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Formulation Development And Evaluation Of Solid Lipid Nanoparticle For The Antihypertensive Chlorthalidone Drug

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Abstract:

The present study aimed to Formulation, Development and Evaluation of Solid Lipid Nanoparticle for the Chlorthalidone Antihypertensive Drug. The solid lipid nanoparticles(SLN's) are submicron colloidal carriers which are composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. SLNs were prepared by the Hot Homogenization Method. The effect of formulation variables, viz. Different glyceryl monostearate ratio, difference stirring speed, concentration of surfactants using Design of experiments (DOE). Formulation was characterized by different evaluation parameters. Chlorthalidone SLN were evaluated for entrapment efficiency, drug content, particle size, zeta potential, in vitro drug release. The Chlorthalidone -loaded optimized SLN formulation was prepared by setting the formulation factors of drug: glyceryl monostearate ratio of (1:0.5), volume of internal phase 5 ml, volume of external phase 30 ml, surfactant concentration (5% w/v) homogenization speed of 1000 rpm, and homogenization time of 90 minutes. The optimized formulation was subjected to differential scanning calorimetry, Fourier transform infrared spectroscopy, and scanning electron microscopy study. Conclusively, Chlorthalidone-SLNs were successfully formulated with higher drug entrapment and could serve as promising delivery for poorly solubledrug.

Keywords: Chlorthalidone, Solid Lipid Nanoparticle, Colloidal Carriers, In-Vitro Drug Release

1. INTRODUCTION

As was previously said, hypertension causes high blood pressure. It is a condition where the heart's pumping causes blood pressure to rise above normal limits. The heart exerts great effort as it pumps blood into the arteries from the top of the head to the bottom of the feet to help blood reach distant organs. The force that blood applies to the artery walls as it circulates through the body is known as blood pressure. Blood pressure peaks as it leaves the heart through the aorta and subsequently decreases as it travels through progressively smaller blood vessels. (1,2)

The Systole, when the heart beats and the sphygmomanometer reads higher, and the Diastole, when the heart is at rest and the sphygmomanometer reads lower, are the two distinct stages of the human heartbeat. Systolic and diastolic blood pressure averages can be as high as 120 and 80 millimetres of mercury, respectively. While a risk appears to rise even above 120/80 mm Hg, the World Health Organization and the International Union of Hypertension (WHO and ISH, 2003) guidelines define hypertension as 140 mm Hg systolic and 90 mm Hg diastolic. (3,4,5)

Solid lipid nanoparticles (SLNs), which have a number of potential uses in drug delivery and research, are at the forefront of the quickly evolving field of nanotechnology and were originally introduced in 1991. SLNs, which range in size from 50 to 1000 nm and are sub-micron colloidal carriers, are made of physiological lipids dispersed in water or aqueous surfactant solutions.

The lipid core of SLNs is stabilised by surfactants and has a solid lipid core matrix that can solubilize compounds that are lipophilic. The capacity of nanoparticles to pass through various anatomical barriers, prolonged release of their contents, and stability at nanometre size are all necessary for their successful application in drug delivery. Many biocompatible or biodegradable lipids can be found that are solid at room temperature, very pure, generally regarded as safe (GRAS), and affordable. Triglycerides, carnauba wax, beeswax, cetyl alcohol, emulsifying wax, cholesterol, and cholesteryl butyrate are a few of the frequently utilised solid lipids.It is possible to formulate both BCS Class II and IV pharmaceuticals as well as biologics using nano- and micro-particles formed of these lipids that are suspended in water, potentially resolving the problems with shelf-life stability, cost, and toxicity associated with the use of organic solvents. (16,19)

2. METHOD AND MATERIALS

2.1. MATERIALS

Chlorthalidone is given by Umedica laboratories as gift sample, Glyceryl monostearate, Stearic acid, Tewwn-80, Ethanol, Distilled water is obtained from Vishal chem, Mumbai.

2.2. METHODS

2.2.1. SCREENING OF LIPIDS

The medication's solubility in lipid is one of the most important factors in determining the drug loading capacity of the sln. The three lipids tested for chlorthalidone solubility were bees wax,

stearic acid, and gms; gms had the best solubilization capability. According to this study, gms can load chlorthalidone more readily than other lipids. (20)

2.2.2. SOLUBILITY

Solubility of drug in various vehicles are very important for preparing solid lipid nanoparticles. It shows the compatibility between drug and vehicles. (21)

2.2.3. FORMULATION OF SOLID LIPID NANOPARTICLES

1) Take two beakers first. Put the lipid in one beaker while heating the distilled water in the other. The medicine should be dissolved in the lipid once both have achieved 70–80 °C.

2) And in another beaker, add the surfactant.

3) Then add the ethanol to the lipid solution.

4) Using a syringe and maintaining heat, slowly incorporate the lipid solution into the aqueous solution to create a pre-emulsion. The material is then hot homogenized for the necessary amount of time and rpm to generate a colloidal emulsion.

5) The mixture should be cooled to room temperature. Dry the solution after filtering it. (22)

2.2.4. EXPERIMENTAL DESIGN: 3^2 FULL FACTORIAL DESIGN

A Full factorial design was used for optimization and to evaluate the relationship between the independent variables like drug to Conc of Lipid (A), String speed (B) and dependent (responses) variables, i.e., Particle Size (R1), Entrapment Efficiency(R2), Drug release(R3). Therefore, by fixing the homogenization time (60 min), stirring speed (1200 rpm), the selected variables (A) and (B) were studied at three different levels as low (–1), medium (0) and high (+1). Different batches were prepared and data were substituted in design expert software (version 12). The generated polynomial equation was used to draw the conclusion after considering the magnitude of coefficient and mathematical sign it carries, i.e., positive or negative. The polynomial regression results were expressed using 3D plots. The criteria of selection of optimum formulations were primarily based on minimum particle size, maximum entrapment efficiency. (64)

Contour Plot

Contour plot is a diagrammatic representation of the values of the response and it is helpful in explaining visually the relationship between independent and dependent variables. The reduced model was used to plot two-dimension contour plot using demo version of Design Expert 12 software. (65)

Response Surface Plot

Understanding the primary and interaction impacts of factors during the formulation development is aided by the response surface plot. The relevant response surface plot can be used to understand how the level of the independent variable affects the response parameter. (65)

Optimization of SLNs formulation using overlay plot by Design Expert software

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses. The desirability procedure involves two steps:

(1) Finding the levels of the independent variables that simultaneously produce the most desirable predicted responses on the dependent variables.

(2) Maximize the overall desirability with respect to the controllable factors. (66)

2.2.5.CHARACTERIZATION OF SOLID LIPID NANOPARTICLES

Particle size: - The average particle size the solid lipid nanoparticles were determined using Zetasizer Nanoseries Nano-ZS, Malvern Instruments, Malvern, UK. In-built dynamic light scattering, DLS, and Laser Doppler Electrophoresis were used for the determinations of particle size and for zeta potential. The samples were put in 'folded capillary cells and results obtained for size and zeta-potential were recorded. (66)

Zeta Potential: - The zeta potential of Zaleplon loaded SLNs were determined by using Malvern zeta sizer (Nano ZS, Malvern Instrument). Same method for preparing test samples was followed 7,8. Results was taken and the values was reported.(66)

Entrapment efficiency: - The amount of free drug in the supernatant following centrifugation of a known amount of nanoparticulate dispersion at 10000 RPM for 10 minutes using a freeze centrifuge (BL – 135 R) was quantified spectrophotometrically at 227 nm to determine entrapment efficiency. The efficiency of trapping was estimated using.

% Drug entrapment = [Total drug – Free drug / Total drug] × 100

Where, Total drug = Amount of drug added in the formulation,

Free drug = Amount of drug detected by UV-VIS spectrophotometer. (66)

In-vitro drug release: - The in vitro release of chlorthalidone from SLN dispersion was determined using the dialysis bag diffusion technique. An accurately weighed amount of chlorthalidone-loaded chlorthalidone dispersion containing the drug equivalent to 12.5 mg was transfer to a dialysis bag and sealed. The sealed bag was then suspended in a beaker containing 250 ml of phosphate buffer Ph 6.8 and stirred at a constant speed of 50 rpm at 37°C ±0.5°C. Aliquots were withdrawn at predetermined intervals from the receptors compartments up to 6 hours and the same was replaced with fresh buffer. Then the drug content was determined spectrophotometrically by measuring the absorbance 227nm using the phosphate buffer Ph 6.8 as blank, to calculate the amount of drug release from the nanoparticles. (23)

Stability Study: - To determine the drug and formulation stability, stability studies were work according to ICH guidelines. Optimized formulation capsule of SLN of Chlorthalidone were kept I the humidity chamber maintained at 30°C and relative humidity is 65% for 1 months. Among the two Formulation, Formulation (SLN 5) was selected for stability studies based on the physicochemical characterization release characteristics. The sample were analyzed for the

physical appearance, particle size, PDI, % CDR of Chlorthalidone in vitro release profile stability indicating parameters after 30 days. (23)

3. RESULT AND DISCUSSION

3.1. SCREEING OF LIPIDS

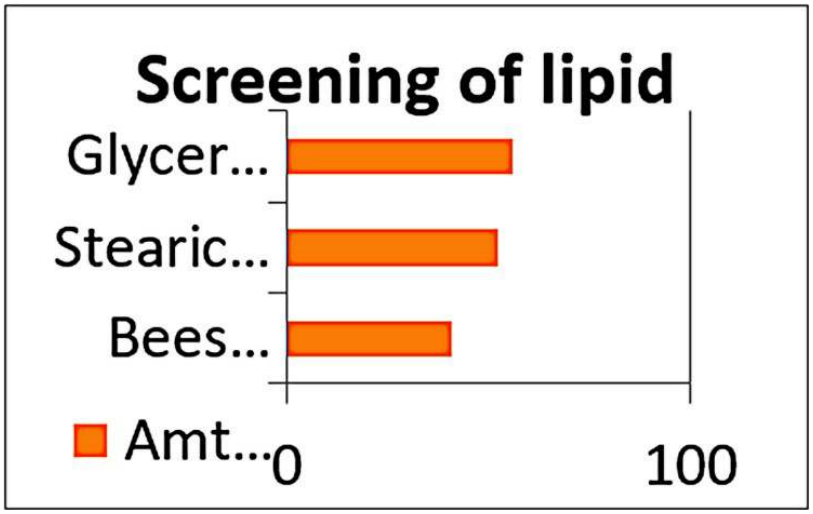

Figure 1 Screening of Lipids

3.2. SOLUBILITY

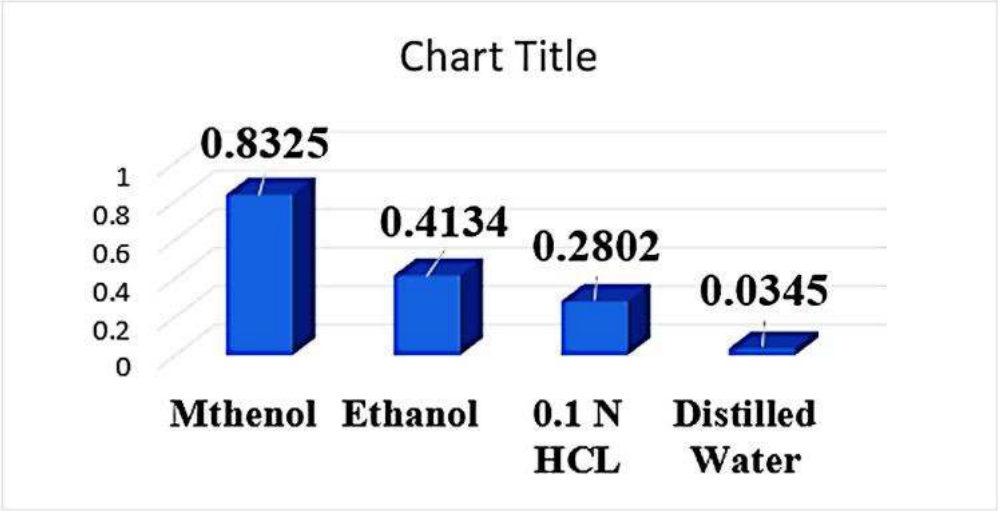

Figure 2 Solubility of Chlorthalidone

3.3. OPTIMIZATION OF FORMULATION USING 32 FULL FACTORIAL DESIGN

The 32 factorial design study is applied for the preparation of Sln considering the factors that affect the stability.

Table 1 Independent Variables and Dependent Variable

Independent Variables				
Code	**Name of factor**	**Low (-)**	**Medium (0)**	**High (+)**
X1	Conc of Lipid (mg	-1	0	+1
X2	Speed (rpm)	-1	0	+1
Dependent Variables				
Y1 = Particle Size				
Y2= % Entrapment Efficiency				
Y3 = % CDR				

Table 2 Evaluation Data of Factorial Batches

Formulation No	F1	F2	F 3	F4	F5	F 6	F7	F8	F9
Particle size(nm)	148	145	136	135	134	129	117	123	125
Zeta potential (mv)	- 29mv	-27mv	-24mv	-22mv	-21mv	-23mv	- 17m v	- 25m v	- 22m v
%Entrapment efficiency	83.48±0.28	84.99±0.56	85.12±0.32	85.74±0.17	86.83±0.45	86.83±0.13	89.28± 0.59	83.340.25	85.12± 0.62
PDI	0.37	0.35	0.32	0.31	0.29	0.27	0.22	0.36	0.32
%CDR	91.5±0.18	92.6±0.24	93.1±0.27	94.4±0.58	95.10±.48	95.3±0.29	99.85± 0.56	93.39± 0.51	93.75± 0.24

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Table 3 Dissolution Study of Factorial Batch

In-Vitro Drug release (%)									
Time (hr.)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	11.4±0.2	12.6±0.91	12.5±0.36	13.7±0.26	10.7±0.23	11.2±0.8	14.6±0.41	10.2±.32	12.1±0.12
2	25.6±0.54	23.9±0.77	26.4±0.48	22.8±0.39	23.4±0.14	24.7±0.36	29.1±0.32	22.8±.22	21.9±0.27
3	43.2±0.77	44.5±0.45	44.8±0.87	45.4±0.65	41.3±0.44	42.1±0.33	47.1±0.46	43.5±.27	41.8±0.23
4	53.6±0.41	62.3±0.73	61.5±0.14	63.9±0.46	64.7±0.29	62.5±0.76	66.7±0.38	55.2±.43	62.5±0.76
5	76.8±0.78	77.5±0.25	78.2±0.26	78.1±0.19	79.6±0.82	79.8±0.58	79.5±0.49	76.4±.47	78.9±0.59
6	91.5±0.18	92.6±0.24	93.1±0.27	94.4±0.58	95.10±.48	95.3±0.29	99.85±0.56	93.39±0.51	93.75±0.24

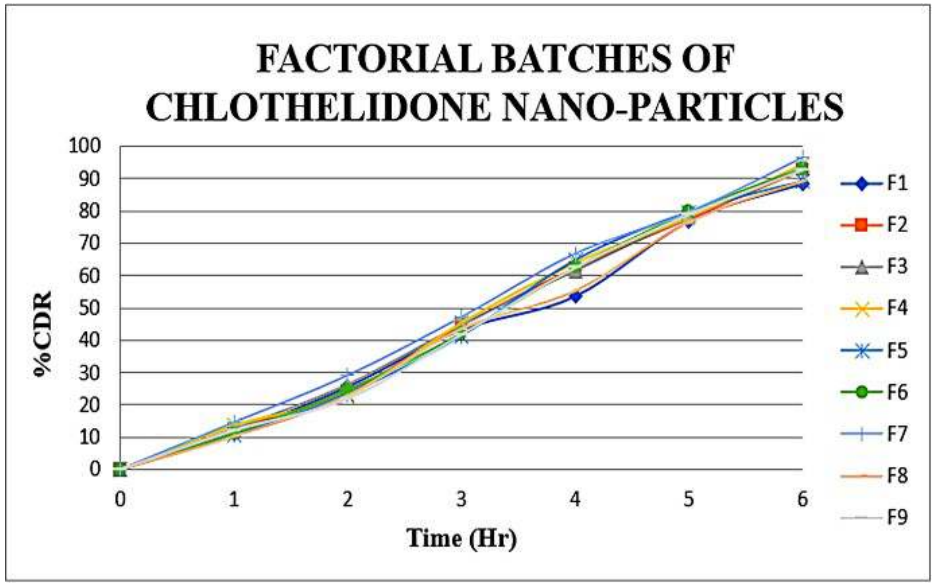

Figure 3 Dissolution Graph

Table 4 Design matrix and response with respective observed response

Factorial Batches	X_1 (Conc.of Glyceryl monostearate)	X_2 Stirring speed(RPM)	Y_1 Particle size(nm)	Y_2 Entrapment efficiency	Y_3 (%CDR at 6hr)
F1	300	800	148	83.48±0.28	91.5±0.18
F2	600	800	145	84.99±0.56	92.6±0.24
F3	900	800	136	85.12±0.32	93.1±0.27
F4	300	1000	135	85.74±0.17	94.4±0.58
F5	600	1000	134	86.83±0.45	95.10±0.48
F6	900	1000	129	86.83±0.13	95.3±0.29
F7	300	1200	117	89.28±0.59	99.85±0.56
F8	600	1200	123	83.34±0.25	93.39±0.51
F9	900	1200	125	85.12±0.62	93.75±0.24

Table 5 Summary of Results of Multiple Regression Analysis for Y_1 and Y_2

Dependent Variables	Y_1 Particle size (nm)		Y_2 Entrapment efficiency		Y_3 (%CDR at 6hr)	
	Coefficients	P-Value	Coefficients	P-Value	Coefficients	P-Value
Intercept	134.44	0.0003	86.87	0.0073	95.19	0.0008
X_1	-3.67	0.0014	0.2533	0.1049	0.3167	0.0242
X_2	-11.00	0.0001	1.19	0.0017	1.95	0.0001
X_1X_2	2.00	0.0138	-0.7000	0.0139	-0.5500	0.0093
X_1^2	-2.67	0.0163	-0.6333	0.0450	-0.3833	0.0599
X_2^2	-0.6667	0.3081	-0.7283	0.0316	-0.5833	0.0206

Table 6 Summary of Quadratic polynomialequation for responses Y1 and Y2

Quadratic Model	
Y_1 (Particle Size)	$134.44-3.67X_1-11.00X_2+2.0X_1X_2-2.67X_1^2-0.6667X_2^2$
Y_2 Entrapment efficiency	$86.87+0.2533X_1+1.19X_2-0.7000X_1X_2-0.6333X_1^2-0.7283X_2^2$
Y_3 (%drug Release)	$95.19+0.3167X_1+1.95X_2-0.5500X_1X_2-0.3833X_1^2-0.5833X_2^2$

Table 7 ANOVA for quadratic model Y1

ANOVA for Quadratic model

Response 1: Particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	837.78	5	167.56	282.75	0.0003	significant
A-glyceryl monostearate	80.67	1	80.67	136.13	0.0014	
B-Stirring speed	726.00	1	726.00	1225.13	< 0.0001	
AB	16.00	1	16.00	27.00	0.0138	
A^2	14.22	1	14.22	24.00	0.0163	
B^2	0.8889	1	0.8889	1.50	0.3081	
Residual	1.78	3	0.5926			
Cor Total	839.56	8				

Table 8 Anova for quadratic model Y2

ANOVA for Quadratic model

Response 2: Entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12.73	5	2.55	35.00	0.0073	significant
A-glyceryl monostearate	0.3851	1	0.3851	5.29	0.1049	
B-Stirring speed	8.52	1	8.52	117.16	0.0017	
AB	1.96	1	1.96	26.95	0.0139	
A^2	0.8022	1	0.8022	11.03	0.0450	
B^2	1.06	1	1.06	14.59	0.0316	
Residual	0.2182	3	0.0727			
Cor Total	12.95	8				

Table 9 ANOVA for quadratic model Y3

ANOVA for Quadratic model

Response 3: Drug Release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	25.60	5	5.12	151.92	0.0008	significant
A-glyceryl monostearate	0.6017	1	0.6017	17.85	0.0242	
B-Stirring speed	22.82	1	22.82	676.93	0.0001	
AB	1.21	1	1.21	35.90	0.0093	
A^2	0.2939	1	0.2939	8.72	0.0599	
B^2	0.6806	1	0.6806	20.19	0.0206	
Residual	0.1011	3	0.0337			
Cor Total	25.70	8				

3.4. CONTOUR PLOTS AND RESPONSE SURFACE ANALYSIS

The relationship between the dependent and independent variables was further explained by constructing contour plots and 3D surface plots based on full factorial design with the help of design Expert 12 software. This type of plot is used for determination of two factors simultaneously on one time.

Effect of X_1 and X_2 on Response Y_1

Two dimensional and three-dimensional plots are shown in Figure and Which showed Particle size Decreases as the levels of Glyceryl Monostearate and Stirring speed were Increased.

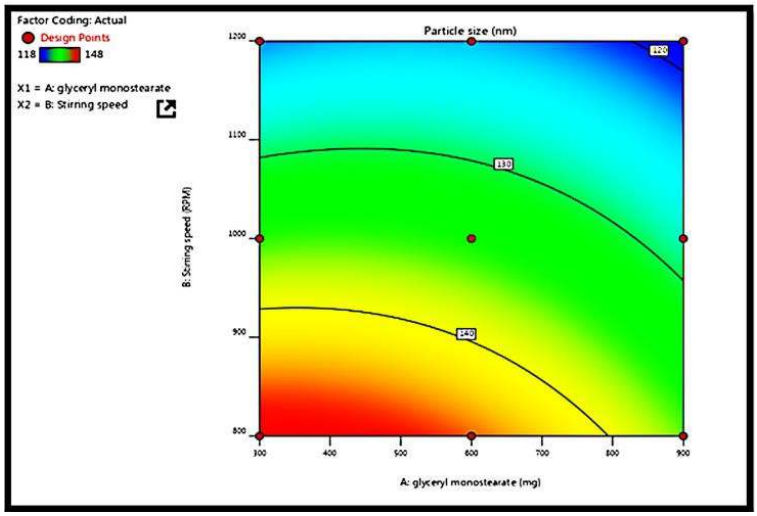

Figure 4 Effect of X_1 and X_2 on Response Y_1

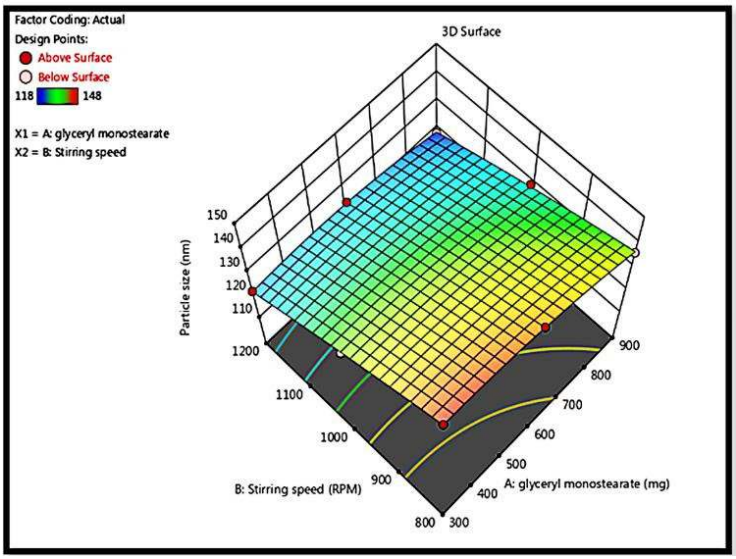

Figure 5 Effect of X_1 and X_2 on Response Y_1

Effect of X_1 and X_2 on Response Y_2

Two dimensional and three-dimensional plots are shown in Figure and which showed Entrapment efficiency Increases as the levels of Glyceryl Monostearate and Stirring speed were Increased.

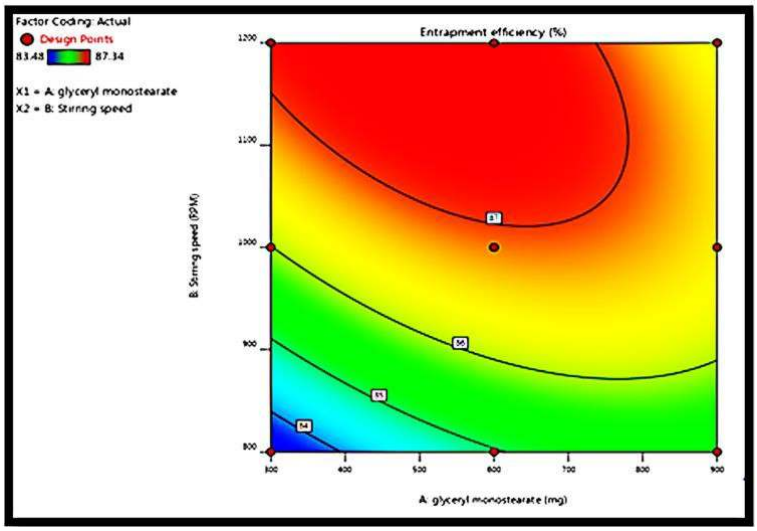

Figure 6 Effect of X_1 and X_2 on Response Y_2

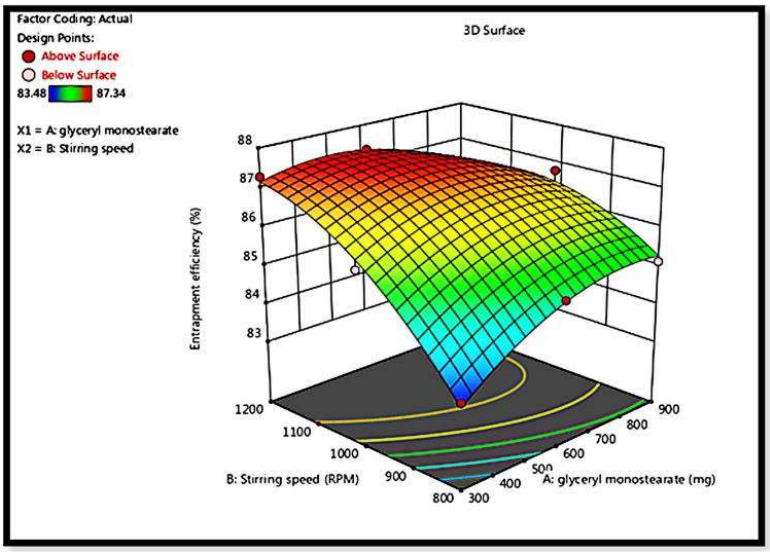

Figure 7 Effect of X_1 and X_2 on Response Y_2

Effect of X_1 and X_2 on Response Y_3

Two dimensional and three-dimensional plots are shown in Figure and which showed %Drug Release Increases as the levels of Glyceryl Monostearate and Stirring speed were Increased.

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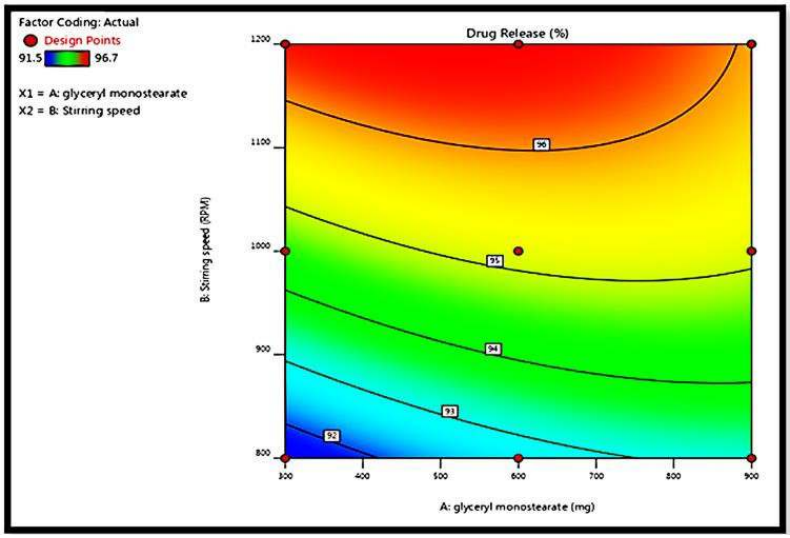

Figure 8 Effect of X_1 and X_2 on Response Y_3

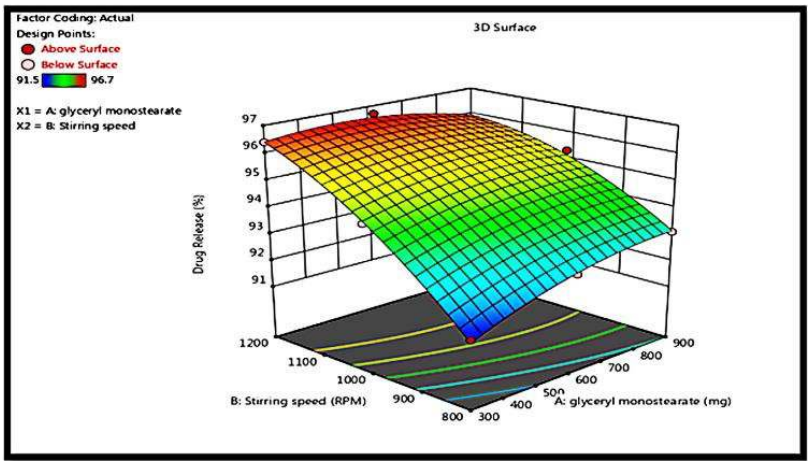

Figure 9 Effect of X_1 and X_2 on Response Y_3

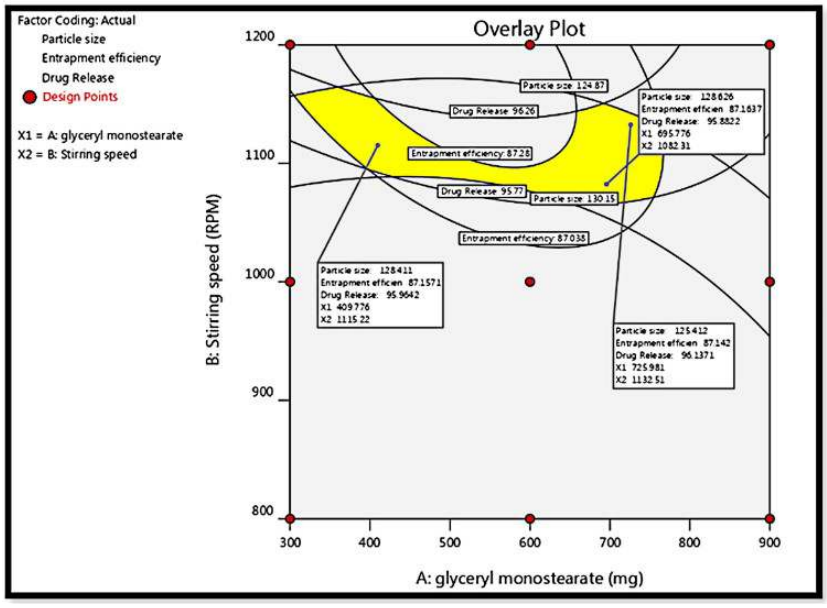

Figure 10 overlay plot

3.5.Check Point Batch Analysis

Three Different Check Point Batches of Chlorthalidone Solid Lipid Nanoparticle Were Prepared according to the levels of factors as shown in Table 31 The check point was evaluated for particle size, % entrapment efficiency, % CDR. From above Batches the Best batch were selected.

Table 10 Evaluation Parameters of checkpoint batches

S.NO	Parameter	Result		
		F1	F2	F3
1	Particle size	126	125	127
2	Zeta potential(mv)	-22mv	-20mv	-21mv
3	% Entrapment efficiency	85.26±0.65	86.88±0.21	86.32±0.83
4	PDI	0.25	0.24	0.26
5	%CDR	94.8±0.54	95.7±0.26	94.2±0.46
6	% Drug Content	99.12±0.53	99.48±0.72	99.26±0.44

3.6.Characterization of optimized formulation

3.6.1. Polydispersity Index and Particle Size

Polydispersity index (PDI) is a measure of particle size homogeneity and it varies from 0.0 to 1.0. Polydispersity is the ratio of standard deviation to mean particle size; hence, it indicates the uniformity of particle size within the formulation. The higher the polydispersity, the lower the uniformity of the particle size in the formulation. The closer to zero the polydispersity value, the more homogenous are the droplets. The polydispersity index of formulation is shown in Fig. The polydispersity index was found to be 0.29 for formulation SLN 5.

Particle Size: - Optimized formulation of Chlorthalidone SLN was shown particle size 125 nm.

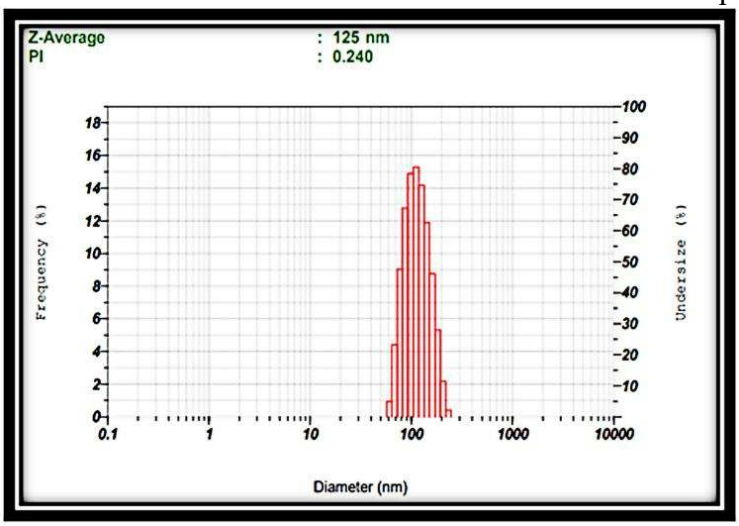

Figure 11 Particle size and PDI

3.6.2. Zeta Potential

Zeta potential is a measure of the charge of particles. If the value of zeta potential is large, the amount of charge on the surface will also large. It represents an index for particle stability. In case of charged particles, as the zeta potential increases, the repulsive interaction are larger leading to the formulation of more stable particles with more uniform size distribution. The Zeta Potential Was Found to be -20.

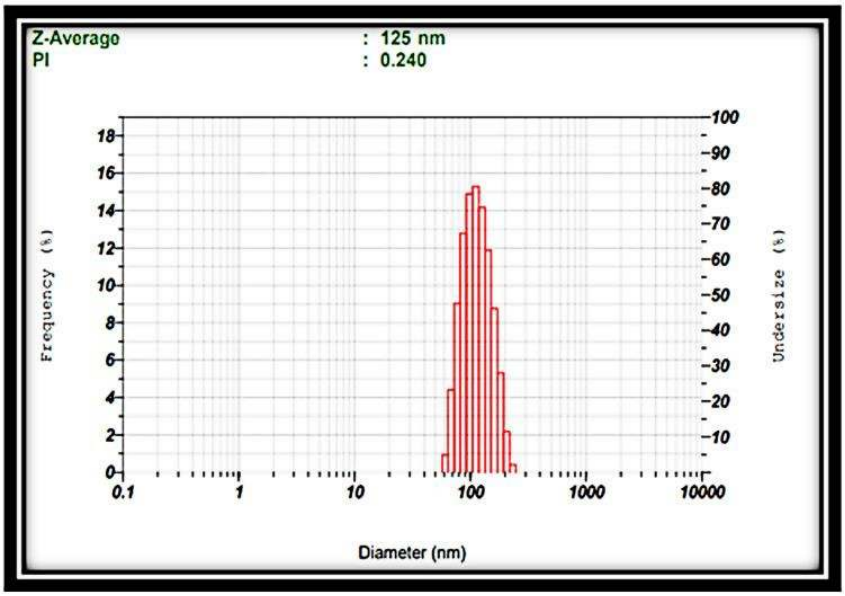

Figure 12 Zeta Potential

3.6.3. Scanning Electron Microscopy

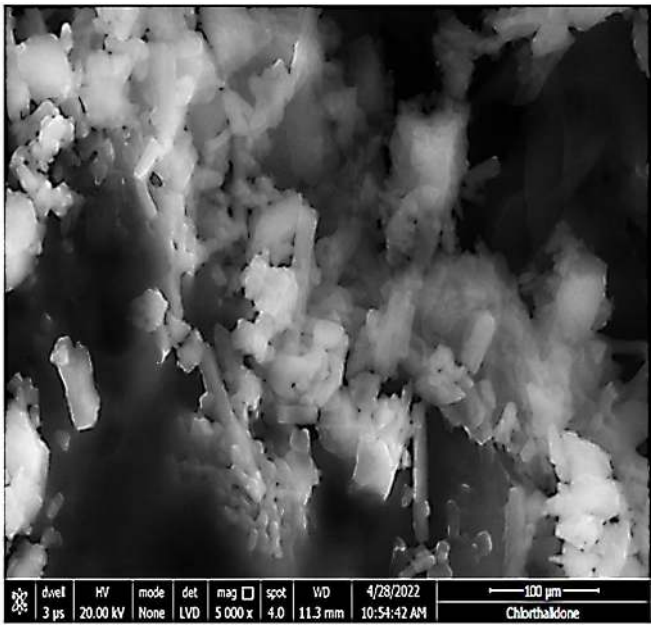

Figure 13 SEM Study of Chlorthalidone API

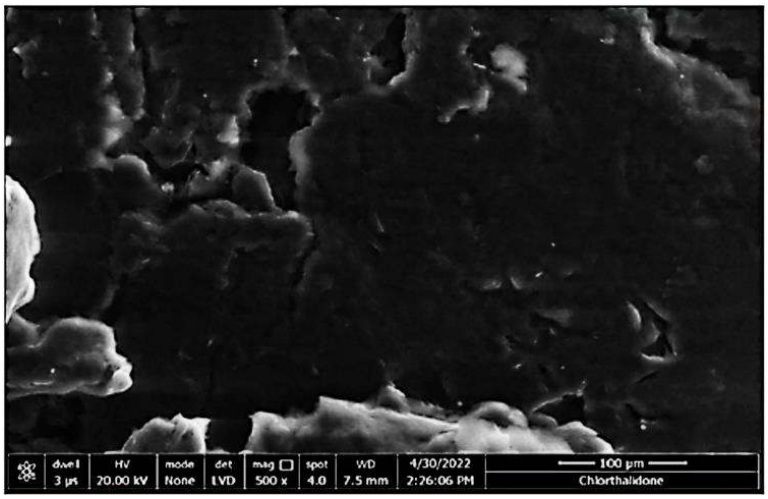

Figure 14 SEM Study of Chlorthalidone Solid Lipid Nanoparticle

3.7. Comparison of *In-vitro* Drug Release between Optimized Formulation and Marketed Formulation.

Table 11 formulation of Comparison

TIME (HR)	Marketed Formulation (CTD-12.5)	FINAL FORMULATION
0	0	0
1	95.4	14.1
2	-	26.9
3	-	44.8
4	-	61.3
5	-	75.8
6	-	94.8

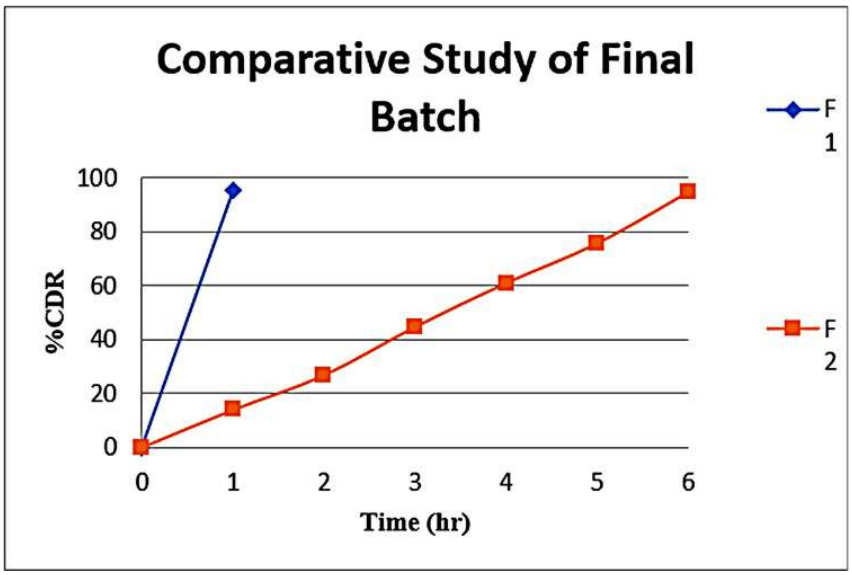

Figure 15 Dissolution of Comparison

3.8.stability Study

- The stability study was carried out based on the ICH guideline Q_2AR1.
- Storage condition was at 30°C ± 2°C/65± 5% RH.

Table 12 Stability Study

Parameters	Accelerated Condition 30±2◦C/65± 5% RH		
	Initial	After15 Days	After30 Days
Particle size	125	125	124
Zeta potential(mv)	-20mv	-22mv	-23mv
% Entrapment efficiency	86.88±0.21	86.85±0.41	86.81±0.83
PDI	0.24	0.24	0.25
%CDR	95.7±0.26	95.1±0.34	94.9±0.89
%Drug Content	99.48±0.72	98.97±0.45	98.83±0.87

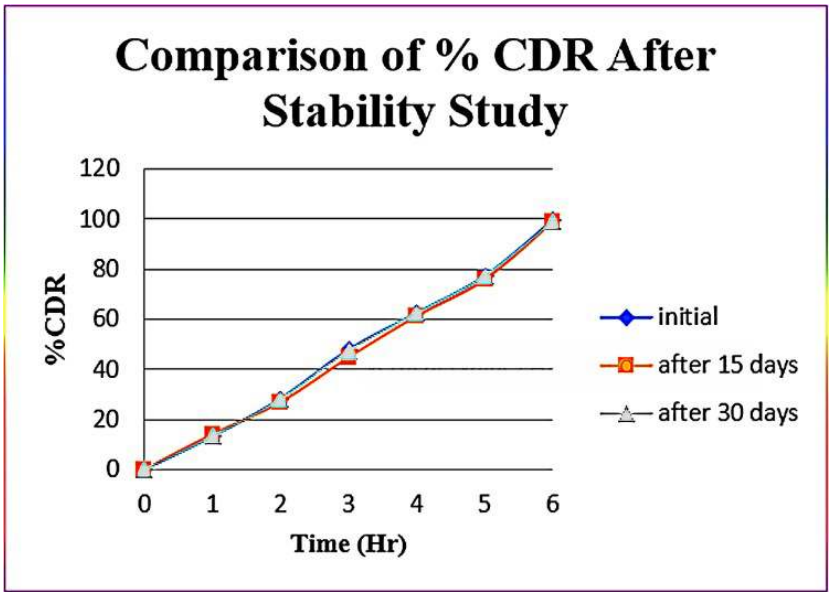

Figure 16 Dissolution of Stability

DISCUSSION

In this study Chlorthalidone loaded SLNs was successfully prepared by using GMS as lipid core and Tween 80 as Surfactant by using Hot Homogenization method. Different formulation parameters, found to influence fabrication of drug into SLNs. The most important parameters were Conc. Of lipid and Stirring time for high %EE, %CDR and minimum particle size. In vitro drug release was observed in controlled drug release profile.

CONCLUSION

The main objective of this project was to developed a Chlorthalidone loaded solid lipid nanoparticles for Oral delivery for the treatment of Hypertension. Solid lipid nanoparticles are most promising delivery systems for the enhancement of bioavailability of highly lipophilic drugs which causing first pass metabolism. Chlorthalidone bioavailability is very low in case of oral delivery because of low solubility and first pass metabolism.

Pre-formulation study including drug identification, Lipid and surfactant selection, and drug-excipient compatibility study, was performed for the selection of suitable formulation components.

Lipid were screened for the development of Chlorthalidone loaded SLN based on drug solubility. Ethanol was selected as solvent based on its ability to dissolve both drug and Lipid at its least quantity.

Chlorthalidone loaded SLNs were prepared by Hot Homogenization method for Optimization. Response Surface plot and contour plot was described to evaluate effect of dependent variable and independent variable. Conc. of Lipid and Stirring Speed was selected as independent variable while Particle size, %EE and % CDR was selected as dependent variable

Optimized batch containing 50 mg Chlorthalidone, 1269 mg GMS as a Lipid, 1.4 ml Tween 80 as surfactant was characterized for morphology, particle size, Zeta potential and % E.E. In-vitro release study, ex-vivo permeability and brain uptake study of optimized batch were carried out. Particle size, Zeta potential and % E.E of optimized batch were found to be 125 nm,-25 mV and 86.88% respectively.

Comparative in-vitro release study was carried out for Drug Tablet and Chlorthalidone loaded SLN. It was found that developed SLNs showed the Controlled release of drug (more than 6 hrs.) as compared to Dispersion due to Drug entrapped in Lipid.

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Availability of data and materials

All data and materials are available upon request.

Declarations Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest regarding publication of the current research work.

SUPPLEMENTARY MATERIAL

Chlorthalidone is given by Umedica laboratories as gift sample, Glyceryl monostearate, Stearic acid, Tewwn-80, Ethanol, Distilled water is obtained from Vishal chem, Mumbai.

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