Nose to brain targeting of donepezil nanostructured lipid carrier insitu gel: Formulation, *In-vitro*, *Ex-vivo*, *in- vivo* pharmacokinetic and Pharmacodynamic characterizations

Table S1. Summary of results of regression analysis for responses Y1, Y2, Y3

Result of Analysis of variance	Particle size (nm)	Drug Loading (%)	Entrapment Efficiency (%)
Regression			
Sum of squares	3.139E+05	4447.51	815.44
Degree of freedom	11	11	11
Mean squares	28537.35	28404.32	74.13
F-value	16.47	15.25	34.32
P-value	0.0206	0.0504	0.041
Inference	significant	significant	significant
Lack of fit test			
Sum of squares	98711.03	1310.70	99.60
Degree of freedom	3	3	3
Mean squares	32903.68	436.90	33.20
R^2	0.9837	0.9506	0.9921
Correlation of validation (% CV)	2.05	2.3	1.58
Adequate Precision	15.2578	6.8979	19.3224
Residual			
Sum of squares	5196.90	231.23	6.48
Degree of freedom	3	3	3
Mean squares	1732.30	77.08	2.16
Standard deviation	41.62	8.78	1.47

Data optimization and response surface methodology validation (RSM)

Based on the values of characteristic parameters like particle size, drug loading and entrapment efficiency the DPZ NLC6 was chosen for further optimization by point prediction i.e., further small changes were made in independent constraints either in single constraints or more than one together. By setting constraints for particle size as minimum, drug loading and entrapment efficiency as maximum the optimization by point prediction was performed . The composition of the batch was 75:25(ratio of solid to liquid lipid), 0.9% surfactant and 12HPH cycle .The value of particle size, drug loading and entrapment efficiency for the selected batch was 112.5± 2.44nm, 58.0±2.13% and 98.7± 4.01%respectively. The obtained size of DPZ- NLC- OPT is appropriate for brain targeting as reported in the previous literature (≤ 200 nm is suitable for the nose to brain delivery). The zeta potential value for DPZ- NLC- OPT shows the highest value i.e.-23.2mV amongst all batches. The model validity was estimated with desirability value 1.

Table S2. Point prediction check point for optimization

Batch	Composition	Actual value			Predicted value		
	SL:LLratio / Surf (%)/ HPH cycle	Particle Size(nm) Y1	Drug Loading (%) Y2	Entrapment Efficiency (%) Y3	Particle Size (nm) Y1	Drug Loading (%) Y2	Entrapment Efficiency (%) Y3
DPZ-NLC6	70:30 /1 /10	123±2.05	57.99±2.02	98.5±3.17	123	57.99	98.5
DPZ-NLCOPT1	75:25 /0.9 /12	112.5± 2.44	58.0±2.13	98.7± 4.01	78.41	59.65	99.45

Table S3**.** In-vitro release kinetic parameters.

Formulation	Zero order		First order		Higuchi model		Korsmeyer –peppas	
DPZ-NLC-OPT	R^2	K0(h^{-1})	R^2	K0(h^{-1})	R^2	K0(h^{-1})	R^2	K0(h^{-1})
	0.7784	3.6009	0.965	0.3828	0.977	10.954	0.9512	0.3556
Pure drug DPZ	0.9383	3.5536	0.8818	0.3499	0.9708	15.492	0.9387	0.2954