

Table 1s: Design of experiments using the central composite design method to optimize the NLCs. (Data are represented as Mean $\pm$ SD, n = 3).

Row	Lipid (W/W%): A	*Lipid ratio: B	Surfactant (W/W%): C	Tween 20 /Span 80: D	Pomegranate Essential oil (W/W%): E	Size (nm) $\pm$ SD	Zeta Potential (mV) $\pm$ SD	Dispersion Index $\pm$ SD
1	4	4	2	1.25	2	252 $\pm$ 3.31	-21.2 $\pm$ 2.52	0.358 $\pm$ 0.021
2	4	4	2	1.25	3	240 $\pm$ 6.31	-22.1 $\pm$ 5.13	0.352 $\pm$ 0.035
3	5	5	1	2	1	521 $\pm$ 5.48	-23 $\pm$ 3.21	0.490 $\pm$ 0.041
4	4	4	2	1.25	2	250 $\pm$ 6.27	-21 $\pm$ 4.35	0.352 $\pm$ 0.045
5	5	5	1	0.5	3	518 $\pm$ 3.81	-22.2 $\pm$ 8.78	0.485 $\pm$ 0.038
6	4	4	2	1.25	2	251 $\pm$ 8.32	-21.1 $\pm$ 2.92	0.350 $\pm$ 0.057
7	4	3	2	1.25	2	248 $\pm$ 5.92	-22.3 $\pm$ 2.87	0.355 $\pm$ 0.038
8	5	4	2	1.25	2	482 $\pm$ 2.12	-21.5 $\pm$ 2.34	0.430 $\pm$ 0.057
9	5	5	3	0.5	1	420 $\pm$ 3.18	-21.4 $\pm$ 2.55	0.420 $\pm$ 0.048
10	4	10	2	1.25	2	258 $\pm$ 4.85	-22.3 $\pm$ 2.87	0.349 $\pm$ 0.035
11	3	3	3	2	3	151 $\pm$ 5.94	-21.7 $\pm$ 3.50	0.231 $\pm$ 0.039
12	4	4	1	1.25	2	282 $\pm$ 4.72	-21.8 $\pm$ 3.55	0.380 $\pm$ 0.027
13	4	4	2	0.5	2	245 $\pm$ 3.70	-21.3 $\pm$ 4.87	0.352 $\pm$ 0.028
14	5	3	3	2	1	415 $\pm$ 2.87	-22.1 $\pm$ 3.52	0.418 $\pm$ 0.039
15	5	3	3	0.5	3	418 $\pm$ 5.95	-22.2 $\pm$ 4.95	0.420 $\pm$ 0.048
16	3	5	3	0.5	3	158 $\pm$ 6.91	-22.6 $\pm$ 3.78	0.228 $\pm$ 0.075
17	3	5	3	2	1	150 $\pm$ 4.98	-22.6 $\pm$ 4.72	0.218 $\pm$ 0.068
18	5	3	1	2	3	521 $\pm$ 9.95	-21.5 $\pm$ 3.95	0.490 $\pm$ 0.069
19	4	4	2	1.25	2	251 $\pm$ 2.32	-21.3 $\pm$ 3.25	0.351 $\pm$ 0.071
20	4	4	2	1.25	2	252 $\pm$ 3.81	-21.2 $\pm$ 3.87	0.350 $\pm$ 0.065
21	4	4	3	1.25	2	250 $\pm$ 5.95	-21.1 $\pm$ 2.91	0.353 $\pm$ 0.077
22	4	4	2	1.25	1	253 $\pm$ 3.90	-22.0 $\pm$ 2.35	0.357 $\pm$ 0.062
23	3	3	1	0.5	1	189 $\pm$ 8.34	-22.3 $\pm$ 2.38	0.250 $\pm$ 0.068
24	4	4	2	2	2	248 $\pm$ 4.95	-22.1 $\pm$ 4.30	0.353 $\pm$ 0.069
25	3	4	2	1.25	2	171 $\pm$ 2.38	-22.3 $\pm$ 3.98	0.235 $\pm$ 0.038
26	3	5	1	2	3	190 $\pm$ 4.87	-21.8 $\pm$ 3.71	0.250 $\pm$ 0.071

*Stearic acid/ grape seed oil

Statistical evaluations

Following model fitting, statistically insignificant terms ($p > 0.05$) were removed using backward elimination to obtain a reduced quadratic model. Thus, particle size (Y) was significantly affected by independent factors A, C and E.

$$Y = 253.17 + 150.81A - 47.83C + 24.17E - 29.57AC + 11.93AE - 38.21BC + 49.29BE - 13.67CD + 13.33DE + 71.83A^2 + 11.33C^2 - 8.17D^2 - 8.17E^2$$

Table 2s : Variance analysis of the quadratic polynomial model for particle size.

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value	
Model	3.528E+05	13	27141.86	1110.67	< 0.0001	significant
A-Lipid (W/W%)	1.955E+05	1	1.955E+05	8000.16	< 0.0001	
C-Surfactant	1556.68	1	1556.68	63.70	< 0.0001	
E-Pomegranate Essential oil (W/W%)	397.34	1	397.34	16.26	0.0020	
AC	2352.31	1	2352.31	96.26	< 0.0001	
AE	382.60	1	382.60	15.66	0.0022	
BC	315.13	1	315.13	12.90	0.0042	
BE	524.44	1	524.44	21.46	0.0007	
CD	493.89	1	493.89	20.21	0.0009	
DE	470.07	1	470.07	19.24	0.0011	
A ²	13280.51	1	13280.51	543.45	< 0.0001	
C ²	330.53	1	330.53	13.53	0.0036	
D ²	171.71	1	171.71	7.03	0.0226	
E ²	171.71	1	171.71	7.03	0.0226	

Zeta potential:

No statistically significant polynomial relationship was observed between the studied formulation variables and zeta potential within the investigated experimental domain.

Polydispersity Index:

The reduced quadratic model developed for PDI was highly significant ($p < 0.0001$) with very low residual error.

$$Y = 0.3484 + 0.1226A - 0.0029B - 0.0415C - 0.0050D + 0.0026E + 0.0352AB - 0.0129AC + 0.0081AD + 0.0103AE - 0.0392BC - 0.0077BD + 0.0072BE - 0.0137CD - 0.0077CE + 0.0013DE - 0.0206A^2 + 0.0035B^2 + 0.0134C^2 - 0.0006D^2 + 0.0014E^2$$

Table 3s : Variance analysis of the quadratic polynomial model for particle PDI.

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value	
Model	0.1587	20	0.0079	1734.34	< 0.0001	significant
A-Lipid (W/W%)	0.0034	1	0.0034	736.19	< 0.0001	
B-*Lipid ratio	0.0000	1	0.0000	3.66	0.1284	
C-Surfactant	0.0004	1	0.0004	84.25	0.0008	

D-Tween 20 /Span 80	5.573E-06	1	5.573E-06	1.22	0.3317	
E-Pomegranate Essential oil (W/W%)	1.558E-06	1	1.558E-06	0.3405	0.5909	
AB	0.0001	1	0.0001	26.48	0.0068	
AC	0.0002	1	0.0002	44.02	0.0027	
AD	0.0001	1	0.0001	17.04	0.0145	
AE	0.0001	1	0.0001	27.89	0.0062	
BC	0.0002	1	0.0002	32.83	0.0046	
BD	5.775E-06	1	5.775E-06	1.26	0.3241	
BE	5.062E-06	1	5.062E-06	1.11	0.3523	
CD	0.0002	1	0.0002	49.27	0.0022	
CE	0.0001	1	0.0001	15.56	0.0169	
DE	2.044E-06	1	2.044E-06	0.4465	0.5406	
A ²	0.0011	1	0.0011	238.64	0.0001	
B ²	3.553E-06	1	3.553E-06	0.7765	0.4280	
C ²	0.0005	1	0.0005	100.51	0.0006	
D ²	9.837E-07	1	9.837E-07	0.2150	0.6670	
E ²	4.901E-06	1	4.901E-06	1.07	0.3592	

Entrapment efficiency

To determine the encapsulation efficiency and the percentage of drug loading in the nanocarriers, an indirect method was employed. For this purpose, a centrifuge and a filter-equipped Falcon tube (Amicon) with a cutoff of 10 kDa were used. Different concentrations of pomegranate essential oil, including 0.5, 1, 1.5, 2, and 2.5 mg.mL⁻¹, were prepared, and their absorbance was measured at the wavelength of 278 nm using UV-Vis spectroscopy. A calibration curve (Figure 1s) was plotted and then, to calculate the loading percentage, 0.5 mL of the selected formulation was transferred to the filter-equipped Falcon tube and centrifuged at 6000 rpm for 30 minutes. The supernatant was collected for further analysis. In the subsequent step, the absorbance of the supernatant was measured, and the amount of pomegranate essential oil in the supernatant was calculated using the calibration curve.

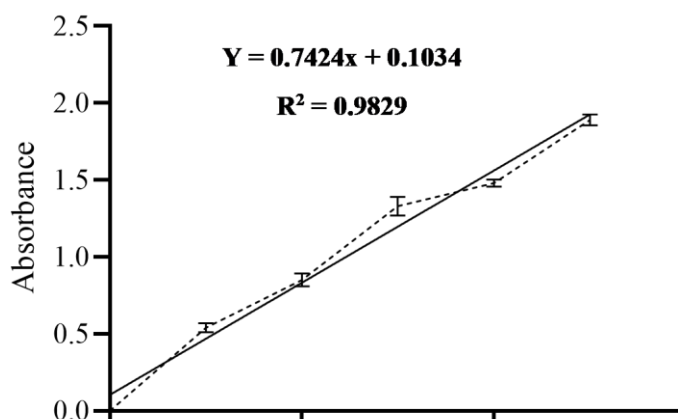


Figure 1s: UV calibration curve of pomegranate essential oil at 278 nm.