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Research Article

DEVELOPMENT, CHARACTERIZATION AND OPTIMIZATION OF SOLID LIPID NANOPARTICLES FROM MICROEMULSION TECHNIQUE USING A BOX-BEHNKEN DESIGN

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ABSTRACT

The objective of this study was to formulate and optimize PA loaded SLNs usingvarious solid lipids. The SLNs were prepared from phase behaviours of hot microemulsions-ultra probe sonication technique.The phase diagrams were prepared from lipid/Smix at 60^{o}c using prosimsoftware. The analogy between design factors and experimental data was studied using response surface methodology(RSM). A statistical technique of RSM with Box-Behnken design wasframed to study and determine the influence of formulation independent factors including a solid lipid (x_1), surfactant/co-surfactant ratio (x_2), sonication time (x_3). The dependent factors were particle size, entrapment efficiency(%EE). Tristearin showed better stability and low particle size hence selected for the study. The prepared nanoparticles were in a spherical shape and average particle size of 24.69 nm andthe polydispersity index, zeta potential and %EE range of -0.274, -28.47mV & 89.82% respectively. In Vitro diffusion studies showed a burst release at the initial stage followed by prolonged release of PA from SLNs up to 15hrs and drug diffusion found to be 94.85%. The release kinetics of the optimized formulation was best fitted the zero order model (R^2- 0.9938).These results concluded that the prednisolone acetate –loaded SLNs could potentially be exploited as a delivery system with improved drug entrapment efficiency, low mean particle size and controlled drug release.

INDRODUCTION

Solid lipid nanoparticles (SLNs) showed better advantages compared to existing drug , delivery systems. The benefits of SLNs are sustained release, increased loading capacity, incorporation of both hydrophobic and hydrophilic drugs etc. Several methods are available for the production of SLNs among them microemulsion method was highly suitable for SLNs preparation at laboratory level. In order to reduce the number of experiments and improve the productivity the SLNs was prepared as per response surface method (RSM). The present research work utilizedbiocompatible solid lipids were monostearin (GMS), pearl stearic (stearic acid), palmitate (palmitic acid), cetylpalmitate, glycerol tristearate (tristearin), glycerol tripalmitate(tripalmitin), and surfactants as polysorbate 60,polysorbate 80, polysorbate 20, klliphor RH 40 and co-surfactants such as polyglycol400, ethanol. The prepared SLNs were analyzed forsurface charge, surface potential, %EE and drug diffusion.

MATERIALS AND METHODS

Materials

Prednisolone acetate (PA) was procured from Sigma-Aldrich chemical Co; Germany.Cremophor RH40 was purchased BASF certified supplier zeel. Tween 20, 60, 80 and PEG400 were brought from chemisol.

Bangalore. Ethanol was purchased from Hi-media, Secunderabad. Solid lipids were from Bros scientifics,Tirupati. All other chemicals and solvents used were of analytical grade.

Preparation of lipid microemulsions

Among the selected solid lipids tristearin showed low zeta size, better zeta potential and stability hence was selected. Initially pseudoternary phase diagrams were constructed using solid lipid (tristearin), surfactant as polysorbate 60, polysorbate 80, polysorbate 20, Kolliphor RH 40 and co-surfactants such as polyglycol 400, ethanol. The weight ratio of surfactant to co-surfactants ratio of 1:1, 2:1, 3:1, 4:1 and lipid to Smix ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (%w/w). From all these pseudoternary phase diagrams had highest area was selected for further studies. The procedure for the preparation of microemulsion, tristearin kept at 65^{o}cto this melt PA followed by polysorbate 60 and ethanol was added. The similar procedure was followed for the remaining surfactants and co-surfactants.

Preparation of SLNs

The formula for the preparation of SLNs was followed as per Box-Behnken design. The prepared lipid microemulsionwas added to cold water under probe sonicator at 200w amplitude with probe 8mm diameter at different time intervals. The obtained liquid SLNs converted into solid SLNs using ScanVac analyzer and its size, potential and PDI was analyzed by zeta sizer.

Experimental design

The 3^3 Box-Behnkendesign was applied to study the effect of independent variables on dependent variables and shown in Table 2.

Zeta size, potential and PDI

The SLNs size, potential and size was measured using zeta sizermentioned in Table 2.

Encapsulation efficiency

The prepared SLNs were centrifuged and amount of drug which was not incorporated analyzed using UV-Visible spectrophotometer from that drug encapsulated determined.

EE %= [(wtotal- wfree)/wtotal]*100

Wtotal = total amount of drug added
Wfree = not encapsulated drug

***In vitro*drug diffusion studies**

The prepared SLNs (5ml) kept in receptor compartment and samples were withdrawn at different time intervals 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 hrs. The following conditions were used for in vitro diffusion studies.
Diffusion medium : SNES
The run speed: 50 rpm
Volume of diffusion medium: 30mL
Temperature: 37±0.5^{o}c

RESULTS AND DISCUSSION

In the present study, a 17 run, 3-factor, 3-level Box-Behnken design was designed to obtain polynomial equations. The response surface plots and polynomial equations was obtained using Design expert soft ware (Trial version9). Independent factors including a tristearin (X_1), Smix ratio (X_2), sonication time (X_3). The dependent factors were particle size (Y_1), entrapment efficiency (Y_2) were shown in Table1. The design expert software 9 used to carry out the experimentation and quantities of independent variables and responses were shown in Table 2.

Table 1: Box-Behnken design variables

Variables	Factors X,Y	Levels used, Actual (Coded)		
		Low (-1)	Medium (0)	High (+1)
Independent variables	___	___	___	___
Tristearin (%w/w)	X_1	3	7.5	12
Surfactant mixture (%w/w)	X_2	50	55	60
Stirring time (min)	X_3	3	10	15
Dependent variables	___	**Constraints**		
Particle size (nm)	Y_1	Minimize		
Entrapment efficiency (%)	Y_2	Maximize		

Table 2: Box-Behnken design matrix and observed responses

RUN	Tristearin (%w/w)	Cremo:Ethanol (3:1), (%w/w)	Sonication time (Min)	Particle size(nm)	Entrapment efficiency (%)	Zeta potential (mV)	PDI
1	3	50	10	94.4	74.26	-15.5	0.535
2	3	55	5	91.23	77.17	-13.5	0.715
3	3	55	15	89.41	77.34	-13.5	0.715
4	3	60	10	87.42	79.35	-12.6	0.767
5	7.5	50	15	79.84	83.12	-22.8	0.522
6	7.5	50	5	80.04	83.01	-13.7	0.522
7	7.5	55	10	27.98	86.43	-16.4	0.901
8	7.5	55	10	27.98	86.43	-16.4	0.901
9	7.5	55	10	27.98	86.43	-16.4	0.901
10	7.5	55	10	27.98	86.43	-16.4	0.901
11	7.5	55	10	27.98	86.43	-16.4	0.901
12	7.5	60	5	26.64	89.78	-22.8	0.281
13	7.5	60	15	24.69	89.82	-28.4	0.275
14	12	50	10	179.1	90.07	-19.8	0.334
15	12	55	15	180.23	91.43	-17.1	0.281
16	12	55	5	183.04	91.56	-17.1	0.639
17	12	60	10	187.63	94.78	-13.7	0.639

Entrapment efficiency

The encapsulation efficiency ranges from 74-94%. The effect of X_1, X_2 and X_3 on encapsulation efficiency explained by quadratic equation.

$$\% \text{ Entrapment efficiency} = +86.43+7.47*A+2.91*B- 0.024*C-0.095*AB-0.075*AC-0.018*BC-1.94A^2+0.12*B^2-0.12*C^2$$

The + values indicated that directly affected by the factors i.e. increased the factor ratio increases the response. The r^2 was found to be 0.9999, indicating good fit in the equation. The P value of X_1andX_2 was $P<0.05$ hence these two factors significantly affect the % entrapment of drug in lipid core. The X_3(sonication time) had – value hence sonication not influences the entrapment of drug in lipid core.

Particle size

The nanoparticle size varied from 24 to 187 nm. From the ramp model various trials were carried out and shown in Fig 3. After comparing the optimized and experimental value showed that the particle size, zeta potential, PDI and %EE was 24.69nm, -28.47mV, 0.274 and 89.82% respectively. The zeta size graph and optimized ,experimental value showed in Fig 1 & 2 and Table 3.

Table 3: Values of optimized and experimental values

parameters at desired level	Optimized value		Exp. value	
	Zeta size, nm	% EE[b]	Zeta size, nm	% EE
Tristearin (lipid) (%) = 11 Surfactant mixture (%) = 58.88 Stirring time (min) =9.33	28.22	90.79	24.69	89.82 (PDI-0.274, zeta potential -28.47mV)

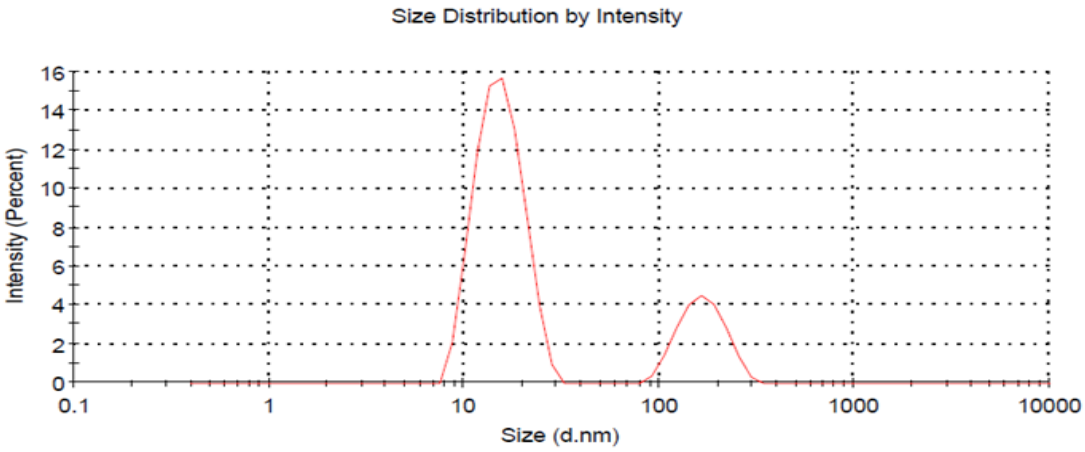

Fig. 1: Zeta size graph of optimized formulation

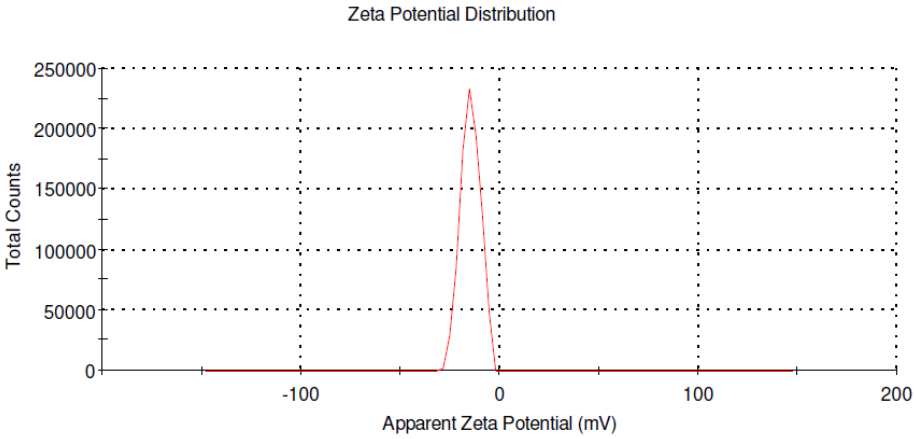

Fig. 2: Zeta potential graph of optimized formulation

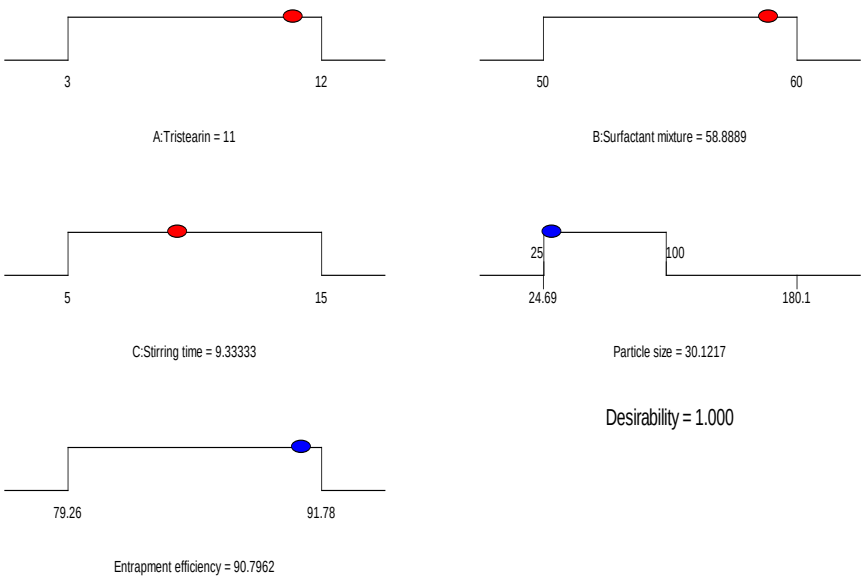

Fig 3: Ramp model of optimized formulation

Optimization by Box Behnken Design

The values were treated with Box-Behnken design and subjected to model adequacy test, model summary statistics and ANOVA these values were mentioned in Table 6, 7 &8 respectively. The effect of independent variables on response was shown in surface plots from fig.

In vitro diffusion studies

The drug release was found up to 15 hrs of 94%. The kinetic release studies showed that drug followed zero order release. The values of drug release and graph was mentioned in table 4 and Fig:4 respectively. The kinetics of drug release was mention in Table 5.

Table 4: Values of *in vitro* diffusion studies

Time	% drug diffused						
	Trial -I	Trial -II	Trial -III	Trial -IV	Trial -V	Trial -VI	Avg ±SD
0	0	0	0	0	0	0	0
0.5	3.93	5.12	3.62	4.2	3.98	4.53	4.23±0.53
1	13.34	16.69	13.67	13.08	13.27	14.82	14.14±1.39
2	20.62	22	20.67	21.35	19.86	21.64	21.023±0.78
3	24.62	28.72	24.16	25.37	24.35	24.97	25.36±1.69
4	28.79	31.22	29.02	28.63	28.73	29.53	29.32±0.98
5	36.3	38.27	35.41	37.87	35.62	36.28	36.62±1.18
6	42.61	43.87	42.94	43.86	41.03	41.93	42.70±1.11
7	48.31	49.10	49.02	48.72	47.19	46.57	48.15±1.04
8	55.62	54.87	55.42	56.71	54.75	54.26	55.27±0.85
9	62.57	65.28	63.48	62.93	61.32	62.35	62.98±1.33
10	68.18	72.07	69.03	68.27	67.26	69.48	69.04±1.66
11	76.62	79.12	77.83	76.91	75.13	76.47	77.01±1.35
12	80.32	84.57	80.16	81.24	79.56	83.27	81.52±1.97
13	84.02	91.38	83.87	84.27	83.93	90.06	86.25±3.48
14	91.32	93.97	91.04	92.48	90.45	91.26	91.75±1.27
15	95.45	94.03	94.56	95.72	96.08	93.27	94.85±1.08

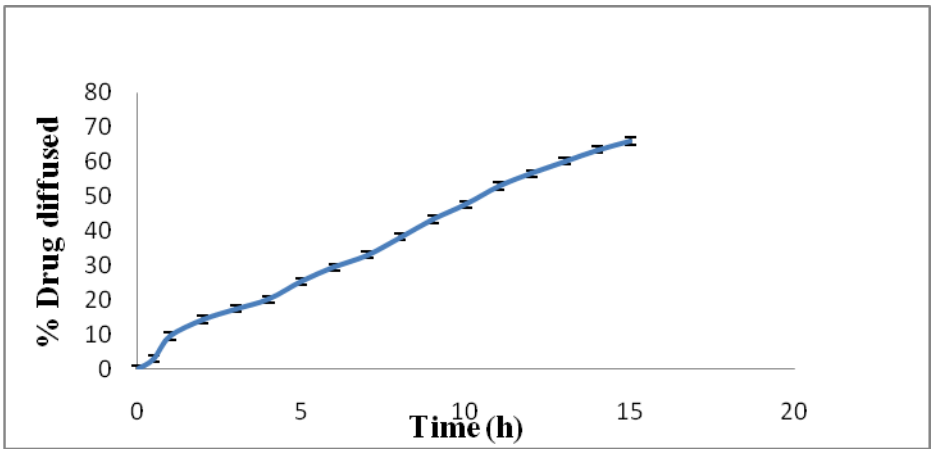

Fig. 4: *In vitro* diffusion studies of optimized formulation

Table 5: Release kinetics of optimized formulation

S.NO.	R^2 Value			Korsemeyerpeppas	
Optimized	Zero order	First order	Higuchi Plot	R^2 Value	n-Value
	0.9938	0.9424	0.949	0.9903	0.75

Optimization by Box Behnken Design

Table 6: Model adequacy tested in the design

Source	Sum of squares	DF	Mean square	F value	P value Prob>F	Remarks
Particle size						
Mean	1.226E+005	1	1.226E+005			
Linear	18322.58	3	6107.53	1.84	0.1904	
2FI	61.15	3	20.38	4.720E-003	0.9995	
Quadratic	41671.10	3	13890.37	64.11	<0.0001	Suggested
Cubic	1516.67	3	505.56			Aliased
Residual	0.000	4	0.000			
Total	1.842E+005	17	10832.56			
Entrapment efficiency						
Mean	1.256E+005	1	1.256E+005			
Linear	293.79	3	97.93	267.35	<0.0001	Suggested
2FI	0.55	3	0.18	0.43	0.7332	
Quadratic	4.18	3	1.39	286.30	<0.0001	Suggested
Cubic	0.034	2	0.017			Aliased
Residual	0.000	5	0.000			
Total	1.259E+005	17	7402.94			

Table 7: regression analysis for Y1 and Y2

Source	Std. Dev	R-squa	Adj.R-squ	Pred. R-squ	PRESS	Remarks
Model summary statistics for Particle size						
Linear	57.68	0.2976	0.1355	-0.2165	74900.38	
2FI	65.72	0.2986	-0.1223	-1.5484	1.569E+005	
Quadratic	14.72	0.9754	0.9437	0.8059	24266.72	Suggested
Cubic	0.000	1.000	1.000			
Model summary statistics for Entrapment efficiency						
Linear	0.61	0.9841	0.9804	0.9704	8.84	
2FI	0.65	0.9859	0.9774	0.9311	20.56	
Quadratic	0.070	0.9999	0.9997	0.9966	1.04	Suggested
Cubic	0.000	1.000	1.000			

Table 8: ANOVA results for Y1 and Y2

Source	Coeff. estim	Sum of Squa.	DF	Stan. error	Mean Squ.	F-Value	P-Value Prob>F	
Zeta size								
Model		60054.82	9		6672.76	30.80	<0.0001	Signf.
Intercept	27.98			6.58				
A-TS	45.94	16885.71	1	5.20	16885.71	77.93	<0.0001	
B-Surfactant	-13.38	1431.13	1	5.20	1431.13	6.61	0.0370	
C-Sonication time	-0.85	1305.75	1	5.20	1305.75	5.27	0.0452	
AB	3.88	0.083	1	7.36	0.083	23.09	0.0020	
AC	-0.25	0.25	1	7.36	0.25	1.131E-003	0.9741	
BC	-0.44	0.77	1	7.36	0.77	3.534E-003	0.9543	
A^2	96.17	38938.73	1	7.17	38938.73	179.72	<0.0001	
B^2	12.99	710.62	1	7.17	710.62	3.28	0.1130	
C^2	11.83	589.38	1	7.17	589.38	2.72	0.1431	
Residual		1516.67	7		216.67			
Lack of fit		1516.67	3		505.56		Not sign.	
Pure error		0.000	4		0.000			
Cor total		61571.49	16					
Encapsulation efficiency								
Model		298.52	9		33.17	6817.33	<0.0001	
Intercept	86.43			0.031				
A-TS	7.47	205.36	1	0.029	205.36	42209.34	<0.0001	
B-Surfactant	2.91	0.56	1	0.025	0.56	115.47	<0.0001	
C-Stirring time	0.024	1.029E-004	1	0.029	1.029E-004	0.021	0.8885	
AB	-0.095	0.096	1	0.035	0.096	19.75	0.0030	
AC	-0.075	1.582E-004	1	0.048	1.582E-004	0.033	0.8620	
BC	-0.018	0.000	1	0.035	0.000	0.000	1.000	
A^2	-1.94	3.92	1	0.038	3.92	805.76	<0.0001	
B^2	0.12	3.550E-005	1	0.038	3.550E-005	7.296E-003	0.9343	
C^2	-0.12	9.680E-004	1	0.038	9.680E-004	0.20	0.6690	
Residual		0.034	7		4.865E-003			
Lack of fit		0.017	2		0.017			
Pure error		0.000	5		0.000			
Cor total			16					

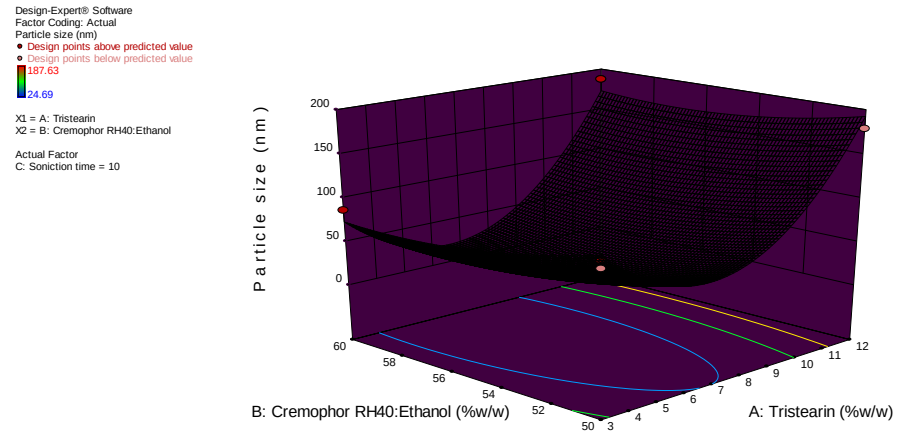

Fig. 5: Response surface plot of optimized formulation

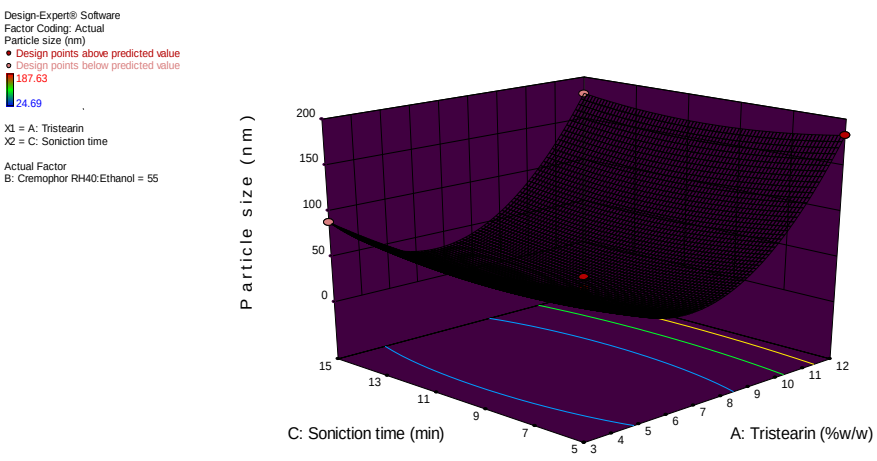

Fig. 6: Response surface plot of optimized formulation

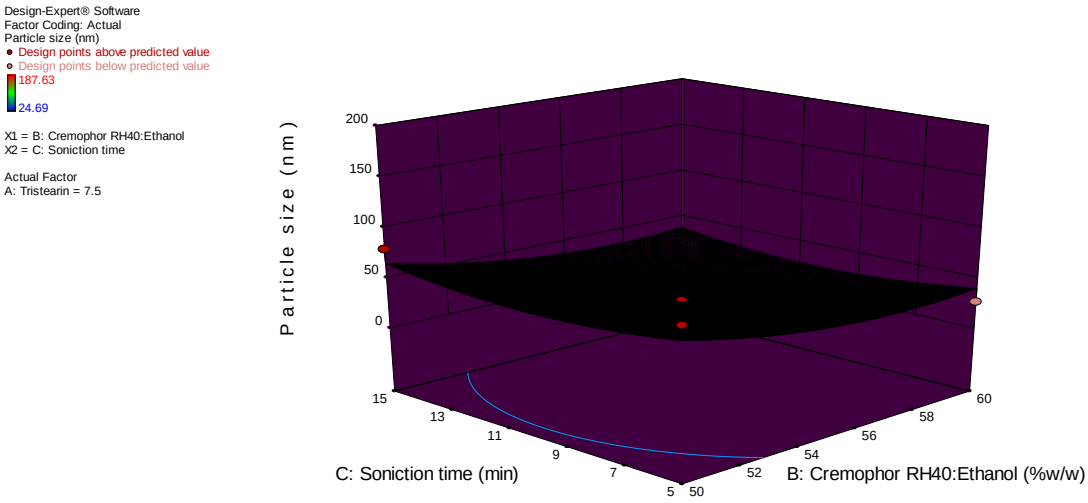

Fig. 7: Response surface plot of optimized formulation

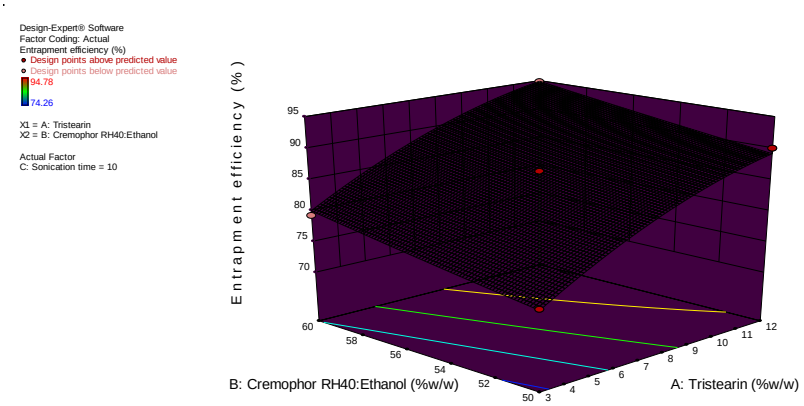

Fig. 8: Response surface plot of optimized formulation

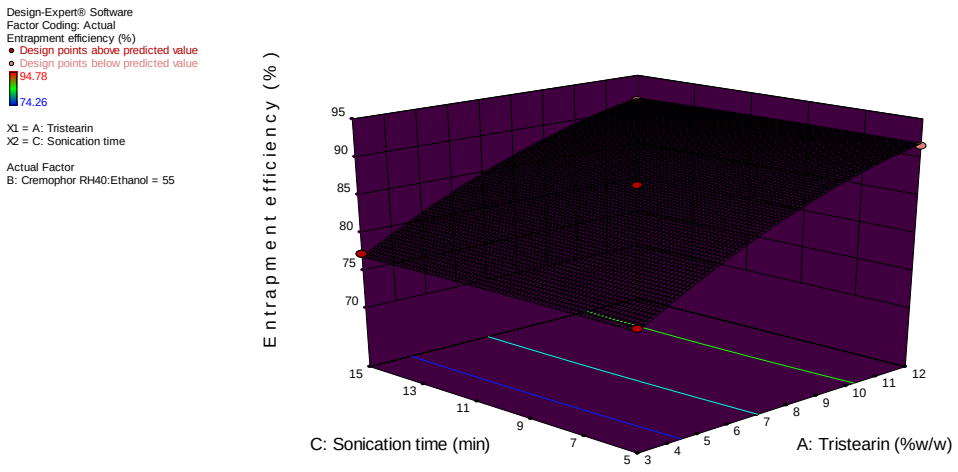

Fig. 9: Response surface plot of optimized formulation

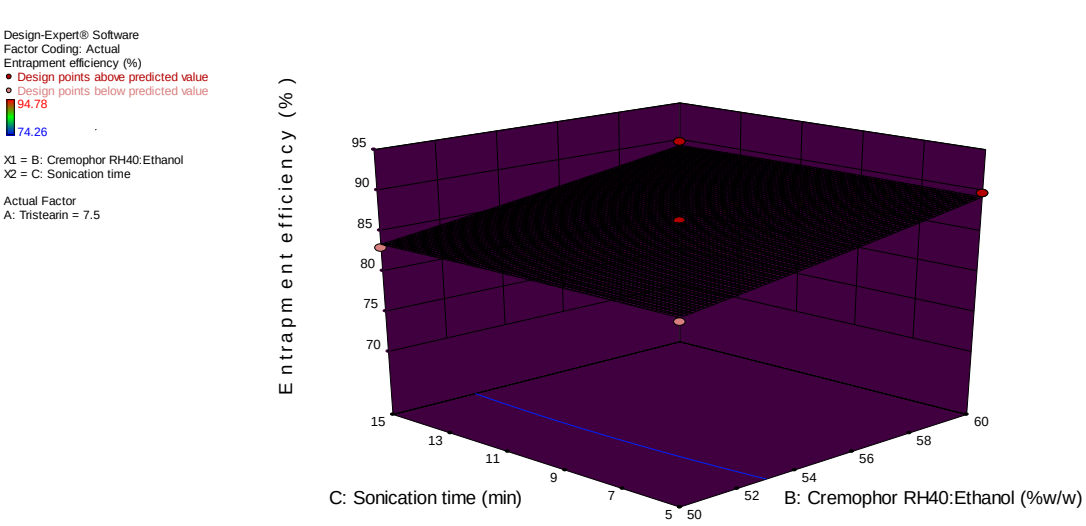

Fig. 10: Response surface plot of optimized formulation

REFERENCES

1. Mukherjee S, Ray S and Thakur RS. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. Ind J Pharm. 2009;71:349-358.
2. ZurMuhlen A, Schwarz C and Mehnert W. Solid lipid nanoparticles (SLN) forcontrolled drug delivery-drug release and release mechanism. Eur JPharm Biopharm. 1998;45(2):149–155.
3. Muller RH, Madar K and Gohla S. Solid lipid nanoparticles for controlled drug delivery-a review of the state of the art. Eur J Pharm Biopharm. 2000;50:161-177.
4. Jannin V, Musakhanian J and Marchaud D. Approaches forthe development of solid and semi-solid lipid-based formulations, Advanced Drug Delivery Reviews. 2008;60(6):734–746.
5. Mehnert W and Mader K. Solid lipid nanoparticles: production, characterization and application, Adv Drug Deliv Rev. 2001;47:165-196.
6. Muller RH, Olbrich C and Kayser O. Lipase degradation of Dynasan 114 and116 solid lipid nanoparticles (SLN) – effect of surfactants, storage timeand crystallinity. Int J Pharm. 2002; 237:119–128.

7. Yang HJ, Cho WG and Park, SN. Stability of oil-in-water emulsions preparedusing the phase inversion composition method. J Ind Eng Chem. 2009;15:331.
8. Basu B, Garala K, Bhalodia R, Joshi B and Mehta K. Solid lipid nanoparticles : A promising tool for drug delivery system. J Pharm Research. 2010;3(1):84-92.
9. Das S and Chaudhury A. Recent advances in lipid nanoparticleformulations with solid matrix for oral drug delivery. AAPS Pharm Sci Tech. 2011;12(1):62–76.
10. Uner M. Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): Their benefits as colloidal drug carrier systems. Pharmazie. 2006;61:375–86.
11. Kaur IP, Bhandari R, Bhandari S and Kakkar V. Potential of solid lipidnanoparticles in brain targeting. J Control Release. 2008;127:97-109.
12. Wissing SA, Kayser O and M¨uller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv. Drug Deliv Rev. 2004;56(9):1257–1272.
13. Almeida AJ, Runge S and Miiller RH. Peptide-loaded solid lipid nanoparticles (SLN): Influence of production parameters. Int J Pharm. 1997;149:255-265.
14. Ketenci A, Basat H and Esmaeilzadeh S. The efficacy of topical thiocolchicoside (Muscoril) in the treatment of acute cervical myofascial pain syndrome: a single-blind, randomized, prospective, phase IV clinical study. Journal of the Turkish Society of Algology. 2009;21(3):95–103.
15. Soonawalla DF and Joshi N. Efficacy of thiocolchicoside in Indian patients suffering from low back pain associated with muscle spasm. Journal of the Indian Medical Association. 2008;106(5): 31–5.