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Research Article

OPTIMIZATION OF FREEZE DRYING CONDITION OF PREDNISOLONEACETATE SOLID LIPID NANPARTICLES USING BOX-BEHNKEN DESIGN

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ABSTRACT

The long term stability of prednisolone acetate solid lipid nanoparticles (PA-SLNs) was increased by freeze drying process. PA-SLNs were utilized for administration through intranasal route hence converted into dry powder. The lipid core tristearin was stabilized using Smix (cremophor RH40: Ethanol). The stability of PA-SLNs particle size was studied by using various types and percentages of cryoprotectants. Trehalose was found to better cryoprotectant to prevent the growth of particle during and after freeze drying process. The particle size of PA-SLNs was slightly increased after freeze drying but which was not significant.

INTRODUCTION

Nanoparticulate carrier system was used to target, improve bioavailability, control the drug release and avoid the enzymatic degradation. Among the nanoparticulates i.e. SLNs are attractive and face the challenges of stability. The major obstacle with the SLNs was agglomeration in order to avoid this problem freeze drying was attempted. In this laboratory process which involves freezing of sample followed by lyophilization. Freeze drying process involves freezing of liquid SLNs in order to remove the bound water which will produce highly concentrated product, but in some conditions there is a possibility of agglomeration of particles to avoid this cryoprotectants were added. The widely used cryoprotectants were sucrose, mannitol, glucose and trehalose and the concentration of these optimized by trial and error method or by any suitable statistical design.

In the present study tristearin loaded prednisolone acetate SLNs was stabilized by cremophor RH40: Ethanol and it was freeze dried to improve the shelf life. In order to reduce the number of experimentation and to save the time and money to dry the SLNs Box-Behnken design was opted. The % and type of cryoprotectant and freeze drying temperature was considered important in freeze drying process hence these were optimized using Box-Behnken design.

MATERIALS AND METHODS

Materials

Prednisolone acetate (PA) was procured from Sigma-Aldrich chemical Co; Germany.Cremophor RH40 was purchased BASF certified supplier zeel.. Ethanol was purchased from Hi-media, Secunderabad. Tristearin was from Bros scientifics,Tirupati. All other chemicals and solvents used were of analytical grade.

Preparation of SLNs

Prednisolone acetate SLNs were prepared by microemulsion method. During this process initially tristearin micro emulsion was prepared followed as melted the tristearin to this PA was added then Smix (cremophor RH40: Ethanol, 3:1). The prepared microemulsion was added to cold water under

probe sonicator at 200w using 8mm probe. The liquid form SLNs was converted into solid form by freeze drying process.

Zeta size, potential and PDI analysis
The particle size, charge and PDI was determined using Malvern zeta sizer (Bangalore).

Freeze drying of SLNs
Initially the sample was freezed at different temperatures -20^{O}C and -80 ^{O}C using scientific freezer a period of 24hrs followed by lyophilized at -40 ^{O}C for 24hrs and pressure at 0.4 bar. The freeze drying process was carried by using cryoprotectants such as sucrose, mannitol and trehalose at different concentrations of 5-20% to achieve the small particle size.

Morphology study
The surface morphology of SLNs was determined using TEM (Transmission electron microscopy).

Drug diffusion studies
The drug diffusion studies were carried out using franz diffusion cell apparatus. Before and after freeze drying the samples were subjected to *in vitro* diffusion studies. Before freeze drying 5ml of liquid SLNs and after freeze drying dried nanoparticles dispersed in distilled water was placed in dialysis membrane (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.

DSC analysis
The pure drug and freeze dried sample was subjected to DSC analysis. Accurately weighed (2mg) sample was placed in aluminum pan and exposed to 0^{O}Cto 500 ^{O}C.

Experimental design
Literature survey indicated that type and concentration of cryoprotectant, freezing temperature were important factors hence these further optimized using Box-Behnken design (RSM). The various variables and its levels was shown in Table 1. The design matrix was shown in Table 2.

Table 1: Variables used to box – behnken design in freeze drying of PA SLNs

Variables	Factors X,Y	Levels used, Actual (Coded)		
		Low (-1)	Medium (0)	High (+1)
Independent variables	___	___	___	___
Type of cryoprotectant	X_1	sucrose	mannitol	Trehalose
Concentration of cryoprotectant (%w/w)	X_2	5		20
Freeze temperature (^{O}C)	X_3	-20^{O}C		-80 ^{O}C
Dependent variables	___	**Constraints**		
Particle size (nm)	Y_1	Minimize		

Table 2: Box-Behnken design in various runs and its response in freeze drying of SLNs of PA

RUN	Type of cryoprotectant	Concentration of cryoprotectant, (%w/w)	Freezing Temperature, (^{O}C)	Particle size(nm)
1	sucrose	20	-50	204.45
2	sucrose	12.5	-80	291.23
3	sucrose	12.5	-20	320.76
4	sucrose	5	-50	387.42
5	Trehalose	12.5	-50	97.18
6	Trehalose	5	-80	180.04
7	Trehalose	5	-20	180.04
8	Trehalose	12.5	-50	97.18
9	Trehalose	12.5	-50	97.18
10	Trehalose	12.5	-50	97.18
11	Trehalose	12.5	-50	97.18
12	Trehalose	20	-80	35.34
13	Trehalose	20	-20	35.34
14	Mannitol	12.5	-20	183.04
15	Mannitol	5	-50	280.23
16	Mannitol	12.5	-80	183.04
17	Mannitol	20	-50	127.63

RESULTS AND DISCUSSION

Optimization of particle size

The results indicated that decreased the particle size by increasing the cryoprotectant concentration. Table 2 showed different parameters which influences the particle size. From the Table 3 the selection of best model was to optimize the particle size. ANOVA results were shown in Table 4. UsingR^2 and predicted R^2 the quadratic model was suitable to determine the response. Zeta size graph was shown if fig 1. The contour plot of RUN-12 showed in fig 6. When compared with sucrose and mannitol low particle size was obtained from trehalose hence RUN-12 was selected for further studies.

Table 3: regression analysis for Y1

Source	Std. Dev	R-squ	Adj. R-squ	Pred. R-squ	PRESS	Remarks
statistics for Zeta size						
Linear	90.43	0.3483	0.1980	-0.1733	1.914E+005	
2FI	79.37	0.6139	0.433	-1.3549	3.842E+005	
Quadratic	76.03	0.9467	0.3822	-4.6248	9.177E+005	Suggested
Cubic	46.62	0.7520	0.7868			

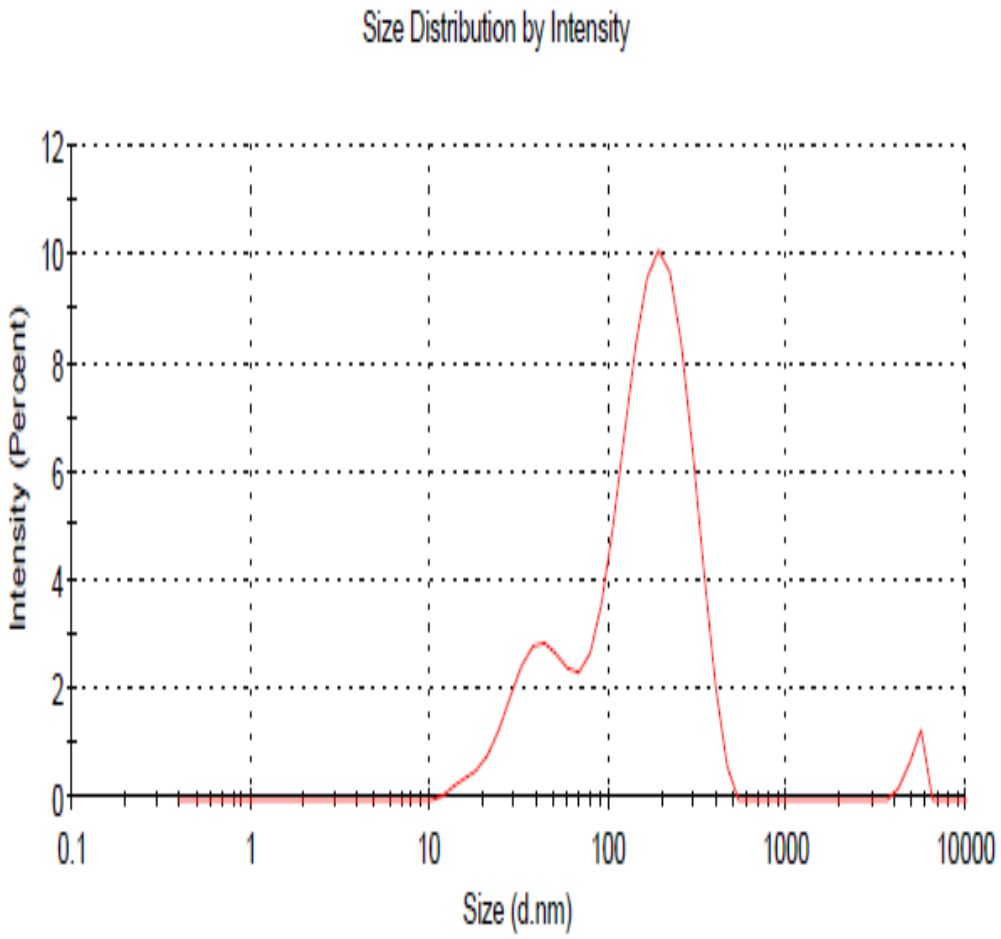

Fig. 1: Zeta size graph of RUN-12

Table 4: ANOVA results for Y1

Source	Coeff. estim	Sum of Squa.	DF	Mean Squ.	F-Value	P-Value Prob>F	
Model		1.227E+005	9	13631.50	2.36	0.0001	**Signf.**
Intercept	122.28						
A-Type of cryo	22.0	1765.65	1	1765.65	0.31	0.5977	
B-Con of cryo	-72.75	28239.28	1	28239.28	4.88	0.0628	
C-Freeze temp	-40.05	7685.25	1	7685.25	1.33	0.2868	
AB	-30.83	1397.81	1	1397.81	0.24	0.6380	
AC	175.90	52984.65	1	52984.65	9.17	0.0192	
BC	-15.42	565.83	1	565.83	0.098	0.7635	
A^2	0.52	0.61	1	0.61	1.050E-004	0.9921	
B^2	91.34	20891.40	1	20891.40	3.61	0.0991	
C^2	2.39	19.30	1	19.30	3.338E-003	0.9555	
Residual		40466.84	7	5780.98			
Lack of fit		31771.34	3	10590.45	4.87	0.0801 Not sign.	
Pure error		8695.50	4	2173.88			
Cor total		1.632E+005	16				

TEM studies

The surface morphology of optimized formulation (RUN-12) was shown in fig 2. The particle shape was found to be spherical.

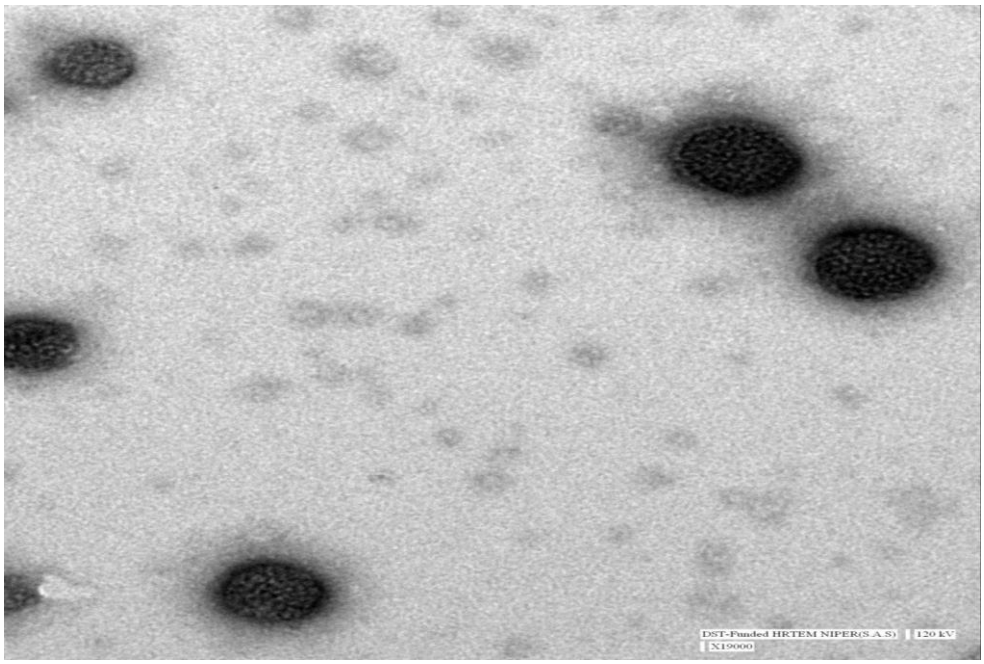

Fig. 2: TEM photo graph of optimized formulation (RUN-12)

Drug release studies

The drug release studies were conducted upto 15 hrs and were found 95.67%. The fig 3. Showed the drug release characteristics indicate no significant difference before freeze drying and after freeze drying.

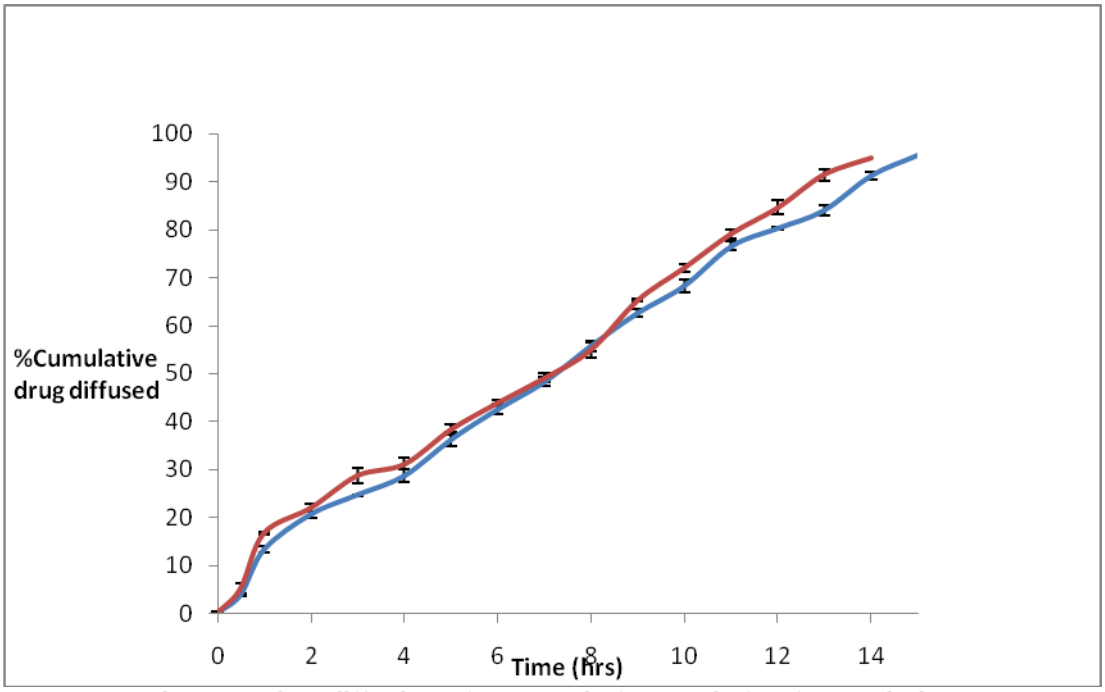

Fig. 3: In vitro diffusion of RUN-12 before and after freeze drying

DSC studies

DSC thermogram of pure drug and optimized formulation showed in fig 4&5. The melting point of pure drug in alone and formulation showed no signficant difference.

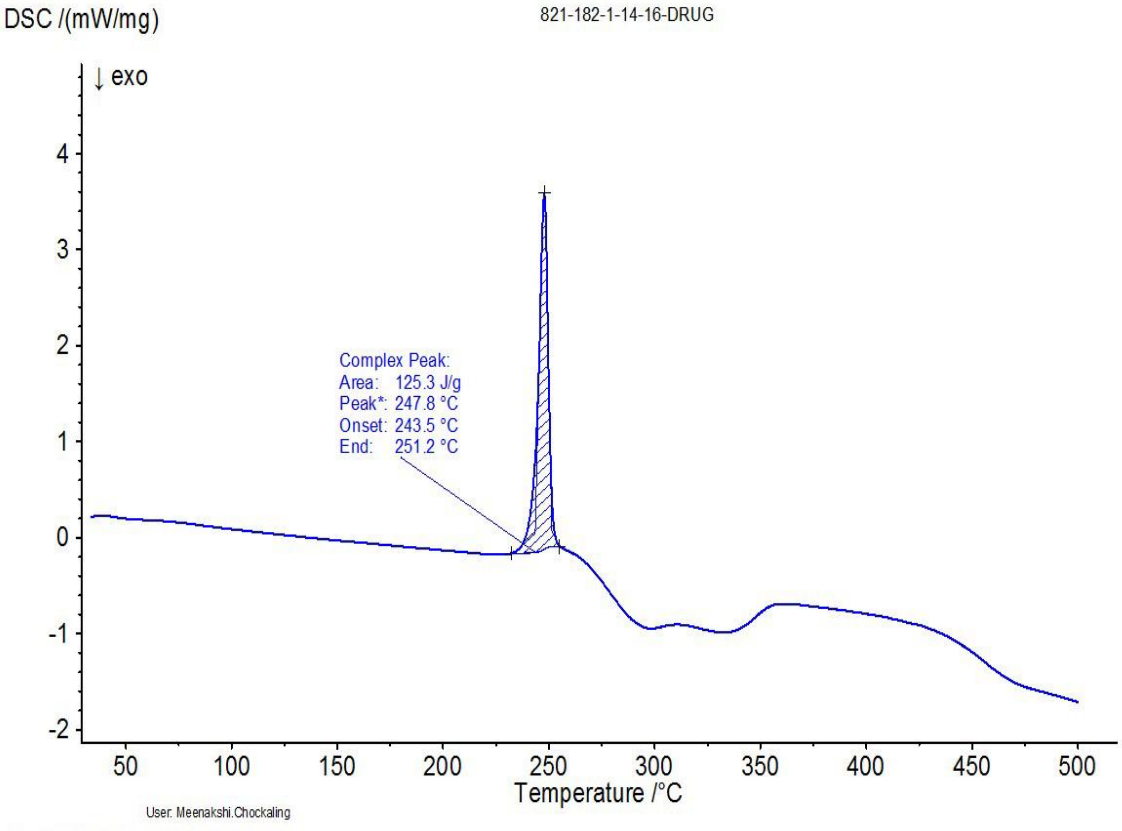

Fig. 4: DSC thermogram of pure drug

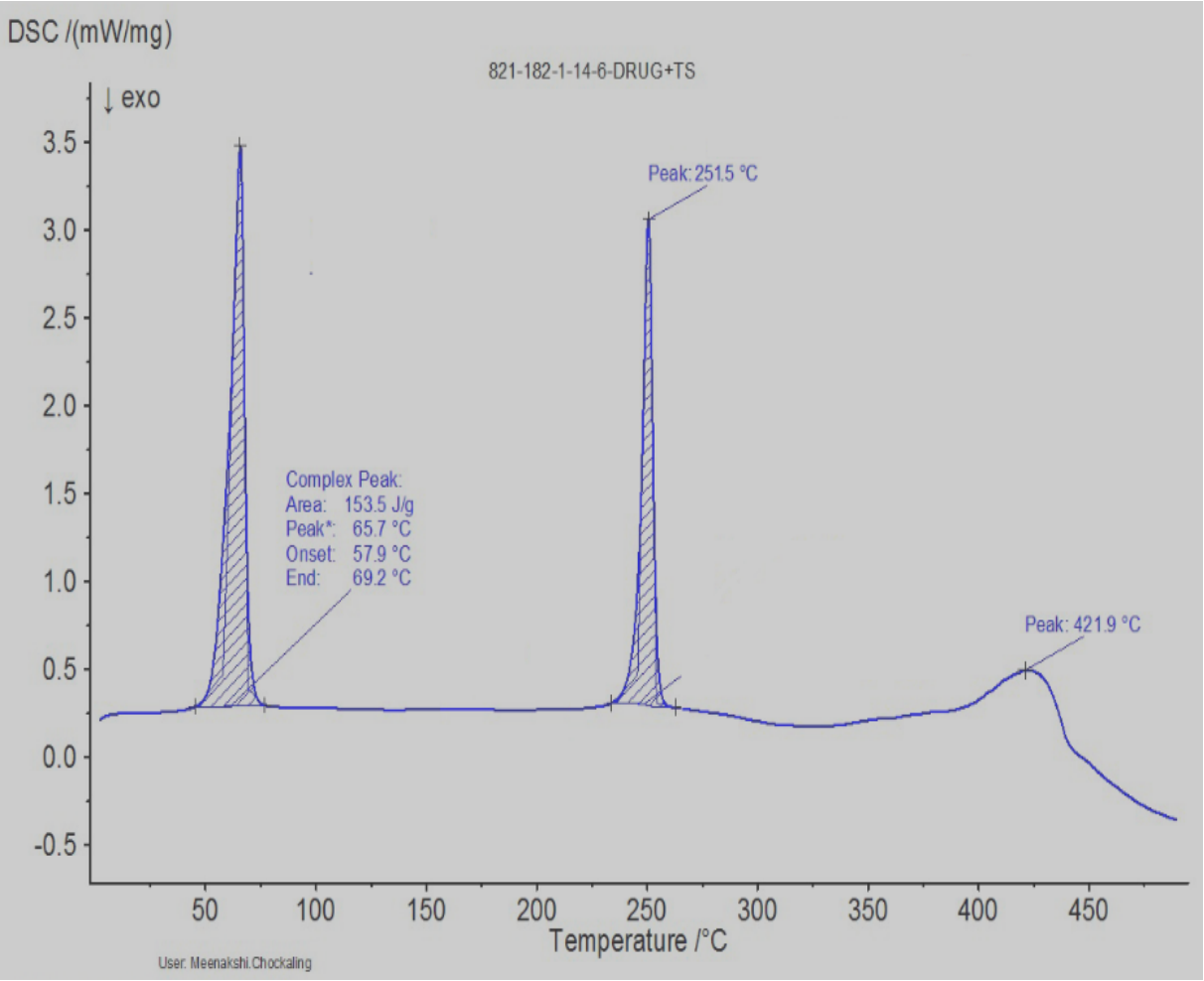

Fig. 5: DSC thermogram of RUN-12

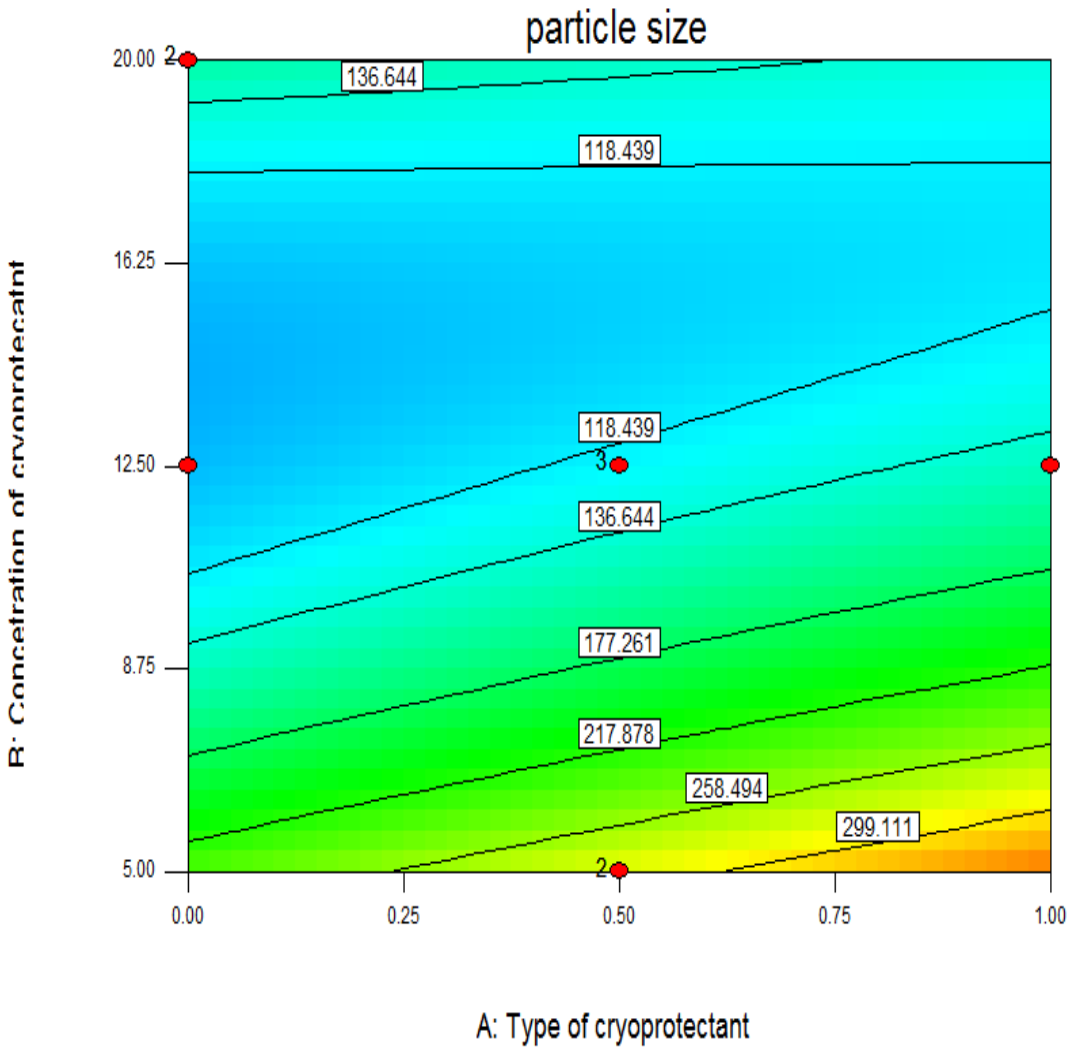

Fig. 6: Contour response plot of RUN -12

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