Dual drug-loaded topical delivery of photodynamically active lipid-based formulation for combination therapy of cutaneous melanoma

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SUPPLEMENTARY MATERIAL

Table T1. Design and characterizations of Dual drug loaded lipidic nanocarriers (DDLN)

Batch No.	Independent variables			Response variables	
	Lipid (mg)	Surfactant (%)	Sonication (min)	Size (nm)	Entrapment Efficiency (%)
1	100	1.50	5	65.33 ± 1.53	45.41 ± 1.52
2	200	1.50	1	214.67 ± 5.03	65.97 ± 1.97
3	100	2.50	3	57.83 ± 1.61	67.47 ± 0.64
4	150	0.50	5	174.33 ± 4.51	45.43 ± 1.19
5	150	1.50	3	84.17 ± 2.02	75.63 ± 2.16
6	150	1.50	3	80.87 ± 2.01	75.60 ± 0.88
7	150	1.50	3	92.90 ± 2.89	71.00 ± 1.74
8	150	2.50	1	193.90 ± 4.55	59.53 ± 0.60
9	150	1.50	3	88.67 ± 3.51	72.78 ± 0.66
10	150	0.50	1	220.00 ± 5.57	43.83 ± 0.35
11	200	2.50	3	89.23 ± 3.61	80.20 ± 2.30
12	150	1.50	3	94.10 ± 4.48	73.80 ± 0.61
13	100	1.50	1	158.67 ± 6.51	45.77 ± 0.67
14	150	2.50	5	54.07 ± 3.66	62.07 ± 0.75
15	100	0.50	3	102.13 ± 3.07	43.73 ± 0.40
16	200	0.50	3	198.23 ± 3.25	67.83 ± 0.58
17	200	1.50	5	90.27 ± 1.59	80.73 ± 3.19

Table T2. ANOVA for regression coefficients for Box Behnken designed effects (linear, quadratic, and interaction) against the CQAs i.e., dependent variables (particle size and entrapment efficiency) to establish the best fitted quadratic equation.

Effect	Variable	Particle size		Entrapment efficiency	
		F- value	P- value	F- value	P- value
Linear effect	**A**	58.37	< 0.0001	118.86	< 0.0001
	B	142.77	< 0.0001	65.71	< 0.0001
	C	236.42	< 0.0001	5.54	< 0.0001
Interaction effect	**AB**	11.64	0.0077	5.21	0.0484
	BC	20.70	0.0014	-	-
	AC	-	-	6.29	0.0334
Quadratic term	**A2**	-	-	-	-
	B2	33.81	0.0003	28.10	0.0005
	C2	97.00	< 0.0001	81.11	< 0.0001

Table T3. Validation batch by numerical method

Responses	Predicted results	Actual results	% Relative
Particle size (nm)	92.12 ± 4.25	94.50 ± 4.58	-0.761
Entrapment efficiency (%)	77.66 ± 1.57	78.73 ± 1.31	-1.382

Table T4. Release mechanism of DDLN formulation and free drugs by curve fitting method.

Kinetic model	**DDLN curcumin**				**Free drug curcumin**			
	R^2	**k**	**AIC**	**MSC**	**R^2**	**k**	**AIC**	**MSC**
Zero order	0.6480	4.962	65.0120	0.7940	0.8167	9.599	53.3398	1.0529
First order	0.9937	0.122	32.7659	4.8248	0.9790	0.213	38.1573	3.2219
Higuchi	0.9333	19.934	51.6998	2.4580	0.9269	27.710	46.9003	1.9729
Koresmeyer peppas	0.9386 n = 0.548	17.727	53.0350	2.2911	0.9408 n= 0.608	22.443	47.4264	1.8977
Kinetic model	**DDLN chlorin e6**				**Free drug chlorin e6**			
	R^2	**k**	**AIC**	**MSC**	**R^2**	**k**	**AIC**	**MSC**
Zero order	0.8448	4.914	58.7186	1.6129	0.9740	13.781	33.8377	2.9149
First order	0.9885	0.103	37.9272	4.2118	0.9545	0.272	37.2053	2.3536
Higuchi	0.9434	19.144	50.6484	2.6217	0.9416	32.899	38.6994	2.1046
Koresmeyer peppas	0.9817 n = 0.647	13.284	43.6279	3.4992	0.9971 n= 0.778	20.663	22.7725	4.7591

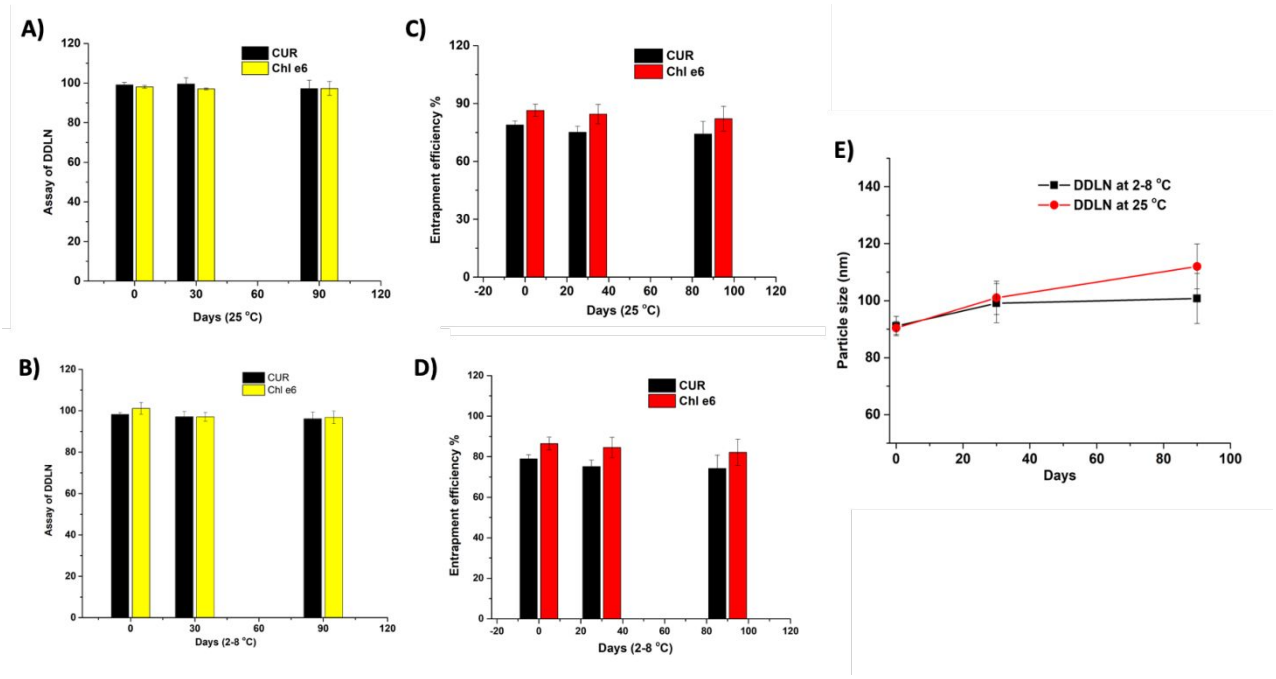

Figure F1. Stability data of DDLN Gel at different storage conditions. Assay of DDLN gel at 25 ^{0}C for 90 days A); Assay of DDLN gel at 2-8 ^{0}C for 90 days B); Entrapment efficiency of DDLN formulation containing CUR and Chl e6 at 25 ^{0}C for 90 days C); Entrapment efficiency of DDLN formulation containing CUR and Chl e6 at 2-8 ^{0}C for 90 days D); DDLN particle size data for 90 days at 2-8 ^{0}C and 25 ^{0}C represents in graph E).