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Research Article

In-vivo Evaluation of Fluvastatin Loaded Self-nanoemulsifying Drug Delivery Systems

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ABSTRACT

The present work aims to formulate fluvastatin SNEDDS, its optimization using Box-Behnken Design (BBD) followed by in-vitro and in-vivo evaluation. The SNEDDS formulation comprises 30% sefsol 218, 50% cremophor RH40 and 35% propylene glycol choosen optimal with maximum drug release of 98.62% compared to a pure drug (13.76 %). The formulations evaluated for physic-chemical parameters and results were found within the acceptable limits. The in vivo pharmacokinetic study conducted on Wistar rats indicates that drug plasma concentration in rats administrated with fluvastatin SNEDDS was higher than that of animals treated with fluvastatin. C_{max} of the Self-nanoemulsifying drug delivery system (SNEDDS) (699.972 ± 2.85 ng/mL) was found significant ($p < 0.05$) when compared to that of a pure drug (225.7 ± 0.57 ng/mL). T_{max} of both fluvastatin SNEDDS and pure drug were 50 ± 0.53 and 75 ± 0.72 minutes. The $AUC_{0-\infty}$ and AUC_{0-t} of SNEDDS was higher than the pure drug indicating higher drug concentrations in the blood resulting in systemic absorption of fluvastatin from developed SNEDDS.

Introduction

Fluvastatin is an anti-hypercholesterolemia drug used in treating cardiovascular diseases, which lower water solubility. This drawback poses a major challenge for formulation scientist as deprived drug solubility also leads to lower drug dissolution and bioavailability.[1-2]

Various solubility enhancement techniques with SNEDDS (Self nano emulsifying drug delivery system) are one such potential approach for successful formulation and bioavailability enhancement of poorly water-soluble drugs. SNEDDS are isotropic mixtures of oils, surfactants and co-surfactants that form oil-in-water (o/w) microemulsion agitation in gastric fluids. They spread quickly in the gastrointestinal tract, where the digestive motility provides required agitation for self-emulsification and spontaneously form nanoemulsion (<100 nm) thus enhancing the dissolution, drug absorption and bioavailability. These SNEDDS can ease the administration by forming droplets of nano range that increase both water solubility physical stability and intestinal permeation of the drug.[3-4] Hence the current work aims to formulating and evaluating fluvastatin SNEDDS using BBD to improve solubility, dissolution rate and in vivo bioavailability.

Material and Methods

Material Used

Fluvastatin was gifted from Aurobindo Pharma Limited, Hyderabad. The polymers sefsol 218, cremophor RH40 and propylene glycol were procured from Gattefose France.

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Methods

Construction of pseudo ternary phase diagram

The excipients sefsol 218 (oil), cremophorRH40 (surfactants), and propylene glycol (co-surfactants) were mixed in varying ratios (1:1, 1:2, 1:3, 2:1, 3:1, and 4:1), and % transmittance of the obtained emulsion was analyzed spectrophotometrically at 304 nm. Ternary phase diagram used to identification of self-emulsifying regions with oil, S_{mix}, and water each of them representing apices of the triangle.[5]

Design of Experiment

A 3^3 BBD was used to optimize the main interaction and quadratic effects of ingredients on SNEDDS. About 17 experiments carried out with dependent and independent variables as specified in Tables 1 and 2. The BBD matrix was generated using Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), the second-order quadratic or polynomial equation can be approximated.[6-7]

Preparation of fluvastatin loaded SNEDDS

Based on the solubility study, the oil phase (sefsol 218), surfactant (cremophor RH40), and co-surfactant (propylene glycol) were chosen for the formulation of fluvastatin SNEDDS. 40mg of drug added to oil at 40°C for complete dissolution followed by addition of *surfactant and co-surfactant and sonicated for 60 minutes. Seventeen such formulation prepared and filled into* size 0 gelatin capsule shells.[8]

Characterization of SNEDDS

Developed fluvastatin SNEDDSs were physicochemically evaluated in terms of droplet diameter, zeta potential (ZP), entrapment efficiency (EE), drug content, cumulative % drug release and in-vitro release studies.[9-13]

Table 1: Diifferent factors and responses in BBD

Independent variables			*Levels*		
Variable	*Name*	*Unit*	*Low (-1)*	*Middle (0)*	*High (+1)*
A	Amount of sefsol 218	Mg	10	20	30
B	Amount of cremophor RH 40	Mg	40	50	60
C	Amount of propylene glycol	Mg	15	25	35
Dependent variables			*Goal*		
Y1	Droplet size	nm	Minimize		
Y2	Zeta Potential	mV	Minimize		
Y3	Drug release after 60 minutes	%	Maximize		

Table 2: The BBD with observed responses

Run	*Amount of Sefsol 218 (mg)*	*Amount of cremophor RH 40 (mg)*	*Amount of propylene glycol (mg)*	*Droplet size (nm)*	*Zeta Potential (-mV)*	*Drug release after 60 min (%)*
1	10	40	25	37.5	9.5	93.77
2	30	40	25	98.6	19.6	87.38
3	10	60	25	81.6	22.3	84.58
4	30	60	25	59.8	16.8	83.48
5	10	50	15	35.1	15.6	87.23
6	30	50	15	60.4	18.3	94.59
7	10	50	35	69.5	21.9	93.54
8	30	50	35	22.1	6.7	98.62
9	20	40	15	41.2	13.5	91.37
10	20	60	15	76.1	25.2	89.25
11	20	40	35	44.5	27.5	86.37
12	20	60	35	78.6	10.1	95.29
13	20	50	25	50.3	27.8	85.11
14	20	50	25	87.5	20.2	90.15
15	20	50	25	65.3	24.1	82.47
16	20	50	25	81.8	14.7	88.45
17	20	50	25	81.6	22.13	84.58

In vivo Pharmacokinetic Studies of Fluvastatin SNEDDS

Animal Preparation

The study was conducted on Wistar rats (150-180 g) that were healthy throughout the experimental study. The animals maintained at restricted environmental conditions (25°C, 45% RH and 12 hour light and dark cycles) with fresh air exchange, continuous supply of power and water. The animals fed with standard diet and water *ad libitum* and the study protocol was approved by institutional animal ethics committee.

Animal Study

All the animals were categorized into two different groups; each group containing of six rats. The treatments as given below were administered to the animals. The rats were not fed 24 h prior to the experiment and were provided with food only after 4h of drug administration.[14]

Group I: Treated with pure fluvastatin suspension prepared in 0.5% methocel

Group II: Treated with fluvastatin optimized SNEDDS prepared in 0.5% methocel by oral administration (0.625 mg).

Blood Sampling[15-16]

200 µL of the blood sample was collected at regular time intervals from the femoral artery till 24 hours post dosage and stored in Eppendorf tubes filled with heparin in retard clotting followed by centrifugation to separate the plasma (5000 rpm, 5-10 minutes). The plasma samples were preserved at −20°C and analyzed by HPLC with Mobile phase: Methanol: phosphate buffer: ACN (5:3:2 v/v), Injection volume: 20 µL with flow rate of 1.2 mL/min at 235 nm.

Pharmacokinetic Analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} T_{max} and $t_{½}$ values, an area under plasma concentration-time curve from zero to the last sampling time (AUC_{0-t}), area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$).

Results and Discusion

Construction of Ternary Phase Diagram[17]

Three-component system chosen for the fluvastatin SNEDDS preparation was sefsol 218-cremophor RH40 - propylene glycol. The phase diagram constructed with each component ranges: 10% ≤ sefsol-218 ≤30%, 40% ≤ Cremophor RH40 ≤60%, and 15% ≤ propylene glycol ≤35%.

Evaluation of SNEDDS

The droplet size of all fluvastatin SNEDDSs ranged between 23.8 nm to 98.6 nm and zeta potential ranging in between -6.7 mV to -27.3mV. The formulation FVT8 displayed droplet size and zeta potential of 23.8 and -6.7mV, respectively. The drug content of all formulations ranged between 96.19 ± 0.21 to 99.42 ± 0.15% with maximum value exhibited by FVT8. All seventeen formulations' entrapment efficiency varies between 93.18 ± 0.067 to 98.26 ± 0.079%, with a maximum value displayed by FVT8.

In vitro Dissolution Testing of Fluvastatin SNEDDS

The result suggested that the SNEDDS formulation significantly enhanced the dissolution of fluvastatin with the highest drug release for FVT8 (98.62 ± 1.47%) in comparison to pure drug (13.76 ± 1.56%) (Figs 1 and 2).

Design of Experiments

A series of experiments performed based on 3^3 BBD. All the responses were fitted into the second quadratic equation to check the model's adequacy by ANOVA test. Stat-Ease Design-Expert ® software V8.0 was utilized to analyze data, get regression equation, regression coefficient, and

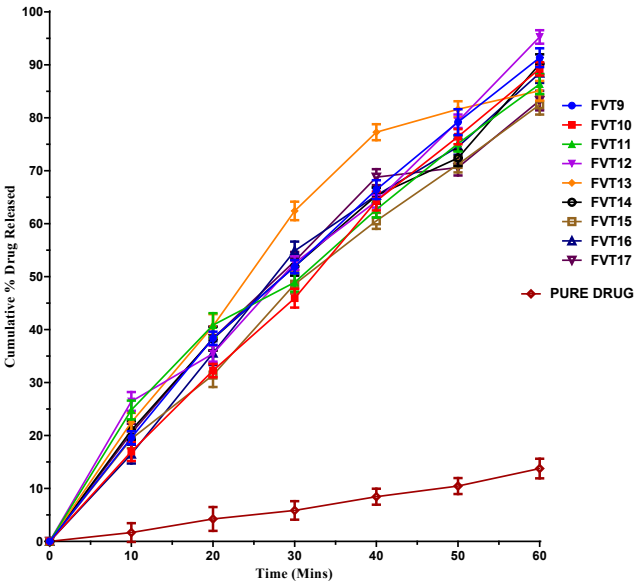

Fig. 1: The drug release profile of Fluvastatin SNEDDS (FVT1-FVT8) and pure drug

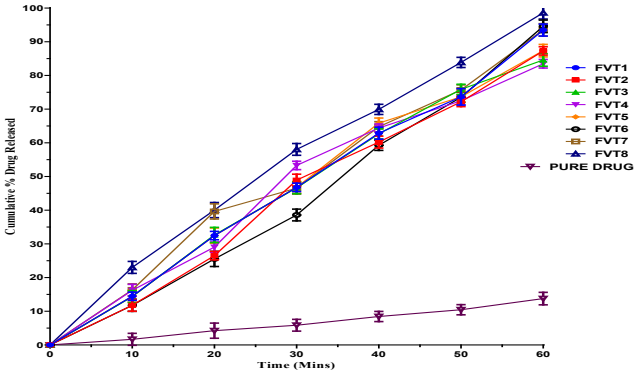

Fig. 2: The drug release profile of Fluvastatin SNEDDS (FVT9-FVT17) and pure drug

analysis of variance (ANOVA).

Droplet size (Y1): The Y1 of the nanoparticles was found to be in the range of 23.8-98.6 nm. The perturbation plot show that B has a major effect on Y1 , A, and C had a moderate effect. The mathematical model obtained for Y1 was found to be significant (Fig. 3)

Zeta potential (Y2): The Y2 of the nanoparticles was found to be in the range of -6.7 to -27.5 mV. The amount of cremophor RH40 and the amount of propylene glycol significantly influence the zeta potential. The mathematical model was found to be significant (Fig. 4).

Cumulative percent drug released (Y3): The Y3 in 60 min from the nano formulations was found to be in the range of 82.47 – 98.62%. The quadratic model generated revealed that the amount of sefsol 218, amount of cremophor RH40 and amount of propylene glycol have a significant influence on the droplet size. The mathematical model generated for Y3 was found to be significant. (Fig. 5)

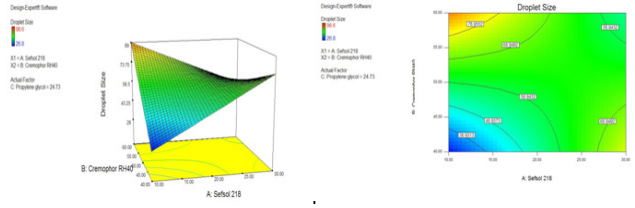

Fig. 3: Response surface and Contour plots showing the influence of amount of sefsol 218 and amount of cremophor RH40 on droplet size fixed level of C

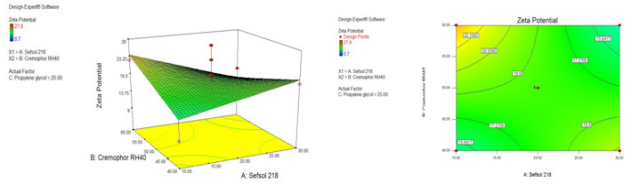

Fig. 4: Response surface and counter plots indicating the influence of sefsol 218 and cremophor RH40 on Y2 level of C

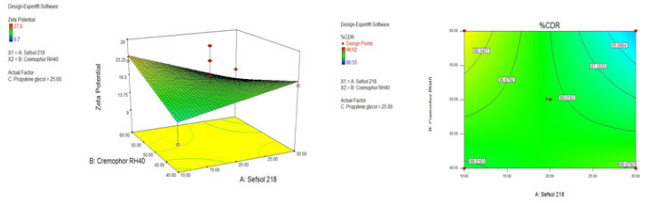

Fig. 5: Response surface and contour plot showing the influence of amount of sefsol 218 and amount of cremophor RH40 on Y3 of C

Optimization by desirability function

The maximum function value was obtained at X1:30, X2:50 and X3:35. To confirm the model adequacy for prediction, three batches of formulations under the optimum composition were prepared, and the three responses were evaluated for each formulation. The results indicate a fine agreement existed between the predicted and observed results indicating the Box–Behnken design's success combined with a desirability function for the evaluation and optimization of fluvastatin SNEDDS formulations (Table 3). The same authors published the formulation and evaluation parameters of fluvastatin SNEDDS.[18]

Pharmacokinetic studies of fluvastatin

At any time point in concentration vs time curve, the drug plasma concentration in animals administrated with SNEDDS formulation is higher than the concentration of animals administrated with pure drug (Figs 6-8).

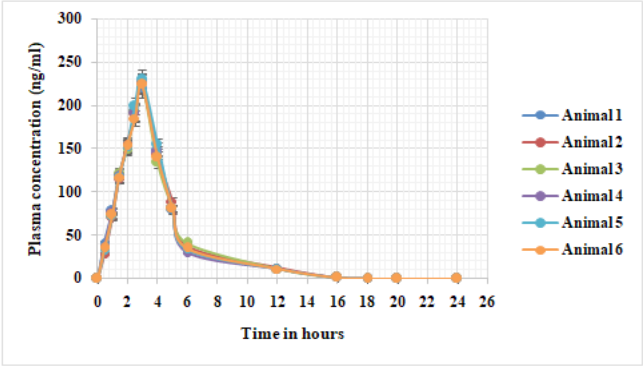

Fig. 6: The drug plasma concentration profile of fluvastatin pure drug in rat plasma

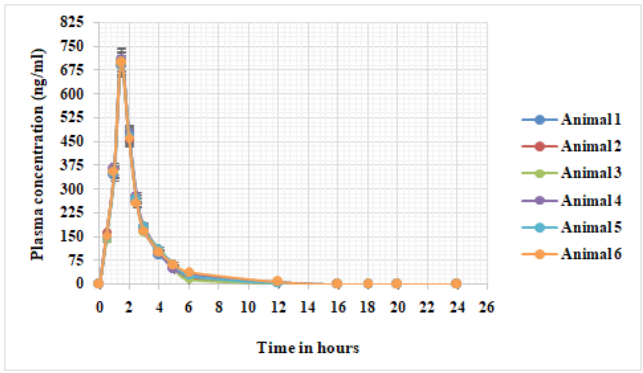

Fig. 7: The drug plasma concentration profile of fluvastatin optimized SNEDDS in rat plasma

Table 3: Optimized values obtained by the constraints applies on y1, y2 and y3

		Predicted values						
Independent variable	*Nominal values*	*Droplet size (Y1) (nm)*	*Zeta Potential (Y2)*	*%CDR (Y3)*	*Batch*	*Droplet size (Y1) (nm)*	*Zeta Potential (Y2)*	*Percent drug release in 15 min (Y3)*
Amount of sefsol 218 (A)	30				1	27.1	-7.5	97.66
Amount of cremophor RH 40 (B)	50	26.8	-6.7	98.62	2	28.6	-6.9	98.23
Amount of propylene	35				3	26.9	-7.2	98.17

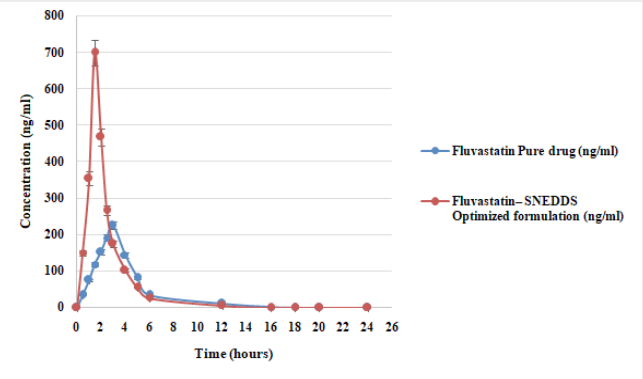

Fig. 8: Mean plasma concentration-time profiles for fluvastatin pure drug and optimized SNEDDS in rats (n = 6)

Table 4: Pharmacokinetic parameters of fluvastatin pure drug and optimised snedds

Pharmacokinetic parameters	*Fluvastatin pure drug*	*Fluvastatin optimized SNEDDS*
C_{max} (ng/mL)	225.7 ± 0.57	699.972 ± 2.85
AUC_{o-t} (ng.h/mL)	640.11 ± 1.02	2065.647 ± 3.21
AUC_{o-inf} (ng.h/mL)	1936.4 ± 1.73	6765.4 ± 2.48
T_{max} (min)	75 ± 0.72	50 ± 0.53
$t_{1/2}$ (h)	3.0 ± 0.02	1.5 ± 0.05

Table 5: Statistical analysis of pkp of fluvastatin

	Fluvastatin pure drug vs. fluvastatin optimized SNEDDS	
Pharmacokinetic parameters	*Significant difference between means (P < 0.05)*	*P*
C_{max}	Yes	<0.0001
T_{max}	Yes	<0.0001
$AUC_{(o-t)}$	Yes	<0.0001
$AUC_{0-\infty}$	Yes	<0.0001

C_{max} of the SNEDDS (699.972 ± 2.85 ng/mL) was significant (p <0.05) when compared to pure drug (225.7 ± 0.57 ng/mL). The T_{max} of both SNEDDS and pure drug were 50 ± 0.53 and 75 ± 0.72 minutes, respectively. The $AUC_{0-\infty}$ infinity for SNEDDS formulation was higher (6765.4 ± 2.48 ng. h/mL) than the pure drug (1936.4 ± 1.73 ng.h/mL). The AUC_{0-t} of fluvastatin SNEDDS (2065.647 ± 3.21 ng h/mL) was higher (p <0.05) than that of pure drug (640.11 ± 1.02 ng h/mL) (Table 4).

The statistical analysis results indicate that there exists a significant variation in PK values of fluvastatin pure drug and SNEDDS formulation (Table 5).

Conclusion

The three-factor, the three-level design approach was applied for optimizing the effects of formulation excipients on (Y1) droplet size in nm, (Y2) zeta potential and (Y3) % CDR. The optimized SNEDDS formulation was evaluated and results show a nanometric particle range with higher drug loading and drug dissolution. The formulation displayed a minimum droplet size of 22.1 nm, zeta potential of -6.7 mV and maximum drug release 98.62 ± 1.47%. The in vivo pharmacokinetic study conducted on Wistar rats indicate that drug plasma concentration in rats administrated with fluvastatin SNEDDS was higher than that of animals treated with fluvastatin. C_{max} of the SNEDDS (699.972 ± 2.85 ng/mL) was found significant (p <0.05) when compared to that of the pure drug (225.7 ± 0.57 ng/mL). T_{max} of both fluvastatin SNEDDS and pure drug were 50 ± 0.53 and 75 ± 0.72 minutes. The $AUC_{0-\infty}$ and AUC_{0-t} of SNEDDS was higher than the pure drug indicating higher drug concentrations in blood, resulting in systemic absorption of fluvastatin from developed SNEDDS. Thus our study confirmed that fluvastatin SNEDDS enhanced the dissolution rate of drug leading to enriched bioavailability.

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