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Supplementary file S1: pilot study for the preparation and optimization of dasatinib-loaded bilosomes (DST-LB) formulas

The formulation step used the Box-Behnken design to optimize Tween 60, Span 60, cholesterol, DSC, and Soluplus, yielding 46 formulas. The resulting formulas, PS, PDI, EE, and the *in vitro* release study for the optimal formula, were the parameters used to choose the optimized DST-LB formula. The Box-Behnken statistical design with five factors (3^5), three levels (-1, 0, 1), and 45 runs was chosen for the optimization analysis. The experimental design theoretically consists of points at the midpoint of each edge and the replicated center point of the multidimensional cube.

The dependent and independent variables in the experimental design of the D-LB, formulated with cholesterol, Span 60, Tween 60, SDC, and Soluplus, are presented in Table S1. Using the software Minitab® 17.1.0, the following equation (quadratic model) was applied for designing the different formulae:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{55} x_5^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{15} x_1 x_5 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{25} x_2 x_5 + \beta_{34} x_3 x_4 + \beta_{35} x_3 x_5 + \beta_{45} x_4 x_5$$

The goal of the DST-LP optimization was to set PS (Y1) to 100 nm, PDI (Y2) to 0.2%, and EE (Y3) to ≤0.8.

Table S1: Independent and dependent variables applied in a Box-Behnken design

Factors	Independent variables		Low (-1)	Medium (0)	High (+1)
X_1	Cholesterol	mg	60	120	180
X_2	Tween 60	mg	50	100	150
X_3	Span 60	mg	100	200	300
X_4	SDC	mg	5	10	15
X_5	Soluplus	mg	50	75	100
Response					
Y_1	Particle Diameter	mm			
Y_2	PDI	%			
Y_3	Encapsulation Efficiency (EE)	%			

All batches of the formulations were produced as scheduled and evaluated for varied responses. The current study employed ANOVA (Analysis of Variance) to examine each variable individually and in conjunction with other responses to enhance the outcomes. The Box-Behnken Design was employed, and outcomes were examined and validated using a significant coefficient with R^2 values throughout the entire experimental region. Furthermore, optimal checkpoint formulations were developed and assessed to identify the optimized formula for the specified experimental domain and equations.

In the current study, optimization was carried out using the Box-Behnken design (BBD), with 46 formulas (3^5) evaluated across five factors at three levels. Table S2 illustrates the results of each formula in terms of response parameters (PS, EE, and PDI), in which PS ranged from 89.8±1.527 to 314.3±1.886 nm, PDI ranged from 0.05±0.001 to 0.515±0.010%, and EE ranged from 0.73±0.004 to 0.95±0.019%.

Table S2: Experimental design and response of DST-LB

F	Span 60	Tween 60	Cholesterol	SDC	Soluplus	PS	PDI	EE%
1	300	100	120	10	50	291±1.746	0.3±0.042	0.91±0.006
2	200	100	120	10	75	89.8±1.527	0.23±0.031	0.82±0.014
3	100	100	180	10	75	111.3±1.557	0.08±0.032	0.79±0.009
4	200	100	120	15	100	151.8±1.670	0.29±0.061	0.85±0.006
5	100	150	120	10	75	106.2±1.381	0.26±0.022	0.82±0.009
6	300	100	120	10	100	268.1±5.094	0.49±0.061	0.92±0.013
7	100	100	120	10	100	139.1±1.947	0.35±0.022	0.76±0.013
8	100	50	120	10	75	205.3±2.874	0.33±0.024	0.77±0.008
9	100	100	120	5	75	175.5±2.984	0.24±0.041	0.8±0.006
10	200	50	60	10	75	172.8±1.901	0.41±0.061	0.73±0.004
11	200	150	180	10	75	97.1±1.262	0.05±0.019	0.82±0.014
12	100	100	120	10	50	123.3±1.356	0.47±0.032	0.75±0.012
13	200	100	120	10	75	94±1.410	0.12±0.028	0.83±0.005
14	200	50	120	10	50	139.1±2.226	0.35±0.033	0.78±0.004
15	200	50	120	10	100	184.3±2.765	0.25±0.042	0.76±0.008
16	200	50	120	15	75	271.3±1.899	0.38±0.044	0.77±0.015
17	200	100	180	10	50	275.5±3.031	0.31±0.052	0.89±0.009
18	200	100	180	10	100	211.8±3.601	0.43±0.061	0.88±0.012
19	200	150	120	15	75	139.1±0.835	0.35±0.053	0.84±0.004
20	200	150	60	10	75	111.6±2.009	0.37±0.063	0.84±0.017
21	100	100	60	10	75	101.4±1.014	0.21±0.034	0.78±0.004
22	200	100	180	5	75	255.4±3.831	0.46±0.052	0.87±0.007
23	200	100	60	10	50	114.7±1.262	0.31±0.037	0.76±0.013
24	200	100	60	10	100	112.1±1.561	0.37±0.024	0.76±0.012
25	200	100	120	5	100	116.4±1.513	0.21±0.042	0.81±0.016
26	200	100	60	15	75	130.4±1.956	0.33±0.024	0.75±0.008
27	200	100	120	10	75	101.9±2.713	0.23±0.024	0.83±0.012
28	200	100	120	10	75	94.2±1.12	0.27±0.072	0.86±0.009
29	200	50	180	10	75	314.3±1.886	0.51±0.044	0.8±0.016
30	200	150	120	10	100	144.2±2.451	0.2±0.029	0.85±0.009
31	200	100	120	10	75	92.9±1.9	0.23±0.043	0.84±0.015
32	300	100	60	10	75	159.3±1.274	0.3±0.044	0.9±0.009
33	200	100	120	15	50	170.8±3.074	0.27±0.044	0.85±0.017

34	100	100	120	15	75	191.5±1.724	0.07±0.019	0.81±0.015
35	200	50	120	5	75	199.3±1.997	0.28±0.034	0.77±0.009
36	300	100	120	15	75	289.8±5.796	0.37±0.043	0.92±0.010
37	300	100	180	10	75	206.4±2.270	0.11±0.029	0.95±0.019
38	200	100	60	5	75	120.8±2.054	0.47±0.080	0.75±0.010
39	300	100	120	5	75	174±2.436	0.07±0.019	0.91±0.013
40	300	150	120	10	75	110.8±2.216	0.24±0.032	0.79±0.012
41	200	150	120	10	50	180±1.901	0.28±0.047	0.89±0.013
42	200	100	120	5	50	119.2±1.550	0.35±0.037	0.82±0.015
43	300	50	120	10	75	120±2.400	0.2±0.032	0.84±0.017
44	200	100	180	15	75	278.1±3.893	0.52±0.018	0.91±0.009
45	200	150	120	5	75	178.5±2.856	0.25±0.041	0.86±0.008
46	200	100	120	10	75	94.7±1.8	0.23±0.019	0.87±0.010

Data presented as mean± SD, n=3

The optimized formula was selected via numerical optimization in Minitab 17.1.0, based on the desirability factor's proximity to 1. The predicted cholesterol (x1 = 95.1515 mg), Tween 60 (x2 = 150 mg), Span 60 (x3 = 110.083 mg), SDC (x4 = 12.2727 mg), and Soluplus (x5 = 100 mg) were acquired, and this was selected as the optimized formula with a desirability of 0.9914, as seen in Figure S1.

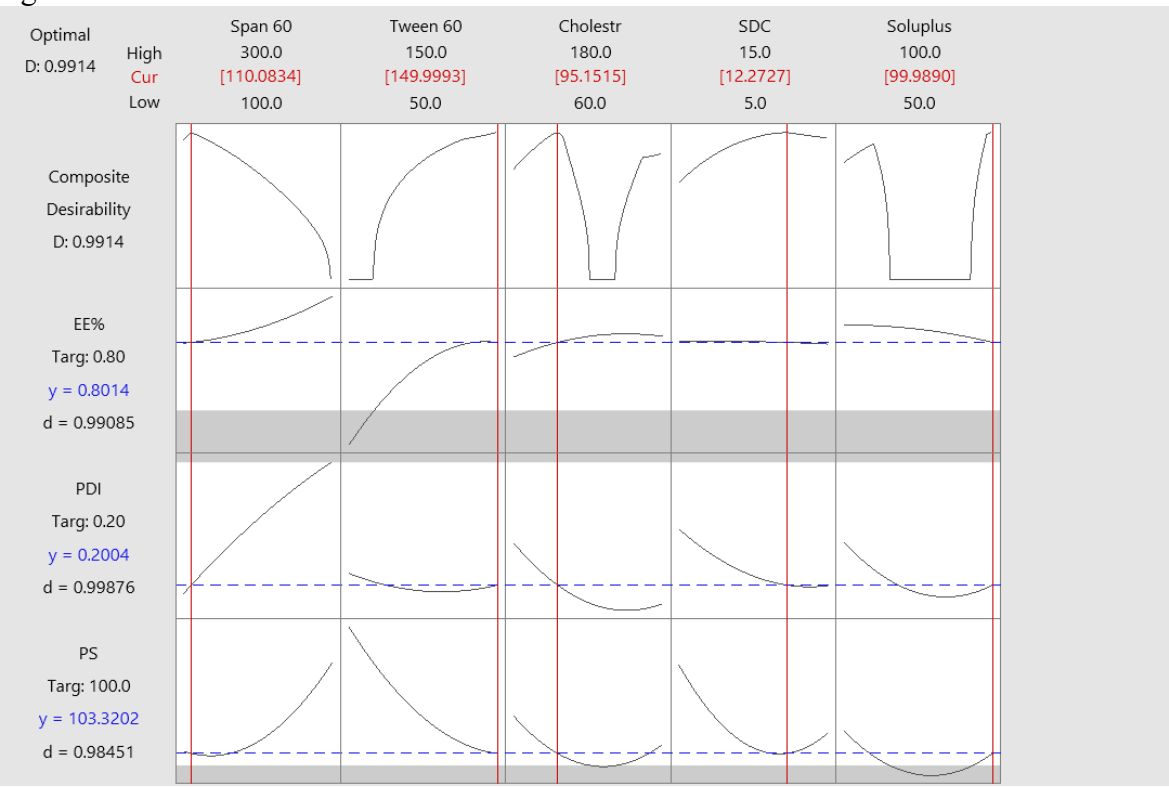

Figure S1: The desirability value for optimized formulation