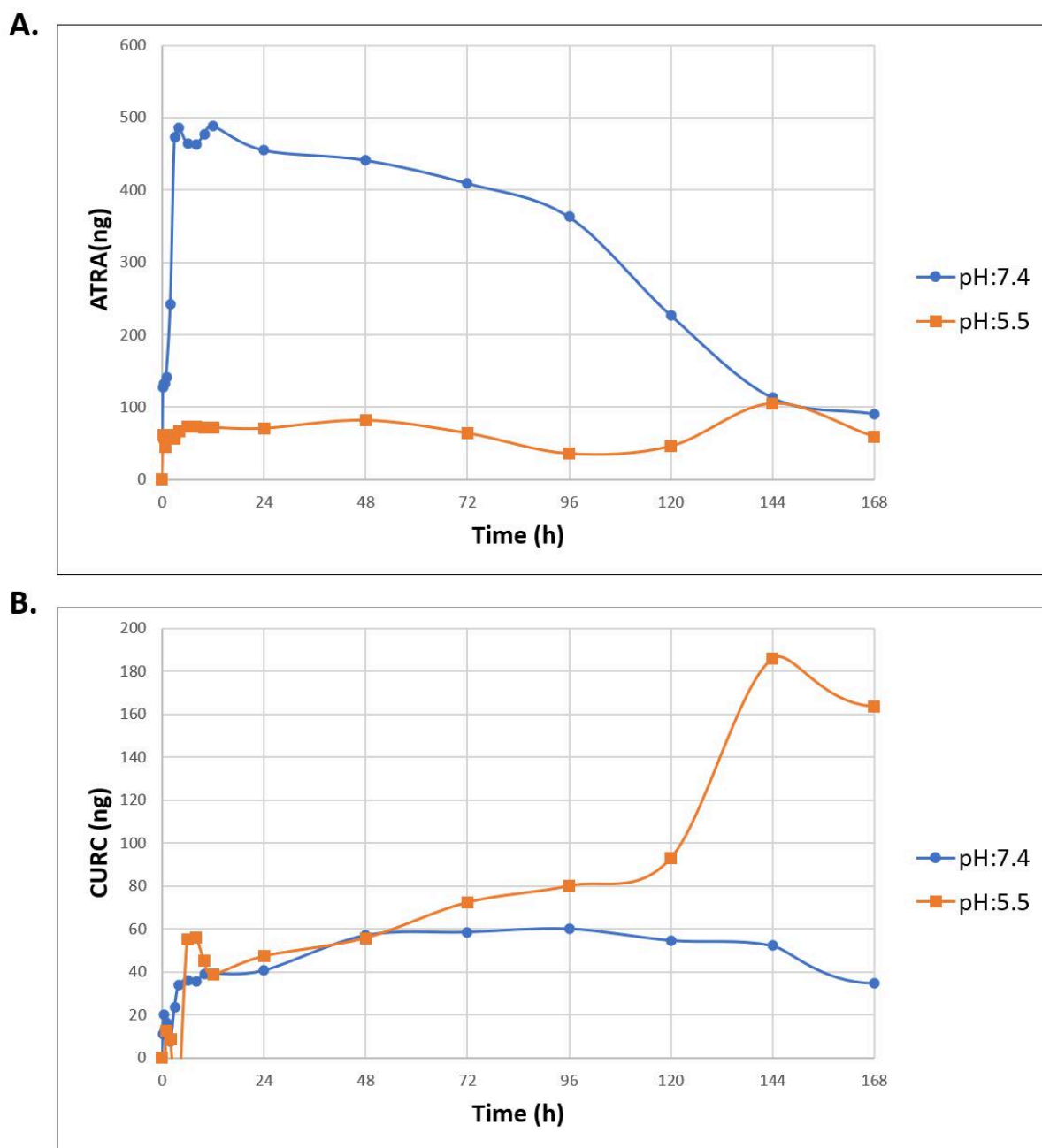


Supplementary Table S1. Formulations prepared during the optimization process (BSA, phosphate buffer, ethanol, glutaraldehyde concentration, stirring speed and reaction time).

	BSA (mg)	Phosphate buffer (mL)	Ethanol (mL)	Glutaraldehyde	rpm	Reaction time
C1	60	1	-	10 mg 20 µl	500	24 hour
C2	30	1	1	10 mg 20 µl	500	24 hour
C3	30	1	0.75	10 mg 20 µl	500	24 hour
C4	30	1	0.5	10 mg 20 µl	500	24 hour
C5	30	1	0.25	10 mg 20 µl	500	24 hour
C6	30	1	0.1	10 mg 20 µl	500	24 hour
C7	30	1	0.75	10 mg 20 µl	200	24 hour
C8	30	1	0.75	10 mg 20 µl	300	24 hour
C9	30	1	0.75	10 mg 20 µl	750	24 hour
C10	30	1	0.75	10 mg 20 µl	1000	24 hour

C11	30	1	0.75	2.5 mg 40 µl	500	24 hour
C12	30	1	0.75	5 mg 40 µl	500	24 hour
C13	30	1	0.75	10 mg 40 µl	500	24 hour
C14	30	1	0.75	15 mg 40 µl	500	24 hour
C15	30	1	0.75	20 mg 40 µl	500	24 hour
C16	30	1	0.75	2.5 mg 30 µl	500	24 hour
C17	30	1	0.75	2.5 mg 50 µl	500	24 hour
C18	30	1	0.75	2.5 mg 75 µl	500	24 hour
C19	30	1	0.75	2.5 mg 100 µl	500	24 hour
C20	30	1	0.75 %50 EtOH	2.5 mg 100 µl	500	24 hour
C21	30	1	0.75 %70 EtOH	2.5 mg 100 µl	500	24 hour

C22	30	1	0.75 %96 EtOH	2.5 mg 100 µl	500	24 hour
C23	30	1	0.75 %99 EtOH	2.5 mg 100 µl	500	24 hour



Supplementary Figure S1. Release profile of combination loaded BSA nanoparticles in PBS medium both pH:5.5 (orange line) and in pH:7.4 (blue line). The released ATRA (A) and curcumin (B) drugs are shown in nanograms.

Supplementary Table S2. Characterization of drug-free formulations prepared during the optimization process (Particle Size, PDI, zeta potential)

Formulation Code	Particle size (nm) $\pm$ SD	Polydispersite Index (PDI) $\pm$ SD	Zeta Potential (mV) $\pm$ SD
C1	2555.2 $\pm$ 1841.9	0.3 $\pm$ 0.0	-30.6 $\pm$ 0.8
C2	649.3 $\pm$ 165.9	0.3 $\pm$ 0.0	-30.6 $\pm$ 0.8
C3	485.1 $\pm$ 70.4	0.3 $\pm$ 0.0	-24.4 $\pm$ 0.5
C4	572.1 $\pm$ 145.1	0.3 $\pm$ 0.1	-15.5 $\pm$ 1.0
C5	616.4 $\pm$ 76.0	0.3 $\pm$ 0.0	-37.8 $\pm$ 8.3
C6	457.7 $\pm$ 50.9	0.3 $\pm$ 0.0	-26.2 $\pm$ 1.8
C7	422.4 $\pm$ 45.1	0.3 $\pm$ 0.0	-29.1 $\pm$ 3.4
C8	585.7 $\pm$ 112.8	0.4 $\pm$ 0.2	-25.5 $\pm$ 1.0
C9	520.8 $\pm$ 114.0	0.3 $\pm$ 0.0	-33.9 $\pm$ 2.4
C10	814.0 $\pm$ 409.4	0.3 $\pm$ 0.0	-28.0 $\pm$ 0.6
C11	154.8 $\pm$ 24.1	0.2 $\pm$ 0.0	-31.7 $\pm$ 1.8
C12	224.5 $\pm$ 5.8	0.2 $\pm$ 0.0	-33.2 $\pm$ 2.1
C13	261.3 $\pm$ 6.8	0.2 $\pm$ 0.0	-32.3 $\pm$ 0.9
C14	399.5 $\pm$ 87.7	0.3 $\pm$ 0.0	-23.3 $\pm$ 6.0
C15	495.8 $\pm$ 98.6	0.3 $\pm$ 0.0	-29.1 $\pm$ 6.0
C16	161.0 $\pm$ 16.3	0.3 $\pm$ 0.2	-0.9 $\pm$ 2.5

C17	94.7±25.1	0.3±0.0	-17.3±9.4
C18	94.2±12.5	0.3±0.0	-23.0±5.2
C19	93.8±4.4	0.2±0.0	-2.4±1.1
C20	74.69±9.,3	0.24±0.0	-23.02±0.8
C21	153.0±2.7	0.2±0.0	-26.0±1.3
C22	208.2±2.6	0.2±0.0	-25.5±1.2
C23	180.2±3.2	0.2±0.0	-24.2±3.6

SD, Standard Deviation.

Supplementary Table S3. Cytotoxicity results of the multiple ANOVA (Sidak) comparison test for the nanoparticle groups. Adjusted p-values obtained from Sidak's multiple comparisons test for U87-MG cell cytotoxicity show the effects of ATRA (75 μ M) NP, curcumin (20 μ M) fNP, and co-loaded nanoparticle groups compared with the negative control group at different time points (0–72 h). “ns” (non-significant) indicates non-significant differences, whereas $p < 0.05$, $p < 0.01$, and $p < 0.001$ indicate statistically significant differences.

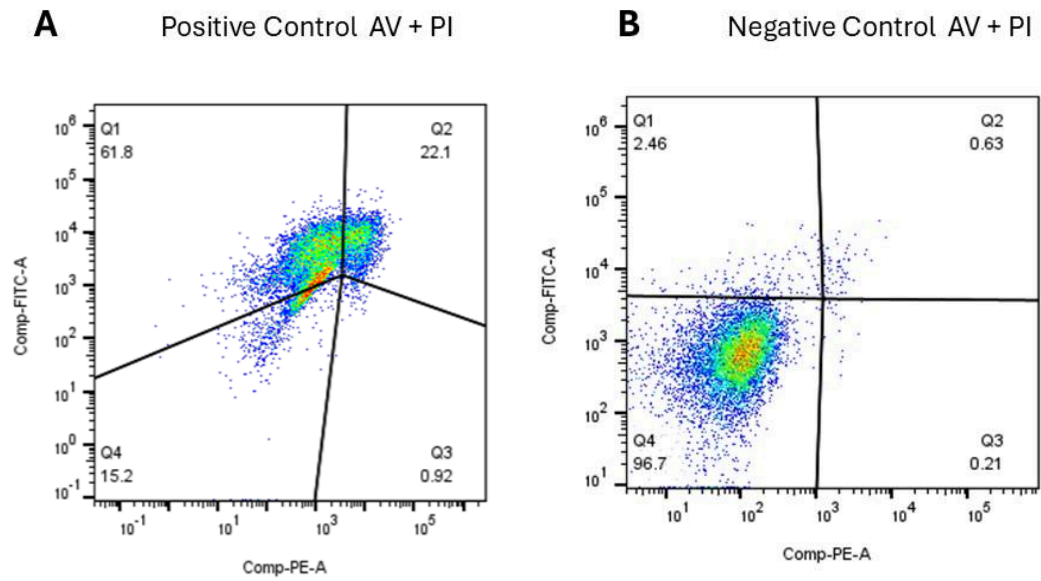
Time (h)	ATRA 75 μ M NP vs Negative Control	CURC 20 μ M NP vs Negative Control	ATRA 75 μ M + CURC 20 NP vs Negative Control
0 h	ns	ns	ns
6 h	0.005**	0.027*	0.004**
12 h	0.002**	ns	0.017*
18 h	ns	ns	ns
24 h	ns	ns	ns
30 h	ns	ns	ns
36 h	ns	ns	ns
42 h	ns	ns	ns
48 h	ns	ns	ns
54 h	ns	ns	ns
60 h	0.003**	ns	ns
66 h	ns	ns	ns
72 h	ns	ns	<0.001***

Supplementary Table S4. Migration results of the multiple ANOVA (Sidak) comparison test for the nanoparticle groups. Adjusted p-values obtained from Sidak's multiple comparisons test for U87-MG cell migration show the effects of ATRA (75 μ M) NP, curcumin (20 μ M) NP, and co-loaded nanoparticle groups compared with the negative control and the drug-free NP group at different time points (0–87 h). “ns” (non-significant) indicates non-significant differences, whereas $p < 0.05$, $p < 0.01$, and $p < 0.001$ indicate statistically significant differences.

Time (h)	ATRA 75 μ M NP vs Negative Control	CURC 20 μ M NP vs Negative Control	ATRA 75 μ M + CURC 20 NP vs Negative Control	Drug-free NP vs Negative Control	ATRA 75 μ M NP vs Drug-free NP	CURC 20 μ M NP vs Drug-free NP	ATRA 75 μ M + CURC 20 NP vs Drug-free NP
0 h - 33h	ns	ns	ns	ns	ns	ns	ns
34 h	ns	0.048*	ns	ns	ns	ns	ns
35 h	ns	0.023*	ns	ns	ns	ns	ns
36 h	ns	0.029*	ns	ns	ns	ns	ns
37 h	ns	0.005**	ns	ns	ns	ns	ns
38 h	ns	0.005**	ns	ns	ns	ns	ns
39 h	ns	0.003**	ns	ns	ns	ns	ns
40 h	ns	0.002**	ns	ns	ns	ns	ns
41 h	ns	0.001**	ns	ns	ns	ns	ns
42 h	ns	0.001**	ns	ns	ns	ns	ns
43 h	ns	0.001**	ns	ns	ns	ns	ns
44 h	ns	0.001**	ns	ns	ns	ns	ns

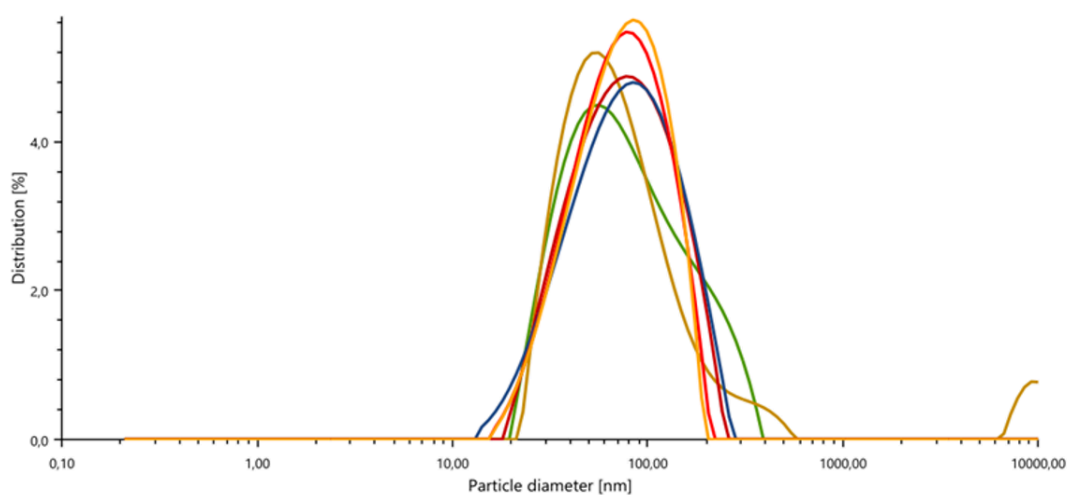
45 h	ns	0.001**	ns	ns	ns	ns	ns
46 h	ns	0.001**	ns	ns	ns	ns	ns
47 h	ns	0.001**	ns	ns	ns	ns	ns
48 h	ns	0.001**	ns	ns	ns	ns	ns
49 h	ns	<0.001***	ns	ns	ns	ns	ns
50 h	ns	<0.001***	ns	ns	ns	ns	ns
51 h	ns	<0.001***	ns	ns	ns	ns	ns
52 h	ns	<0.001***	ns	ns	ns	ns	ns
53 h	ns	<0.001***	ns	ns	ns	ns	0.009*
54 h	ns	0.032*	ns	0.044*	ns	ns	<0.001***
55 h	ns	ns	ns	0.017*	ns	ns	<0.001***
56 h	ns	ns	ns	0.001**	ns	ns	<0.001***
57 h	ns	ns	ns	<0.001***	0.036*	0.008**	<0.001***
58 h	ns	ns	ns	<0.001***	ns	0.002**	<0.001***
59 h	0.024*	ns	ns	<0.001***	ns	0.001**	<0.001***
60 h	0.017*	ns	ns	<0.001***	ns	<0.001***	<0.001***
61 h	0.001**	ns	ns	<0.001***	ns	<0.001***	<0.001***
62 h	<0.001***	ns	ns	<0.001***	ns	<0.001***	<0.001***
63 h	0.001**	ns	ns	<0.001***	ns	0.001**	0.001**

64 h	0.001**	ns	ns	<0.001***	ns	0.002**	0.001**
65 h	0.001**	ns	ns	<0.001***	ns	0.006**	0.002**
66 h	0.004**	ns	ns	<0.001***	ns	0.012*	0.003**
67 h	0.007**	ns	ns	<0.001***	ns	0.027*	0.006**
68 h	0.006**	ns	ns	<0.001***	ns	0.032*	0.029*
69 h	0.005**	ns	ns	<0.001***	ns	0.045*	0.040*
70 h	0.002**	ns	ns	<0.001***	ns	0.037*	0.049*
71 h	<0.001***	ns	ns	<0.001***	ns	0.034*	0.032*
72 h	<0.001***	0.038*	0.043*	<0.001***	ns	0.049*	0.043*
73 h	<0.001***	0.023*	0.014*	<0.001***	ns	ns	ns
74 h	<0.001***	0.008**	0.005**	<0.001***	ns	ns	ns
75 h	<0.001***	0.004**	0.002**	<0.001***	ns	ns	ns
76 h	<0.001***	0.001**	<0.001***	<0.001***	ns	ns	ns
77 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns
78 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns
79 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns
80 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns
81 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns
82 h	<0.001***	<0.001***	<0.001***	<0.001***	ns	ns	ns

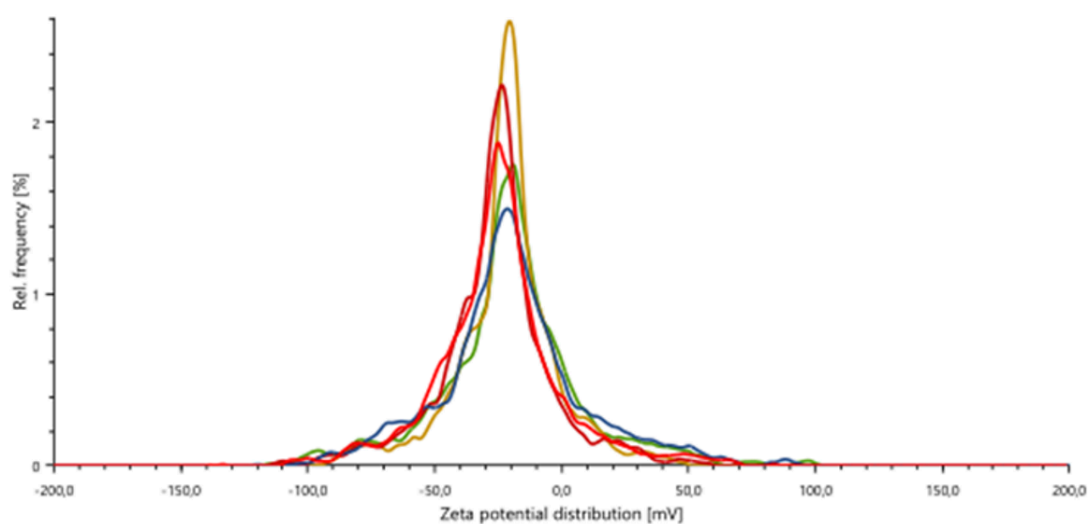


Supplementary Figure S2. Annexin V-FITC/PI analysis of positive and negative control U87 glioblastoma cells. U87 cells were either exposed to an apoptosis-inducing positive control condition or left untreated as a negative control and then stained with Annexin V-FITC and propidium iodide (PI). A: Positive control; B: Negative control.

A. Particle size distribution by intensity



B. Zeta potential distribution



Supplementary Figure S3. The particle size (A) and zeta potential (B) distribution of the C20 (drug-free) formulation.