Research Paper

2^3 Factorial Design: An Approach For Formulation of Solid Lipid Nanoparticles of Etravirine for Oral Administration

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Etravirine is an antiviral belonging to biopharmaceutics classification system class IV due to low aqueous solubility and poor permeability. In this study, to augment the dissolution profile of etravirine, solid lipid nanoparticles have been formulated by utilizing lipid mixture of Compritol® 888 ATO and Gelucire® 50/13 as lipids and poloxamer 188 as surfactant by hot homogenization technique. Effect of variables, ratio of lipids, the concentration of surfactant and sonication time on responses like: Percentage yield, percentage encapsulation efficiency and percentage drug release were studied at 2 different levels and were statistically analyzed using 2^3 full factorial statistical design. The effect of factors on responses was well explained by a significant linear model. The optimized solid lipid nanoparticles preparation was evaluated for zeta potential, size of the particle and polydispersity index. The solid lipid nanoparticles displayed a zeta potential of 36.2 mV, the particle size of 178 nm and polydispersity index value of 0.075 indicating the solid lipid nanoparticles were nano sized and monodispersed. The absence of interaction of drug with excipients was confirmed with differential scanning calorimetry and X-ray powder diffractometer. *In vitro* drug profile of optimized solid lipid nanoparticles formulation was found to be 43.68 % in comparison with pure drug (19.99 %) and the release kinetics for optimum preparation was explained by first-order kinetics. Based on promising *in vitro* results it can be concluded that solid lipid nanoparticles of etravirine can be formulated based on validated factorial design, which may be a potential drug delivery system in the management of human immunodeficiency virus infection.

Key words: Etravirine, solid lipid nanoparticles, 2^3 full factorial design, biopharmaceutics classification system class IV, antiretroviral drugs

Nanotechnology offers many advantages like targeted drug delivery and controlled/programmed release of therapeutic compounds for improved drug pharmacokinetics, biodistribution and pharmacodynamics activity. In addition, the strategy can be implemented to drugs belonging to Biopharmaceutics Classification System (BCS) class II and IV to increase their solubility and permeation through Gastrointestinal (GI) barrier. It can be used to solve the problems associated with poorly soluble drugs, thereby increasing the solubility and bioavailability[1,2]. In recent years lipid-based nanoparticles for drug delivery have gained attention due to their physiochemical diversity, biocompatibility and ability to enhance oral bioavailability of poorly water-soluble drugs[3,4]. Matrix type lipid-based Solid Lipid Nanoparticles (SLN) represent an alternative carrier system to conventional colloidal systems such as emulsions, liposomes and polymeric micro and nanoparticles. SLN are sub-micron colloidal carriers with particle size ranging from 50-1000 nm. SLN is biocompatible and biodegradable and has been used for controlled drug delivery and specific targeting. It also offers a greater surface area, prolonged drug release and instant uptake by the cells. It has the potential to overcome the solubility and bioavailability problem of poorly soluble drugs and enhances the bioavailability of entrapped drugs by enhancing the rate of dissolution and tissue distribution[5,6]. It can be administered by various routes and can incorporate hydrophilic/ hydrophobic drugs with different physicochemical and pharmacological properties[7,8].

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Antiretroviral (ARV) drugs are clinically accepted for effective management of Acquired Immune Deficiency Syndrome (AIDS) by controlling of Human Immunodeficiency Virus (HIV). Etravirine (ETR) is a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) developed for the treatment of HIV-1 infection. It belongs to BCS class IV compound with low solubility and low permeability. With LogP>5, it is practically insoluble over a wide range of physiological pH and metabolized by cytochrome P450[9,10].

Novel drug delivery system has taken a new direction to reach the goals of ARV therapy in the management of HIV/AIDS[11]. SLN has been explored for antiviral drugs for targeting the lymphatic system[12-14] to enhance oral bioavailability by resolving solubility issues and to minimize drug toxicity and extensive first-pass metabolism. Therefore, these SLN holds great promise for reaching the goal of controlled and site-specific drug delivery.

Lipids in SLN preparation should be biocompatible and remain solid at body temperature. Compritol® 888 ATO (COM) has gained attention in the preparation of SLN for its ability to solubilize and entrap lipophilic drug due to its complex structure which gives more space for drug loading[15]. Gelucire® 50/13 (GEL) chemically named as stearoyl polyoxyl-32 glycerides, is a lipid that can enhance drug solubility and bioavailability due to its unique composition. It is blended with other lipids in SLN drug delivery system, for stabilizing lipid nano system and to increase drug loading[16-18].

Since not much work is reported on ETR-SLN preparation, in this work ETR-SLN was prepared using lipids COM and GEL, based on 2^3 factorial design and to evaluate the effect of variables on *in vitro* responses.

MATERIALS AND METHODS

ETR was obtained as a generous gift sample from Apotex Research Private Limited (Bengaluru, India). GEL and COM are gift samples obtained from Gattefosse India Private Limited (Mumbai, India), poloxamer 188 from Sigma Aldrich, and the rest of the analytical grade chemicals and reagents were procured from Merck (Mumbai, India) and SD Fine Chem Limited (Mumbai, India).

Solubility study of ETR in lipids:

The specific amount of lipid was taken in a vial and melted at a temperature greater than its melting point on a hot plate. ETR was added in increments of 10 mg to the melted lipid and vortexed until the complete ETR was solubilized in the molten lipid. This is continued till the saturation level. The amount of drug solubilized in the lipid was determined analytically. Solid lipids such as COM, GEL, stearic acid and glyceryl monostearate were screened to determine the saturation solubility of ETR[13-16].

Formulation of SLN:

Hot homogenization technique was followed for preparation of SLN followed by probe ultra-sonication. The SLN formulation was prepared using GEL and COM as lipids and poloxamer 188 as surfactant. A weighed amount of solid lipids were taken and melted at 70° on a hot plate and ETR was dissolved in a molten lipid mixture. An aqueous phase was prepared separately in a beaker by dissolving poloxamer 188 in distilled water and heated up to 70°. Once the lipid mixture melts the hot surfactant aqueous solution was added to the molten lipid, it is homogenized at 15 000 rpm for 10 min in IKA T18 digital Ultra-Turrax. During this homogenization process, the temperature was maintained constant at 70°. The resultant oil/water (o/w) emulsion was ultra-sonicated by probe sonicator-PUS-250 at 100 W for 5 and 10 min[12,19,20]. The obtained milky nano emulsion was cooled down to room temperature to form SLN suspension. The SLN suspension was centrifuged at 14 000 rpm and the supernatant solution was separated. After centrifugation, the dispersion of SLN is stored in an airtight container and dried in a vacuum desiccator.

ETR-SLN formulations by 2^3 full factorial design:

ETR-SLN formulations were prepared as per 2^3 factorial design. The independent variables selected were lipid ratio (A), surfactant concentration (B) and sonication time (C). Percentage (%) yield (Y1), % Entrapment Efficiency (% EE) (Y2), and % Drug Release (% DR) (Y3) were taken as the response parameters and are considered as dependent variables. Levels of independent variables are shown in Table 1 and the codes +1 and -1 indicates the high and low values of the independent variables. A total of eight formulations ET1 to ET8 with two levels of each factor were prepared according to a 2^3 full factorial experimental as shown in Table 2.

TABLE 1: INDEPENDENT VARIABLES AND LEVELS

Factor	Coded value	
	-1	+1
Lipid ratio (g)	1:1	1:2
Surfactant concentration	1 %	2 %
Sonication time	5 min	10 min

TABLE 2: LIST OF SLN FORMULATIONS WITH CODED VALUES

Formulation	ET1	ET2	ET3	ET4	ET5	ET6	ET7	ET8
Drug, ETR (mg)	100	100	100	100	100	100	100	100
COM:GEL (g)	1:1 (-1)	1:1 (-1)	1:2 (+1)	1:2 (+1)	1:1 (-1)	1:2 (+1)	1:1 (-1)	1:2 (+1)
Poloxamer 188 (%)	1 (-1)	1 (-1)	2 (+1)	2 (+1)	2 (+1)	1 (-1)	2 (+1)	1 (-1)
Distilled water (ml)	50	50	50	50	50	50	50	50
Sonication time (min)	5 (-1)	10 (+1)	10 (+1)	5 (-1)	10 (+1)	5 (-1)	5 (-1)	10 (+1)

Physicochemical characterization of SLN:

Percentage yield and % EE of ETR-SLN: The % yield (% Y) of the dried ETR-SLN was calculated by determining actual yield and theoretical yield[21]. Where actual yield is the amount recovered after preparation and theoretical yield is the yield calculated based on ingredients added in preparation. The % EE of ETR-SLN was determined by the centrifugation method[13]. A fixed quantity of SLN dispersion was centrifuged at 20 000 rpm (Refrigerated micro centrifuge ELTEK RC 4815 F) for 25 min and the sample from supernatant solution was diluted using Dimethyl Sulfoxide (DMSO) and further quantified for free ETR using Ultraviolet-Visible (UV-VIS) spectrophotometer (Shimadzu UV-VIS 1800 spectrophotometer) at 315 nm.

% EE=Weight of initial drug-Weight of free drug/ Weight of initial drug×100

***In vitro* drug release of ETR from ETR-SLN:** *In vitro* drug release from ETR-SLN was performed by United States Pharmacopeia (USP) apparatus II (Paddle type) using 900 ml of 0.01 M Hydrochloric acid (HCl) with 1 % Sodium Lauryl Sulfate (SLS) at 37°±0.5° and 50 rpm. An aliquot of sample was withdrawn at regular time intervals of 30 min and after each sampling, the equivalent volume is replaced with fresh media and sample volume is filtered by 0.45 µm membrane filter[19]. The dissolution was performed for 8 h and the amount of drug released was analyzed using a UV-VIS spectrometer at 315 nm.

Statistical analysis and model validation: The responses obtained after *in vitro* evaluation were subjected to Design-Expert 12.0 for statistical analysis and model validation.

Characterization of ETR-SLN:

Particle size analysis, Polydispersity Index (PDI) and zeta potential: ETR-SLN preparations were dispersed in distilled water and stirred for 5 min to form dispersion and then PDI and mean particle size was measured by dynamic light scattering using a zeta sizer (Malvern Zetasizer). The surface charge was determined by measuring the zeta potential of ETR-SLN. Zeta potential measurements were carried out at 25º with an electric field strength of 23 V/cm[18].

Differential Scanning Calorimetry (DSC): DSC was analyzed for dry samples of the SLNs using DSC 60-Shimadzu. Samples were accurately weighed and placed on the aluminum pan and heated at 25°-300° below nitrogen flow. The flow of heat was measured as a sample temperature and used to study the thermal behavior of nanoparticles[17].

X-ray Powder Diffraction (XRPD): X-ray scattering measurements were obtained by employing X-ray powder diffractometer; generated at 40 kV and current of 30 mA with the diffraction angle (2θ) between 5° and 80° at 25° temperature. XRPD studies were carried out for drug and lipid mixtures, the physical mixture containing drug and excipients, and optimized SLN formulation[19].

Release kinetics: *In vitro* drug release data obtained from release studies were subjected to modeling to assess the kinetics of drug release. Attempts were made to fit the dissolution data into Higuchi model, Korsmeyer-Peppas model and Hixson-Crowell model to determine the mechanism of drug release. The drug release mechanism is explained in terms of the highest correlation coefficient; the highest values of correlation coefficient suggest the release mechanism[20].

RESULTS AND DISCUSSION

Solubility of the drug in lipid is a prerequisite for high drug loading in preparation of SLN[13]. To select suitable lipid for the present study, saturation solubility of ETR was done in various lipids and lipids GEL and COM were selected for preparation of SLN. Good solubility of ETR in GEL (stearoyl macrogol glycerides) is due to high Hydrophilic-Lipophilic balance (HLB) value and amphiphilic property[17] and solubility in COM (glyceryl behenate) is attributed to its chemical structure of lipid[15].

Preparation of SLN by hot homogenization technique followed by probe sonication gave uniform dispersed suspension, which was further processed to get dried ETR-SLN powder. ETR-SLN formulations ET1 to ET8 were evaluated for % Y and % EE and results are tabulated in Table 3. The % EE was found to be in the range of 55.6±1.15 to 89.3±1.29 and % Y in the range of 63 % to 83 %. To understand the effect of independent variables on drug dissolution, formulations were subjected to *in vitro* release studies in comparison with pure drug. The results are depicted in fig. 1a and fig. 1b and ETR-SLN exhibited sustained drug release.

TABLE 3: *IN VITRO* CHARACTERIZATIONS OF SLN FORMULATIONS

Formulation	% Y (Y1)	% EE (Y2)	% DR at 8th h (Y3)
ET1	63	58.4±1.20	21.73±2.90
ET2	69	55.6±1.15	22.50±3.44
ET3	83	87.1±1.57	43.68±2.84
ET4	75	89.3±1.29	41.50±3.18
ET5	76	65.5±1.59	17.66±2.82
ET6	70	81.8 ±1.16	30.47±2.8
ET7	70	63.9±1.41	19.70±2.85
ET8	73	79±1.75	43.05±1.57

Note: Y1, Y2 and Y3 are responses of SLN formulations

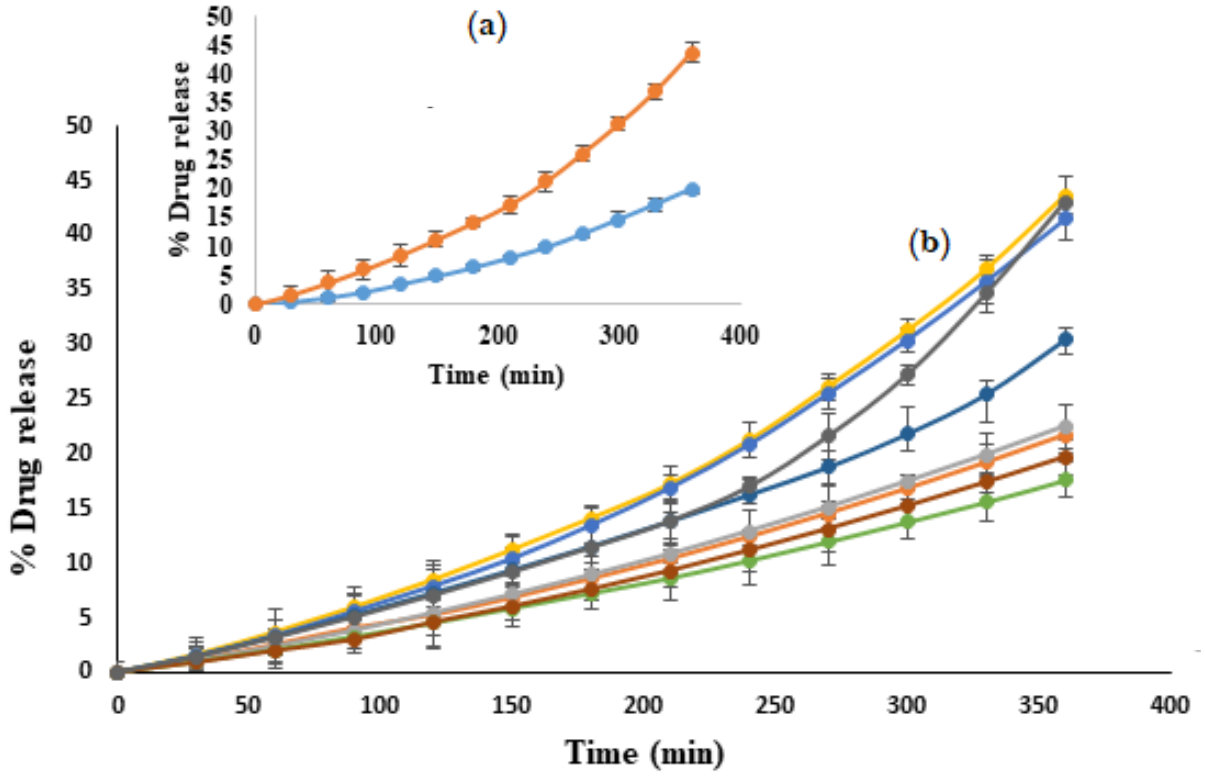

Fig. 1: *In vitro* drug release, (a) *In vitro* drug release of SLN formulation ET3 in comparison with pure drug, (—) Pure drug; (—) ET3 and (b) *In vitro* drug release of SLN formulations ET1 to ET8, (—) ET1; (—) ET2; (—) ET3; (—) ET4; (—) ET5; (—) ET6; (—) ET7; (—) ET8

Formulations were prepared according to a 2^3 full factorial experimental design (Table 2) and the observed responses for all formulations are shown in Table 3. Multiple linear regression analysis was done to estimate the effect of factors on responses by generating a polynomial equation:

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{12}AB+\beta_{23}BC+\beta_{13}AC+\beta_{123}ABC$$

Where Y is the response parameter for each factor level; β_0 is an intercept. A, B and C are the coded levels of independent variables. The polynomial equation was used to determine the coefficients and the mathematical sign positive and negative implies a synergistic effect and antagonistic effect. To identify the best fit model, results were subjected to several regression models; Linear, Two-Factor Interactions (2FI) and Three-Factor Interactions (3FI) in the design expert.

Based on the p value, R^2 value, predicted R^2 value and Predicted Residual Error Sum of Squares (PRESS) value represented in Table 4 and results of Analysis of Variance (ANOVA) represented in Table 5, the best fit significant model for all the responses was found to be linear model represented by the equations as shown in Table 4.

TABLE 4: SUMMARY OF RESULTS OF REGRESSION ANALYSIS FOR RESPONSES Y1, Y2 AND Y3

Reponses	R^2	Adjusted R^2	Predicted R^2	Precision	SD	% CV	p
% Yield (Y1)	0.97	0.9534	0.8934	26	1.27	1.76	0.0013
% Encapsulation (Y2)	0.99	0.9904	0.9781	26.96	1.3	1.79	<0.001
% DR (Y3)	0.88	0.7914	0.5232	416.22	5.1	16.98	0.0256

Note: SD: Standard Deviation; CV: Coefficient of Variation; P: Probability

TABLE 5: RESULTS OF ANOVA FOR MEASURED RESPONSES

Parameters	DF	SS	MS	F
% Yield				
Model	3	237.38	79.13	48.69, significant
Residual	4	6.5	1.63	-
Total	7	243.83	-	-
% Encapsulation				
Model	3	1224.73	408.24	242.28, significant
Residual	4	6.74	1.69	-
Total	7	1231.74	-	-
% DR				
Model	3	768.86	256.29	9.85, significant
Residual	4	104.05	26.01	-
Total	7	872.91	-	-

Note: DF: Degrees of Freedom; SS: Sum of Square; MS: Mean Sum of Square and F: Fischer's ratio

As shown in Table 4, the polynomial linear equation generated for responses are as follows.

Y1=72.38+2.87A+3.62B+2.87C,

Y2=72.58+11.72A+3.87B-0.77C,

Y3=30.04+9.64A+0.59B+1.69C

From the generated linear equation and its coefficient values, it is observed that there is no interaction effect (ABC) on the responses. The effect of independent variables A and B shows a positive significant effect on all the response variables Y1, Y2 and Y3. This indicates that the lipid ratio and surfactant concentration has a significant effect on % Y, % EE and % DR. But variable C has a positive effect on % Y and % DR and a negative effect on % EE. For a better understanding of the effect of independent variables on dependent variables and the significance of the effect, surface response plots and Pareto charts were generated for all the responses. Surface response plots and Pareto charts are represented in fig. 2 and fig. 3 for all the responses. During this study, the volume of the continuous phase and the processing variables such as stirring speed and time is kept constant. From fig. 2a it is observed that all three independent factors are responsible for an increase in the % Y, which is significant according to Pareto charts. This indicates that % Y is affected by the concentration of lipids, the ratio of COM:GEL mixture and sonication time.

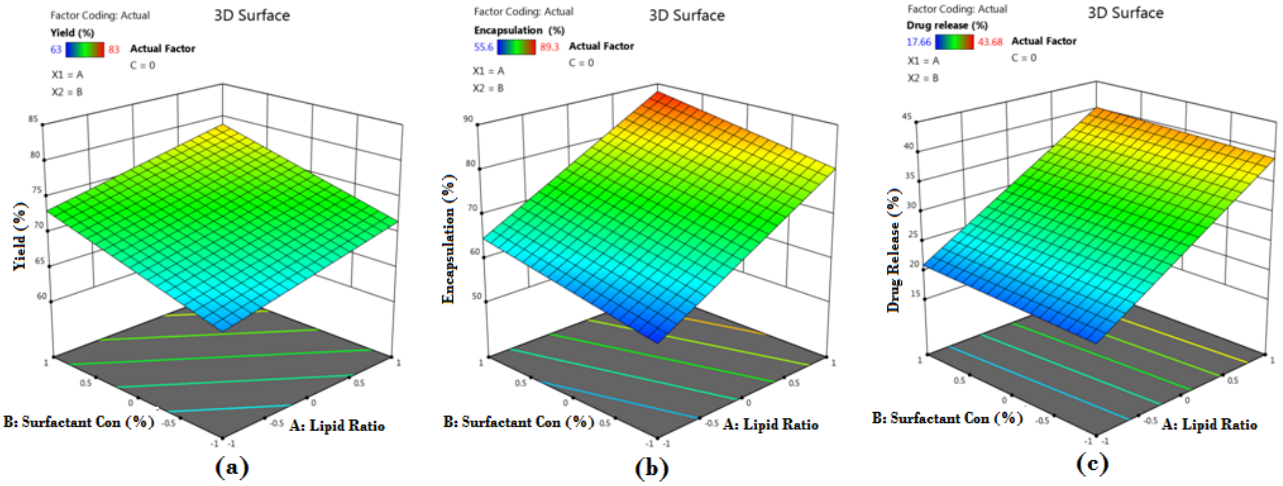

Fig. 2: Response surface plot, (a) Effect of factors on % yield (Y1); (b) Effect of factors on EE (Y2) and (c) Effect of factors on drug release (Y3) responses

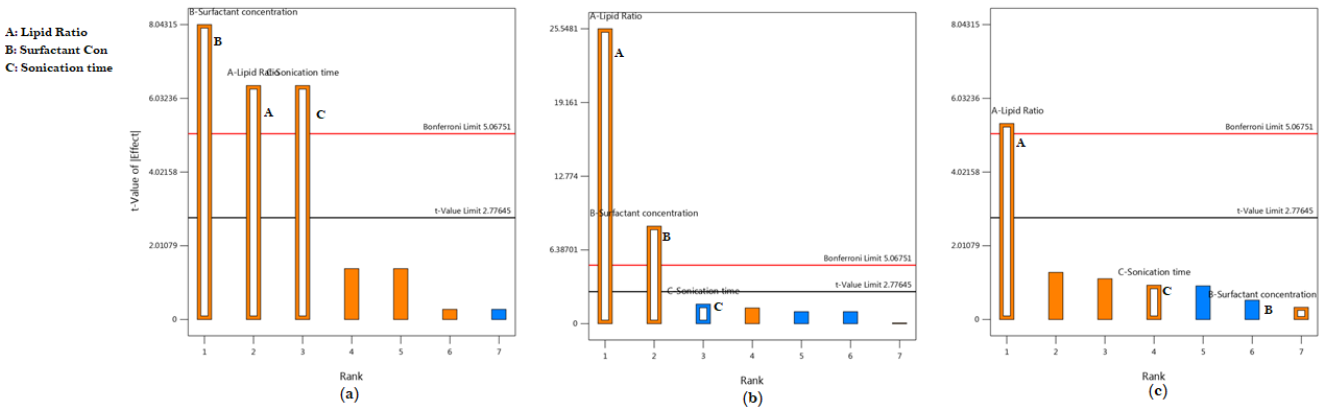

Fig. 3: Pareto chart, (a) Effect of factors on % yield (Y1); (b) Effect of factors on EE (Y2) and (c) Effect of factors on drug release (Y3) responses, where A: Lipid ratio; B: Surfactant concentration and C: Sonication time; ( ) Positive effect and ( ) Negative effect

Fig. 2b and fig. 3b represents the response surface plot and Pareto chart of % EE. Lipid type and its concentration affect drug % EE. In the present study, it is observed that an increase in lipid ratio (COM:GEL) and surfactant concentration (poloxamer-188) increases % EE. This may be due to high lipid concentration containing a mixture of mono, di and triglyceride which tend to form less perfect crystals which result in greater drug loading capacity[13] and in the presence of poloxamer 188 rapid solubility of the drug occurs in lipid phase enhancing drug encapsulation[8]. However, at the same time, it was observed that an increase in surfactant concentration also resulted in decreased % EE, which may be due to increased solubility of the drug in the aqueous phase during the preparation of SLN in the presence of surfactant[21].

Two responses were observed for the effect of factors on % DR, sustained release of the drug and increase in drug dissolution. Sustained release is attributed to the formation of rigid solid particles due to increased lipid concentration and increased viscosity of the medium and aggregation of particles which occurs at higher sonication speed. Both factors result in decreased drug diffusion to the dissolution medium[22]. Another effect observed was change in the lipid ratio which increases drug release. This effect was due to the lipid GEL, which has the properties of reducing interfacial tension and enhancing the solubilization of poorly soluble drugs[17].

For generating optimized formulation numerical optimization was used by stating the range for independent and dependent variables[23]. Formulation with greater % Y, % EE and % DR was selected as optimized formulation, therefore ET3 with % Y 87 %, % EE 87.1 % and % DR 43.68 % was selected as an optimum formulation with a desirable value of 1. Observed values and predicted values of ET3 were in close relation with predicted values of formulation ET3. The model was validated by preparing ET3 formulation in triplicate and evaluating the response. Predicted average experimental values of ET3 were subjected to conformation study and observed that the average data of the experimentation is within the confirmation view indicating the validity of the model (Table 6). From this result, it indicates that generated linear model describes the effect of independent variables on dependent variables and therefore an optimization technique is an appropriate tool for optimization of ETR-SLN.

Particles with submicron sizes, specifically smaller than 400 nm, may increase the bioavailability of drugs due to decreased particle size[24]. In the present study size of the particle for optimum ET3 was found to be 178.8 nm as shown in fig. 4. This result shows that though a high amount of lipid COM is used, the presence of non-ionic hydrophilic surfactant stabilizes particles in the nano size range[25]. PDI of dispersed particles was found to be 0.075 as shown in fig. 4. PDI is a sign of measurement of the particle size distribution; its value specifies the quality of dispersion. PDI values≤0.1 indicate the highest quality of dispersion[26] and in the present study, the PDI value was observed to be 0.075 indicating a homogenous distribution of SLNs. An increase in concentration of surfactant was found to decrease the PDI value. Homogenization facilitates the particle partition and decreases surface area with an increase in surfactant concentration[27]. Positive or negative zeta potential value signifies more repellant forces and repulsion among the particles with like electrical charges and avoids aggregations of particles and therefore, shows a simple distribution[28]. Zeta potential was measured for ET3 formulation and found to be -36.2 mV as shown in fig. 5, which confirms good physical stability.

DSC is used to detect the possible interactions among the components. DSC spectra of drug lipid mixture, physical mixture and optimized preparation of SLN are shown in fig. 6a-fig. 6d.

TABLE 6: CONFIRMATION TABLE INDICATING THE VALIDITY OF THE MODEL

Run 3 response	Predicted mean	Predicted median	Observed	SD	n	SE pred	95 % PI low	Average data of formulation (ET3)	95 % PI high
Yield	81.75	81.75	83	1.2747	3.00	1.1636	78.5191	81.4967	84.9809
Encapsulation	87.4	87.4	87.1	1.298	3.0	1.1849	84.11	84.3967	90.69
Drug release	41.96	41.96	43.68	5.100	3.0	4.6559	29.033	42.2667	54.887

Note: SD: Standard Deviation; SE: Standard Error and PI: Prediction Interval

		Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 178.8	Peak 1:	195.2	100.0	59.47
PdI: 0.075	Peak 2:	0.000	0.0	0.000
Intercept: 0.955	Peak 3:	0.000	0.0	0.000
Result quality Good	D(0.1): 124	D(0.5): 186	D(0.9): 284	

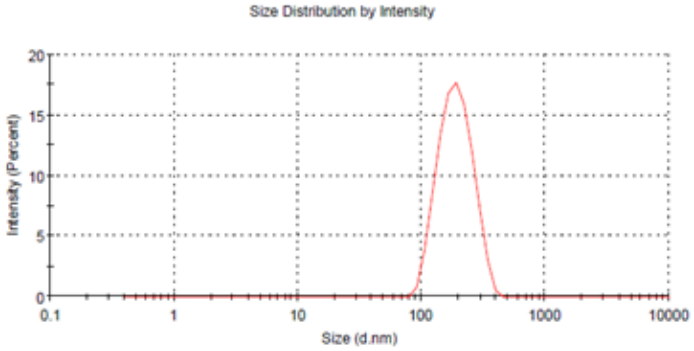

Fig. 4: Size distribution of SLN formulation ET3

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -36.2	Peak 1:	-36.2	100.0	5.43
Zeta Deviation (mV): 5.43	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0190	Peak 3:	0.00	0.0	0.00
Result quality Good				

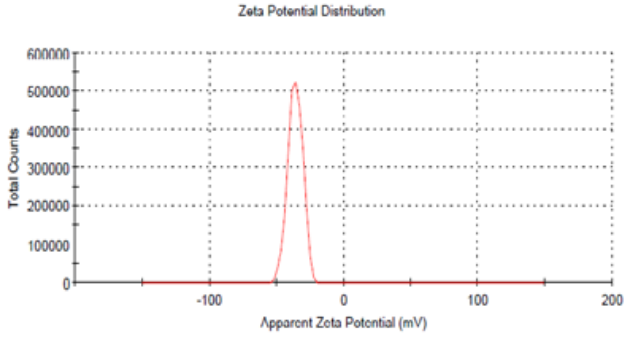

Fig. 5: Zeta potential of SLN formulation ET3

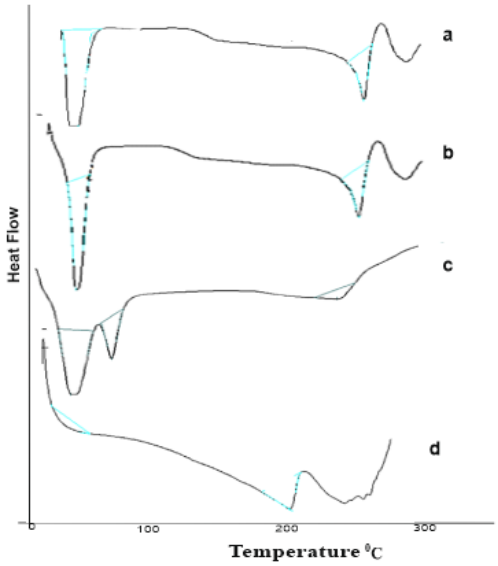

Fig. 6: DSC endothermic peaks, (a) Physical mixture of ETR:GEL; (b) Physical mixture of ETR:COM; (c) Physical mixture of ETR:GEL:COM and (d) ET3 SLN formulation

The sharp endothermic peaks in fig. 6a and fig. 6b at 280°, 75.01° and 50.93° indicate endotherm of ETR, COM and GEL, and the absence of any interaction between drug and lipids. A decrease in intensity of peak or absence of endothermic peak within the melting point range of ETR in the physical mixture and ET3 preparation confirms the conversion of the crystalline state to an amorphous state of ETR. Literature supports such conversion of drugs from crystalline to amorphous when GEL acts as a carrier in SLN dispersion[13,29].

For the assessment of crystallinity of lipid matrices, XRPD was carried out for drug lipid mixture, physical mixture and SLN formulation ET3, and results are depicted in fig. 7a-fig. 7d. As depicted in fig. 7a sharp peaks in drug lipid mixture at about 2θ-scattered angles indicated the crystalline nature of ETR and absence of interaction. In the XRPD pattern of the physical mixture and ET3 SLN fig. 7d, it was observed that reduced intense peaks of ETR present at the same position, which indicated the absence of interaction and ETR was not in crystalline form in SLN. The amorphous state of ETR in the lipid matrix is the probable reason for the better solubility and dissolution of ETR. These results confirmed that ETR existed in an amorphous state in the SLN formulation[27,30].

To study the release kinetics, data obtained from *in vitro* drug release studies were subjected to various kinetic models. It was found that the *in vitro* drug release kinetics of formulation ET3 was best explained by first-order kinetics with the highest linear correlation coefficient (r^2=0.9704) followed by zero-order (r^2=0.9708), Higuchi (r^2=0.909) respectively as shown in Table 7. From Korsmeyer's plots, n value was observed to be 0.6219, indicating drug release followed by Non-Fickian diffusion[24,31].

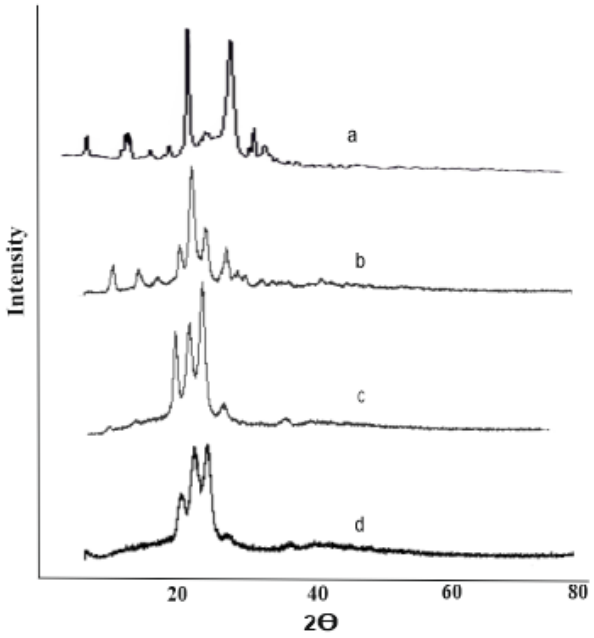

Fig. 7: XRD pattern, (a) Physical mixture of ETR:GEL; (b) Physical mixture of ETR:COM; (c) Physical mixture of ETR:GEL:COM and (d) ET3 SLN formulation

TABLE 7: RELEASE KINETICS OF SLN FORMULATION ET3

Models	Slope (n)	Regression coefficient (r)
Zero order	7.87	0.9664
First order	0.2068	0.9704
Higuchi	26.587	0.9107
Hixson Crowell	-0.0932	0.9557
Korsmeyer-Peppas	0.6219	0.8614

In conclusion, ETR loaded SLNs were successfully prepared by a hot homogenization method followed by probe sonication employing 2^3 full factorial design. From optimization, it was observed that drug release, % encapsulation and % yield are significantly affected by lipid ratio, surfactant concentration and sonication time. The increase in drug release is attributed to nano sized particles and the presence of GEL and surfactant concentration. Hence prepared nano sized ETR-SLN particles can be effectively targeted to lymphatic circulation for the destruction of the viral reservoir. Further, *in vivo* studies are required to confirm the benefits of ETR-SLN particles in the management of HIV infection.

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Conflict of interests:

The authors declared no conflict of interest.

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