Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Formulation Development and Evaluation of Solid Self Microemulsifying Drug Delivery System of Azelnidipine

Anuradha P. Prajapati*, Pratik S. Patel, Neha S. Vadgama, Sachin Narkhede, Shailesh Luhar, Shivani J. Gandhi

Department of Pharmaceutics, Smt. BNB Swaminarayan Pharmacy College, Vapi, Gujarat, India

ARTICLE INFO

Article history:

Received: 20 July, 2022

Revised: 30 April, 2023

Accepted: 01 May, 2023

Published: 30 May, 2023

Keywords:

Self-emulsifying drug delivery systems, Azelnidipine poorly soluble, Pseudo-ternary phase diagram.

DOI:

10.25004/IJPSDR.2023.150303

ABSTRACT

This study aimed to develop a self-micro emulsifying drug delivery system (SMEDDS) for poorly soluble azelnidipine using Capryol 90 as the oil, Tween 80 as the surfactant, and transcutol-HP as the co-surfactant. A factorial design was used to optimize the formulation, and Neusilin UFL2 was used as an adsorbent to convert the liquid SMEDDS to solid SMEDDS. The optimized formulation had a particle size of 80.5nm, a transmittance of 98.2%, a zeta potential of -3.1 mV, and a polydispersibility index of 0.226. The solid SMEDDS tablet exhibited improved drug release (99.4% in 60 minutes) compared to the marketed tablet (67.09.75%) and pure drug (26.17%). This study demonstrates the potential of the SMEDDS approach to enhance the solubility and *in-vitro* drug release of poorly soluble drugs such as azelnidipine.

Introduction

Pharmaceutical for the chronic treatment of human diseases, the oral route has been the primary route of drug delivery. However, oral administration of 50% of the drug compound has drawbacks because of the high lipophilicity.[1]

These days, a growing number of medications are classified as class- II medications by biopharmaceutical classification systems (BCS) due to their poor water solubility and high lipophilicity.[2-5] Poor oral bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality are common side effects of class-II medications. Therefore, it is crucial to create appropriate formulations to increase such drugs' solubility and bioavailability.[6-8]

The most prevalent antihypertensive medication is a calcium channel antagonist, which is also used to treat hypertension, the most common chronic disease. In the past 30 years, there has been an increase from 650 million to 1.28 billion adults aged 30 to 79 who have hypertension, according to a WHO report. There are more than 700 million undiagnosed cases of hypertension worldwide.[9-12] 57% of stroke deaths and 24% of deaths from coronary heart disease in India are attributed to hypertension. In India, about 33% of urban Indians and 25% of rural Indians suffer from hypertension.[13-15]

Azelnidipine is a third-generation and long-acting dihydropyridine calcium channel antagonist I. A series of research has demonstrated that azelnidipine produced an effective antihypertensive effect in patients with essential hypertension.[16-18]

***Corresponding Author:** Dr. Anuradha P. Prajapati

Address: Department of Pharmaceutics, Smt. BNB Swaminarayan Pharmacy College, Vapi, Gujarat, India

Email ✉**:** anupatel03@gmail.com

Tel.: +91-9913051223

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The main mechanism by which azelnidipine reduces blood pressure is by inhibiting transmembrane Ca^{2+} influx through the vascular smooth muscle's voltage-dependent channels. Azelnidipine favors L-type Ca^{2+} channels specifically. Strong lipophilicity and affinity for vascular smooth muscle cell membranes are properties of azelnidipine.[19]

The most common approach for delivering drugs is to incorporate them into inert lipid carriers, such as oils, surfactant dispersions, liposomes, microemulsions, and nanoemulsions. The emphasis is on self-emulsifying drug delivery systems (SEDDS), which are stirred to form small droplets ranging in size from 10–100 nm. Due to their ability to increase the interfacial surface area, small droplets improve drug absorption. The drug dissolves in this system's oil, solvent, or surfactant, enhancing its bioavailability and efficacy. SEDDS has been demonstrated to improve the oral bioavailability of poorly water-soluble drugs, making them a promising option for drug delivery. Overall, the use of SEDDS is an effective method for improving the solubility and absorption of drugs.[20]

Formulating drugs with low water solubility, especially those in BCS class II or IV, is complex. Self-emulsifying drug delivery systems (SMEDDS) have become a favored solution in recent years, particularly with the rise of lipid-based oral pharmaceuticals. This study is centered on creating solid SMEDDS for azelnidipine, an antihypertensive medication classified as BCS class II because of its poor solubility. SMEDDS have been shown to improve drug solubility, absorption, and bioavailability, making them a promising alternative for poorly soluble drugs. Developing solid SMEDDS for azelnidipine could potentially enhance its therapeutic efficacy and reduce side effects.

A phase diagram is a graphical representation that displays the relationship between the phase behavior of a mixture and its composition. In the case of a ternary phase diagram, it shows the phase behavior of a micro-emulsion system that consists of oil, surfactant, and co-surfactant. Each corner of the diagram represents 100% of that particular component.

Predetermined amounts of oil, surfactant, and co-surfactant were used to construct pseudo-ternary phase diagrams. The mixtures of surfactant and co-surfactant were formulated in different ratios, such as 3:1, 2:1, and 1:1. The ratio of oil to Smix (Surfactant: co-surfactant) was also varied from 9:1 to 1:9. water was gradually added drop by drop to a predetermined amount of oily mixture under constant magnetic stirring. These mixtures were left to equilibrate overnight, and micro-emulsions were identified through visual observation and polarized light microscopy.

L-SMEDDS can be solidified into S-SMEDDS using different techniques, such as capsule filling and adsorption onto solid carriers. Capsule filling is a simple and common method that offers high drug-loading potential and suitability for low-dose potent drugs. Adsorption onto solid carriers involves mixing L-SMEDDS with suitable carriers to form free-flowing powders that can be filled into capsules. The adsorption technique offers content uniformity and can accommodate high levels of L-SMEDDS (up to 70% w/w) onto the carriers. These solidification techniques can improve the bioavailability and stability of poorly soluble drugs, such as azelnidipine.[21]

Our research aims to develop a self-micro emulsifying drug delivery system (SMEDDS) for azelnidipine, as no existing formulation exists for this drug. This innovative approach is intended to improve Azelnidipine oral bioavailability, thus increasing its effectiveness as a treatment. To support this aim, we will review and analyze previously published manuscripts related to SMEDDS formulations and their impact on drug delivery and bioavailability, providing a solid foundation for our research. Ultimately, our goal is to contribute to the development of more effective drug delivery systems and improve patient outcomes.

Materials and Methods

Material

Azelnidipine API was gifted from Pure Chem Ltd., Ankleswar. Capryol-90 and Transcutol- HP were generous gift from Gattefose for research. Kolisolv GTA, Kolisolv MCT, Koliphor RH-40 were given as gift samples by BASF, Mumbai. Acrysol EL 135 and Acrysol K-140 were given as gift samples by Corel Pharma, Ahmedabad. Tween 80, Tween 20, propylene glycol, PEG-400, Neusilin, aerosil were obtained from S.D. Fine Chem, Mumbai. Captex-355 was gift from Abitech Corporation, Mumbai.

Methods

Solubility Study

Solubility of azelnidipine in various oils (Capryol 90, Kolisolv GTA, Kolisolv MCT, Captex-355), surfactants (tween 20, tween 80, Koliphor RH-40, Acrysol EL-135, Acrysol K-140), azelnidipine was dissolved in excess in 2 mL of each of the chosen oils, surfactant, and co-surfactant in stoppered vials to determine the co-surfactant (Propylene glycol, polyethylene glycol 400, and Transcutol-HP. The mixtures were shaken at 37°C for 72 hours to achieve equilibrium and continuously stirred for 10 minutes in a vortex mixer. The samples were centrifuged at 3000 rpm for 15 minutes with the equilibrated state, and the supernatant was then filtered through a 0.45 m membrane filter and diluted with a suitable solvent. Using a UV-vis spectrophotometer, the amount of drug was measured.[22-24]

Screening of Surfactant

The solubilizing and emulsifying properties of various surfactants must be considered in order to select the best surfactant (tween 20, tween 80, Koliphor RH-40,

Acrysol EL-135, Acrysol K-140). Investigation was done with the screened oil. Weighing and vortexing 10 mL of oil phase and 10 mL of surfactant for two minutes was followed by 30 seconds of warming at 40 to 45°C. We can thus produce an isotropic mixture. In a volumetric flask, 1-mL of the isotropic mixture was diluted with 100 mL of double-distilled water before being filtered through a 0.45 m membrane filter. A clear emulsion was visually seen to form after a number of flask inversions. The resulting emulsions were permitted to stand for 2 hours after which transmittance measurements were made at 650 nm. A clear emulsion with fewer inversions and greater transmittance was created using the surfactant that was chosen.[25-27]

Screening of Co-surfactant

After screening an oil, the ability of various co-surfactants (such as Propylene glycol, Poly ethylene glycol 400, and Transcutol-HP) to emulsify the screened oil was examined in order to find an appropriate co-surfactant with good solubilizing capacity. Prior to warming at 40 to 45°C for 30 seconds, 10 mL of oil phase and 10 mL of co-surfactant were weighed, vortexed for two minutes, and then combined. We can thus produce an isotropic mixture. In a volumetric flask, 100 mL of double-distilled water that had previously been filtered through a 0.45 m membrane was added to 1-mL of the isotropic mixture to dilute it. Visual observation revealed a number of flask inversions that eventually formed a clear emulsion. The resulting emulsions were permitted to stand for 2 hours after which transmittance measurements were made at 650 nm. The co-surfactant with the fewest inversions and the highest transmittance that forms a clear emulsion was chosen.[28-30]

Construction of Pseudo Ternary Phase Diagram

The purpose of phase diagrams is to determine the proportion of components that will result in the greatest microemulsion existence area. These diagrams were created using the water titration method at room temperature with oil, surfactant/co-surfactant, and water. The procedure involved preparing solutions with various surfactant-to-co-surfactant weight ratios such as 1:1, 2:1, 3:1, and so on, which were then vortexed for 5 minutes and placed at 50°C for one hour to produce an isotropic mixture. Each of these solutions was used to prepare a mixture containing oil and Smix (surfactant and co-surfactant mixture) in the following weight ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, which was then vortexed for 5 minutes before being placed in an oven at 50°C.[31] Following that, all of the mixtures were left at room temperature for 24 hours. The appearance of the mixtures with water in them ranged from 5 to 95%. (turbid or clear). The formation of a coarse emulsion might be indicated by turbid samples, whereas the formation of a micro emulsion would be indicated by clear isotropic solutions. A ternary phase diagram was created using the percentage of oil, Smix, and water at which a clear mixture formed. To plot a pseudo-ternary phase diagram, use Prosim 4.1.[32,33]

Formulation of Liquid SMEDDS

The surfactant to co-surfactant ratio was optimized based on the ternary phase diagram. Then, various formulations were created by altering the oil to Smix ratio. SMEDDS were made by adding 8 mg of drug to mixtures of precisely weighed amounts of Smix and oil in a glass beaker. Formulations were made by first creating an optimized ratio of Smix. The components were first stirred with a magnetic stirrer to create a homogeneous mixture, then vortexed with a cyclomixer, and heated on a water bath at 60°C. During storage at 37°C, the SMEDDS were monitored for homogeneity, changes in color and transparency, and phase separation.[34,35]

Experimental Design: 3^2 Full Factorial Design

3^2 Full Factorial Design

To investigate and optimize the main effects interaction effects, and quadratic effects of the formulation ingredients on the *in-vitro* performance of liquid SEDDS, a 3^2 full factorial design factor was used. The Design-Expert software was used to create and evaluate a total of 9 experimental runs at the center (version 12.0.2.0, Stat-Ease Inc., Minneapolis, USA). The replication was performed to estimate experimental error and improve precision by computing model-independent estimate of the process standard deviation. Zeta potential (Y1), self-emulsification time (Y2), and percent transmittance were the important response variables investigated for evaluating the quality of the SEDDS formulation (Y3). The data collected following each response was fitted to a quadratic polynomial model and explained by the following non-linear equation: $Y = \beta0 + \beta1X1 + \beta2X2 + \beta12X1X2 + \beta1X12 + \beta2X22 + E$, where Y is the response of the dependent variables, 0 to 2 are the regression coefficients, and X1, X2 are independent variables. The range constraints were fixed, and all three responses were optimized using the desirability function approach by reducing the zeta potential (Y1), self-emulsification time (Y2), and transmittance percentage (Y3).[36]

Contour Plot

The relationship between independent and dependent variables can be explained visually using a contour plot, which is a diagrammatic representation of the response's values. Using the Design Expert 12 software demo, the reduced model was used to create a two-dimensional contour plot.[37]

Response Surface Plot

Understanding the main and interaction effects of variables during formulation development is aided by the response surface plot. The corresponding response

surface plot can be used to understand how the level of the independent variable affects the response parameter.[38]

Optimization of SMEDDS Formulation using Overlay plot by Design Expert Software

The desirability function approach is a technique for simultaneously determining the best settings for input variables that can determine the best levels of performance for one or more responses. Two steps make up the desirability process:

- Determining the independent variable levels that produce the dependent variable predictions at the same time that are as desirable as possible.
- Increase overall desirability while taking into account the variables under your control.[39]

Characterization of Liquid SMEDDS

Visual Assessment

In 100 mL of purified water was used to dilute the azelnidipine liquid SMEDDS and a magnetic stirrer was used to gently stir the mixture. The ideal temperature is 37°C.[40,41]

Dispersibility Test

It is shown in Table 1 to determine compatibility to dispersing into an emulsion and the size of the resulting globules. The SMEDDS polydispersity test was conducted. Using a standard USP paddle type 2 dissolution test apparatus, 500 mL of water at 37°C was added to the formulation, and the paddle was rotated at 50 rpm. The SMEDDS formulation produces a mixture of different types upon titration with water. Depending on how the formulation's in vitro performance can be evaluated.[42,43]

Determination of Self-emulsification Time

The emulsification time of SMEDDS was determined using dissolution apparatus. One mL of each formulation was added dropwise to 500 mL of distilled water at 37 ± 0.5°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. Emulsification time was assessed visually.[44-46]

Thermodynamic Stability Studies

The performance of a lipid formulation largely depends on its physical stability because drug precipitation in the excipient matrix may have a negative impact. A formulation's bioavailability and therapeutic effectiveness can be impacted by excipient phase separation caused by poor physical stability. Additionally, incompatibilities between the formulation and the capsule's shell may result in brittleness, softness, deleted disintegration, or insufficient drug release. The subsequent cycles were run for these studies.[47-49]

Heating Cooling Cycle

For 100 times distilled water was used to dilute the improved SMEDDS formulations. Six cycles between cooling (4°C) and heating (45°C) temperatures were carried out, with exposure at each temperature lasting at least 48 hours. After that, a centrifugation test was performed on that formulation, which was stable.[50-52]

Centrifugation Test

To estimate metastable systems the optimized SMEDDS formulations were diluted with 100 times distilled water. Centrifuged at 3500 rpm for 30 minutes after passing through heating and cooling cycles. The freeze-thaw stress test is performed on formulations that do not exhibit any phase separation.[53-55]

Freeze-thaw Cycle

This test was carried out to assess the accelerated stability of a micro-emulsion formulation. In this study, three freeze-thaw cycles of formulations were exposed to temperatures ranging from 21 to 25°C for a total of 48 hours. Six such cycles should be run for each batch of formulation to improve the estimation of accelerated stability studies. The formulations with the highest stability were chosen for further investigation.[56-58]

Cloud Point Measurement

In a beaker, dilute the formulation 1-mL with 1000 mL of water and place it on a water bath, gradually increasing the temperature until the diluted formulation becomes cloudy or turbid. It indicates the stability of the microemulsion at body temperature.[59-61]

Table 1: Dispersibility test

S. No.	*Dispersibility and appearance*	*Grade*	*Time to self emulsify (Minutes)*
1	Rapidly forming (with in 1 min) Nano or microemulsion having a clear or bluish appearance.	A	Within 1
2	Rapidly forming, slightly clear emulsion having a bluish white Appearance	B	Within 1
3	Fine milky emulsion that formed with in 2 minutes.	C	Within 2
4	Dull, grayish white emulsion having a slightly oily appearance that is slow to emulsify (longer than two minutes).	D	Within 2
5	Exhibit poor or minimal with large oil droplets present on the surface.	E	Within 3

Percentage Transmittance

A UV spectrophotometer is used to measure the system's percent transmittance while using distilled water as a reference. One mL of the formulation is diluted with 100 mL of distilled water to test the stability of the microemulsion formulation with respect to dilution. Transmittance is then measured using a UV spectrophotometer. At 650 nm, the samples' transmittance is measured, and three replicate assays are run for each sample.[62,63]

Particle Size Distribution (PSD) and ζ-potential Analysis

Using distilled water at a temperature of 37 ± 0.5°C the SMEDDS formulation was diluted 100 times. Using a magnetic stirrer, gentle agitation for 10 minutes produced the desired emulsions. Malvern zeta sizer was used to calculate the final microemulsion's PSD and ζ-potential. [64-66]

Drug Content in L-SMEDDS

SMEDDS liquid that contained 8 mg of azelnidipine was diluted in the appropriate amount of methanol. The sample was thoroughly mixed before being stirred to help the drug dissolve in the methanol. Through a 0.45 µm membrane filter, the solvent extract is filtered. The amount of the drug was determined by comparing the UV spectrophotometer absorbance to the drug's standard solvent solution.[67]

Stability of Azelnidipine SMEDDS

Azelnidipine SMEDDS samples were placed in glass vials with rubber stoppers in stability chambers for 1-month at 25 ± 0.5˚C/60 ± 5% RH and 40 ± 0.5˚C/75 ± 5% RH. Duplicate samples were taken at 0, 15, and 30 days to assess their physical and chemical stability. Visual inspection for physical changes (such as phase separation and drug precipitation) was used to assess physical stability, and a particle size analyzer was used to determine the mean particle size after dilution with water.[68]

Conversion of Liquid SMEDDS into Solid S-SMEDDS

The solid carriers (adsorbing agents) used for adsorption were made of materials with a high surface area and good binding properties to liquid. Fujicacin, colloidal silicon dioxide (Aerosil 200), and Neusiline UFl2 (NU2) were among the solid carriers tested (NU2). The optimized L-SMEDDS formulation was added drop by drop on 2 g of adsorbing agents in a broad porcelain dish, and the mixture was homogenized using a glass rod after each drop of L-SMEDDS to ensure uniform distribution of formulation.[69]

Invitro Drug Release from S-SMEDDS

The *in-vitro* drug release of prepared S-SMEDDS was measured in triplicate using a USP dissolution Type II apparatus (Paddle type) at 37 ± 0.5˚C. In 900 mL of dissolution medium, S-SMEDDS containing 8 mg equivalent of drug was placed (0.1 N HCL). The paddle's revolution speed was kept constant at 100 rpm. To maintain sink conditions 5 mL of dissolution medium was collected, filtered, and the same volume of fresh dissolution medium was replenished at predetermined time intervals. A UV-vis spectrophotometer set to 257 nm was used to determine the drug concentration in the samples.[70]

Result and Discussion

Selection of Oil, Surfactant and Cosurfactant

It is tried solubility with different solvents which is mentioned in Table 2.

The results of the azelnidipine solubility screening in various vehicles are shown in Table 2. Azelnidipine had significantly higher solubility in capryol 90 (223 ± 1.76%) other than Kolisolv GTA, captex-355, kolisolv MCT. Among surfactant and co-surfactants, tween 80 (130 ± 3.22%), transcutol-HP (270 ± 0.27%) showed highest solubility. Based on solubility studies, capryol 90 was chosen as the oil phase, tween 80 as the surfactant, and transcutol-HP as the co-surfactant.

Screening of Surfactant and Co-surfactant[71]

Screening of Surfactants with Capryol 90

The %transmittance values and number of inversions required for uniform emulsion, of various dispersions are given in Table 3.

Tween-80 has good ability to emulsify capryol-90. Also the number of inversions required for the formation of the uniform emulsion was less. Thus selected as surfactant.

Screening of Co-surfactant with Capryol-90

Transcutol - HP emulsifies capryol 90 well and requires fewer inversions to form a uniform emulsion, so it. was chosen as a co-surfactant, shown in Table 4.

Table 2: Solubility study in various vehicles

S. No.	*Solvent*	*Solubility (mg/mL)*
1	Capryol-90	223 ± 1.76
2	Kolisolv GTA	31.1 ± 0.26
3	Captex-355	128 ± 2.19
4	Kolisolv MCT	20.7 ± 1.32
5	Koliphor RH-40	22.47 ± 1.2
6	Tween 80	130 ± 3.22
7	Tween 20	35 ± 1.32
8	Acrysol EL-135	52.1 ± 1.78
9	Acrysol K-140	38 ± 1.64
10	PEG 400	9.39 ± 0.94
11	Propylene Glycol	13.6 ± 1.1
12	Transcutol-HP	270 ± 0.27

Table 3: Emulsification efficacy of surfactant with capryol 90

S.No.	*Surfactant*	*%Transmittance*	*Number of inversions*
1	Tween 20	92	12
2	Koliphor RH-40	95	9
3	Tween 80	99	7

Table 4: Screening of co-surfactant with capryol-90

S.No.	*Co-surfactant*	*%Transmittance*	*Number of inversions*
1	Transcutol-HP	97	8
2	Propylene glycol	94	13
3	PEG-400	93	14

Construction of Pseudo ternary Phase Diagram

To determine optimum oil surfactant and co-surfactant concentration, pseudo-ternary phase diagrams were constructed using CHEMIX software. SMEDDS form microemulsion when titrated with water under agitation condition. The presence of surfactant facilitates this process. It forms a layer around the oil globule in such a way that polar head lies towards aqueous and non-polar tail pull out oil, thereby reducing the surface tension between oil and aqueous phases. Another factor affecting micro-emulsion formation is the ratio of surfactant and co-surfactant. Since surfactant and co-surfactant absorb at the interface and provide a mechanical barrier to coalescence, selection of oil, surfactant, co-surfactant and mixing ratio of surfactant to co-surfactant plays an important role in emulsion formation. The pseudo-ternary phase diagrams were initially constructed at S/CoS (km) 1:1, 2:1, 3:1 and 1:2 ratios. Initially, the surfactant ratio was checked for emulsion formation and fixed. Then by keeping the surfactant fixed amount, it was checked by varying the ratios of co-surfactants and evaluating best- formed formulations and in the concentration of oil taken was maximum, i.e., 90%, and the amount of S/CoS was kept minimum, i.e., 10%. Gradually, oil concentration was decreased and that of S/CoS was increased. It was observed during these experiments that a high concentration of oil forms poor emulsion with a requirement of very less amount ofwater upon dilution. Another observation was that as the concentration of S/CoS increases, the time estimated to form micro-emulsion decreases. The area of micro-emulsion in different ratios of oil: Smix is shown in Fig. 1. The yellow region is where the emulsion shows the highest stability. The region around the yellow region shows less stability of the emulsion. The emulsion has no stability in the lighter region at the corners of the phase diagram. The highest stability was observed L-SMEDDS containing capryol-90, Tween 80 and Transcutol HP at 2:1 Smix ratio. The oil concentration was found to be a rate-limiting factor; in all aspects, high oil concentration resultedin poor emulsion region. The yellow boundary coversthe micro-emulsion region. At any point beyond this boundary, if formed initially, micro-emulsion becomes turbid on further dilution of solution. The more stable formulations that resulted in fine emulsion are further subjected to evaluation parameters.

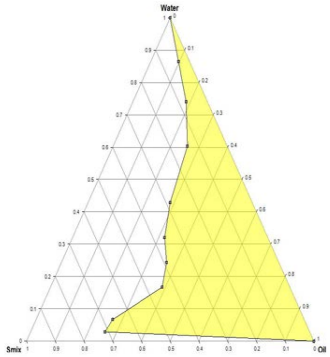

Fig. 1: Pseudo-ternary phase diagram of Capryol 90, Tween 80, Transcutol-HP and Water at 2:1

Optimization of Formulation Using 3^2 Full Factorial Design

When making medications, the 3^2 factorial design study is used to consider the variables that impact both stability and emulsification time.[72]

The study's two independent variables were as follows:

- X1 = capryol 90 concentration
- X2 = Smix (2:1) tween 80 + Transcutol HP concentration

The responses were chosen based on the preliminary studies, and it was discovered that the dependent variables chosen were the zeta potential, percent transmittance, and emulsification time.

Contour Plots and Response Surface Analysis

With the aid of design expert 12 software, contour plots and 3D surface plots based on full factorial designs were created to further explain the relationship between the dependent and independent variables. This kind of plot is employed to simultaneously determine two variables.

Effect of X1 and X2 on Response Y1

As the levels of capryol 90 and S-mix were raised, zeta potential decreased, as shown by two- and three-dimensional plots in Figs. 2 and 3.

Effect of X1 and X2 on Response Y2

As the levels of capryol 90 and S-mix were increased, emulsification time decreased, as shown by two- and three-dimensional plots Figs. 4 and 5.

Table 5: Selection of independent variables

Independent variables	*Levels*	
	Coded value	*Actual Value (mL)*
Concentration Capryol 90 (X1)	-1	0.15
	0	0.20
	+1	0.25
Concentration of Smix (X2)	-1	0.75
	0	0.80
	+1	0.85

Table 6: Design matrix and response with respective observed response

Formulation No	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*
Zeta potential(mv)	-9	-7	-5	-4	-4	-2	-3	-3	-3
PDI	0.37	0.34	0.31	0.28	0.26	0.24	0.21	0.25	0.23
Cloud point (°C)	68	68	68	69	69	70	70	72	72
% Transmittance	94.1	94.5	95.2	96.7	97.5	97.1	98.1	97.9	96.6
Emulsification time (sec)	30	29	27	26	25	25	24	26	26
% Drug Content	96.21 ± 0.27	97.25 ± 0.38	99.01 ± 0.14	99.32 ± 0.25	99.67 ± 0.33	99.78 ± 0.61	99.32± 0.17	99.69 ± 0.38	99.17± 0.53

Table 7: Respective response

Factorial Batches	*X_1 (Conc. of Capryol 90) (mL)*	*X_2 (Conc. of S- mix) (mL)*	*Y_1 Zeta potential (mv)*	*Y_2 Emuls-ification time (sec)*	*Y_3 % Trans-mittance (%)*
F1	0.15	0.75	-9	30	94.1
F2	0.2	0.75	-7	29	94.5
F3	0.25	0.75	-5	27	95.2
F4	0.15	0.8	-4	26	96.7
F5	0.2	0.8	-4	25	97.5
F6	0.25	0.8	-2	25	97.1
F7	0.15	0.85	-3	24	98.1
F8	0.2	0.85	-3	26	97.9
F9	0.25	0.85	-3	26	96.6

Table 8: Summary of Quadratic Polynomial Equation for Responses Y1, Y2 and Y3

`	*Quadratic polynomial equation*
Y_1 (Zeta potential)	$-3.56+1.00X_1+2.00X_2-1.00X_1X_2+0.3333X_1^2-1.67X_2^2$
Y_2 (Emulsification time)	$25.56-0.3333X_1-1.67X_2+1.25X_1X_2-0.3333X_1^2+1.67X_2^2$
Y_3 (% Transmittance)	$97.11-0.0333X_1+1.37X_2-0.6500X_1X_2-0.0667X_1^2-1.07X_2^2$

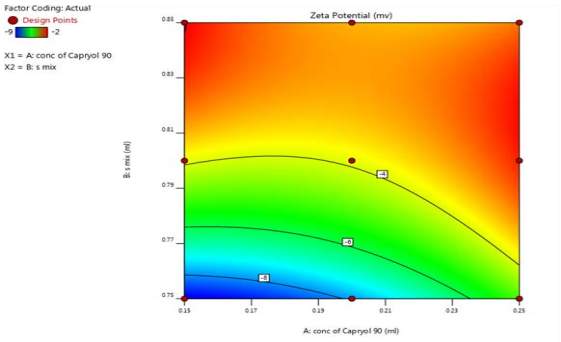

Fig. 2: Contour plot for the effect of zeta potential

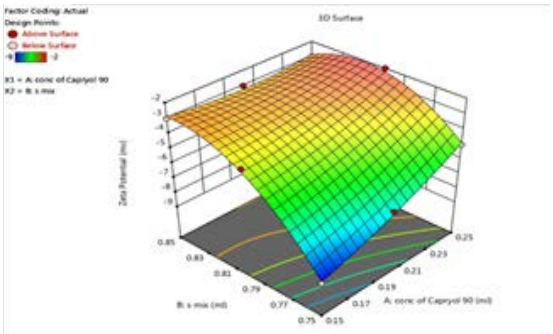

Fig. 3: 3D Surface plot for the effect of zeta potential

Effect of X1 and X2 on Response Y3

As the levels of capryol 90 and the S-mix were raised, the two-dimensional and three-dimensional plots in Figs. 6 and 7 showed an increase in the percentage of transmittance.

Optimization

From design expert 12, the optimized batch was found. The overlay plot, where clearly shows the value for X1 (conc. of capryol 90) and X2 (conc. of Smix), i.e., 0.19 and 0.83 mL, respectively for the desired value of zeta potential,

Table 9: Summary of Results of Multiple Regression Analysis for Y1, Y2 and Y3

Dependent variable	*Y_1 Zeta potential (mv)*		*Y_2 Emulsification time (sec)*		*Y_3 % Transmittance (%)*	
	Coefficients	*p-value*	*Coefficients*	*p-value*	*Coefficients*	*p-value*
Intercept	-3.56	0.0039	25.56	0.0159	97.11	0.0012
X_1	1.00	0.0079	-0.3333	0.2249	-0.0333	0.6400
X_2	2.00	0.0010	-1.67	0.0047	1.37	0.0002
X_1X_2	-1.00	0.0138	1.25	0.0186	-0.6500	0.0037
X_1^2	0.3333	0.3081	-0.3333	0.4437	-0.0667	0.5917
X_2^2	-1.67	0.0088	1.67	0.0218	-1.07	0.0024

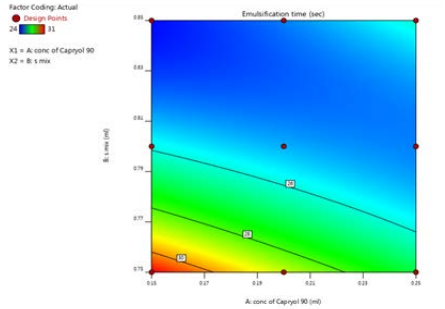

Fig. 4: Contour plot for the effect of self-emulsification time

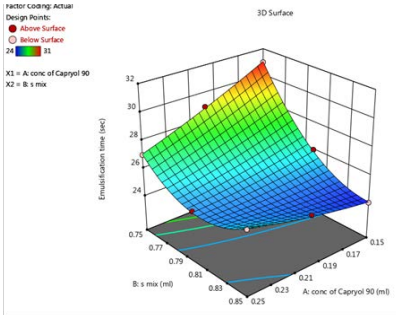

Fig. 5: 3D surface plot for the effect of self-emulsification time

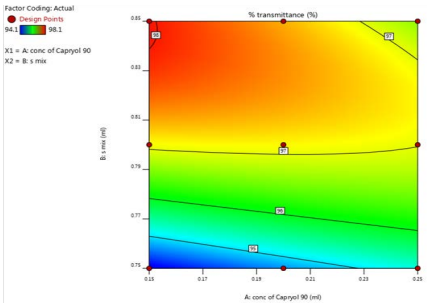

Fig. 6: contour plot for the effect of % transmittance

emulsification time, and %transmittance, was obtained from the contour and response surface plots of the factorial batches. The optimized batch was created in accordance with Fig. 8, where the optimized region is shown in yellow.

Check Point Batch Analysis

Azelnidipine SMEDDS were prepared in three different checkpoint batches (P1, P2, and P3) based on the levels of the factors listed in Table 10. The zeta potential, self-emulsification time, and transmittance percentage of the checkpoints were assessed. The values for zeta potential, self-emulsification time, and transmittance percentage The optimized formulation was examined, and the outcomes were compared to the expected values as shown in Table 11. The outcomes from the optimized batch were comparable and close to the expected values. As a result, we can say that the statistical model is sound mathematical. P2 formulation produces better results than other checkpoint batches. Consequently, chosen for optimized formulation.

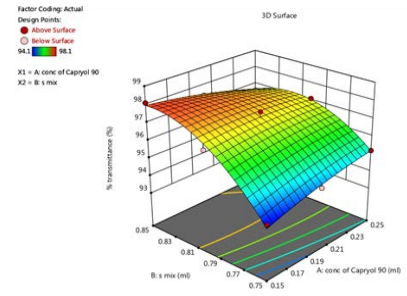

Fig. 7: 3D surface plot for the effect of % transmittance

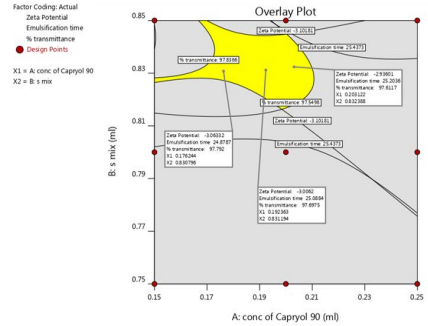

Fig. 8: Overlay plot

Characterization of Optimized Formulation

Globule Size Analysis and Polydispersibility Index

The optimized L-SMEDDS for Azelnidipine were found to have a globule size of 80.5 nm seen in Fig. 9. It was discovered that the polydispersibility index was 0.226. The optimized formulations' polydispersity index was discovered to be lower than 1, indicating that globules were distributed uniformly throughout the formulation. According to these results, the optimized L-SMEDDS generated fine microemulsion with a small mean size and a condensed particle size distribution.

Zeta Potential

The optimized formulation's zeta potential was discovered to be (-3.1 mV) is seen in Fig. 10. This low zeta potential points to increased drug permeability as well as formulation stability and, consequently, formulation efficacy.

Thermodynamic Stability

Physical stability of SMEDDS was essential to its performance, which can be affected by the precipitation of the drug. In addition, the formulation having poor physical stability can affect its performance and lead to phase separation. Hence, thermodynamic stability studies were performed by heating, cooling cycles, and centrifuge tests. it was found that optimized SMEDDS showed good stability without phase separation, creaming, or cracking

Transmittance

The optimized L-SMEDDS formulation exhibits a very close to 100% transmittance percentage of around 98.5%. This

Table 10: Checkpoint batch analysis

Batches	*P1*	*P2*	*P3*
X1	0.17	0.20	0.19
X2	0.83	0.83	0.83
Response	Predicted	Predicted	Predicted
Zeta Potential (mV) Y1	-3.06	-2.93	-3.00
Self-Emulsification Time (sec) Y2	24.87	25.20	25.08
%Transmittance Y3	97.79	97.61	97.09

Table 11: Evaluation of Checkpoint Batches

S.No.	*Parameter*	*Result*		
		P1	*P2*	*P3*
1	Zeta potential(mv)	-4	-3.1	-3.5
2	PDI	0.25	0.23	0.22
3	Cloud point	69°C	70°C	70°C
4	%Transmittance	98.4	98.5	97.27
5	Emulsification time (sec)	25.24	24.47	24.53
7	%Drug Content	99.15 ± 0.27	99.83 ± 0.44	99.05 ± 0.13

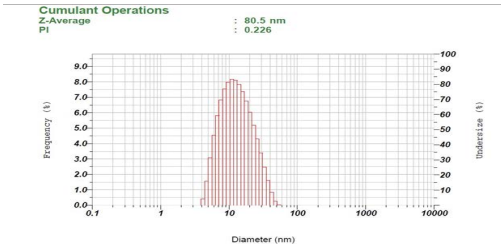

Fig. 9: Globule size of optimized formulation

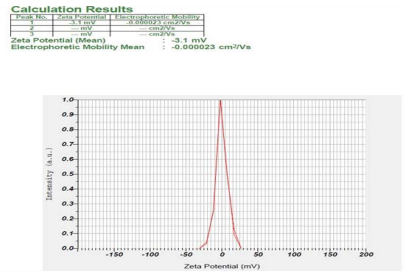

Fig. 10: Zeta potential of optimized formula

suggests a very clear formulation, which is also a sign that the medication is entirely soluble in the body.

pH

The pH of the optimized formulation was 5.9 ± 0.5, indicating the formulation's acidic nature, which is crucial for patient compliance. The formulation's mild acidity also helps to reduce the likelihood of gastric irritation.

Self-emulsification Time

The optimized formulation took 24.47 seconds to self-emulsify. A homogeneous dispersion of the preconcentrate of azelnidipine SMEDDS is made in less than 30 seconds, which is a crucial condition for *in-vitro* dissolution.

Drug Content

Azelnidipine was detected in the methanol extract of L-SMEDDS, which was used to determine the drug content of the formulation. 99.83%. was found to be the drug content.

Cloud Point

The cloud point was found to be 70°C which indicates better stability of L-SMEDDS.

Conversion of L-SMEDDS to S-SMEDDS[73]

Based on evaluation parameters and studies the optimized liquid SMEDDS in Table 12 were converted into free-flowing powder by adsorption into solid carriers selection.

Holding Capacity of Adsorbents

The amount of carrier to be used in the formulation was calculated by the holding capacity and the Lf factor. The results showed that Neusilin US2 had a higher flowability when compared to Neusilin, Fujicalin and Aerosil 200.

Evaluation of Flow Properties for S-SMEDDS Formulations

Neusilin has a very large specific surface area and high oil and water adsorption capacity. Neusilin® is superior in compressibility, enabling hard tablets at low compression force. It can also improve the hardness of other fillers and binders of low concentration. Optimized formulations were studied for their flow properties like angle of repose, bulk density, tapped density, Hausner's ratio and compressibility index. Angle of repose < 30° indicates free flow property while angles > 40° indicate poor flow. From the above formulations, it was observed that formulation with Neusilin US2 has the least flow property when compared formulations as shown in the table above. All the formulations were within the Indian Pharmacopoeia (IP) limits. These formulations were compressed into tablets using 12 mm punch. The tablets were evaluated for physio-chemical properties.

In-vitro Dissolution Studies

Dissolution studies were carried out with US apparatus II (paddle type). All the formulations were subjected to *in-vitro* dissolution studies in 900 mL of 0.1 N HCl. The tablet dissolution studies were done. The S-SMEDDS formulations contain carrier materials Neusilin US2. The drug release profile of the final S-SMEDDS

Table 12: Adsorbent selection

Adsorbent	*Amount of Liquid SMEDDS (mL)*	*Amount of adsorbent required to get free flow powder (mg)*
Neusilin	1	260
Aerosil 200	1	370
Fujicalin	1	425

Table 13: Flow properties of various adsorbent

Adsorbent	*Parameters*				*Inference*
	Bulk density (gm/mL)	*Tapped density (gm/mL)*	*Carr's index %*	*Hausner's Ratio*	
Aerosil 200	0.397	0.542	14.6	1.36	Passable
Neusilin	0.602	0.749	20.16	1.24	Excellent
Fujicalin	0.417	0.601	13.2	1.27	Passable

formulation containing Neusulin showed a better drug release (97%) within 60 minutes when compared to the other formulations (Fig. 11).

Comparison of *In-vitro* Drug Release between Optimized Formulation of S-SMEDDS and Marketed Formulation

It is possible to compare the dissolution profiles of the optimized formulation and the marketed preparation because a conventional tablet was already on the market. Using oral Tablets, the drug release study was carried out (Azovas 8 mg). Azelnidipine capsule S-SMEDDS was examined for *in-vitro* dissolution. For one hour, S-SMEDDS capsules were tested in 0.1N HCL. S-SMEDDS releases 95.4% of the drug in an hour, whereas the market formulation only releases 67.09% seen in Fig. 12. S-SMEDDS offers superior dissolution compared to the commercial formulation.

Accelerated Stability Study[74]

When stored at 40°C ± 2°C/75 ± 5% RH and at the stability study was carried out based on the ICH guideline Q2AR1. Storage condition was at 40°C ± 2°C/75 ± 5% RH accelerated temperature. The stability studies of the optimized formulation showed no significant changes in the physical parameters. There are no interactions seen in the FTIR spectra. Over the course of 30 days, there was no discernible decrease in the amount of the active drug. Consequently, the formulation's 30 day stability was discovered it, was seen in Table 14.

Table 14: Accelerated stability study

Parameters	*Accelerated condition 40°C ± 2°C/75 ± 5% RH*		
	Initial	*After 15 Days*	*After 30 Days*
Zeta potential(mv)	-2.93	-2.87	-2.78
PDI	0.22	0.22	0.23
Cloud point	70°C	70°C	70°C
%Transmittance	98.5	97.91	97.78
Emulsification time (sec)	24.47	24.25	25.11
%CDR	95.4 ± 0.65	94.9 ± 0.78	94.6 ± 0.53
%Drug content	99.05 ± 0.83	98.56 ± 0.41	98.31 ± 0.64

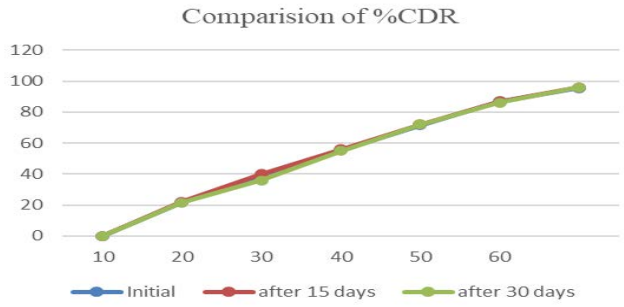

Fig. 11: Comparison study of % CDR

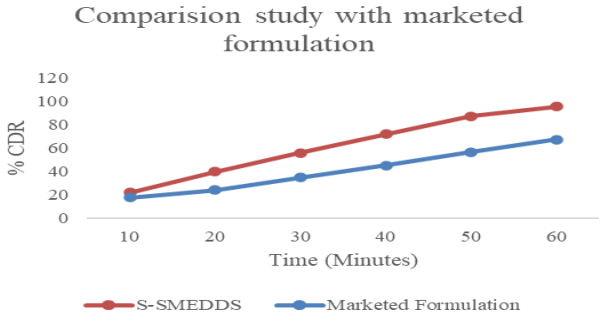

Fig. 12: Comparative study of final batch

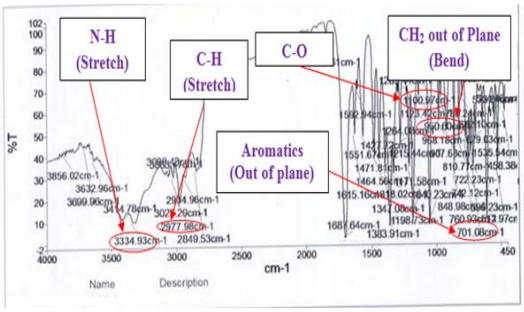

Fig. 13: FTIR spectra of L-SMEDDS after stability study

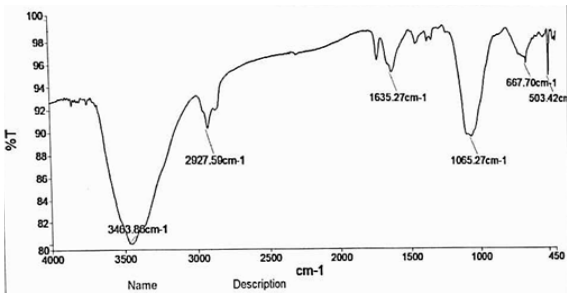

Fig. 14: FTIR spectra of S-SMEDDS after stability study