

Supporting Information

Nanostructured lipid carriers (NLC)-based novel hydrogels as potential carriers for nepafenac applied after cataract surgery for the treatment of inflammation: design, characterization and in vitro cellular inhibition and uptake studies

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Table S1 Independent variables and their corresponding levels in NP-NLC preparation.

Independent variables	Levels				
	-1.682	-1	0	1	1.682
X ₁ : nepafenac concentration (%)	0.07	0.08	0.10	0.12	0.13
X ₂ : liquid lipid concentration (%)	0.56	0.70	0.90	1.10	1.24
X ₃ : the CRE/SOY ratio	1.16	1.50	2.00	2.50	2.84

A three-factor, five-level central composite design was generated to optimize the NP-NLC preparation. The study design involved investigating the effects of three independent variables, including the NP concentration (X₁), the MIG concentration (X₂), and the CRE/SOY ratio (X₃). The dependent variables were the mean particle size (PS, Y₁), polydispersity index (PI, Y₂) and entrapment efficiency (EE, Y₃).

Table S2 Coded levels and measured responses for the 20 experiment formulation runs.

	Coded levels of factors			Measured responses		
	X ₁	X ₂	X ₃	Y ₁	Y ₂	Y ₃
Factorial points						
1	-1	-1	-1	99.75±2.02	0.27±0.02	95.01±0.34
2	+1	-1	-1	156.18±2.72	0.48±0.08	59.39±1.89
3	-1	+1	-1	134.25±1.45	0.35±0.01	84.79±1.03
4	-1	-1	+1	156.47±2.88	0.59±0.00	63.54±1.20
5	+1	+1	-1	97.31±0.87	0.23±0.04	91.07±0.91
6	+1	-1	+1	100.01±1.98	0.43±0.00	84.25±0.76
7	-1	+1	+1	112.13±3.01	0.41±0.02	75.46±1.26
8	+1	+1	+1	107.79±1.36	0.31±0.02	78.43±0.45
Axial points						
9	-1.682	0	0	113.95±1.34	0.31±0.01	78.43±0.33
10	+1.682	0	0	152.85±5.22	0.37±0.03	55.29±2.03
11	0	-1.682	0	95.79±1.11	0.33±0.03	95.03±0.33
12	0	+1.682	0	126.91±2.09	0.36±0.01	88.43±0.42
13	0	0	-1.682	156.83±1.83	0.58±0.04	82.13±0.84
14	0	0	+1.682	91.37±1.73	0.31±0.02	92.73±0.69
Centre points						
15	0	0	0	110.43±1.88	0.30±0.04	85.47±1.03
16	0	0	0	103.15±2.35	0.30±0.00	84.27±0.89
17	0	0	0	105.65±1.45	0.32±0.02	84.78±1.24
18	0	0	0	107.55±2.02	0.31±0.03	85.41±1.30
19	0	0	0	107.49±1.82	0.31±0.01	86.03±1.39
20	0	0	0	105.65±1.56	0.30±0.04	85.13±0.95

X₁ is factor of nepafenac concentration (%), X₂ is factor of liquid lipid concentration (%), and X₃ is factor of the CRE/SOY ratio. Y₁ is response of the mean particle size (PS), Y₂ is response of polydispersity index (PI) and Y₃ is response of entrapment efficiency (EE).

Table S3 Fit summary for of measured responses.

	Sum of squares			df			F-value			P-value (prob>F)					
	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃			
sequential model sum of squares															
Linear	6567.20	0.08	868.15	3	3	3	13.15	4.58	3.21	0.0001	0.0169	0.0511			
2F1	1037.08	0.027	636.32	3	3	3	2.76	1.71	3.43	0.0843	0.2134	0.0494			
Quadratic	1403.96	0.037	784.20	3	3	3	20.99	4.14	127.30	0.0001	0.0377	<0.0001			
Cubic	154.81	0.030	10.26	4	4	4	3.41	123.53	1.50	0.0880	<0.0001	0.3130			
Residual	68.12	0.0004	10.27	6	6	6	-	-	-	-	-	-			
Total	283400	2.74	137600	20	20	20	-	-	-	-	-	-			
	Sum of squares			df			F-value			P-value (prob>F)					
	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃			
Lack-of-fit															
Linear	2633.91	0.093	1439.20	11	11	11	39.84	127.28	353.60	0.7544	0.6598	0.6093			
2F1	1596.83	0.067	802.88	8	8	8	33.21	125.25	271.23	0.8216	0.7234	0.6402			
Quadratic	192.87	0.030	18.68	5	5	5	6.42	88.78	10.10	0.8912	0.7891	0.6731			
Cubic	38.06	0.00003	8.42	1	1	1	6.33	0.39	22.77	0.7834	0.5618	0.4250			
Pure	30.05	0.00033	1.85	5	5	5	-	-	-	-	-	-			
Error															
	R-squared			Adjusted R-square			Predicted R-square			PRESS			Std. deviation		
	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃
Model summary statistics															
Linear	0.7114	0.4618	0.3760	0.6573	0.3609	0.2589	0.5206	0.0727	-0.1327	4425.55	0.16	2615.57	12.90	0.077	9.49
2F1	0.8238	0.6143	0.6515	0.7424	0.4363	0.4907	0.5929	-0.5210	0.2807	3758.24	0.26	1661.10	11.19	0.072	7.87
Quadratic	0.9759	0.8281	0.9911	0.9541	0.6733	0.9831	0.8211	-0.4174	0.9354	1651.69	0.25	149.10	4.72	0.055	1.43
Cubic	0.9926	0.9979	0.9956	0.9766	0.9935	0.9859	0.0864	0.9647	0.1947	8433.93	0.006	1859.56	3.37	0.008	1.31

df: degree of freedom; PRESS: predicted residual error sum of squares. Y₁: average particle size; Y₂: PI; Y₃: encapsulation efficiency.

Table S4 ANOVA for response surface quadratic model of measured responses.

	Sum of squares			df			F-value			P-value (prob>F)		
	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃	Y ₁	Y ₂	Y ₃
ANOVA												
NP% (X ₁)	1485.47	0.031	593.86	1	1	1	69.40	10.37	289.20	<0.0001	0.0092	<0.0001
MIG% (X ₂)	881.62	0.007	61.67	1	1	1	41.19	2.21	30.03	<0.0001	0.1681	0.0003
C/S (X ₃)	4200.11	0.043	212.63	1	1	1	196.23	14.29	103.55	<0.0001	0.0036	<0.0001
Residual	235.44	0.030	20.53	11	10	10	-	-	-	-	-	-
Lack-of-fit	205.39	0.030	18.68	6	5	5	5.70	88.78	10.10	0.8379	0.7899	0.6120
Pure error	30.05	0.0003	1.85	5	5	5	-	-	-	-	-	-

Y₁: average particle size; Y₂: PI; Y₃: encapsulation efficiency.