Supporting Information

Evaluation of Anti-Cancer and Anti-Metastatic Effects of Folate-PEGylated Niosome for Co-Delivery of Letrozole and Ascorbic Acid on Breast Cancer Cells

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SI-1. Experimentation

Table S1. Compositions of different formulations (L-Niosome, A-Niosome, LA-Niosome, FA-LA-Niosome, Empty Niosome)

Systems	Formulations	Surfactant: Cholesterol (molar ratio)	Span60:Tween60 (molar ratio)	Cholesterol (mg)	Span 60 (mg)	Tween 60 (mg)	Drug Concentration (mg/mL)
Letrozole-Loaded Niosome (L-Niosome)	F1	1:1	0.5:1	58.00	21.22	131.66	1.5
	F2	2:1	0.5:1	38.28	28.43	176.42	1.5
	F3	1:1	1:1	58.00	32.15	98.25	1.5
	F4	2:1	1:1	38.28	43.08	131.66	1.5
	F5	1:1	1.5:1	58.00	38.58	78.60	1.5
	F6	2:1	1.5:1	38.28	51.70	105.32	1.5
Ascorbic Acid-Loaded Niosome (AA-Niosome)	F7	1:1	0.5:1	58.00	21.22	131.66	1.5
	F8	2:1	0.5:1	38.28	28.43	176.42	1.5
	F9	1:1	1:1	58.00	32.15	98.25	1.5
	F10	2:1	1:1	38.28	43.08	131.66	1.5
	F11	1:1	1.5:1	58.00	38.58	78.60	1.5
	F12	2:1	1.5:1	38.28	51.70	105.32	1.5
Letrozole/Ascorbic Acid-co-Loaded-Niosome (LA-Niosome)	F13	1:1	1:1	58.00	32.15	98.25	1.5/1.5
	F14	2:1	1:1	38.28	43.08	131.66	1.5/1.5
	F15	2:1	1.5:1	38.28	51.70	105.32	1.5/1.5
Empty Niosome (without drug)		2:1	1:1	38.28	43.08	131.66	-
Folate-PEGylated LA-Niosomes (FA-LA-Niosome)		2:1	1:1	38.28	43.08	131.66	1.5/1.5

Lipid is the total amount of cholesterol and surfactant (Span 60 and Tween 60); Sonication time (5 min).

Table S2. Primers and their sequences used in the real time PCR.

Gene	Primer sequence
MMP2	F: 5’- F: TTG ACG GTA AGG ACGGAC TC-3’ R: 5’- CAT ACT TCA CAC GGA CCA CTTG -3’
cyclinD	F: 5'- CAGATCATCCGCAAACACGC-3’ R: 5'- AAGTTGTTGGGGCTCCTCAG-3'
b-actin	F: 5’- TCCTCCTGAGCGCAAGTAC-3’ R: 5’- CCTGCTTGCTGATCCACATCT-3’
MMP9	F: 5'- GCACGACGTCTTCCAGTACC -3’ R: 5'- CAGGATGTCATAGGTCACGTAGC -3'
Caspase3	F: 5'- CATACTCCACAGCACCTGGTTA-3’ R: 5'- ACTCAAATTCTGTTGCCACCTT-3'
Caspase9	F: 5’-CATATGATCGAGGACATCCAG-3 R: 5’-TTAGTTCGCAGAAACGAAGC-3’

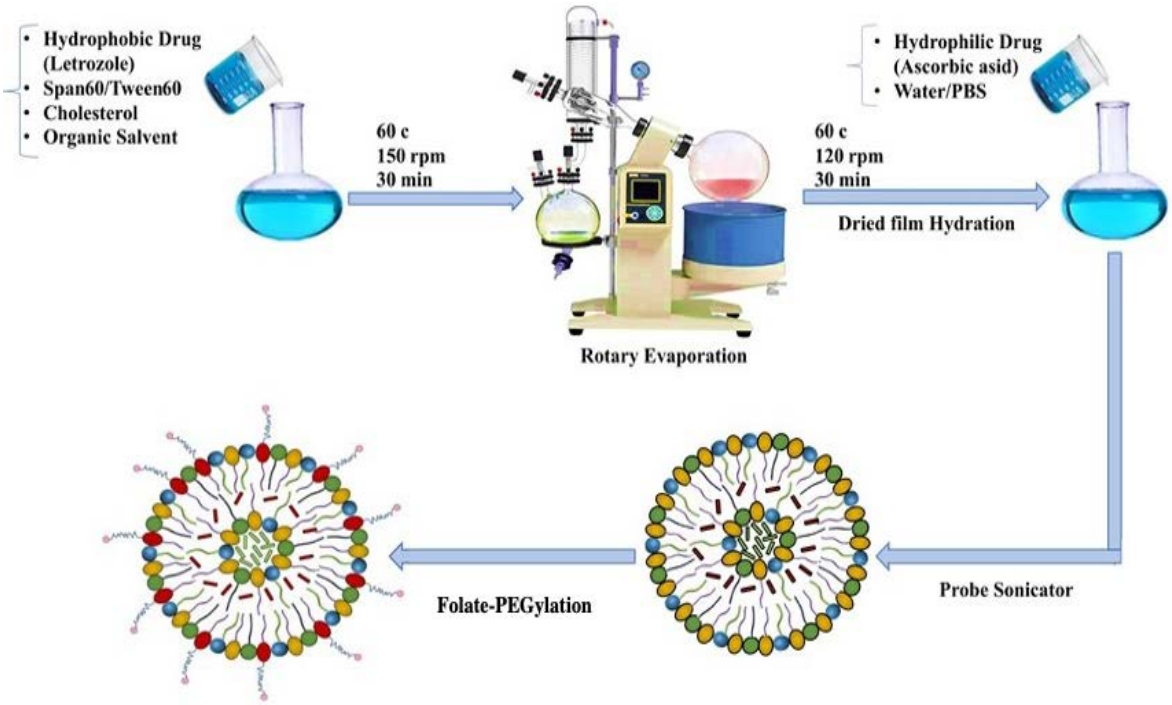

Figure S1. Schematic illustration of the steps in preparing the folate-PEGylated niosomes as drug delivery nanocarriers.

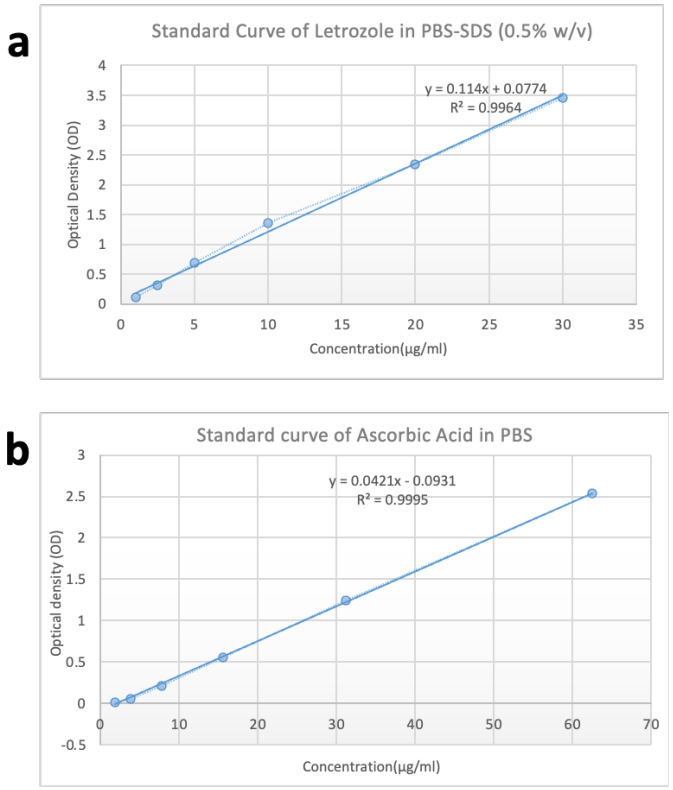

Figure S2. The extinction coefficients (slopes) and calibration curves used to determine the concentration of (a) letrozole and (b) ascorbic acid at the 240 nm and 520 nm wavelength, respectively.

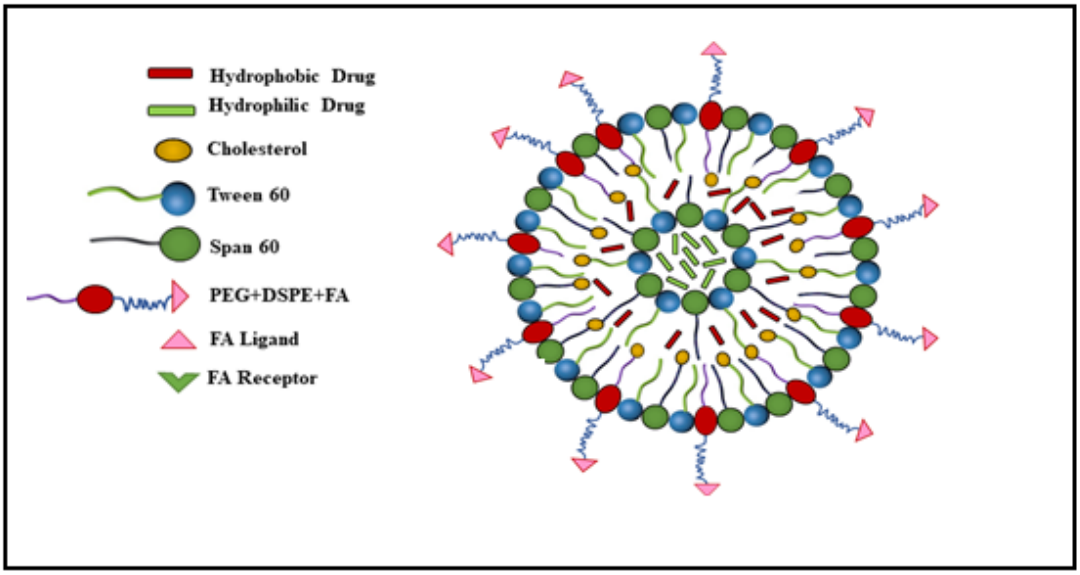

Figure S3. Folate-PEGylated niosomes and drugs positions within the nanostructure

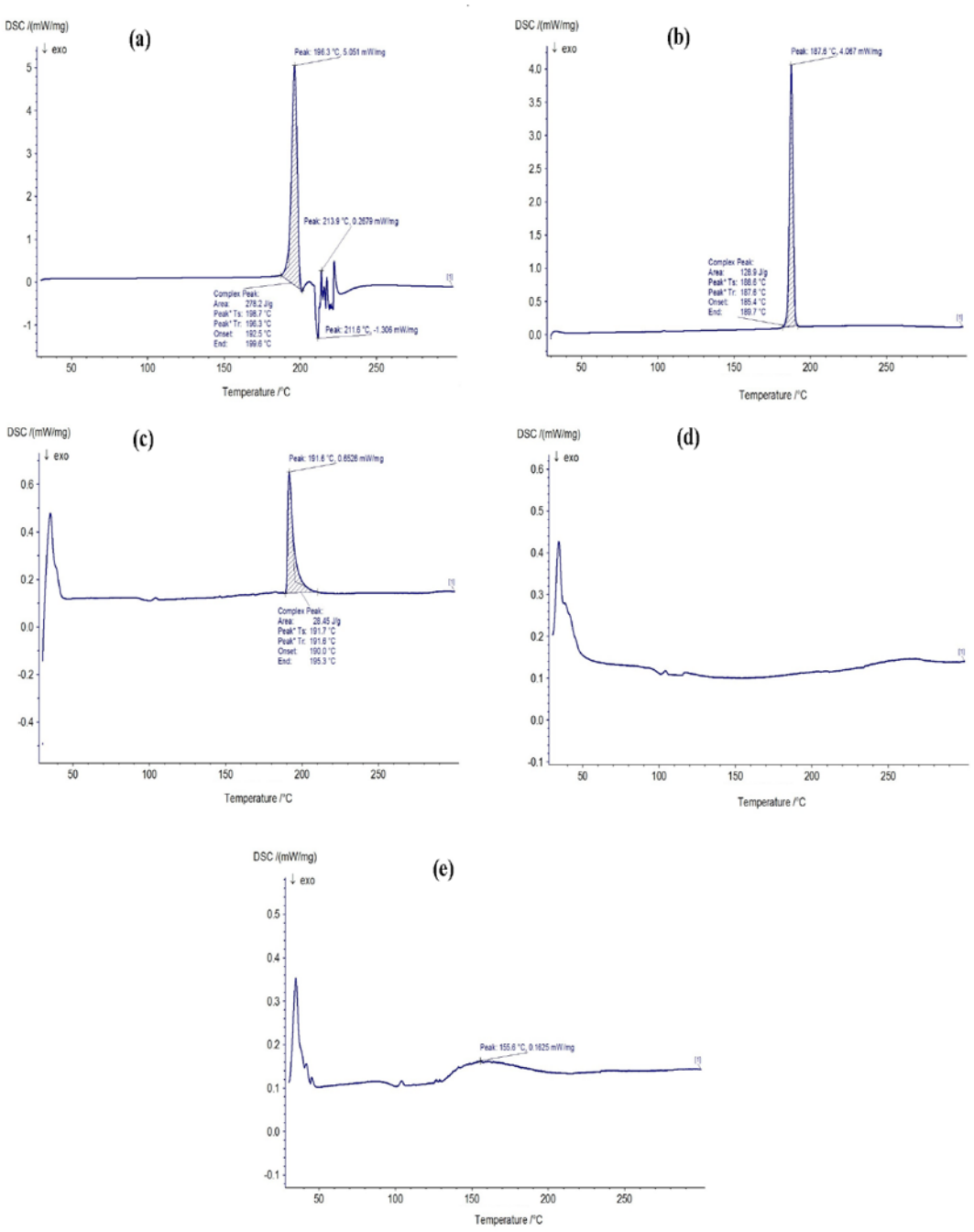

Figure S4. DSC thermograms of (a) Ascorbic acid, (b) Letrozole, (c) AA-Niosome, (d) L-Niosome and (e) LA-Niosome

Table S3. The release kinetic models and parameters obtained for niosomal formulations.

		Kinetic Models				
Sample	Released Drug	Zero-Order	Korsmeyer-Peppas		First-Order	Higuchi
		R^2	R^2	n	R^2	R^2
L(aq)	Letrozole	0.63	0.88	0.44	0.92	0.81
A(aq)	Ascorbic Acid	0.57	0.8	0.38	0.91	0.76
L(aq)/ A(aq)	Letrozole	0.60	0.85	0.45	0.92	0.79
L(aq)/ A(aq)	Ascorbic Acid	0.60	0.89	0.42	0.91	0.79
L-Niosome	Letrozole	0.70	0.89	0.45	0.79	0.87
A-Niosome	Ascorbic Acid	0.75	0.94	0.43	0.86	0.90
LA-Niosome	Letrozole	0.75	0.91	0.50	0.82	0.90
LA-Niosome	Ascorbic Acid	0.71	0.93	0.43	0.82	0.87
FA-LA-Niosome	Letrozole	0.82	0.97	0.45	0.91	0.94
FA-LA-Niosome	Ascorbic Acid	0.76	0.95	0.43	0.91	0.91
			*data fitted for release < 60%			

The kinetic models are defined as below:	
Zero-order model $\boldsymbol{C_t = C_0 + K_0 t}$	Where C_t represents the amount of drug released at time *t*, C_0 is the initial concentration of drug released, which is generally zero. In this model, the release process occurs at a constant rate and is independent of the initial drug concentration
First-order model $\boldsymbol{Log\ C = LogC_0 - \frac{Kt}{2.303}}$	Where C_0 is the initial concentration of the drug, k is the first-order rate constant, and t is the time. C is the drug remaining in the carrier at time t. Log C and t have s linear relationship, and K/2.303 is the slope of the straight line. This model can be used to describe water-soluble drugs in porous matrices.
Higuchi model $\boldsymbol{Q = K_H\sqrt{t}}$	Where, K_H is the Higuchi constant, and it is obtained from the slope of the line. The data obtained were plotted as cumulative percentage drug release versus square root of time. This model can be useful in the case of matrix tablets containing water-soluble drugs.
Korsmeyer-Peppas model $\boldsymbol{M_t/M_\infty = Kt^n}$	Where M_t/M_0 represents the fraction of drug released at time t, k represents the release rate, and n represents the release exponent. For cylindrical-shaped matrices, the n value is used to characterize the various releases. For spherical tablets: n ≤ 0.43: Fickian diffusion mechanism 0.43 < n <0.85: non-Fickian transport. n = 0.85: Case II (relaxational) transport. n > 0.85: super case II transport.

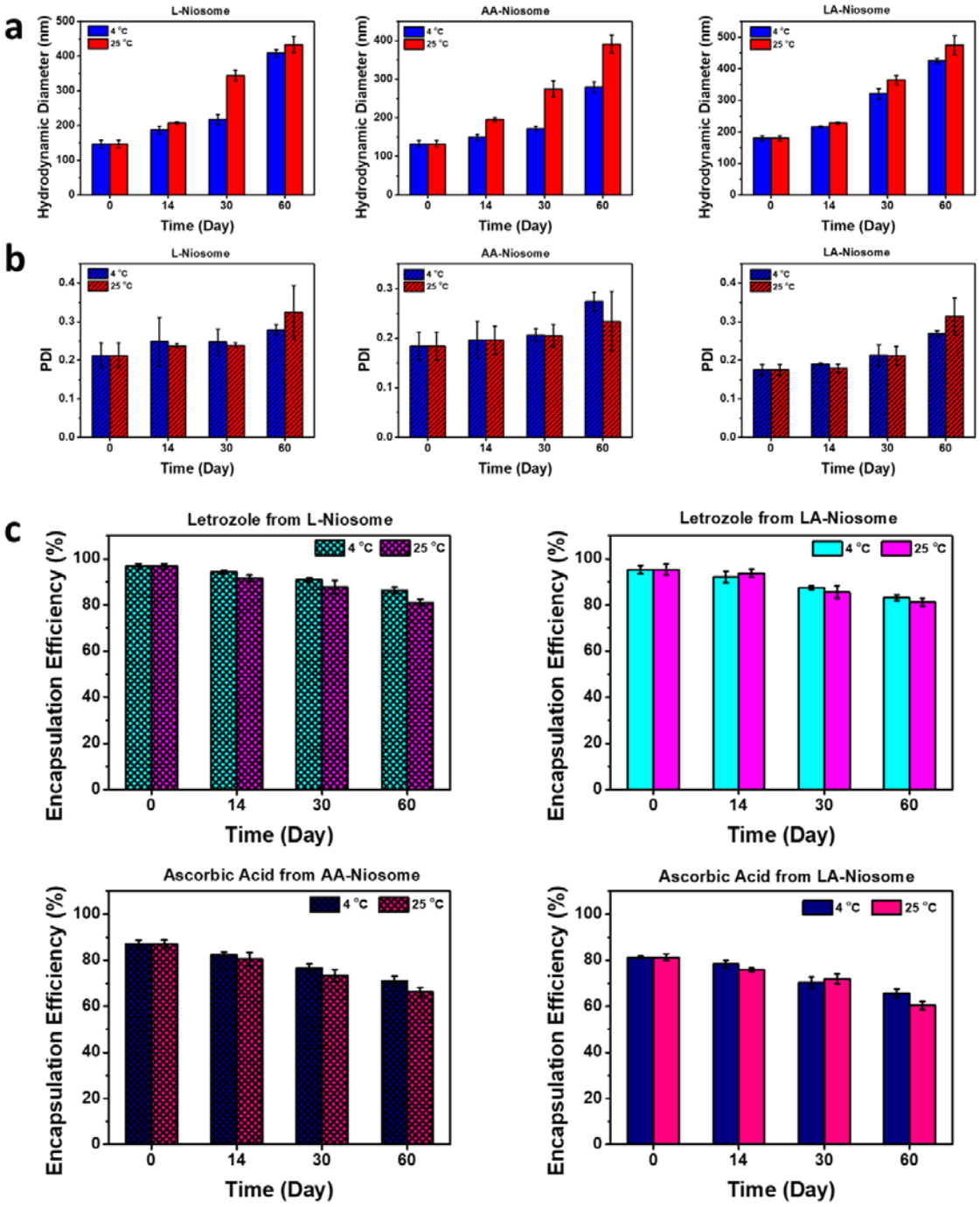

Figure S5. Stability of optimum formulations stored (L-Niosome, A-Niosome and LA-Niosome) during 2 months of storage at 4 ± 2 °C, and 25 ± 2 °C (Data presented as average ± SD) based on changes in their hydrodynamic diameter (a) and polydispersity index (b). c) The change in encapsulation efficiency of letrozole (top row) and ascorbic acid (bottom row) from different niosomal formulations.

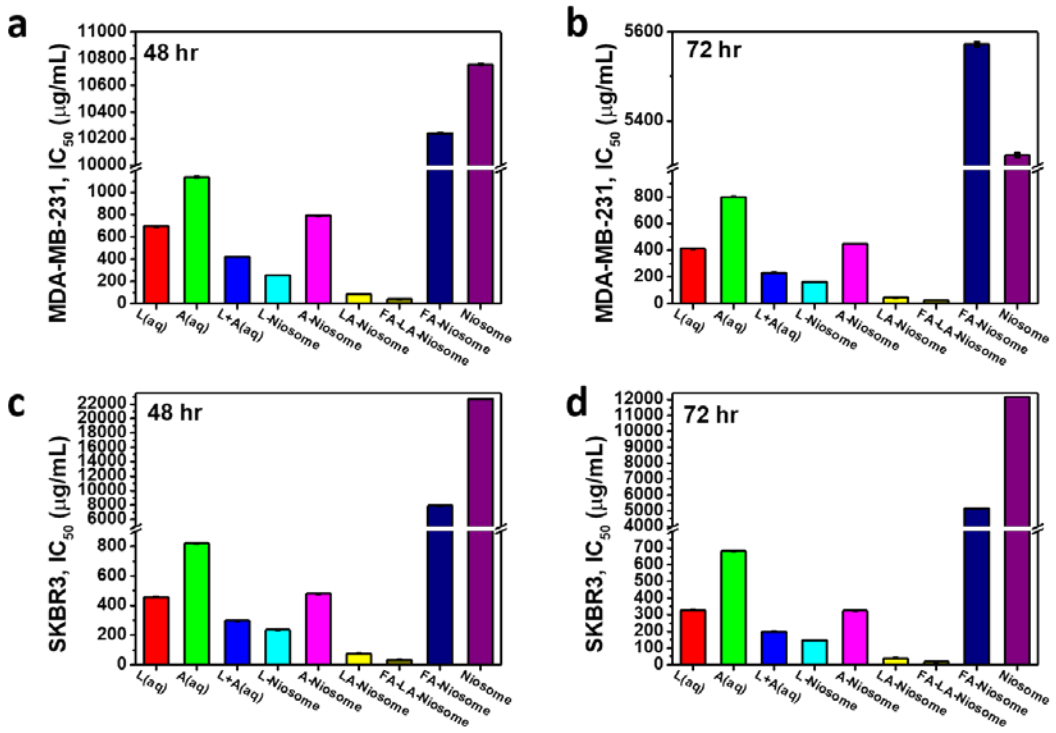

Figure S6. Comparison of IC_{50} value (y-axis) in different samples (x-axis from left to right: L(aq), A(aq), L+A(aq), L-Niosome, A-Niosome, LA-Niosome and FA-LA-Niosome, FA-Niosome, and Niosome). The results were obtained at 48hr and 72 hr in the MDA-MB-231(a, b) and SKBR3(c,d) cell lines respectively using MTT assay. Equal concentrations of drugs were used for the measurement of IC_{50} values for the respective niosomal drug formulation.

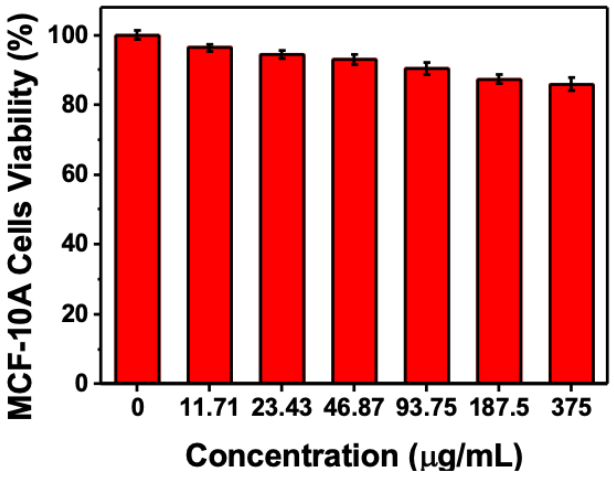

Figure S7. The viability of MCF-10 cell lines after incubation with different concentrations of FA-LA-Niosome for 72 hr. The concentration (X-axis value) refers to concentration of drugs that could potentially be loaded into the niosomal nanocarriers.