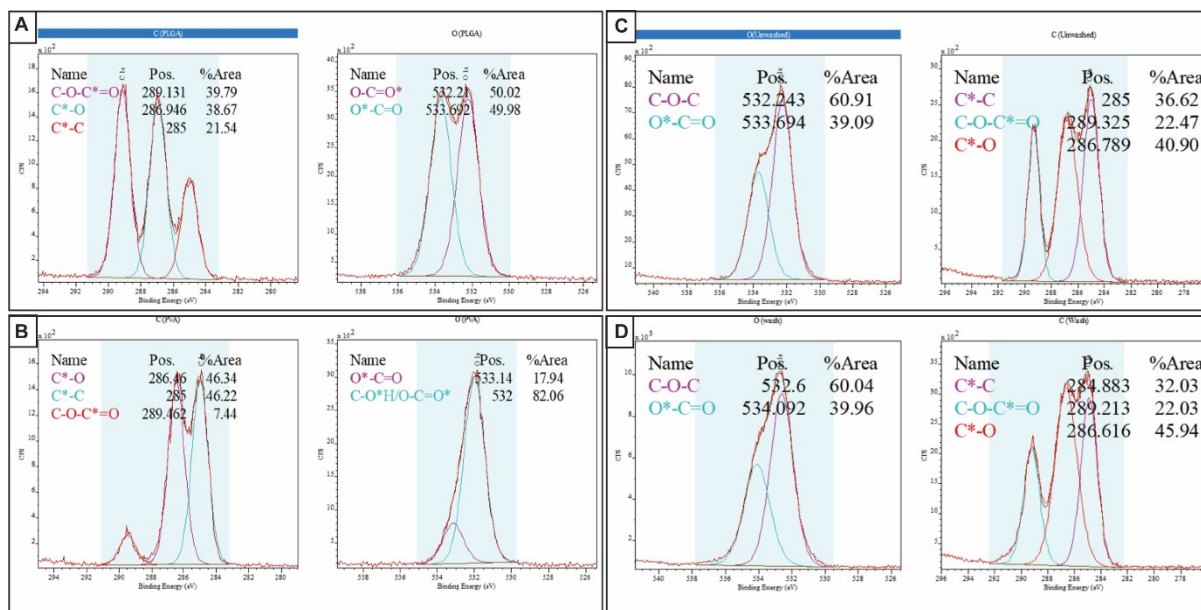
**Table S2.** Temperature measurements during sonication.

Sonication Time (s)	Initial Temp (°C)	Final Temp (°C)	Temp Change (°C)
40	3	9	6
80	2.5	7.5	5
120	3	8.5	5.5
160	3	7.5	4.5
200	3	8	5
240	3	8	5
280	3	7.5	4.5

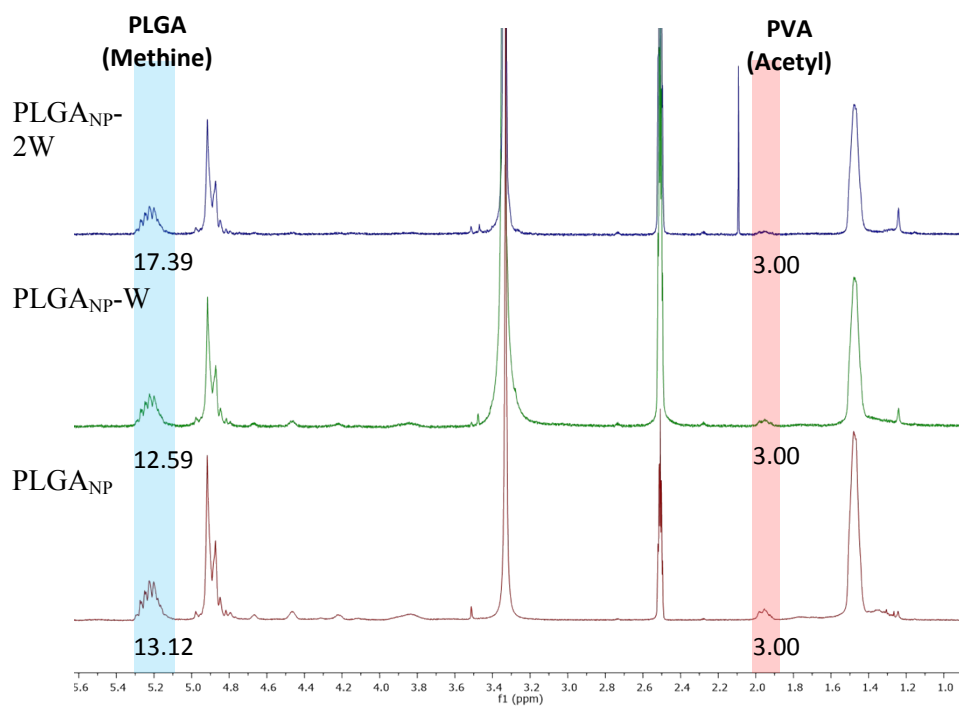
**Figure S4:** Size distribution by intensity of 3 runs from same sample (PLGA-E1-PVA-H-cur) using homogeniser.



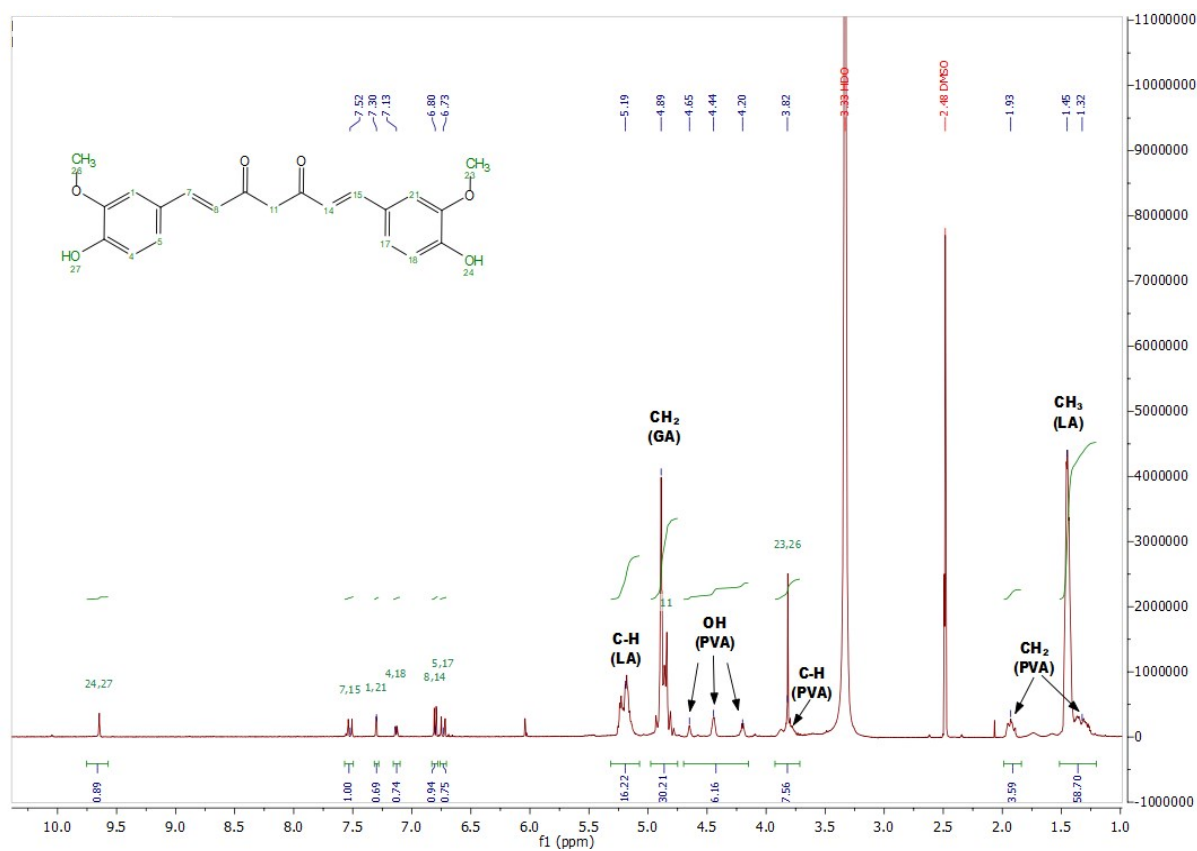
**Figure S5A.** XPS narrow scan spectra of PLGA (A), PVA (B), and PLGA-A1-PVA-S NPs unwashed (C) and PLGA-A1-PVA-S NPs washed (D).



**Figure S5B.**  $^1\text{H}$  NMR of PLGA-A1-PVA-S dissolved in d-DMSO (bottom) without washing (PLGA<sub>NP</sub>) (middle) washed 1x (PLGA<sub>NP</sub>-W) (top) washed 2 x (PLGA<sub>NP</sub>-2W) in d-DMSO.



**Figure S6.** Assigned NMR of PLGA-A1-PVA-s-Cur and calculation of encapsulation efficiency determination.



Example Calculation:

$$\text{Molar Ratio } (M_R) = \frac{\frac{\int_{Drug}}{\#P_{Drug}}}{\frac{\int_{Polymer}}{\#P_{Polymer}}}$$

$$M_R = \frac{1}{\frac{2}{16}} = 0.031$$

Where;

- $\int_{Drug}$  = integral of drug peak,
- $\int_{Polymer}$  = integral of polymer peak,
- $\#P_{drug}$  = no. of protons corresponding to drug peak
- $\#P_{polymer}$  = no. of protons corresponding to polymer peak

$$\text{Exp. Mass Ratio } (Em_R) = M_R \times \frac{MW_{Drug}}{MW_{Monomer}}$$

Where;

- $MW_{Drug}$  = molecular weight of Drug
- $MW_{Monomer}$  = molecular weight of repeat unit

$$Em_R = 0.031 \times \frac{368 \text{ g/mol}}{130 \text{ g/mol}} = 0.087$$



$$\text{Theor. Mass Ratio } (Tm_R) = \frac{m_{Drug}}{m_{Polymer}}$$

Where;

$m_{Drug}$  = mass of Drug

$m_{Monomer}$  = molecular weight of monomer

$$Tm_R = \frac{0.001 \text{ g}}{0.01 \text{ g}} = 0.1$$

$$\text{Encapsulation Efficiency \% (EE\%)} = \frac{Em_r}{Tm_r} \times 100\%$$

$$EE\% = \frac{0.087}{0.1} \times 100\% = 87\%$$