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Formulation Development, Optimization, and Characterization of Cilnidipine-Loaded Self-microemulsifying Drug Delivery System

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Abstract

Cilnidipine, a 2, 4-dihydropyridine antihypertensive, is poorly bioavailable and belongs to Biopharmaceutical Classification System Class II. The present study was carried out to develop and evaluate a cilnidipine-loaded self-microemulsifying drug delivery system (SMEDDS) using food grade oil for enhanced pharmacokinetic parameters. The SMEDDS was prepared by low-energy method. A pseudo-ternary phase diagram was developed using triacetin, Tween 20, and Transcutol HP as oil, surfactants, and cosurfactants, respectively. The statistically optimized formulation was obtained and was evaluated for relevant *in vitro* characterizations. Globule size, zeta potential, and polydispersity index (PDI) of the optimized formulation were found to be 9.045 nm, −2.32 mv and 0.203, respectively, indicating stable and uniformly distributed microemulsion nature of the formulation. Developed SMEDDS of viscosity 31 cps was found to be clear in 500 times dilution in water and phosphate buffer pH 1.2. Selection of the optimized SMEDDS was followed by various formulation characteristics, including goat intestinal membrane permeability. The *in vitro* dissolution study of optimized SMEDDS exhibited much better result as compared to the marketed tablet of cilnidipine.

Keywords: Biopharmaceutical classification system Class II, Cilnidipine, Design expert, Goat intestinal membrane permeability, Pseudo-ternary phase diagram, Self-microemulsifying drug delivery system

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Introduction

Drugs falling under Biopharmaceutical Classification System (BCS) Class II and IV are known to have very low absorption, which is difficult to overcome using conventional dosage forms. Accordingly, lipoidal drug delivery systems might become helpful to overcome the above-mentioned constraints and may achieve desired clinical benefits at a lower dose with the added advantage of reduced toxicities. Cilnidipine is one of the recently approved drugs entity for better antihypertensive management in comparison to other calcium channel blockers.[1] In the present study, self-microemulsifying drug delivery system (SMEDDS) of cilnidipine, a newer congener with problems characteristic of the BCS Class II group, was developed, investigated, and evaluated. SMEDDS, a novel drug delivery system, is an isotropic mixture of oil, surfactant, cosurfactant, and drug. SMEDDS has the ability to form oil-in-water (O/W) microemulsion in a spontaneous manner under mild agitation in gastrointestinal tract fluids after oral administration. Enhancement in the solubility and permeability of the BCS Class II drugs, spontaneous formation, thermodynamic stability, improved bioavailability, and feasibility of the preparation are among the primary advantages of SMEDDS.[2] Here, the presence of triglyceride in composition of self-microemulsifying pre-concentrate is expected to enhance the bioavailability by improving lymphatic transport bypassing the portal circulation, which provides a greater interfacial area for absorption and improvement of the physical and chemical stability of drugs.[3,4]

Materials and Methods

Chemical Reagent

Triacetin (glycerol triacetate) and diethylene glycol monoethyl ether (Transcutol HP) were purchased from Sigma-Aldrich. Tween 20, Tween 80, and Span 80 were purchased from Merck Millipore.

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Labrasol was a gift sample from Gattefosse, India. Analytical grade sodium dodecyl sulfate (SDS) was purchased from E-Merck, India. Glycerol, isopropyl alcohol, and PEG 400 were purchased from Merck, India. Cilnidipine was a gift from PURE CHEM PVT. LTD., Gujarat, India, lercanidipine, α-napthol, and ketoconazole were obtained as a gift sample. All chemicals used were of analytical grade. Milli-Q water was used for the present study.

Methods

Selection of oil

Selection of oil for the formulation of cilnidipine was done on the basis of solubility of the cilnidipine in various oils. Oil, safe for chronic use, and low cost was also a concern. Hence, solubility of cilnidipine was determined in various oils (olive oil, almond oil, castor oil, triacetin, sesame oil, flaxseed oil, rice bran oil, and Capmul MCM).

Cilnidipine was added to an excess amount in 2 mL of the oil of each kind separately in a 5 mL capacity Eppendorf tube and mixed using a Cyclomixer. All the tubes were kept at 25 ± 1.0°C in an isothermal shaker for 72 h to reach equilibrium. The samples were then removed from the shaker and centrifuged at 10,000 rpm for 15 min. The supernatant was withdrawn and filtered through a membrane filter of pore size 0.22 μm. Multiple dilution of supernatant was done using methanol for spectroscopic study. The concentration of cilnidipine was determined in different types of oil using ultraviolet (UV)/visible spectroscopy at 240 nm (Intech, Model No: 295).

Selection of Surfactant and cosurfactant

For the present formulation of SMEDDS, surfactant and cosurfactant were selected step by step.

Selection of surfactants

In the present study, the list of surfactants available for the selection and then formulation of desired SMEDDS include Tween 20, Tween 80, Span 80, and Labrasol. In the investigation for selecting the suitable surfactant first of all in water, 2.5 mL of 15 % by weight surfactant solution was prepared and then 4 μL of selected oil (triacetin) was added with continuous forceful vortexing. After observing one-phase clear solution, the addition of the oil was reciprocated again and again until the solution became hazy and cloudy.[5]

Selection of cosurfactants

Selected surfactant was mixed with different solubilizers as cosurfactants, namely, glycerol, isopropyl alcohol, PEG 400, Transcutol HP, and propylene glycol. At a fixed mix ratio of 1:1, the pseudo-ternary phase diagrams were constructed. Ten different combinations in different weight ratios of oil and S_{mix}, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 1:3.5 were taken so that maximum ratios were covered to outline the boundaries of phases formed exactly in the phase diagrams.[5]

Development of pseudo-ternary phase diagram

The pseudo-ternary phase diagram was developed using various ratios of oil and S_{mix}. For each of the phase diagram, a specific ratio of surfactants and cosurfactant (1:1, 2:1, 3:1, and 4:1) was mixed with the selected oil in various ratios (1:9–9:1, i.e., 1:9, 2:8, 1:3.5, 3:7;4:6,5:5,6:4,7:3, 8:2, and 9:1) in 10 ml Borosil glass tubes. Pseudo-ternary phase diagram was constructed using water titration method. Titration was made using Milli-Q water in increasing proportion and a change in the mixture was noted with each step. Water was added step by step using vortex mixer at 25°C. With this process, phase diagrams were prepared using software and then selection of the appropriate S_{mix} was done observing best microemulsifying region. Design of the formulation was also obtained with the same region. Here, in the obtained pseudo three-component phase diagram, one axis represents the aqueous phase, the other represents oil phase, and the third one represents the mix surfactant phase, that is, S_{mix}.[5,6]

Method of selection of formulation composition

Following the pseudo-ternary phase diagram, the region of highest emulsification where maximum dilution of the emulsion is possible with water would be considered as the formulation composition. Further following the same composition, a formulation design was generated using Design-Expert™ (Stat-Ease Inc.) v7.0 following D-Optimal design and point exchange algorithm and all formulations evaluated for relevant physicochemical characterizations. On the basis of obtained parameters, the optimized formulation was selected and further evaluated for relevant *in vitro* characterizations.

Thermodynamic stability study[7]

The 16 developed formulations (cilnidipine SMEDDS) were subjected to different thermodynamic stability tests to assess their physical stability and observations were made for instability such as phase separation or turbidity.

1. Heating-cooling cycle: All the formulations were kept at 4°C (refrigerated temperature) and 45°C with storage at each temperature of not less than 48 h. A total of six cycles of the same process were repeated. The formulations were examined for stability at these temperatures
2. Centrifugation test: All the developed formulations were centrifuged at 10,000 rpm for 15 min and observed for phase separation phenomenon
3. Freeze-thaw cycle: The formulations were given three consecutive freeze-thaw cycles between −20°C and +25°C storage temperature for not less than 48 h.

Robustness to dilution in GI lumen

Robustness of cilnidipine SMEDDS to dilution was studied by diluting it 125, 250, and 500 times with various dissolution media, namely, water, phosphate buffer pH 1.2, and phosphate buffer pH 6.8. Then, the diluted SMEDDS was kept for 6, 12, 24, and 72 h and observed for drug precipitation and phase separation like phenomena. Five hundred times diluted SMEDDS formulations were checked at 640 nm using Milli-Q water and other media as blank in UV spectroscopy.[8]

Method of globule size, zeta potential (surface charge), polydispersity index (PDI), and measurement

The mean globule size of the developed SMEDDS was determined by dynamic light scattering method (DLS-nano ZS, Zetasizer, Nanoseries, Malvern Instruments). The same instrument was used for assessing PDI, which indicates the broadness of the size distribution of globules and surface charge (zeta potential) of the formulated SMEDDS.[9,10]

pH

The pH of the optimized SMEDDS was measured using pH meter (Sartorius PB-11) at 25°C.

Refractive index

The refractive index of the SMEDDS samples and placebo samples (SMEDDS without drug) was measured using an Abbe refractometer at 25°C. Refractive index is used to assess the isotropic nature of SMEDDS.

Viscosity

All the formulated SMEDDSs were studied for viscosity using Brookfield Viscometer DV II+ Pro (Brookfield Engineering Laboratories, Inc., MA) with spindle CPE 41. Here, 2 mm height of the SMEDDS formulation was used for viscosity determination. The speed of the spindle was adjusted to 10 rpm and a single run was performed at a temperature of 25 ± 0.5°C. A stabilization period of 15 min was provided.[11]

Electrical conductivity

The electrical conductivity of all the formulations was measured to check whether the type of the emulsion formed is O/W or W/O along with percolation effect. Here, electrical current was passed through the samples. A deviation in the digital conductivity meter with cell, (Systronics 304) is expected for the O/W emulsion type.[12]

Method validation for in vitro dissolution and membrane permeability study in high-performance/pressure liquid chromatography (HPLC)

The *in vitro* dissolution studies and goat intestinal membrane permeability of designed formulations were determined by HPLC method. The system consists of Shimadzu series with a UV detector. All the samples were analyzed using an octadecyl silane column (5 µm, 4.6 × 250 mm) with thermostat set at 25°C. For method development acetonitrile, methanol and water were used as mobile phases. Various flow rates were checked as 1 ml/min, 0.8 ml/min, and 0.5 ml/min. Both isocratic and gradient separation methods were studied for this purpose. For selection of internal standard, lercanidipine, α-napthol, and ketoconazole were used.

In vitro dissolution of designed formulations

To select the optimized formulation, all the statistically designed formulations were evaluated for drug release with the sample and separate method.[13] In this method, the developed self-microemulsifying formulations were directly added into the release medium (phosphate buffer 6.8) kept in 900 ml basket of USP apparatus II at rpm 100 and maintained at 37 ± 0.5°C. The samples were collected at time intervals of 1, 5, 10, 15, 20, and 30 min followed by centrifugation at 10,000 rpm. Then, the supernatant was filtered with 0.22 µm membrane filter. Then, the filtrate was measured with the help of HPLC. Following this method, the release of the cilnidipine from the developed formulations was studied for different time points.

Ex vivo permeation studies using goat intestinal membrane

For investigation of the highest permeable SMEDDS among all developed formulations by establishing the individual permeation capability, intestinal permeation studies were conducted. For the study, fresh goat intestine was brought from the nearby butchery and kept at −2°C. The intestinal portion used for the experimental work was washed with Krebs-Ringer solution and the mucous was removed along with unwanted intestinal contents. A bag-shaped structure of the intestine membrane was prepared using both the ends where one end is open. Cilnidipine powder suspension with 0.2% SDS and developed formulations of SMEDDS equivalent to 2 mg of drug was put into the luminal part and was tightly closed. After placing the tissue in an organ bath on RPM 50 maintained with the help of a magnetic stirrer with continuous aeration and maintaining the room temperature of 37 ± 0.5°C, the permeation study was carried out. The receptor compartment consists of 250 ml of phosphate buffer pH 6.8 with 0.5% SDS. Samples were collected at regular time intervals of 5 min, 30 min, 60 min, 1 h, 2 h, 3 h, and 4 h and blank 6.8 pH phosphate buffer was added time to time for maintaining sink condition. This *ex vivo* permeation study was done using Lab Companion (Model No. IST-3075R). Further, the collected samples were filtered through 0.22 mm and quantified using HPLC.[14]

Results

Selection of Oil

Based on maximum drug, solubility as well as therapeutic suitability triacetin were selected as oil for formulation. Triacetin is a short chain triglyceride and GRAS listed oil used as food ingredient with no limitation.[15] Solubility of cilnidipine in various oils is shown in Table 1.

Selection of Surfactant and Cosurfactant

Tween 20 and Transcutol HP were selected as surfactant and cosurfactants, respectively.

Development of Pseudo-ternary Phase Diagram and Design of the Formulation

After components selection, it was required to fix the ratio of the selected oil (triacetin), surfactant (Tween 20), and cosurfactants (Transcutol HP) for formulation development. To fix the ratio, four different pseudo-ternary diagrams were constructed with varying ratio of S_{mix} to oil using water titration method. Further to understand the stability of mixtures formed after water titration to the mixture of oil and S_{mix}, all the tubes were kept for observation up to 48 h. Now considering the maximum emulsifying area obtained in different phase diagrams, acceptable daily intake and extent of homogeneous behavior of mixtures after 48 h as most significant and critical reasons S_{mix} ratio of 1:1 with triacetin (selected oil) were selected for best fitted diagram [Figure 1]. The variable for the formulation design obtained from pseudo-ternary phase diagram is found and depicted in Table 2. Further, 16 formulations were then designed using Design-Expert™ (Stat-Ease Inc.) v7.0 following D-Optimal design and point exchange algorithm and subjected to various characterization studies to select the best one. Results of characterization study are shown in Table 3.

Table 1: Solubility of cilnidipine (presented in mean±SD n=3) in various drugs

Oil	Mean solubility with SD (mg/ml)
SOYA	2.16±0.1587
CMCM	31.71±1.575
TCTN	59.53±3.541
FL	1.241±0.07967
ALM	1.596±0.1021
RB	2.563±0.2014
SES	1.767±0.1193
OL	2.227±0.193

Soya: Soybean oil, CMCM: Capmul MCM, TCTN: Triacetin, FL: Flaxseed oil, ALM: Almond oil, RB: Rice bran oil, SES: Sesame oil, OL: Olive oil

Selection of Optimized Formulation

All 16 formulations were characterized for globule size, zeta potential, PDI, viscosity, percentage transmittance, self-emulsification time, *in vitro* permeability, *in vitro* dissolution, and conductivity. All found data were analyzed for optimization using Design-Expert v7.0. Appropriate response equations were generated and numerically optimized. Responses and optimized formulation are depicted in Figure 2.

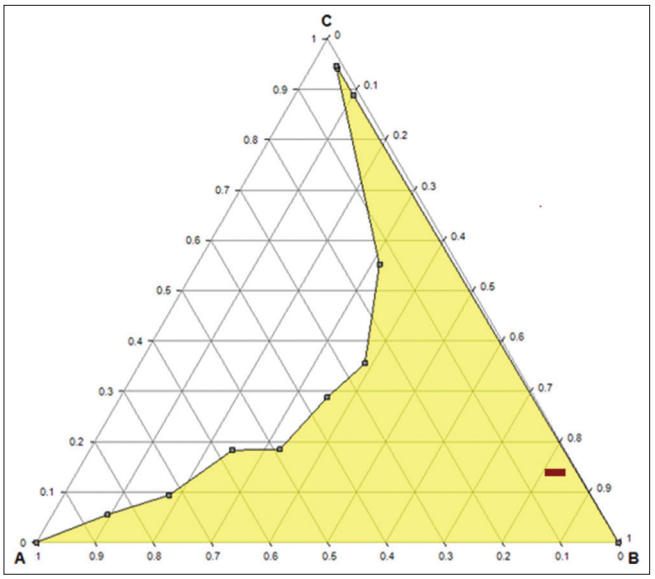

Figure 1: Selected pseudo-ternary phase diagram: 1:1. Where A: Oil, B: Smix, and C: Water. Area depicted in red is desirable composition of components

Table 2: Formulation variable of the SMEDDS

Name	Lower limit	Upper limit
Oil	5	10
S_{mix}	80	85
Water	10	15

SMEDDS: Self-microemulsifying drug delivery system

Formulation of SMEDDS

Considering the data available from statistical optimization study and permissible daily intake of the components of formulation as significant basics, the pre-concentrate of optimized SMEDDS was prepared with formulation components as shown in Table 4.

Thermodynamic Stability Study

All the statistically designed formulations were found thermodynamic stable.

Robustness in GI Solution

After 125, 250, and 500 times dilution of SMEDDS in different media and observation of the same for 24 h, no phase separation was observed. Hence, it can be concluded that all media are robust to the optimized SMEDDS.

Globule Size, PDI, and Zeta Potential

Globule size, PDI, and zeta potential of a SMEDDS are critical characteristics and significant for drug release after post-systemic absorption. Low zeta potential is an indication of better stability of nano sized formulation.[16] As an important fact, low PDI indicates the quality of size distribution, that is, uniformity of dispersed system such as microemulsion.[17] We observed in the experimental procedures that even 150 times, dilution of SMEDDS globule size was remarkably unchanged. The optimized formulation was found with a globule size of 9.025 nm and with a PDI of 0.203. Zeta potential of the optimized formulation was measured with Zetasizer (DLS-nano ZS, Nanoseries, Malvern Instruments). All these findings depict that the optimized SMEDDS of cilnidipine had negative zeta potential (−2.32 mv). This negative zeta potential indicates greater facilitation of drug permeability as well as formulation stability and hence effectiveness of the formulation.[18,19]

Conductivity

Conductivity of the optimized formulation was found as 157 µs. The high conductivity of the optimized SMEDDS compared

Table 3: Results of characterization of statistically design formulations

Formulations	Size (nm)	PDI	ZP (mv)	Transmittance	Conductivity (µs)	Viscosity (cps)	SET (sec)
F1	8.825	0.134	−1.62	97.949	168	33	24
F2	8.763	0.183	−0.58	97.27	106	28	26
F3	8.81	0.121	−0.865	97.78	166	26.5	24
F4	8.38	0.108	−0.58	95.27	158	27	23
F5	8.463	0.153	−0.342	98.8553	190	25	25
F6	8.45	0.169	−0.432	98.4	136	32	24
F7	8.596	0.125	−0.432	93.1108	100.8	26	24
F8	8.636	0.164	−1.28	97.72	176	25	22
F9	23.75	0.134	−1.8	97.72	177.6	24	27
F10	8.144	0.152	1.81	97.72	168.4	28	22
F11	8.693	0.112	−1.38	96.38	184	26	23
F12	9.281	0.226	−0.353	97.35	173.8	26	24
F13	8.88	0.179	−0.78	98.34	167	24	25
F14	8.3	0.187	−1.87	97.37	176	24	26
F15	8.523	0.141	−0.703	98.3	149	29	25
F16	8.237	0.197	0.167	97.72	164	31	26

PDI: Polydispersity index

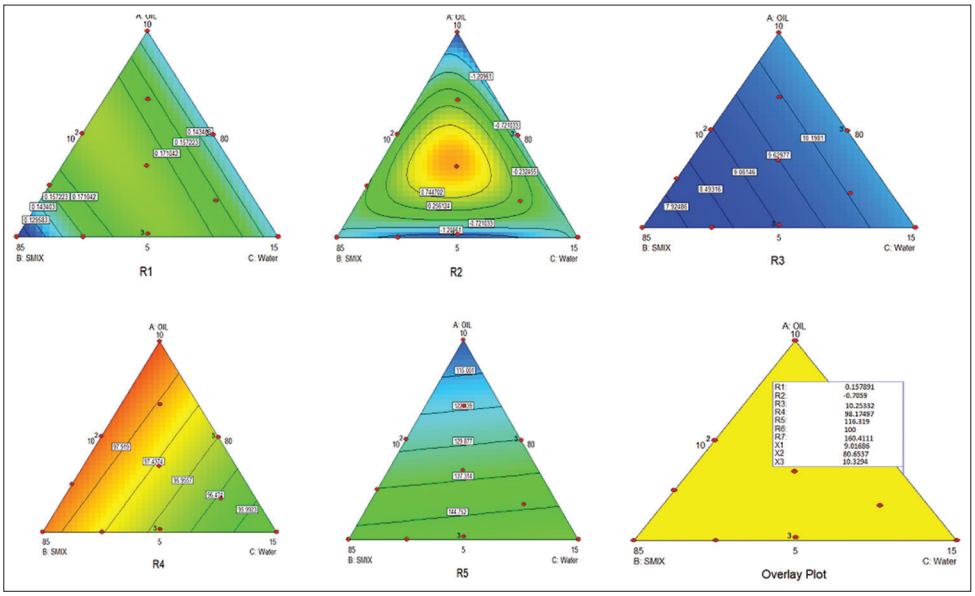

Figure 2: Contour plots. Where (R1) polydispersity index, (R2) zeta potential, (R3) globule size, (R4) transmittance, (R5) conductivity and overlay plot is shown

Table 4: Composition of optimized SMEDDS

Ingredients	Weight in milligram
Cilnidipine	10 mg
Triacetin (selected oil):	130 mg
Tween 20 (selected surfactant)	500 mg
Transcutol HP (selected cosurfactants)	550 mg
Total	1190 mg

SMEDDS: Self-microemulsifying drug delivery system

to that of the dispersion medium indicates the presence of percolation effect, wherein the charge is supposed to jump from one surface to another, leading to sudden increase in conductivity.[20]

Refractive Index

The refractive index of 100 times diluted optimized SMEDDS was 1.378, 1.396, and 1.382 at 25°C. These values are almost similar and constant and near to the value of water (R.I of water 1.333). The constant value of refractive index signifies the thermodynamic stability of the formulation as well as homogeneity and isotropic nature of the delivery system.[21]

Viscosity

The optimized SMEDDS shows viscosity of 31 cps. As far as the patient compliance and manufacturing of dosage forms are concern, lower value of viscosity of the optimized SMEDDS is certainly advantageous.

Self-Emulsification Time

Self-emulsification time of the optimized formulation was 26 s. Time less than 30 s indicates that within 24 s, the pre-concentrate of cilnidipine SMEDDS makes a homogeneous dispersion, which is a critical requirement for the *in vitro* and *in vivo* dissolution.[6,22]

pH

pH of the optimized formulation was 5.9 ± 0.5 indicating the acidic nature of the formulation and important for patient compliance. The mild acidic nature of the formulation is also convenient towards lesser chances of gastric irritation.

Percentage Transmittance

The optimized formulation shows the percentage transmittance of around 98.95 which is very close to 100. This implies very clear formulation, which also is an indication of the drug being completely soluble in the system. Complete drug solubility in the system is essential for the improvement of the bioavailability, which will be studied subsequently, both *in vitro* and in *in vivo*.[23]

Result of HPLC Method Development

Ammonium acetate and water were selected as mobile phase (70:30, v/v) and delivered at a flow rate of 0.8 ml/min. Different concentrations of cilnidipine were recorded at 254 and 237 nm wavelength. Ketoconazole was taken as an internal standard.

In vitro Dissolution of Statistically Designed Formulations

All 16 statistically designed formulations show 100% release in 15 min.

In vivo Permeation through Goat Membrane

Permeability of the drug through intestinal membrane is an important concern. It signifies the presence and effect of oil and surfactants in the formulation. This proof of concept study using through fresh goat membrane significantly shows that suspension of cilnidipine powder drug with 0.2% SDS did not passed though intestinal membrane in 4 h whereas all the statistically designed formulations show permeation through the

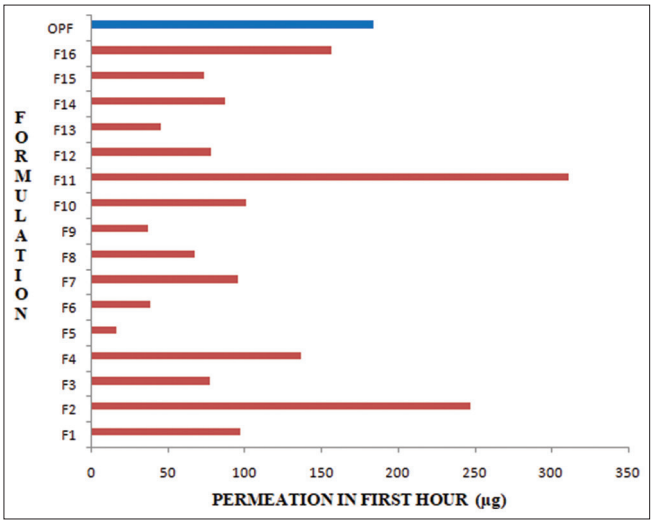

Figure 3: *Ex vivo* permeation of different developed formulation (in µg) from goat intestinal membrane. Here, F indicates number of formulation. OPF is optimized formulation

membrane. This study reflects the importance of composition of the formulations and further helps to find out the optimized formulation. Permeation study was run for 4 h only keeping the integrity of the intestinal membrane as an important concern. Optimized formulation shows permeation of 184 µg cilnidipine through goat intestinal membrane in the 1st h. Result of permeability study of all designed formulations along with optimized SMEDDS is shown in Figure 3.

Comparative *In vitro* Dissolution Study of Optimized Formulation and Marketed Tablet

In comparative *in vitro* dissolution study of optimized formulation and marketed tablet, it was found that the optimized SMEDDS of cilnidipine shows 100% release in 10 min where tablet shows less than 30% in 30 min. This result significantly indicates the enhanced bioavailability of the developed SMEDDS in compare to the conventional tablet dosage form.

Conclusion

In the present study, we have developed a statistically optimized L-SMEDDS of cilnidipine, a BCS Class II and very poorly water-soluble drug. The newly developed formulation of cilnidipine is a pre-concentrate composition of cilnidipine, triacetin, Tween 20, and Transcutol HP as drug, oil, surfactant, and cosurfactant, respectively. The total weight of the optimized pre-concentrate is around 1190 mg consisting 10 mg of antihypertensive CCB cilnidipine. The surfactant and cosurfactants are used in the ratio 1:1 and the amount of the surfactants is kept very low to the acceptable daily intake.[24-26] Triacetin is GRAS listed and fulfills the criteria for the chronic use of the antihypertensive dosage form.[15] Results of goat intestinal membrane permeability study of all designed formulations, various physicochemical characterization studies of optimized SMEDDS, and comparative *in vitro* dissolution study of optimized formulation with marketed tablet significantly indicate toward a thermodynamically stable and desirable SMEDDS and most significantly a better platform for enhanced bioavailability for Cilnidipine in compare to other conventional dosage forms.

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